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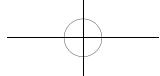
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Proceedings of Anticancer Research

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Analysis of the Clinical Value of Immunohistochemical Testing in the Pathological Diagnosis of Breast Cancer

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Abstract: *Objective:* To investigate the clinical value of immunohistochemistry (IHC) detection in the pathological diagnosis of breast cancer. *Methods:* Eighty breast cancer patients admitted to Baoding No. 1 Central Hospital from June 2022 to June 2023 were selected as study subjects. The samples were divided into a positive group (40 cases) and a negative group (40 cases) according to ER and PR test results. Immunohistochemistry was performed on all patients to compare the differences between the two groups in C-erbB-2 positive expression and axillary lymph node metastasis. *Results:* The positive expression rate of C-erbB-2 in the positive group (35.00%) was significantly lower than that in the negative group (80.00%), with a highly significant difference ($P < 0.001$). The axillary lymph node metastasis rate in the positive group (40.00%) was significantly lower than that in the negative group (75.00%), with a significant difference ($P < 0.05$). *Conclusion:* Immunohistochemical detection in breast cancer pathology enhances diagnostic accuracy, predicts prognosis, and supports personalized treatment by identifying ER, PR, and C-erbB-2. It is worth being widely adopted in clinical practice.

Keywords: Breast cancer; Pathological diagnosis; Immunohistochemical detection

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1. Introduction

Breast cancer, as a malignant tumor in general surgery, has a very high incidence rate, particularly affecting women, with more than 90% of breast cancer cases occurring in female patients. This malignant tumor originates from the epithelial tissues of the mammary glands, which not only threatens women's health but also directly affects their quality of life ^[1]. Regarding the prognosis of breast cancer, the current research consensus indicates that early diagnosis and treatment often result in a better prognosis, higher survival rate, and more optimistic quality of life. However, once the disease progresses to an advanced stage, it becomes significantly more difficult to treat, and the prognosis is often less favorable.

The specific challenge of advanced breast cancer lies in the extensive metastasis of cancer cells, with lymphatic metastasis being the most common pathway. If not controlled in time, cancer cells can invade the local

lymphatic system, such as the thoracic lymph nodes, and may even migrate to various organs throughout the body, posing a serious threat to the patient's health and even life ^[2]. Therefore, early screening and accurate diagnosis of breast cancer, as well as timely and effective treatment strategies, are of great significance for improving the prognosis of patients.

At present, the level of medical technology in China is constantly improving, and immunohistochemistry (IHC) testing has been widely used in clinical diagnosis due to its significant advantages, such as high diagnostic accuracy and specificity. Immunohistochemistry is a powerful biochemical technique used to detect specific protein molecules in tissue sections. The technique relies on antibodies to identify and label target proteins and subsequently visualize these molecules through a chromogenic reaction, enabling the study of localization and expression patterns of biomolecules at the tissue level. This technique is effective in providing accurate diagnostic results in the early stages of disease and can accurately classify different types of disease, thus providing a scientific basis for subsequent personalized treatment.

Through immunohistochemistry testing, it is possible to improve the accuracy of disease management, shorten the diagnostic cycle, enhance treatment efficiency, and win valuable treatment time for patients. This positively affects the improvement of patients' quality of life and survival rate ^[3]. Based on this, the purpose of this paper is to discuss the clinical application value of immunohistochemistry in the pathological diagnosis of breast cancer, and to provide a more scientific and efficient method for the diagnosis and treatment of breast cancer.

2. Materials and methods

2.1. General information

Between June 2022 and June 2023, 80 cases of breast cancer patients admitted to Baoding No. 1 Central Hospital were selected as study subjects. Inclusion criteria: (1) Patients were diagnosed with breast cancer by pathological examination ^[4], and the pathological data were complete; (2) All participants voluntarily joined the study and understood the content of the study; (3) The study was approved by the Ethics Committee of the hospital. Exclusion criteria: (1) The presence of other serious diseases or pathological states that affect the diagnosis and treatment decisions of breast cancer; (2) Those with severe organic diseases, abnormal liver and kidney functions; (3) Those with severe cognitive impairment that prevented them from collaborating in the study; (4) Those who were involved in specific treatments that may affect the results of immunohistochemistry detection, such as immunotherapy, radiotherapy, chemotherapy.

2.2. Methods

Breast cancer was diagnosed using consistent immunohistochemistry methods in both groups, utilizing archived hospital wax slice samples. After selecting the samples, 4-micron sections were prepared. The steps are as follows: (1) Fix the lesion tissue in 10% formalin to maintain cell structure; (2) Treat the specimen with paraffin to complete the embedding; (3) Use a microtome to create 4-micron continuous sections; (4) Apply the ABC method and HE staining, followed by DAB color development and hematoxylin re-staining for a comprehensive observation of the pathological characteristics of the samples.

2.3. Observation indexes

- (1) C-erbB-2 positive expression rate: According to standard guidelines, the presence of positive cells below 10% was judged as negative, while a proportion of positive cells reaching or exceeding 10% was confirmed as positive C-erbB-2 expression. The C-erbB-2 positive expression rate between the two groups of samples was compared to reveal differences in physiological or pathological characteristics.

- (2) Assessment of axillary lymph node metastatic status: The axillary lymph node metastasis status of the two groups of patients was compared by analyzing the size of the metastasis, the degree of damage to the lymph node structure, and the number of metastases. This quantification of metastasis between different groups helps to understand the extent and severity of metastasis in-depth and provides a scientific basis for clinical decision-making.

2.4. Statistical methods

SPSS 26.0 software was used to process the data analysis, and data were expressed as either [*n* (%)] or mean \pm standard deviation (SD), and compared using the *t* or chi-squared test. When the *P* value was less than 0.05, it indicated that the observed differences were statistically significant.

3. Results

3.1. General information

As shown in **Table 1**, the general information of patients in the two groups (including gender, average duration of disease, and average age) was compared with *P* values of 0.369, 0.398, and 0.446, respectively, which was greater than 0.05, hence the groups were comparable.

Table 1. Comparison of general information of patients in two groups

Group	Gender		Average duration of illness (mean \pm SD, months)	Average age (mean \pm SD, years)
	Male	Female		
Control group (<i>n</i> = 40)	20	20	1.81 \pm 0.53	54.37 \pm 10.67
Observation group (<i>n</i> = 40)	24	16	1.90 \pm 0.41	56.17 \pm 10.34
χ^2 / <i>t</i> -value	0.808		0.850	0.766
<i>P</i> -value	0.369		0.398	0.446

3.2. C-erbB-2 positive expression rate

The rate of C-erbB-2 positive expression in the positive group (14/40, 35.00%) was significantly lower than that in the negative group (32/40, 80.00%), and the difference showed a highly significant relationship ($\chi^2 = 16.573$, *P* < 0.001), as shown in **Table 2**.

Table 2. Comparison of C-erbB-2 positive expression rate between the two groups of patients [*n* (%)]

Group	C-erbB-2 positive	C-erbB-2 negative
Negative group (<i>n</i> = 40)	32 (80.00%)	8 (20.00%)
Positive group (<i>n</i> = 40)	14 (35.00%)	26 (65.00%)
χ^2 -value	16.573	
<i>P</i> -value	< 0.001	

3.3. Axillary lymph node metastasis

Table 3 shows that the axillary lymph node metastasis in the positive group (16/40, 40.00%) was significantly lower than that in the negative group (30/40, 75.00%), and the difference showed a significant correlation ($\chi^2 =$

10.026, $P < 0.002$).

Table 3. Comparison of axillary lymph node metastasis between the two groups of patients [n (%)]

Group	Transferred	Non-transferred
Negative group ($n = 40$)	30 (75.00%)	10 (25.00%)
Positive group ($n = 40$)	16 (40.00%)	24 (60.00%)
χ^2 -value	10.026	
P -value	0.002	

4. Discussion

The implementation of immunohistochemistry testing varies significantly across regions, healthcare institutions, departments, and even among testers. This variability is mainly due to the lack of uniform and specific protocols in the field. Such variability reflects a lack of standardization, leading to practical issues such as reagent incompatibility, inconsistent interpretation of results, and non-standardized procedures. Therefore, to ensure the efficacy and reliability of immunohistochemistry testing, standardization, normalization, and traceability should be core objectives. Key aspects such as quality control, tissue testing operations, and result evaluation should be strictly managed to ensure each step is properly executed ^[5].

When analyzing the antigenic expression of normal tissues, physiological characteristics and developmental stages should be considered. It is essential to recognize that fluctuations in the expression of the estrogen receptor (ER) and progesterone receptor (PR) during the normal cycle are largely influenced by the menstrual cycle. During HE staining, where any omission may impact the results, it is crucial to note that proteins within the cells of pathological tissues are often prone to cross-linking with aldehyde groups. This cross-linking can result in the closure of antigenic determinants, preventing antibodies from binding to them ^[6]. This process affects the specific binding of antibodies to the corresponding antigens and may cause important information to be overlooked in subsequent immunohistochemical tests, reducing the accuracy of the test results.

In clinical medicine, research on the *C-erbB-2* gene oncogene and its product P185 has primarily focused on breast cancer because C-erbB-2 plays a crucial role in the diagnosis and pathological analysis of breast cancer. Such studies allow early and accurate determination of the pathological type of breast cancer, which is essential for selecting therapeutic regimens, assessing disease prognosis, and developing personalized diagnostic and treatment strategies ^[7]. Protein P185, encoded by the *C-erbB-2* gene, is not only an important regulator of the proliferation, survival, and metastasis of breast cancer cells but also plays a key role in the cell cycle regulation of breast cancer. Therefore, studying this protein contributes to the early diagnosis of breast cancer and provides important clues to its biological mechanisms, offering new possibilities for the development of clinical medicine and the enhancement of cancer treatment effectiveness. It is widely recognized in clinical practice that the positive expression of the *C-erbB-2* gene protein product can serve as an independent indicator marker, providing an important reference for the prognosis of breast cancer treatment ^[8]. The expression level of this gene product significantly impacts breast cancer growth, progression, and sensitivity to specific treatments, making it an important biomarker for clinical screening and prediction of patient prognosis. By detecting and analyzing the expression of the *C-erbB-2* gene protein product, physicians can more accurately assess the response of different breast cancer patients to therapeutic strategies, as well as the expected disease progression and quality of patient survival.

In this study, the rate of positive C-erbB-2 expression was significantly lower in the positive group compared to the negative group. This finding suggests that immunohistochemistry may provide an important prognostic tool

in assessing breast cancer patients, and the significant variability in C-erbB-2 expression indicates that this marker may be valuable in understanding the behavior of cancer cells and the variability in patient response to treatment. Therefore, by quantifying the expression of C-erbB-2 using immunohistochemistry, it is possible to more accurately assess the prognostic status of patients and help optimize treatment strategies, providing a scientific basis for the diagnosis and treatment of breast cancer.

The results of this study showed that the rate of axillary lymph node metastasis in the positive group was significantly lower than in the negative group. This finding points to an important inference: breast cancer patients with positive ER (estrogen receptor) and PR (progesterone receptor) have a relatively low risk of axillary lymph node metastasis. This suggests that the expression levels of ER and PR are not only closely related to the pathogenesis of breast cancer but also serve as effective diagnostic and prognostic assessment tools. By detecting ER and PR expression levels in patients, clinicians can more accurately predict the prognosis of patients. Relevant studies suggest that when the expression of ER and PR is positive in breast cancer patients, it usually indicates a more optimistic prognosis^[9]. This is mainly due to the positive effects of ER and PR, which can influence tumor growth and differentiation and promote cell cycle regulation, thus slowing down the aggressiveness and spread of tumors to a certain extent. On the contrary, if the expression of ER and PR is negative, it may indicate a higher degree of malignancy of breast cancer, and the prognosis of patients may be less satisfactory.

In conclusion, the application of immunohistochemistry in the pathological diagnosis of breast cancer not only improves diagnostic accuracy but also provides scientific guidance for the formulation of personalized treatment plans and anticipates the possible direction of the patient's condition. This approach is of great value in improving the therapeutic efficacy and prognostic management of breast cancer patients.

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References

- [1] Li J, Shi Z, Hou Y, et al., 2019, Clinical, Pathological and Immunohistochemical Basis of Ultrasound Features in Invasive Triple-Negative Breast Cancer. *Chinese Journal of Cancer*, 29(1): 37–44.
- [2] Wang L, Ren F, Fan Y, et al., 2023, Correlation Analysis of VEGF, ALDH1, BCRP Expression in Breast Cancer Tissues and Pathological Features of Breast Cancer. *Clinical Medical Engineering*, 30(5): 721–722.
- [3] Chen Y, Li H, Wang C, 2022, Diagnostic Efficacy of Ultrasound Elastography and Ultrasonography for Breast Cancer and Its Correlation with Immunohistochemical Parameters. *Journal of Clinical and Pathological Sciences*, 42(10): 2420–2426.
- [4] Oncology Committee of Chinese Association of Integrative Medicine, Integrative Medicine Committee of Beijing Breast Disease Prevention and Treatment Association, Beijing Integrative Medicine Chronic Disease Prevention and Control, et al., 2021, Consensus on Diagnosis and Treatment of Breast Cancer with Integration of Traditional and Western Medicine. *Chinese Journal of Frontiers of Medicinal Science (Electronic Edition)*, 13(7): 44–64.
- [5] Huang Q, Huang Y, Li M, et al., 2019, Analysis of the Correlation between High Expression of Vascular Endothelial

Growth Factor (VEGF) and Low Expression of Platelet-Responsive Protein 1 (TSP-1) and the Prognosis of Breast Cancer Patients. *Journal of Cellular and Molecular Immunology*, 35(9): 828–831.

- [6] Li W, Bai J, Xie X, et al., 2020, Effect of Neutrophil-to-Lymphocyte Ratio on Clinical Prognosis of Triple-Negative Breast Cancer and with Ki-67 Expression. *Modern Preventive Medicine*, 47(12): 2287–2291 + 2304.
- [7] Wei J, Xing X, Wang F, et al., 2022, Comparison of the Value of Immunocytochemical P16/Ki-67 Double Staining, P16INK4 α Single Staining and High-Risk Human Papillomavirus Test for Screening High-Grade Cervical Lesions. *Oncology Research and Clinics*, 34(3): 180–183.
- [8] Wang Z, Zhou H, Wu H, et al., 2020, Impact of Epidermal Growth Factor Receptor Protein and Clinicopathological Features on the Effect and Prognosis of Neoadjuvant Chemotherapy for Breast Cancer. *Hebei Medicine*, 42(3): 325–329.
- [9] Yu X, Qin H, 2018, Expression of ER, PR, C-erbB-2, P53, and Ki-67 in Breast Cancer Tissues and Correlation with Their Clinicopathological Features. *Journal of Practical Cancer*, 33(1): 39–42.

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The Value of MRI and CT in the Diagnosis of Retroperitoneal Tumours

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Abstract: *Objective:* This study aimed to investigate the effectiveness and value of MRI and CT in the diagnosis of retroperitoneal tumours. *Methods:* 60 patients with retroperitoneal tumours admitted to our hospital between July 2022 and March 2023 were selected as the study subjects. All of them received MRI and CT examinations. The detection of the two examination methods was compared and analyzed using the pathological findings as the standard. *Results:* The detection rate of MRI (58/60, 96.67%) was significantly higher than that of CT (50/60, 83.33%), and the difference was significant ($P = 0.015 < 0.05$). *Conclusion:* Both MRI and CT have important application values in the diagnosis of retroperitoneal tumours. MRI has advantages in observing soft tissue structures, nerve tissues, etc., and can provide more detailed anatomical structure information, which can help differentiate the retroperitoneal tumours and locate them accurately. CT, on the other hand, has unique advantages in observing the skeletal structure and the density of certain tumours, etc. It can quickly obtain comprehensive imaging information, which helps to determine the extent and invasion of the tumour.

Keywords: MRI; CT; Retroperitoneal tumour

Online publication: July 11, 2024

1. Introduction

Retroperitoneal tumours are tumours that originate from the posterior wall of the abdominal cavity or metastasize to the peritoneum and are classified into two types: benign and malignant. These tumours lack obvious clinical manifestations in the early stage, which often makes it difficult for doctors and patients to detect them in time. Due to the relatively low overall prevalence of retroperitoneal tumours, they are easily misunderstood as other diseases or overlooked, resulting in delayed diagnosis and treatment. In particular, some benign tumours may not cause symptoms for a long period of time, increasing the risk of being overlooked. Retroperitoneal tumours occur relatively infrequently, and this disease is not considered to be common in clinical practice; however, despite their low incidence, retroperitoneal tumours are unique due to the variety of tissue types they involve ^[1]. These tissues include benign ones, such as cystic tumours and adenomas, and malignant ones, such as sarcomas and carcinomas. The complexity of these tumours multiply the challenges

of diagnosis and treatment. Once a patient is diagnosed with a retroperitoneal tumour, his or her health is at serious risk. These tumours can cause compression of adjacent organs, blood vessels and nerves, resulting in pain, dysfunction and even life-threatening conditions. Due to the vast space of the retroperitoneum and the hidden location of the tumour, diagnosis often requires advanced imaging techniques. Therefore, retroperitoneal tumours not only pose a threat to the physical health of patients but may also have a significant impact on psychological and social functions. At this stage, surgical pathology is considered the most reliable method of tumour identification. Nonetheless, this process involves making an incision in the patient and confirming the type of tumour and, therefore, has certain operational drawbacks. Although effective, this method poses non-negligible challenges and risks, especially for the patient's body and postoperative recovery. Therefore, screening and diagnosis of retroperitoneal tumours is crucial, and the detection rate can be improved with the help of imaging techniques such as MRI and CT so that timely and effective therapeutic measures can be taken to reduce the deterioration of the condition and the risk of complications. In clinical practice, magnetic resonance imaging (MRI) and computed tomography (CT) are frequently used imaging tests. These two examination methods have numerous advantages in assessing the patient's condition, such as noninvasiveness, ease of operation, and reproducibility ^[2-3]. Through MRI and CT examinations, doctors can obtain detailed information about internal tissue structure, which helps to diagnose and formulate appropriate treatment plans accurately. The wide application of MRI and CT technologies provides important technical support for medical diagnosis and provides patients with more comprehensive and detailed medical services.

In clinical practice, Magnetic Resonance Imaging (MRI) is a non-invasive imaging method that produces three-dimensional images of the inside of the body with high resolution by utilizing a magnetic field and harmless radio waves. Compared to traditional X-rays and CT scans, MRI is able to show soft tissue structures such as the brain, spine and joints more clearly. This technique does not require radiation, so there is no risk of radiation to the patient and it is suitable for the diagnosis of certain specific conditions. On the other hand, Computed Tomography (CT) is an imaging method in which multiple X-ray images are taken at different angles and then reconstructed by computer into volumetric data with high resolution. CT scans have high imaging speed and accuracy and can be used to evaluate a variety of diseases, such as tumours, bone fractures, and cerebrovascular disease. Because of the fast-imaging speed of CT scanning, it is particularly important for diagnosing patients in emergency situations. Based on this, this study aims to investigate the effect and value of magnetic resonance imaging (MRI) and computed tomography (CT) in the diagnosis of retroperitoneal tumours and to promote the application and dissemination of imaging technology in clinical practice.

2. Data and methods

2.1. General information

The study subjects were 60 patients with retroperitoneal tumours between July 2022 and March 2023, of which 41 were male and 19 were female. The age range was from 32 to 58 years old, and the mean age was 48.36 ± 8.67 .

Inclusion criteria: (1) All participants presented with different degrees of symptoms such as abdominal mass, abdominal pain and abdominal distension; (2) Those who had a complete record of their medical history; (3) All participants were examined by MRI and CT, and the pathological findings were in line with the clinical diagnostic criteria for retroperitoneal tumours ^[4]; (4) All participants and their families had fully understood the content of the study and had signed an informed consent form.

Exclusion criteria: (1) Patients with other serious diseases or co-morbidities affecting the results of the study; (2) Patients with psychiatric and cognitive disorders, unable to communicate normally; (3) Women in

pregnancy; (4) Patients with contraindications to MRI or CT examination; (5) Patients with systemic disorders.

2.2. Methods

2.2.1. MRI detection

Before MRI detection, patients are required to take off metal objects, such as jewelry and watches, and put on medical clothing. The medical staff will ask if metal implants or other factors may affect the MRI detection. The patient is asked to lie down on a specialized test bed, ensuring that the body is aligned with the magnetic field and remains still. The medical staff will assist in adjusting the posture to ensure that a clear image is obtained. Once the test begins, the patient is wheeled into the MRI machine, which emits a magnetic field and harmless radio waves to obtain images of the inside of the body. During the scanning process, the medical staff may ask the patient to hold their breath or keep their body still to minimize image blurring. After the scan is completed, the physician analyzes and interprets the MRI images to assess the location, size, morphology, and other characteristics of the retroperitoneal tumour and compares them with pathology results to assist in clinical diagnosis and treatment decisions.

2.2.2. CT detection

Before CT detection, patients must remove metal objects from their bodies and put on medical garments. The medical staff will ask the patient if they have any metal implants or other factors that may affect the test. Next, the patient is asked to lie down on the CT test bed in the correct body position and remain still. The medical staff will help the patient adjust his or her posture to ensure that a clear image is obtained. Once the test begins, the CT machine rotates around the patient and emits X-rays to obtain tomographic images of different parts of the body. During the scan, the patient is required to remain still and not move. After the scan is completed, the doctor will analyze and interpret the CT images to assess the morphology, size, location and other characteristics of the tumour and make a comprehensive analysis with other test results.

2.2.3. Observation index

The results presented by two different imaging methods, MRI and CT, are compared.

2.3.4. Statistical methods

SPSS 26.0 software was used for statistical analysis of the data, and the count data were expressed as n (%), and the chi-square test was performed, with $P < 0.05$ indicating statistical significance.

3. Results

3.1. Tumour types of study subjects

After MRI, CT and expert consultation, the tumour types of 60 cases of retroperitoneum are shown in **Table 1**.

Table 1 Tumour types of patients

Type	Neurogenic tumour	Lymphoma	Malignant fibrous histiocytoma	Lymphangioma	Mature teratoma	Other
Number of cases (n)	20	15	13	4	3	5

3.2. MRI and CT detection results

The MRI detection rate (58/60, 96.67%) was significantly higher than the CT detection rate (50/60, 83.33%),

and the difference was statistically significant ($\chi^2 = 5.926$, $P = 0.015 < 0.05$). Refer to **Table 2**.

Table 2 Comparison of MRI and CT detection results

Detection method	Detected	Not detected	Detection rate (%)
MRI ($n = 60$)	58	2	58 (96.67%)
CT ($n = 60$)	50	10	50 (83.33%)
χ^2	-	-	5.926
P	-	-	0.015

4. Conclusion

MRI and CT scanning, as common clinical imaging diagnostic techniques, play a good application in determining the location of tumours, observing tumour characteristics, analyzing the type and extent of tumours, and judging the characteristics of tumour growth [5–6]. These two imaging techniques can provide accurate tumour localization information, help doctors understand the morphology and size of the tumour and enable detailed observation and analysis of the tissue structure and blood supply of the tumour. Through MRI and CT scans, doctors can better assess the degree of malignancy of the tumour, thus providing an important basis for the development of personalized treatment plans. At the same time, these imaging techniques can also track the dynamic changes in tumour growth, which can help monitor the effectiveness of treatment and predict the development trend of the tumour.

Computed tomography (CT scan) is an imaging technique that generates detailed cross-sectional images by rotating X-rays through different parts of the body. CT scan uses X-rays in different directions to obtain density information of tissues in the body and then generates high-resolution images through computer processing. CT images can show the bone structure, soft tissues and blood vessels and are important diagnostic tools in detecting tumours, bone fractures, hemorrhages and other lesions. Bleeding and other lesions have important diagnostic significance. In CT diagnosis of retroperitoneal tumours, it is necessary to pay close attention to the characteristics and location of the lesions. Benign tumours usually show clear boundaries, uniform density, regular morphology and obvious fat gaps in the image, on the contrary, malignant tumours have blurred boundaries, are difficult to distinguish from the surrounding organs and tissues, and sometimes appear to be displaced by organ compression. In addition, benign tumours usually do not invade the surrounding organs, while malignant tumours may cause some organs to be invaded. Localization signs are also important for diagnosis; compression and displacement of organs and whether the bowel is located behind the tumour can provide important information. A comprehensive analysis of the characteristics and location of the lesion helps the doctor to accurately diagnose the nature of the tumour and the patient's condition.

Magnetic Resonance Imaging (MRI) is a medical imaging technique that uses a magnetic field and radio waves to produce detailed images of the body's internal structures. Using a powerful magnetic field and harmless radio waves, MRI can produce high-resolution images showing the structure and lesions of various tissues in the body (e.g., brain, spine, joints, abdomen, etc.). MRI images have excellent contrast and can clearly show soft tissue structures to help doctors diagnose tumours, injuries, and diseases. Compared with CT scanning, MRI technology can more clearly show the fluid, hematoma and oedema within the tumour, etc. It also has higher imaging resolution, which is conducive to a finer examination of the tissue components and thus more accurately determines the nature of the retroperitoneal tumor. In addition, MRI also has the function of evaluating the clinical staging and prognosis of retroperitoneal tumours, which helps to provide comprehensive

diagnostic information through detailed observation of tumour morphology, lymph node metastasis, etc., and provides important support for the formulation of treatment plans and prognosis assessment of patients. MRI provides richer histological information, which helps doctors to have a more comprehensive understanding of the biological characteristics of the tumour, and provides a more reliable basis for the formulation of treatment plans for patients. Therefore, when diagnosing retroperitoneal tumours, combining the features and advantages of MRI can improve the diagnostic accuracy and therapeutic effect of the condition. Various studies have shown that combining both options can achieve better diagnostic results ^[7-8].

In this study, the detection rate of MRI scan was significantly higher than that of CT scan, and the CT image was significant in describing the characteristic tumour components, which were more clearly demonstrated by the influence of low-density fat. In clinical diagnosis, the presence of fat is of positive significance in determining the extent of the lesion ^[9]. In CT scans, patients with lesions with clear margins and distinct segregation sometimes observe fat signals, which may show weak and heterogeneous enhancement, while the degree of tumour differentiation may affect the results of CT display ^[10]. CT flat scans show that the borders of the tumour and the peritoneal mass are clearly visible and regular in shape. However, when CT enhancement scans were performed, the margins of the tumour were blurred and the retroperitoneum showed a soft tissue mass with irregular morphology accompanied by large areas of necrosis. This irregular feature may suggest the malignancy of the lesion, and surgical pathology can be used to ultimately confirm the diagnosis and assess the nature and progression of the tumour ^[11].

In summary, the organic combination of MRI and CT can provide patients with more accurate diagnostic results, and provide more powerful support for the development of treatment plans and prognosis assessment. Therefore, for the diagnosis of retroperitoneal tumours, the combination of MRI and CT imaging has important application value, which can help to improve diagnostic accuracy and therapeutic effect and bring better clinical prognosis and quality of life for patients.

Disclosure statement

The author declares no conflict of interest.

References

- [1] Yang F, 2020, A Retrospective Study of Imaging Diagnosis, Surgery and Postoperative Complications of Retroperitoneal Tumours, thesis, Southern Medical University.
- [2] Liu Y, 2020, Discussion on the Application Value of Ultrasound in the Differential Diagnosis of Pelvic Mass and Pelvic Retroperitoneal Tumour. *Imaging Research and Medical Application*, 4(19): 242–243.
- [3] Huang R, 2020, Analysis of the Clinical Value of CT and MR in the Diagnosis of Retroperitoneal Primary Tumours. *Imaging Research and Medical Application*, 4(9): 149–150.
- [4] Wang J, 2022, Pathologic Diagnosis and Differential Diagnosis of Retroperitoneal Tumours. *Surgical Theory and Practice*, 27(6): 500–505.
- [5] Qian L, Lu C, Ge Y, 2019, Application of Specific CT Signs in the Differential Diagnosis of Primary Retroperitoneal Tumours. *Imaging Research and Medical Application*, 3(17): 115–116.
- [6] Jia X, Li W, 2019, CT Diagnosis of Primary Retroperitoneal Tumour. *Electronic Journal of Cardiovascular Diseases of Integrative Medicine and Western Medicine*, 7(24): 91.
- [7] Zhou X, Li H, Deng X, 2019, CT Diagnosis and Differential Diagnosis of Primary Common Abdominopelvic and Retroperitoneal Tumours. *Imaging Research and Medical Application*, 3(15): 41–43.

- [8] Feng Y, Zhang W, Li Q, et al., 2020, Study on the Diagnostic Value of Primary Retroperitoneal Tumours by MR-DWI and MSCT. *Journal of Hebei Medical University*, 41(11): 1326–1330.
- [9] Jia H, 2019, Application Value of Multislice Spiral CT in the Diagnosis and Differential Diagnosis of Primary Retroperitoneal Tumours. *Systemic Medicine*, 4(6): 111–113.
- [10] Lin J, Diao D, Liao W, et al., 2022, CT Three-Dimensional Reconstruction and 3D Printing Technology in Complex Retroperitoneal Tumour Surgery. *Colorectal and Anal Surgery*, 28(2): 119–123.
- [11] Wang Z, Ren W, Zhang H, et al., 2022, CT-Guided Iodine-125 Particle Implantation for Retroperitoneal Tumours. *Hebei Medicine*, 44(3): 388–391.

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Targeted Therapy of CEA-CAR-NK Cells Against Colorectal Cancer Cells

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Abstract: *Objective:* Investigate the cytotoxic effect of CAR-NK cells targeting CEA on colorectal cancer cells with positive CEA expression. *Methods:* The mRNA and protein levels of CEA in different CRC cell lines were detected by qRT-PCR and Western blot analysis. Lentiviral transduction was used to construct CAR-NK cells and empty vector CON-NK cells targeting CEA. Fluorescence microscopy and WB were used to determine whether the cells successfully constructed and expressed CAR structures. The effector NK cells were co-cultured with target cells, and the levels of LDH, IFN- γ , and GM-CSF were detected. The killing rate of effector cells was calculated, and the release of cytokines during the killing of target cells by different effector cells was compared. *Results:* The expression level of CEA in colorectal cancer patients was significantly higher than that in normal samples and other tumor samples, and the prognosis survival time of patients with high CEA expression was lower than that of CRC patients with low or no CEA expression ($P < 0.05$). The CEA expression of the HT29 cell line was significantly higher than that of the SW1116 cell line at both the mRNA and protein levels. CEA-CAR-NK92 cells and CON-NK92 cells expressed green fluorescence under a microscope, and WB results showed that CEA-CAR-NK92 cells successfully expressed the CAR structure. Compared with CON-NK92 cells and NK92 cells, CEA-CAR-NK92 cells effectively killed HT29 cells ($P < 0.05$). CEA-CAR-NK92 cells secreted a large amount of IFN- γ and GM-CSF during the killing of HT29 cells, while the cytokine secretion of CON-NK92 cells and NK92 cells was not significant ($P < 0.05$). *Conclusion:* CAR-NK92 cells targeting CEA can effectively kill CEA-positive colorectal cancer cells.

Keywords: Colorectal cancer; Chimeric antigen receptor; Natural killer cells; Carcinoembryonic antigen; Immunotherapy

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1. Introduction

Colorectal cancer (CRC) is the third most common cancer globally, with approximately 150,000 new cases diagnosed each year^[1]. It is the second leading cause of cancer-related deaths, following lung cancer. Therefore, finding new treatment methods has become one of the key issues in the field of colorectal cancer treatment.

Natural killer (NK) cells are a type of lymphocyte with innate cytotoxic activity, capable of recognizing and killing tumor cells, and are an important part of the immune system^[2]. As an “off-the-shelf” cell, NK cells can be expanded in vitro and used for treatment. During the killing process, the cytokines and growth factors

released by NK cells do not cause severe adverse reactions, making them a safer option^[3]. CAR-NK cells not only rely on antigen targets but also exert their effects through classical cytotoxicity generated by antibody-dependent cellular cytotoxicity (ADCC) and cytokine pathways, thus reducing the risk of relapse due to antigen loss. Based on the various advantages of NK cells, CAR-NK cell therapy is expected to become a new research direction following chimeric antigen receptor (CAR) T-cell therapy.

Carcinoembryonic antigen (CEA) is an antigenic glycoprotein that is present in low levels in the blood circulation of healthy adults but is overexpressed in about 80% of colorectal cancers. Therefore, CEA could serve as a therapeutic target for NK cells, enhancing their cytotoxic effects against colorectal cancer cells and providing a new therapeutic strategy. This study investigates the cytotoxic effects of CAR-NK cells *in vitro*, laying the groundwork for future research on the *in vivo* killing mechanisms of CAR-NK cells and evaluating their potential application in clinical treatment.

2. Materials and methods

2.1. Materials

2.1.1. Cell lines

Colorectal cancer cell lines HT29 and SW1116 were purchased from Zhongqiao Xinzhou, NK92 cells were obtained from the ATCC cell bank in the USA, and 293T cells were preserved by the Marshall Experimental Center.

2.1.2. Major reagents and consumables

- (1) The construction and identification of the lentiviral vector were completed by Henan Jiurui Biotechnology Co., Ltd.
- (2) WB antibodies, including rabbit CEA antibody, mouse GAPDH antibody, sheep anti-mouse secondary antibody, and goat anti-mouse secondary antibody, were all purchased from Wuhan Sanying Biotechnology Co., Ltd.
- (3) The reverse transcription kit was purchased from Suzhou JinAn Protein Technology Co., Ltd.
- (4) The lactate dehydrogenase detection kit was purchased from Beyotime Biotechnology Co., Ltd.
- (5) Human GM-CSF and IFN- γ ELISA detection kits were purchased from Hangzhou Lianke Biotechnology Co., Ltd.

2.2. Cell line culture

HT29, SW1116, NK92, and 293T cell lines were cultured in RPMI-1640 medium containing 10% fetal bovine serum and 1% penicillin-streptomycin. The cells were incubated at 37°C in a humidified atmosphere with 5% CO₂.

2.3. Construction of CEA-targeted CAR-NK92 cells

293T cells were seeded in a 6-well plate and cultured until the cell density reached approximately 70%. Transfection plasmids and packaging plasmids (psPAX2 and pMS2.G) were added to jetPRIME buffer and then introduced into the 6-well plate containing the cells. After 48 hours of culture, fluorescence was observed, and the cell supernatant was collected. The culture medium was replenished, and the cells were cultured for an additional 72 hours. The viral supernatant was then collected. NK92 cells were transduced with the viral supernatant and polybrene. After culturing, the cells were centrifuged, the supernatant was discarded, and a complete medium with 5 μ g/mL puromycin was added to select and screen the cells. Finally, stable CEA-CAR-

NK92 and CON-NK92 cells were obtained.

2.4. Western blot

Proteins were extracted from HT29 and SW1116 cells. The marker and protein samples were loaded into the gel wells and run at a constant voltage of 120 V for 1.5 hours. After electrophoresis, the proteins were transferred to an NC membrane using a wet transfer method at a constant current of 400 mA for 45 minutes. The membrane was then blocked, incubated with primary and secondary antibodies, and placed in an exposure instrument to observe the development results.

2.5. Quantitative fluorescent PCR

RNA was extracted from colorectal cancer cells HT29 and SW1116 using the TRIzol method. Genomic DNA was removed and cDNA was obtained using ChamQ Universal SYBR qPCR Master Mix (gDNA Purge). The reaction system was prepared as follows: Mix 5 μ L, Primer1 (10 μ M) 0.5 μ L, Primer2 (10 μ M) 0.5 μ L, cDNA 4 μ L. The reaction was then performed on a qPCR instrument.

2.6. Lactate dehydrogenase (LDH) release assay

HT29 and SW1116 cells were seeded into 96-well cell culture plates. Effector-to-target ratios of 10:1, 5:1, and 1:1 were set up, and the cells were co-cultured for 24 h. After 23 h of culture, LDH release reagent was added to the “maximum enzyme activity control well” and incubated for 1 hour. The supernatant was collected by centrifugation, LDH detection working solution was added, and absorbance was measured at 490 nm using a microplate reader.

2.7. Enzyme-linked immunosorbent assay

HT29 and SW1116 cells were seeded in 6-well plates. CEA-CAR-NK92, CON-NK92, and NK92 cells were added to each well and co-cultured for 24 hours. The supernatant was collected by centrifugation. Enzyme-linked immunosorbent assay (ELISA) kits for human GM-CSF and human interferon-gamma (IFN- γ) were used for the assay, and OD values were measured at a wavelength of 450 nm using a microplate reader.

2.8. Statistical analysis

Statistical analysis was performed using SPSS 26.0 software, and graphs were generated using Adobe Photoshop and GraphPad Prism 9.0 software. Measurement data are expressed as mean \pm standard deviation (SD). Depending on the data type, *t*-tests or one-way analysis of variance (ANOVA) were used for comparisons. A *P*-value of < 0.05 was considered statistically significant.

3. Results

3.1. Differences in CEA expression among different colorectal cancer cell lines

HT29 and SW1116 cell lines were selected for this study. Quantitative analysis of CEA at the mRNA level was performed using qRT-PCR. The results showed that the expression level of CEA in the HT29 cell line was significantly higher than that in the SW1116 cell line, with a statistically significant difference ($P < 0.001$, **Figure 1A**). To further verify this result, Western Blot analysis was conducted to detect CEA at the protein level. The results confirmed that the HT29 cell line expresses CEA protein, whereas the SW1116 cell line does not express CEA protein (**Figure 1B**).

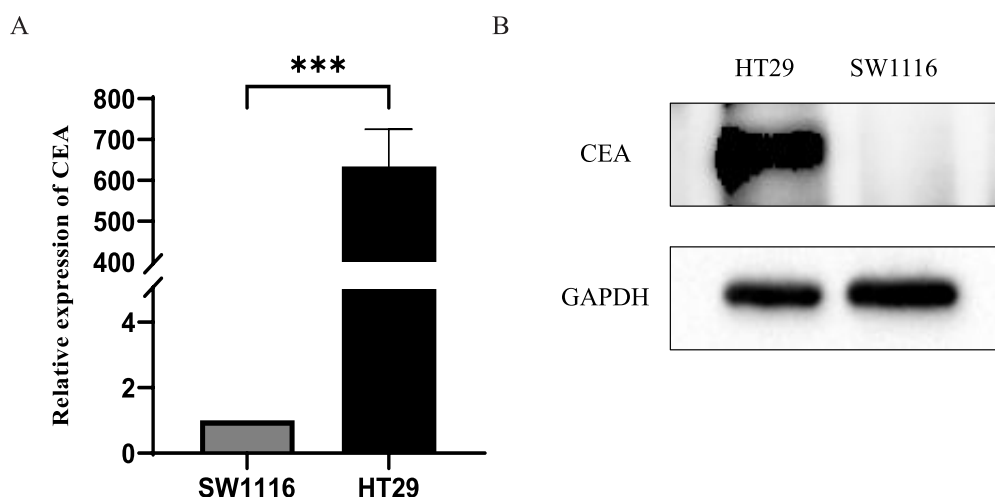


Figure 1. Expression of CEA in Two Cell Lines. (A) Relative expression levels of CEA in colorectal cancer cell lines HT29 and SW1116 as detected by qRT-PCR; (B) Expression of CEA protein in HT29 and SW1116 cell lines as detected by Western blot. *** $P < 0.001$, $n = 3$

3.2. Construction and identification of CEA-CAR-NK92 cells

3.2.1. Identification of CEA-CAR-NK92 cells

NK92 cells were transduced with either CEA-CAR lentivirus or GFP empty vector lentivirus, resulting in CEA-CAR-NK92 cells and CON-NK92 cells. After a period of selection, cells were observed under a confocal microscope through the GFP channel. The presence of green fluorescence indicated successful transfection (Figure 2).

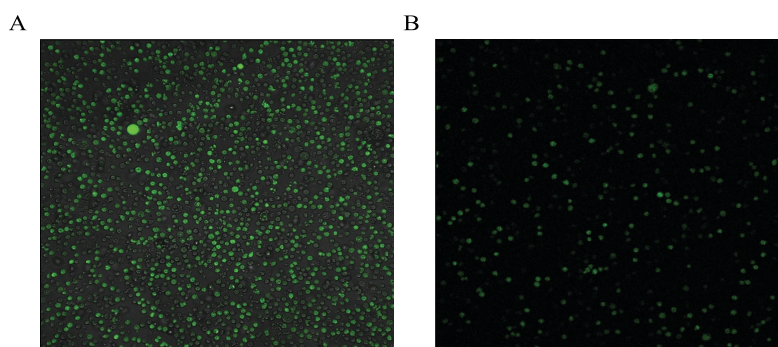


Figure 2. Results of lentiviral transduction of NK92 cells. (A) Observation of CEA-CAR-NK92 cells under a confocal microscope (10×); (B) Observation of CON-NK92 cells under a confocal microscope (10×)

3.2.2. Expression of CD3ζ in NK92 cells

Western blot analysis showed high expression of CD3ζ protein in CEA-CAR-NK92 cells, whereas CON-NK92 and NK92 cells did not express CD3ζ protein (Figure 3). This result confirms that CEA-CAR-NK92 cells were successfully transfected and expressed the CEA-CAR structural protein.

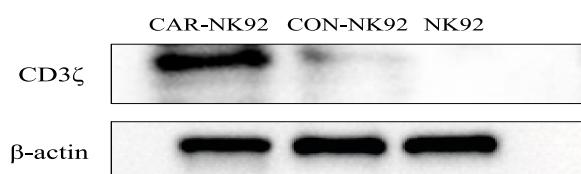


Figure 3. Western blot detection of CD3ζ expression in CEA-CAR-NK92, CON-NK92, and NK92 cell lines

3.3. Cytotoxic effect of CEA-CAR-NK92 cells on colorectal cancer cells

3.3.1. LDH assay for detecting the cytotoxic ability of CEA-CAR-NK92 cells

CEA-CAR-NK92, CON-NK92, and NK92 cells were co-cultured with target cells for 24 hours, and the LDH content in the supernatant was measured. The results showed that, compared to the two control groups (CON-NK92 and NK92), CEA-CAR-NK92 cells exhibited significant cytotoxicity against CEA-positive HT29 cells. Additionally, the cytotoxic effect of CEA-CAR-NK92 cells increased with a higher effector-to-target ratio, with statistically significant differences observed at effector-to-target ratios of 10:1 and 5:1 ($P < 0.0001$), and at a ratio of 1:1 ($P < 0.01$, **Figure 4A**). However, at different effector-to-target ratios, there was no significant difference in the cytotoxic effects of the three types of effector cells on CEA-negative SW1116 cells ($P > 0.05$, **Figure 4B**). This indicates that CEA-CAR-NK92 cells specifically target and kill colorectal cancer cells via the CEA target and that increasing the proportion of CEA-CAR-NK92 cells can enhance the cytotoxic effect.

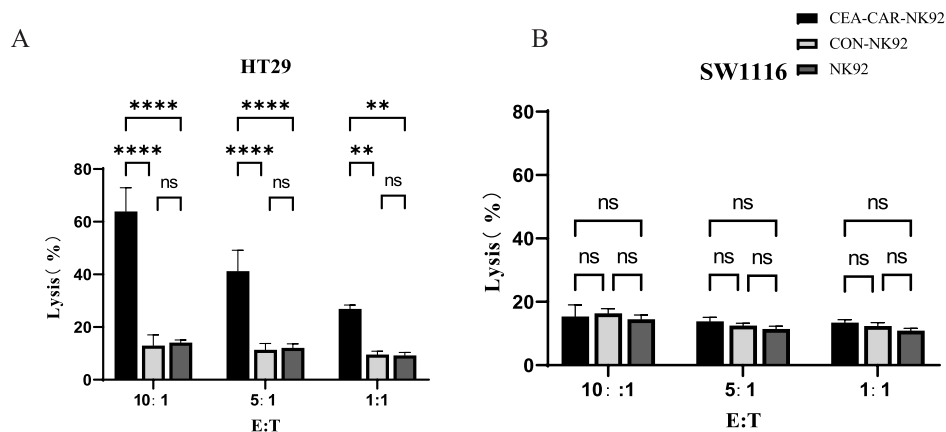


Figure 4. LDH assay for detecting the cytotoxic ability of CEA-CAR-NK92 cells. **(A)** Cytotoxic efficiency against CEA-positive HT29 cells; **(B)** Cytotoxic efficiency against CEA-negative SW1116 cells. ** $P < 0.01$, **** $P < 0.0001$, $n = 3$

3.3.2. Cytokine release by CEA-CAR-NK92 cells detected by ELISA

Different effector cells were co-cultured with HT29 cells at an effector-to-target ratio of 5:1 for 24 hours. The cytokine secretion in the supernatant was measured using ELISA. As shown in **Figure 5**, the release of IFN- γ and GM-CSF by CEA-CAR-NK92 cells was significantly higher than that by CON-NK92 and NK92 cells, with a statistically significant difference ($P < 0.0001$). This indicates that CEA-CAR-NK92 cells may enhance their antitumor effect through cytokine release.

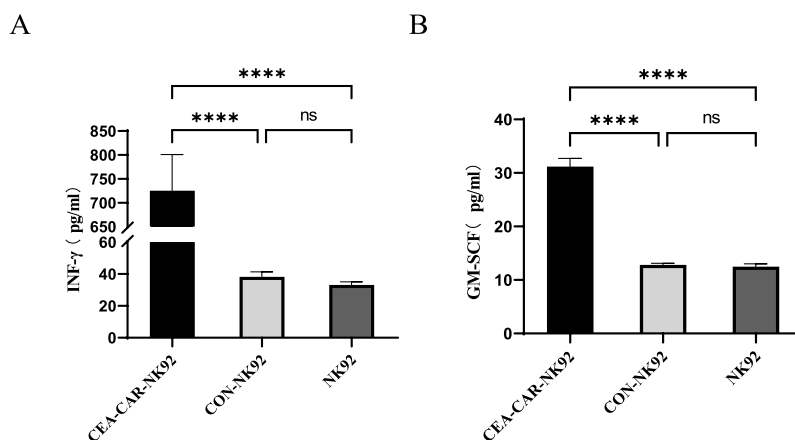


Figure 5. Cytokine release detected by ELISA. **(A)** Measurement of cytokine IFN- γ ; **(B)** Measurement of cytokine GM-CSF. **** $P < 0.0001$, $n = 3$

4. Discussion

In recent years, the incidence and mortality rates of CRC have been increasing in China. In 2020, newly diagnosed CRC cases in China accounted for 28.8% of the global total, and CRC-related deaths accounted for 30.6% of the global total ^[4]. This places a significant burden on the healthcare system. Therefore, this study aimed to explore new treatment methods for CRC through immunotherapy, bringing hope to CRC patients.

The clinical activity of NK cells is currently limited by tumor immune evasion and low NK cell activity ^[5]. Thus, CAR gene reprogramming is considered a strategy to enhance the antitumor efficacy of NK cells. To date, most CAR-NK studies, including many clinical trials, have been conducted using the NK92 cell line. NK92 cells lack the ability to proliferate *in vivo*, and the infused cells are cleared after seven days. While multiple infusions are required to ensure therapeutic efficacy, this approach has a higher safety profile ^[6].

In this study, a second-generation chimeric antigen receptor (CAR) structure was used to successfully construct a CEA-CAR-NK92 cell line via lentiviral transduction, which could stably express the CAR structure. Compared to CON-NK92 cells (empty vector-transduced) and unmodified NK92 cells, CEA-CAR-NK92 cells effectively eliminated CRC cells *in vitro*. However, for CEA-negative SW1116 cells, the killing effect was not significant among the three types of effector cells, indicating that NK cells primarily exert their cytotoxic effects through specific targeting of the CEA antigen. Furthermore, co-culturing CEA-CAR-NK92 cells with HT29 cells resulted in increased levels of cytokines such as IFN- γ and GM-CSF in the supernatant. This finding aligns with other studies, suggesting that CEA-CAR-NK cells can indirectly enhance immune function by secreting cytokines in addition to directly killing target cells.

Other studies have used electroporation to construct NK cells that can transiently express CAR, significantly enhancing the tumor lysis activity of NK cells against CRC cell lines *in vitro* ^[7]. This study chose lentiviral vectors to integrate the CAR gene into the NK cell genome, enabling permanent CAR expression and reducing the need for multiple infusions due to short CAR expression duration.

This study successfully constructed second-generation CAR-NK cells and demonstrated their specific targeting and killing ability against colorectal cancer cells. Our results suggest that CEA could be a potential target for CAR-NK therapy in CRC. However, this study has limitations, such as the absence of animal model experiments, resulting in a lack of data on the systemic responses of CAR-NK cells *in vivo*. Future research should continue to address these limitations and lay the groundwork for the clinical application of CEA-CAR-NK cells in CRC patients.

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- (2) Henan Province 2022 Science and Technology Development Plan "Study on Pyroglutamate Targeting DJ-1 to Trigger ROS-Induced Cell Death and Protective Autophagy in Pancreatic Cancer" (Grant No. 222102310725)

Disclosure statement

The authors declare no conflict of interest.

References

- [1] Yan H, Talty R, Johnson CH, 2023, Targeting Ferroptosis to Treat Colorectal Cancer. *Trends Cell Biol*, 33(3): 185–188. <https://doi.org/10.1016/j.tcb.2022.11.003>
- [2] Laskowski TJ, Biederstädt A, Rezvani K, 2022, Natural Killer Cells in Antitumour Adoptive Cell Immunotherapy. *Nat Rev Cancer*, 22(10): 557–575. <https://doi.org/10.1038/s41568-022-00491-0>
- [3] Klingemann H, 2014, Are Natural Killer Cells Superior CAR Drivers? *Oncoimmunology*, 3: e28147. <https://doi.org/10.4161/onci.28147>
- [4] Sung H, Ferlay J, Siegel RL, et al., 2021, Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin*, 71(3): 209–249. <https://doi.org/10.3322/caac.21660>
- [5] Hu W, Wang G, Huang D, et al., 2019, Cancer Immunotherapy Based on Natural Killer Cells: Current Progress and New Opportunities. *Front Immunol*, 10: 1205. <https://doi.org/10.3389/fimmu.2019.01205>
- [6] Wang W, Jiang J, Wu C, 2020, CAR-NK for Tumor Immunotherapy: Clinical Transformation and Future Prospects. *Cancer Lett*, 472: 175–180. <https://doi.org/10.1016/j.canlet.2019.11.033>
- [7] Xiao L, Cen D, Gan H, et al., 2019, Adoptive Transfer of NKG2D CAR mRNA-Engineered Natural Killer Cells in Colorectal Cancer Patients. *Mol Ther*, 27(6): 1114–1125. <https://doi.org/10.1016/j.ymthe.2019.03.011>

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The Role of *ZNF207* in Liver Hepatocellular Carcinoma: Expression Analysis and Prognostic Implications

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Abstract: *Objective:* To analyze the expression and clinical significance of the zinc finger protein *ZNF207* gene in liver hepatocellular carcinoma (LIHC) based on The Cancer Genome Atlas (TCGA) database. *Methods:* The mRNA sequencing data of 371 cases of primary liver cancer, 50 cases of normal tissues, and 3 cases of recurrent liver cancer were downloaded from the TCGA database. The corresponding clinical information of the 371 cases of hepatocellular carcinoma was subsequently analyzed. The difference in *ZNF207* expression between normal and tumor tissues was analyzed using the UALCAN online database. The impact of *ZNF207* expression on survival prognosis was assessed using the Kaplan-Meier method in R software. The GO and KEGG pathways of *ZNF207* were analyzed. The Cox proportional hazards model was used to evaluate the prognostic factors of patients with LIHC. RT-qPCR was employed to verify the expression of *ZNF207* in LIHC cells. *Results:* *ZNF207* was highly expressed in LIHC tissues and HepG2 cells, with a significant difference ($P < 0.05$). Multivariate Cox regression analysis revealed that patients with high *ZNF207* expression had a significantly shorter overall survival time compared to those with low *ZNF207* expression (HR = 1.466, 95% CI: 1.011–2.126, $P < 0.05$). GO enrichment analysis suggested that *ZNF207* may influence the onset and progression of hepatocellular carcinoma by regulating mRNA splicing and mRNA transcription processing through the spliceosome. KEGG pathway enrichment analysis indicated that *ZNF207* might affect the onset and progression of hepatocellular carcinoma through mitophagy, mRNA surveillance, homologous recombination, spliceosome, and nuclear-cytoplasmic transport. *Conclusion:* The expression of *ZNF207* may be an independent predictor of the prognosis of patients with LIHC and could influence the development of hepatocellular carcinoma through various gene functions and pathways. It has the potential to serve as a novel molecular marker for predicting the prognosis of hepatocellular carcinoma.

Keywords: *ZNF207*; Hepatocellular carcinoma; TCGA database

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1. Introduction

Liver cancer is one of the most common types of malignant tumors, with various pathogenic factors contributing to its occurrence and development. Hepatocellular carcinoma (LIHC) is the most prevalent form of primary liver cancer^[1].

In countries with a high prevalence of hepatitis B virus, there is a significant progression from hepatitis to liver cirrhosis and eventually to liver cancer. The clinical symptoms and signs of liver cancer are often not obvious, with the disease typically being discovered at a late stage. Many patients with advanced liver cancer, who are less likely to undergo surgical resection, have limited treatment options and often resort to local or palliative treatments ^[2]. Early detection, diagnosis, and treatment are essential to reduce the mortality rate of liver cancer. Consequently, many researchers have conducted extensive studies to explore potential biomarkers of LIHC. Early diagnosis of hepatocellular carcinoma is crucial, and identifying these biomarkers provides a strong foundation for it.

ZNF207 is a protein-coding gene located on human chromosome 6p21.3 and is a member of the zinc finger protein family. *ZNF207* has been shown to regulate embryonic stem cell self-renewal and pluripotency ^[3]. Additionally, *ZNF207* can inhibit the immune microenvironment of hepatocellular carcinoma ^[4]. Zhou Chenghui *et al.* demonstrated that *ZNF207* is highly expressed in hepatocellular carcinoma ^[5]. However, due to the limited sample size and lack of ethnic diversity in studies of *ZNF207* expression, the clinical prognosis for LIHC patients remains uncertain. Furthermore, there is a lack of research on the function and pathway of *ZNF207* in hepatocellular carcinoma. This study aims to investigate the expression of *ZNF207* in LIHC, its clinical significance, and the function and pathways associated with *ZNF207*, using gene expression data from The Cancer Genome Atlas (TCGA) database and corresponding clinical information.

2. Materials and methods

2.1. Cell sample source and data download and collation

HepG-2 and Lo2 cells, obtained from the Shanghai Institute of Cell Biology, were used in this study at the Chinese Academy of Sciences. The RNA-Seq data of 50 normal tissues and 374 hepatocellular carcinoma tissues (including three patients with recurrent hepatocellular carcinoma) were retrieved and downloaded from The Cancer Genome Atlas (TCGA) database (<https://portal.gdc.cancer.gov/>), along with clinical data of 371 patients with hepatocellular carcinoma.

2.2. UALCAN database analysis

The UALCAN database (UACAN.path.uab.edu/home) integrates clinical information and transcriptional data from the TCGA database of various tumors to enable visual online analysis ^[6]. The UALCAN database was used to analyze the difference in *ZNF207* expression between normal and tumor tissues.

2.3. Survival analysis

Using the “ggplot2,” “ggpubr,” “survminer,” and “survival” R packages in the R language, a graph illustrating the relationship between the expression of *ZNF207* and total survival time (K-M curve) was created.

2.4. Human Protein Atlas database analysis

The Human Protein Atlas (HPA) is a database (<https://proteinsatlas.org/>) that integrates data from proteomics, transcriptomics, and systems biology to map tissues, cells, and organs. The protein expression data includes both tumor and normal tissues, and the survival curve of tumor patients can also be consulted ^[7]. The difference in *ZNF207* expression between normal tissues and LIHC tissues was investigated using the gene expression immunohistochemical staining map.

2.5. Relationship between ZNF207 expression level and clinical characteristics and survival status of LIHC

Clinical information and corresponding *ZNF207* expression data were removed for 2 patients with missing survival status. Using the “pheatmap” and “ggplot2” R packages in the R language, the elevation of *ZNF207* expression was plotted. This plot corresponds to the heatmap of clinical features and the dot plot of survival status.

2.6. Gene function enrichment analysis

Using Pearson correlation analysis, the most relevant gene set of *ZNF207* was selected (Pearson product-moment correlation coefficient $\text{cor} > 0.5$). The gene set was uploaded to The Database for Annotation, Visualization, and Integrated Discovery (DAVID, V2021). *Homo sapiens* was selected as the species, and the results of Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were obtained. The first five places with the largest *P*-values are shown as $P = -\text{Log}_{10}(P\text{Value})$.

2.7. The relationship between ZNF207 and clinical common immune checkpoints

Using the “Circlize” R package, chords representing the correlation degree between *ZNF207* and common tumor immunosuppression targets such as “CD200R1,” “CD47,” “CTLA4,” “Tim-3,” “PD-1,” “TIGIT,” and “HVEM” were plotted. The correlation degree was determined using Pearson correlation analysis ($\text{COR} > 0$).

2.8. RT-qPCR verification of ZNF207 gene expression

Total RNA was isolated and extracted using the TRIZOL method, and the quality of the extracted RNA was assessed. Total RNA was reverse transcribed into cDNA using the PrimeScript RT Master Mix (Perfect Real Time) reverse transcription kit. RT-qPCR reaction assays were meticulously performed using the TB Green Premix Ex Taq II (TLI RNaseH Plus) fluorescent PCR kit, following specific instructions. Using β -actin as an internal reference, the relative expression of the *ZNF207* gene was determined by the $2^{-\Delta\Delta\text{CT}}$ method. The primers used were Forward: GCCTCAACTTCATTT-CAGCCACAG and Reverse: CATTCTGGTGCTCCTGGTACTG. The reaction conditions were as follows: 95°C for 30 seconds (one cycle), 95°C for 5 seconds, and 60°C for 30 seconds (39 cycles), followed by 95°C for 10 minutes, 65°C for 5 seconds, and 95°C for 5 minutes.

2.9. Statistical analysis

The Pearson correlation between *ZNF207* and other genes was calculated using R software. In IBM SPSS Statistics 29.0, a univariate Cox proportional hazards model was used to assess the association of *ZNF207* expression and clinical characteristics with prognosis in patients with LIHC. To provide statistical evidence for the aforementioned studies, Prism 9 (version 9.4.1) was utilized to demonstrate the expression of *ZNF207*. Additionally, χ^2 was employed to assess the correlation between the expression of *ZNF207* and the clinical features of 371 patients with LIHC after multiple imputations. The difference was statistically significant with $P < 0.05$.

3. Results

3.1. Expression of ZNF207 in LIHC

The UALCAN database showed that *ZNF207* was significantly upregulated in hepatocellular carcinoma compared to normal tissues, with a statistically significant difference ($P < 0.05$; **Figure 1A**). Immunohistochemical staining from the HPA database revealed a higher density of stained granules in LIHC

tissues than in normal tissues, indicating elevated *ZNF207* expression in LIHC (**Figure 1B**).

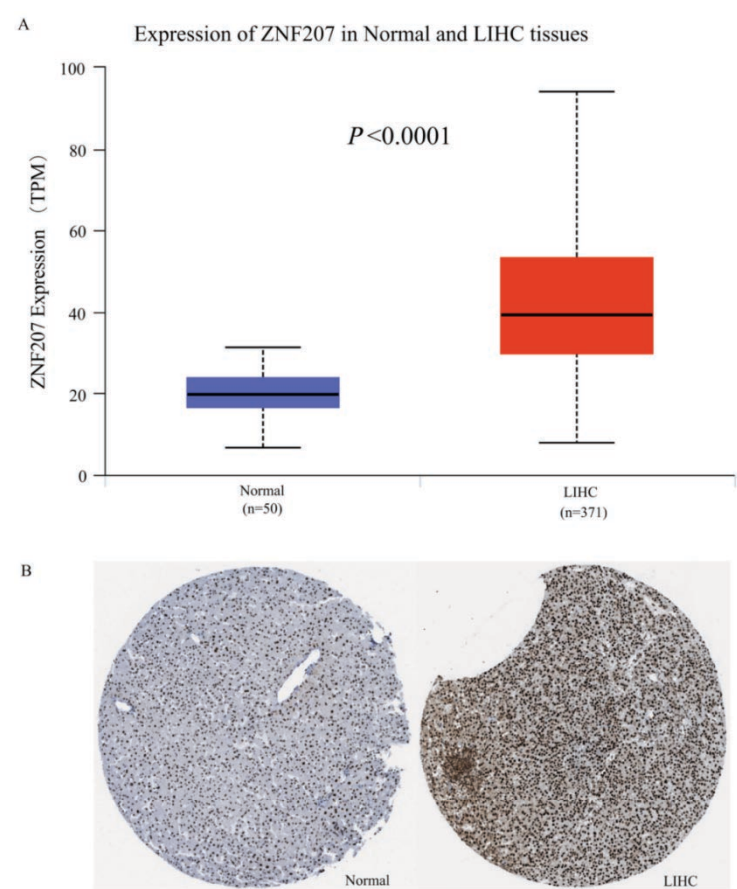


Figure 1. Expression of *ZNF207* in normal and LIHC tissues. **(A)** Comparison of *ZNF207* expression levels in normal tissues and LIHC tissues using the UALCAN database (ualcan.path.uab.edu/home/); **(B)** *ZNF207* immunohistochemical staining in normal and LIHC tissue as seen on the HPA online database (The Human Protein Atlas, <https://proteintlas.org/>).

3.2. Influence of different expressions of *ZNF207* on survival prognosis

The Kaplan-Meier curve illustrating the impact of varying *ZNF207* expression on survival prognosis was generated using R software. As depicted in **Figure 2**, the overall survival rate of the high-expression *ZNF207* group was significantly lower than that of the low-expression group, with a statistically significant difference ($P < 0.05$).

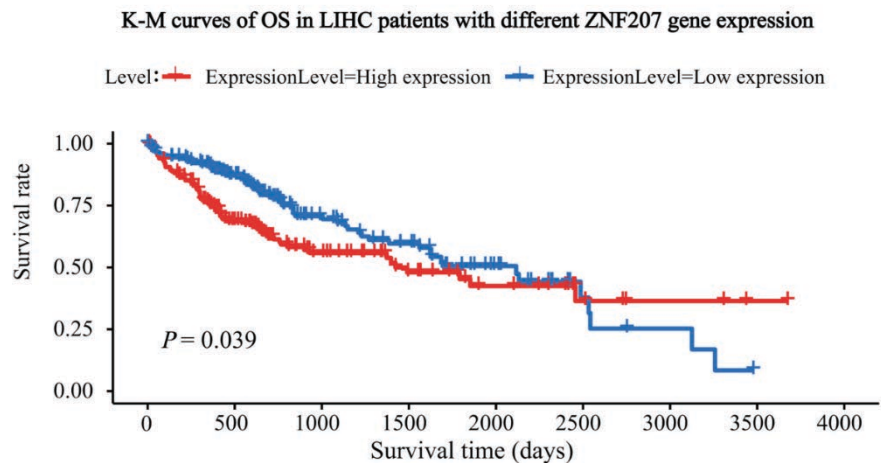


Figure 2. Relationship between *ZNF207* expression and survival prognosis

3.3. Relationship between ZNF207 expression and clinical characteristics and survival status in LIHC

As *ZNF207* expression increased, the rate of cell death also rose, suggesting a synchronous relationship between *ZNF207* and LIHC, as shown in **Figure 3A**. Additionally, changes in the T stage of the TNM classification in LIHC were synchronized with changes in *ZNF207* expression. However, there were no significant changes in age, sex, and race in the clinical characteristics of LIHC with increased *ZNF207* expression, as shown in **Figure 3B**.

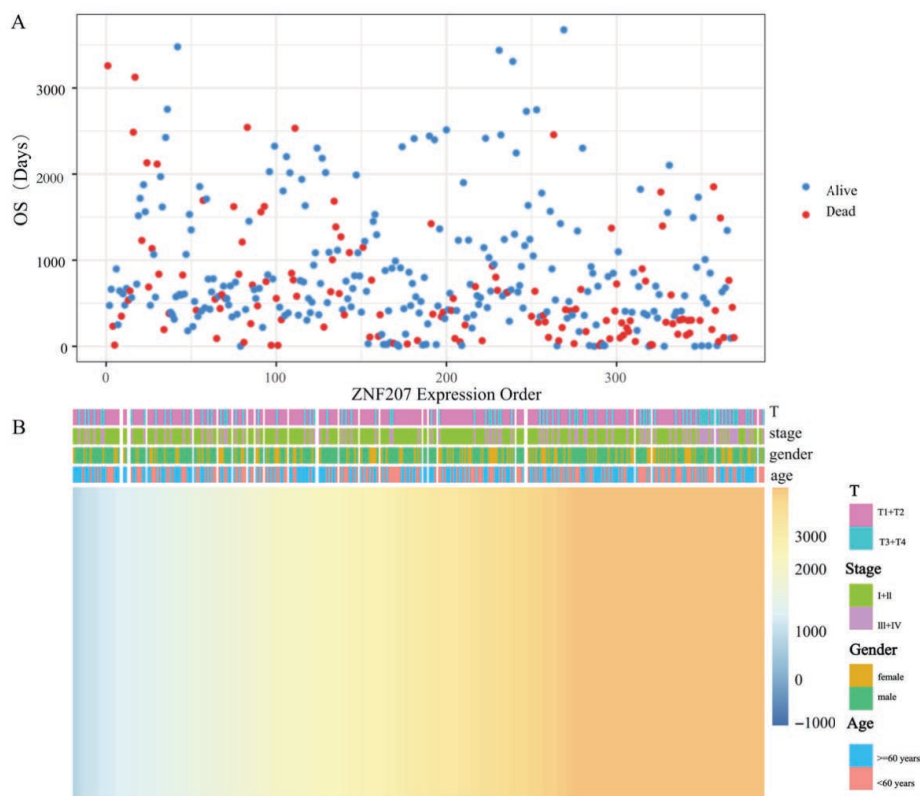


Figure 3. *ZNF207* elevated expression correlates with changes in patient survival status and clinical characteristics. **(A)** Scatter plot showing patient survival in relation to the increase in *ZNF207* expression; **(B)** Heatmaps illustrating patients' clinical features as *ZNF207* expression increases.

3.4. ZNF207 expression correlated with clinical features

As shown in **Table 1**, *ZNF207* expression was not correlated with sex or age 60 or older in patients with LIHC ($P > 0.05$). However, it was associated with the clinical stage (I + II vs. III + IV) and TNM stage (T1 + T2 vs. T3 + T4) in patients with LIHC ($P < 0.05$).

3.5. Gene enrichment analysis results

The biological processes involved in the enrichment analysis of the *ZNF207* gene ontology (GO) mainly include the negative regulation of mRNA splicing by the spliceosome, regulation of RNA splicing, RNA splicing, mRNA processing, and splicing of mRNA by the spliceosome (**Figure 4A**). The cellular components mainly include catalytic Step II spliceosomes, nuclear speckles, ribonucleoprotein complexes, nuclei, and cytoplasm (**Figure 4B**). Molecular functions mainly include nucleic acid binding, transcription cofactor activity, protein binding, mRNA binding, and RNA binding (**Figure 4C**). In the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of *ZNF207*, the main pathways identified were mitophagy-animal, mRNA surveillance, homologous recombination, spliceosome, and nucleoplasmic transport (**Figure 4D**).

Table 1. Relationship between *ZNF207* expression and clinical characteristics of patients

Clinical features	Cases	Expression level of <i>ZNF207</i> /case		<i>P</i>
		Low (<i>n</i> = 185)	High (<i>n</i> = 186)	
Gender				
Male	250	128	122	0.460
Female	121	57	64	
Age (years)				
< 60	170	81	89	0.432
≥ 60	201	104	97	
Clinical stage				
I + II	273	145	128	0.037*
III + IV	98	40	58	
TNM stage				
T1 + T2	278	147	131	0.045*
T3 + T4	93	38	55	

**P* < 0.05

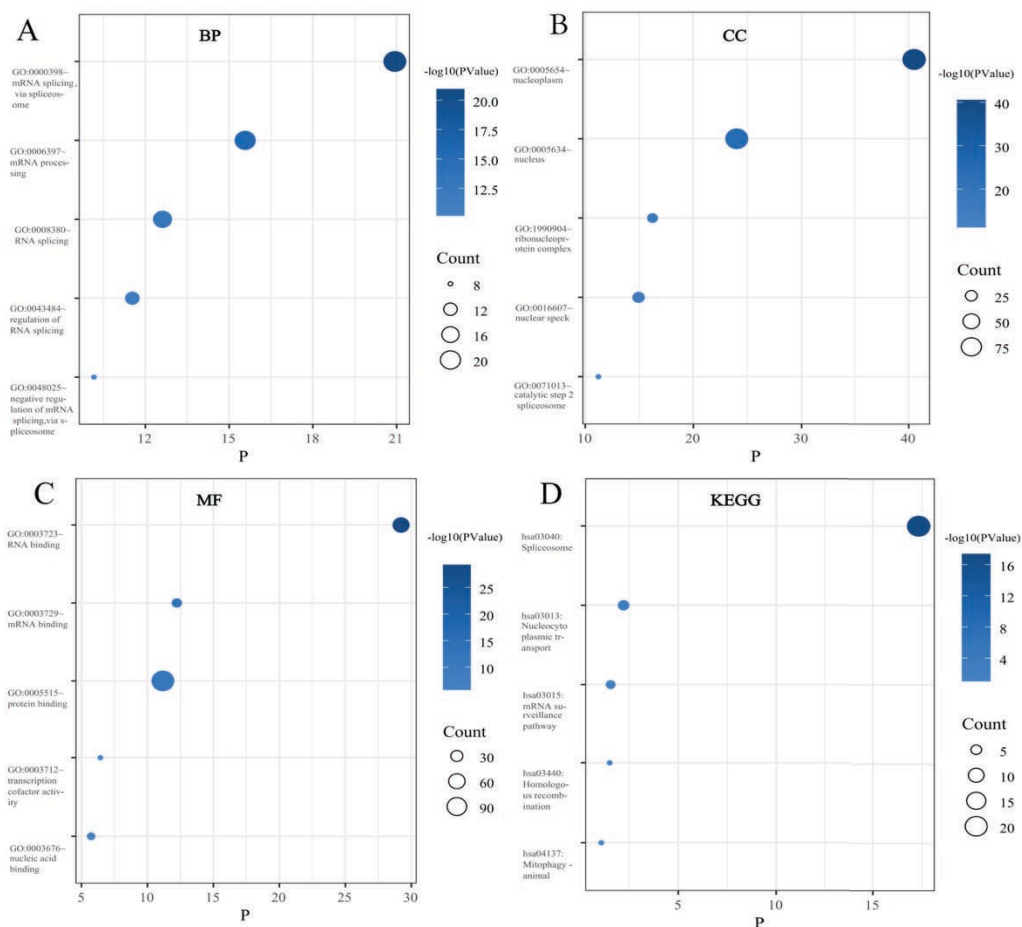


Figure 4. Results of gene enrichment analysis. **(A)** Results of enriched biological processes in Gene Ontology (GO) analysis; **(B)** Results of enriched cellular components in GO analysis; **(C)** Molecular function results enriched in GO analysis; **(D)** Pathway results from KEGG enrichment analysis.

3.6. ZNF207 and common clinical immune checkpoints

ZNF207 showed a positive correlation with CD200R1, CD47, CTLA4, Tim-3, PD-1, TIGIT, and HEVM (correlation coefficient > 0), with the strongest correlation observed with CD47. See **Figure 5**.

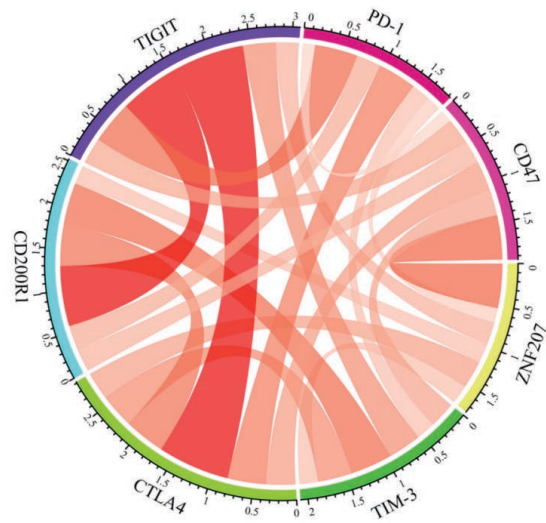


Figure 5. ZNF207 correlates with common clinical immune checkpoints

3.7. Results of univariate and multivariate Cox proportional hazards models

Univariate Cox proportional hazards model assessment suggested that T stage in TNM and ZNF207 expression levels were significantly associated with the prognosis of patients with LIHC, as depicted in **Figure 6A**. The multivariate Cox proportional hazards model suggested that high ZNF207 expression was an independent prognostic factor for LIHC, as depicted in **Figure 6B**.

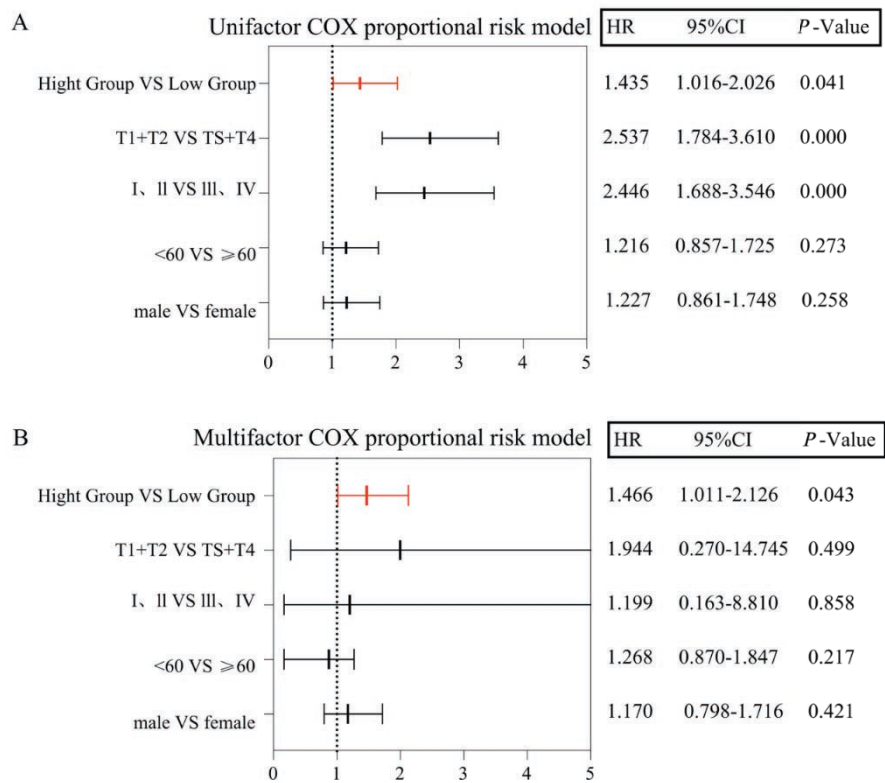


Figure 6. Results of univariate and multivariate Cox proportional hazards models

3.8. RT-qPCR verification of ZNF207 gene expression

The results showed that *ZNF207* expression was significantly higher in HepG-2 cells compared to Lo2 cells ($P < 0.05$), as depicted in **Figure 7**.

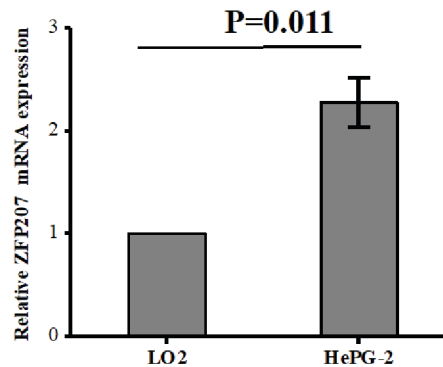


Figure 7. *ZNF207* expression in normal cells and LIHC (verified by RT-qPCR)

4. Discussion

Currently, approximately 383,000 people die from liver cancer in China each year, representing 51% of the global liver cancer deaths. This dire situation imposes a significant burden on our society and healthcare system^[8]. The traditional methods for diagnosing and screening LIHC primarily include a combination of ultrasound imaging (US), computed tomography imaging (CT), magnetic resonance imaging (MRI), and serum alpha-fetoprotein (AFP) levels. Serum AFP levels ≥ 400 $\mu\text{g/L}$ strongly indicate liver cancer, provided that pregnancy, chronic or active liver disease, germ cell embryonal tumors, and digestive tract tumors have been ruled out^[9]. However, biomarkers are needed to supplement ultrasound in the early detection of liver cancer, as AFP is not the optimal choice^[10]. Even the American Association for the Study of Liver Diseases' liver cancer screening guidelines have removed AFP from screening recommendations due to its overall underperformance^[11]. Thus, discovering tumor diagnostic markers that can diagnose early hepatocellular carcinoma with high sensitivity and specificity has become a key focus and challenge in basic and clinical research.

Zinc finger protein (ZNF) was first discovered in *Xenopus* oocyte transcription factor IIIA^[12]. ZNF is the largest transcription factor family in the human genome, with various combinations and functions of zinc finger motifs providing these proteins with a broad range of applications in biological processes such as development, differentiation, metabolism, and autophagy^[13]. *ZNF207*, a member of the zinc finger protein family, was first discovered in human vascular smooth muscle cells (VSMC)^[14].

The authors believe that the results of this study warrant further exploration of the specific mechanisms of *ZNF207* in hepatocellular carcinoma. According to the results, *ZNF207* expression in LIHC tissues was significantly higher than in normal tissues. High *ZNF207* expression significantly decreased the overall survival of patients. The immunohistochemistry results from the HPA database showed higher *ZNF207* expression in LIHC than in normal tissues, consistent with Zhou Chenghui's findings^[5]. The main biological processes involved in gene enrichment include negative regulation of mRNA splicing by the spliceosome, regulation of RNA splicing, RNA splicing, mRNA processing, and mRNA splicing by the spliceosome. The cellular components involved are the catalytic step II spliceosome, nuclear speckles, ribonucleoprotein complex, nucleus, and cytoplasm. The molecular functions include nucleic acid binding, transcription cofactor activity, protein binding, mRNA binding, and RNA binding. The enriched pathways included mitophagy-animal, mRNA surveillance, homologous recombination, spliceosome, and nuclear-cytoplasmic transport. *ZNF207* has

been shown to inhibit the immune microenvironment of LIHC. The authors further analyzed the correlation between *ZNF207* and common clinical immune checkpoints (CD200R1, CD47, CTLA4, Tim-3, PD-1, TIGIT, HVEM) in hepatocellular carcinoma. The related mechanisms warrant further exploration. The univariate and multivariate proportional hazards models suggested that high *ZNF207* expression could be an independent prognostic factor in patients with LIHC. These results align with previous studies, indicating that the prognosis of hepatocellular carcinoma patients is not influenced by their sex or age. The high expression of *ZNF207* in LIHC patients was confirmed through Western blot assay, RT-qPCR assay in HepG-2 cells, TCGA database, UALCAN database, and HPA database. These findings provide a theoretical foundation for investigating the role of *ZNF207* in hepatocellular carcinoma and lay the groundwork for early screening and diagnosis, clinical gene therapy, and the development of anti-tumor drugs for LIHC patients.

In conclusion, the role of *ZNF207* in embryonic stem cell self-renewal, pluripotency, and tumor immunity in the development of hepatocellular carcinoma warrants further investigation. Based on the data presented, *ZNF207* was found to be highly expressed in LIHC and correlated with prognosis. This finding could potentially broaden the sample study and provide reliable tumor diagnostic markers for the early detection of LIHC.

Disclosure statement

The authors declare no conflict of interest.

References

- [1] Jiang Y, Han QJ, Zhang J, 2019, Hepatocellular Carcinoma: Mechanisms of Progression and Immunotherapy. *World J Gastroenterol*, 25(25): 3151–3167. <https://doi.org/10.3748/wjg.v25.i25.3151>
- [2] Lin J, Wu L, Bai X, et al., 2016, Combination Treatment Including Targeted Therapy for Advanced Hepatocellular Carcinoma. *Oncotarget*, 7(43): 71036–71051. <https://doi.org/10.18632/oncotarget.11954>
- [3] Fang F, Xia N, Angulo B, et al., 2018, A Distinct Isoform of ZNF207 Controls Self-Renewal and Pluripotency of Human Embryonic Stem Cells. *Nat Commun*, 9(1): 4384. <https://doi.org/10.1038/s41467-018-06908-5>
- [4] Wang X, Zhou T, Chen X, et al., 2022, System Analysis Based on the Cancer-Immunity Cycle Identifies ZNF207 as A Novel Immunotherapy Target for Hepatocellular Carcinoma. *J Immunother Cancer*, 10(3): e004414. <https://doi.org/10.1136/jitc-2021-004414>
- [5] Zhou C, Li N, 2019, Expression and Significance of ZNF207 in Hepatocellular Carcinoma. *Journal of Central South University Medicine*, 44(4): 406–412.
- [6] Chandrashekar DS, Bashel B, Balasubramanya SAH, et al., 2017, UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. *Neoplasia*, 19(8): 649–658. <https://doi.org/10.1016/j.neo.2017.05.002>
- [7] Uhlén M, Fagerberg L, Hallström BM, et al., 2015, Proteomics. Tissue-Based Map of the Human Proteome. *Science*, 347(6220): 1260419. <https://doi.org/10.1126/science.1260419>
- [8] Lu G, Chen L, Wang H, 2015, The Present Situation and Prospect of Liver Cancer Research in Our Country. *Life Sciences*, 27(3): 237–248. <https://doi.org/10.13376/j.cbbs/2015034>
- [9] Department of Medical Administration, National Health and Health Commission of the People's Republic of China, 2020, Diagnostic and Therapeutic Criteria for Primary Liver Cancer (2019 Edition). *Chinese Journal of Practical Surgery*, 40(2): 121–138. <https://doi.org/10.3760/cma.j.issn.1007-3418.2020.02.004>
- [10] Lok AS, Sterling RK, Everhart JE, et al., 2010, Des-Gamma-Carboxy Prothrombin and Alpha-Fetoprotein as Biomarkers for the Early Detection of Hepatocellular Carcinoma. *Gastroenterology*, 138(2): 493–502. <https://doi.org/10.1053/j.gastro.2010.02.004>

org/10.1053/j.gastro.2009.10.031

- [11] Bruix J, Sherman M, 2005, Practice Guidelines Committee, American Association for the Study of Liver Diseases. Management of Hepatocellular Carcinoma. *Hepatology*, 42(5): 1208–1236. <https://doi.org/10.1002/hep.20933>
- [12] Miller J, McLachlan AD, Klug A, 1985, Repetitive Zinc-Binding Domains in the Protein Transcription Factor IIIA from *Xenopus* Oocytes. *EMBO J*, 4(6): 1609–1614. <https://doi.org/10.1002/j.1460-2075.1985.tb03825.x>
- [13] Jen J, Wang YC, 2016, Zinc Finger Proteins in Cancer Progression. *J Biomed Sci*, 23(1): 53. <https://doi.org/10.1002/j.1460-2075.1985.tb03825.x>
- [14] Pahl PM, Hodges YK, Meltesen L, et al., 1998, ZNF207, A Ubiquitously Expressed Zinc Finger Gene on Chromosome 6p21.3. *Genomics*, 53(3): 410–412. <https://doi.org/10.1006/geno.1998.5442>

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Research Progress on the Bone Metastasis Mechanism of Prostate Cancer and Bone-Targeted Drugs

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Abstract: Prostate cancer is a common male malignant tumor, and bone metastasis is one of the common complications in the late stage of prostate cancer. The mechanism of prostate cancer bone metastasis is a complex process involving multiple factors and steps. In recent years, with in-depth research on the mechanism of prostate cancer bone metastasis and the development of new drugs, important progress has been made in the treatment of prostate cancer bone metastasis. Based on this, this article introduces the mechanism of prostate cancer bone metastasis and the research progress of several bone-targeted drugs to provide reference and inspiration for future research.

Keywords: Prostate cancer; Bone metastasis; Mechanism; Bone targeting drugs; Cancer cell

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1. Introduction

As a malignant tumor, the incidence rate of prostate cancer has increased year by year in recent years ^[1,2]. For this large group of people, bone metastasis has become an important factor affecting their survival. The incidence of bone metastasis in patients with prostate cancer is generally high. According to statistics, the incidence of bone metastasis in patients with advanced prostate cancer is as high as 90% ^[3,4]. Once bone metastasis occurs, the patient's quality of life and life expectancy will be seriously affected ^[5]. Therefore, it is of great significance to study the mechanism of prostate cancer bone metastasis and find effective treatments. Many studies have shown that the interaction between prostate cancer cells and bone matrix is one of the important causes of bone metastasis. Treatment methods for prostate cancer bone metastasis usually include

chemotherapy, radiotherapy, surgery, etc., but the treatment effect is still limited. Bone-targeted drugs are a new type of treatment method that has attracted much attention due to their advantages of strong targeting and low side effects ^[6]. At present, some bone-targeting drugs such as radium-223, strontium-89, and bisphosphonates have gradually been proven to achieve good clinical results in the treatment of prostate cancer bone metastasis.

2. Mechanism of prostate cancer bone metastasis

2.1. Colonization

Colonization of prostate cancer cells in bone is the first step in prostate cancer bone metastasis. Colonization refers to the process in which cancer cells enter the bone through the blood circulation or lymphatic vessels and survive and proliferate in the bone matrix. Research generally agrees that a variety of factors influence this process. Some studies believe that certain components in the bone matrix can promote the adhesion and proliferation of prostate cancer cells. For example, CXCL12 in the bone matrix can bind to CXCR4 expressed by prostate cancer cells, promote cell adhesion and proliferation, and migrate to the bone marrow ^[7,8]. In addition, some growth factors (such as insulin-like growth factor, fibroblast growth factor) and cytokines, such as interleukin-6 (IF-6) ^[9], tumor necrosis factor- α (TNF- α), prostate-specific antigen (PSA), free prostate-specific antigen (fPSA), and interleukin 17A (IF-17A) can also stimulate the proliferation and migration of prostate cancer cells, thus promoting the colonization process ^[10]. These factors are expressed in blood circulation and bone matrix and can affect the growth and metastasis of tumor cells. Of course, more studies have shown that the colonization of prostate cancer cells in bone is promoted by cytokines synthesized in bone matrix release and bone turnover ^[11,12].

2.2. Dormancy

After colonization, prostate cancer cells often enter a dormant state to adapt to their new environment and avoid immune system attack. Cancer cells in a dormant state no longer proliferate but remain in a “dormant” state, waiting for the right time to activate again. Although the dormant cancer cells appear to have been cured, this state can still contribute to cancer recurrence, so this stage should also be highly valued ^[13]. During the dormancy process, the roles of some molecules and signaling pathways are very important. Research by He Ziqiu *et al.* ^[14] believes that activation of the Wnt signaling pathway can reduce the degradation of β -catenin and promote the dormancy and proliferation of cancer cells. Liao Zhuangwen *et al.* ^[15] found that alkaloids may inhibit the epithelial-mesenchymal transition of PC-3 cells by inhibiting the Wnt/ β -catenin/Snail signaling pathway.

Prostate cancer cells avoid immune system attack while in a dormant state. Immune escape means that tumor cells evade or inhibit the recognition and attack of the immune system through various mechanisms, thereby growing and metastasizing in the body ^[16]. Tumor cells can evade immune attack by expressing immunosuppressive molecules, producing immunosuppressive factors, or inhibiting the activation and function of immune cells through other mechanisms. For example, Zhou Rubao *et al.* ^[17] co-cultured PC-3 cells overexpressing miR-335-5p with NK-92MI cells and found that the levels of TNF- α and IFN- γ in the cell supernatant were significantly increased. This means that overexpression of miR-335-5p can kill PC-3 cells by promoting the release of TNF- α and IFN- γ from NK-92MI cells, thereby inhibiting immune escape. Huang Lin *et al.* ^[18] believe that the PD-1/PD-L1 pathway plays an important role in inducing effector T cell apoptosis, inhibiting T cell activation, and inhibiting the body's anti-tumor immune response and tumor immune escape.

2.3. Reactivation

When certain signals stimulate dormant cancer cells, they reactivate and begin to proliferate. These stimulating

factors may include cell adhesion molecules, hypoxic bone microenvironment, RAI14^[19], etc. The reactivated cancer cells begin to proliferate and produce more cancer cells through cell division. In this process, cancer cells' genome instability and immune evasion ability are key factors.

2.4. Proliferation

As cancer cells proliferate, surrounding bone tissue is destroyed and resorbed. This process involves the action of some osteoclasts and osteoblasts. Osteoclasts can break down the organic components and minerals in the bone matrix, while osteoblasts can synthesize new bone tissue to repair the damaged bone matrix^[20]. This dynamic balance of destruction and repair will continue for some time until a relatively stable microenvironment is formed. In this microenvironment, cancer cells survive and continue to proliferate.

3. Research on targeted drugs for prostate cancer bone metastasis

3.1. Radionuclides

3.1.1. Radium-223

Radium-223 is a potential prostate cancer bone metastasis-targeting drug. It is a radioactive isotope that emits alpha particles with high energy and penetrating ability, killing cancer cells and inhibiting their growth^[20]. The targeting properties of radium-223 enable radiation therapy to target tumor cells directly while reducing damage to surrounding normal tissue. For patients with prostate cancer bone metastasis, radium-223 can precisely target tumor cells in the bones and relieve pain and other related symptoms^[21]. As a radioactive substance, the use of radium-223 often brings concerns. However, a study showed that the ambient gamma radiation dose caused by ²²³Ra injection to treat bone mCRPC is relatively low, and no special external radiation protection measures are required^[22].

3.1.2. Strontium-89

Strontium-89 is also a targeted drug used to treat bone metastases from prostate cancer. It is also a radioactive isotope that emits beta particles, killing cancer cells and inhibiting their growth. In preclinical studies, strontium-89 has shown significant therapeutic effects against prostate cancer bone metastases. For example, to help patients suppress pain. Hong Liwei^[23] found in the study that the observation group treated with strontium chloride [⁸⁹Sr] injection had an overall higher effective lesion treatment rate than the control group, and the patient's body pain improved more significantly. Shan Yangang's study^[24] also believed that ⁸⁹Sr can significantly alleviate patients' clinical symptoms and achieve satisfactory results in the treatment of patients with prostate bone metastasis.

3.2. Bisphosphonate drugs

Bisphosphonates are targeted drugs commonly used to treat prostate cancer bone metastases. It is a synthetic compound^[25] that can combine with hydroxyapatite crystals in bones to inhibit the growth and metastasis of tumor cells in bones. Bisphosphonates can inhibit osteoclast activity on the surface of tumor cells and reduce bone destruction and resorption^[26]. At the same time, it can also inhibit the combination of tumor cells and bone matrix and reduce the adhesion ability of tumor cells, thereby inhibiting the growth and metastasis of tumors in bones. Miao Zhixiong *et al.*^[27] compared the changes in various indicators before and one year after treatment with 99-technetium-methylene diphosphate in 76 patients with prostate cancer bone metastases. They found that 99-technetium-methylene diphosphate can promote bone proliferation, inhibit bone resorption, increase bone density, and prevent osteoporosis. The research by Zhang Qifeng *et al.*^[28] also confirmed this. Liu Yongjiang *et al.*^[29] studied that bisphosphonate drugs have a positive effect on reducing pain in patients with prostate cancer bone metastasis and rarely increase other side effects except nausea. Most notably, bisphosphonate drugs have

broad prospects in treating prostate cancer bone metastasis.

4. Conclusion

With the continuous development of science and technology and the continuous advancement of new drug research and development, the treatment methods for prostate cancer bone metastasis will become more and more abundant and effective. Bone-targeted drugs will also become one of the important means of treating prostate cancer bone metastasis in the future. In addition, with a deeper understanding of the bone metastasis mechanism of prostate cancer, it will be possible to discover more therapeutic targets and breakthroughs in the development of new drugs. At the same time, it is also necessary to pay attention to the progress of clinical trials and the effects of actual application to ensure the safety and effectiveness of new drugs. We look forward to achieving a more precise, safe, and effective method to treat prostate cancer bone metastasis in the near future, bringing patients better quality of life and longer survival.

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Disclosure statement

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References

- [1] Zhou Q, Tian X, Chang D, et al., 2022, Chinese Expert Consensus on Prostate Cancer Diagnosis, Treatment, and Health Management Using Integrated Traditional Chinese and Western Medicine. *Chinese Journal of Andrology*, 28(10): 941–953.
- [2] Chen W, Zheng R, Baade PD, et al., 2016, Cancer Statistics in China, 2015. *CA Cancer J Clin*, 66(2): 115–132. <https://doi.org/10.3322/caac.21338>
- [3] Tian Z, Heng L, Dong J, et al., 2023, Bioinformatics Analysis of Key Genes for Prostate Cancer Bone Metastasis. *Chinese Journal of Immunology*, 39(9): 1885–1892.
- [4] Duan X, Cui Y, 2023, Progress in Diagnosis and Treatment of Prostate Cancer Bone Metastasis. *Chinese Medical Journal*, 58(6): 594–596.
- [5] Li X, Chen B, Wu J, et al., 2023, Clinical Study of Denosumab in the Treatment of Prostate Cancer Bone Metastasis Pain. *Modern Chinese Physicians*, 61(18): 14–17 + 56.
- [6] Chen C, Zhou J, Xiang S, 2021, Hot Spots, Challenges, and Opportunities in Diagnosing and Treating Prostate Cancer Bone Metastasis. *Journal of Modern Genitourinary Oncology*, 13(6): 321–324 + 335.
- [7] Gupta N, Duda DG, 2016, Role of Stromal Cell-Derived Factor 1 α Pathway in Bone Metastatic Prostate Cancer. *J Biomed Res*, 30(3): 181–185. <https://doi.org/10.7555/JBR.30.20150114>
- [8] Conley-LaComb MK, Semaan L, Singareddy R, et al., 2016, Pharmacological Targeting of CXCL12/CXCR4 Signaling in Prostate Cancer Bone Metastasis. *Mol Cancer*, 15(1): 68. <https://doi.org/10.1186/s12943-016-0552-0>
- [9] Sun B, Liu J, Cai T, et al., 2018, Analysis of the Correlation Between Serum TGF- β 1, VEGF, and IL-6 Levels and Prostate Cancer Bone Metastasis. *Journal of Clinical and Experimental Medicine*, 17(19): 2086–2089.

- [10] Mao L, Wu W, Huang Y, et al., 2021, Expression Levels and Clinical Significance of PSA, IL-6, TNF- α , and IL-17A in Peripheral Blood of Patients with Prostate Cancer. *Chinese Medical Equipment*, 18(2): 77–81.
- [11] Deng X, He G, Liu J, et al., 2014, Recent Advances in Bone-Targeted Therapies of Metastatic Prostate Cancer. *Cancer Treat Rev*, 40(6): 730–738. <https://doi.org/10.1016/j.ctrv.2014.04.003>
- [12] Ye L, Kynaston HG, Jiang WG, 2007, Bone Metastasis in Prostate Cancer: Molecular and Cellular Mechanisms (Review). *Int J Mol Med*, 20(1): 103–111.
- [13] Zhang X, 2019, Interactions Between Cancer Cells and Bone Microenvironment Promote Bone Metastasis in Prostate Cancer. *Cancer Commun (Lond)*, 39(1): 76. <https://doi.org/10.1186/s40880-019-0425-1>
- [14] He Z, Du D, Fu H, et al., 2020, Research Progress on the Correlation Between the Wnt Signaling Pathway and Prostate Cancer. *Chinese Journal of Laparoscopic Urology (Electronic Edition)*, 14(4): 312–315.
- [15] Liao Z, Liang C, Chen C, et al., 2021, The Effect of Alkaloids on Prostate Cancer Bone Metastasis PC-3 Cells Through the Wnt/ β -Catenin Signaling Pathway. *Journal of Practical Medicine*, 37(3): 298–303.
- [16] Peng W, Fan C, Zhang H, 2020, The Impact of HIF-1 α on Immune Escape of A549 Lung Cancer Cells and the NCR1/NKP46 Pathway by Regulating miR-544. *Journal of Clinical Pulmonology*, 25(8): 1246–1251.
- [17] Zhou R, Guo Z, Yang J, et al., 2023, Study on the Mechanism of miR-335-5p Targeting Galectin-3 to Regulate Immune Escape of Prostate Cancer Cells. *Modern Oncology*, 31(13): 2431–2437.
- [18] Huang L, He J, 2020, Research Progress on PD-1/PD-L1 Inhibitors and Prostate Cancer Immunotherapy. *Chinese Journal of Andrology*, 26(10): 944–948.
- [19] Fan H, Lai Y, Zhou J, et al., 2023, High Expression of RAI14 Promotes Bone Metastasis of Prostate Cancer and is Associated with Poor Prognosis. *Lingnan Modern Clinical Surgery*, 23(2): 127–134.
- [20] Han Y, Ren M, 2023, Research Progress on Prostate Cancer Bone Metastasis Mechanism and Bone-Targeted Drugs. *Journal of Practical Oncology*, 37(3): 252–256.
- [21] Yang X, Li X, Wu K, et al., 2022, Preliminary Study on the Treatment of Bone Metastasis of Prostate Cancer with Radium-223. *Journal of Modern Urology*, 27(2): 133–136.
- [22] Sun H, Zhao G, Yan P. 223Ra Injection in the Treatment of Bone Metastasis and Castration-Resistant Prostate Cancer Caused by Radiation Effects. *Chinese Interventional Imaging and Therapeutics*, 19(9): 575–578.
- [23] Hong L, 2021, Clinical Effect of Strontium Chloride [89Sr] Injection in the Treatment of Prostate Cancer Bone Metastasis Pain. *Chinese and Foreign Medical Research*, 19(34): 152–155.
- [24] Shan Y, 2019, Treatment of Prostate Cancer Bone Metastasis with Radionuclide 89Sr. *Chinese and Foreign Medicine*, 38(22): 184–186.
- [25] Yu X, Kong W, 2012, Bisphosphonates and the Treatment of Giant Cell Tumor of Bone. *Chinese Journal of Clinicians (Electronic Edition)*, 6(17): 4991–4994.
- [26] Cao S, 2018, The Efficacy and Nursing Care of Diphosphonates in Treating Bone Pain in Patients with Metastatic Bone Tumors. *Strait Pharmacy*, 30(2): 213–214.
- [27] Miao Z, Zhu X, 2018, Observation on the Efficacy of 99 Technetium-Methylene Diphosphonate in the Treatment of Prostate Cancer Bone Metastasis. *Guangzhou Medicine*, 49(3): 94–96.
- [28] Zhang Q, Yan S, 2012, Analysis of the Efficacy of 99 Technetium-Methylene Diphosphate in the Treatment of Osteoporosis. *Chinese Journal of Practical Diagnosis and Treatment*, 26(4): 380–381.
- [29] Liu Y, Lu J, 2009, A Systematic Review of Bisphosphonates in the Treatment of Prostate Cancer Bone Metastasis. *Chinese Journal of Andrology*, 23(8): 33–37 + 44.

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Clinical Application Analysis of Laparoscopic-Assisted Total Gastrectomy in the Surgical Treatment of Gastric Cancer

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Abstract: *Objective:* To investigate the clinical application effect of laparoscopic-assisted total gastrectomy in the surgical treatment of gastric cancer. *Methods:* The clinical data of 86 COPD patients included in the study were collected and divided into 43 cases each in Groups A and B using the randomization method, with open total gastrectomy in Group A and laparoscopic-assisted total gastrectomy in Group B. The clinical indexes, pain levels, and complications of patients in the two groups were observed in combination with the indexes. *Results:* The baseline data of the two groups of patients were not statistically significant (all $P > 0.05$); the operation time, incision length, first flatulence time, and hospitalization time of patients in Group B were shorter than those in Group A (all $P = 0.000$); the NRS scores of patients in Group B on the 1st postoperative day and the 2nd postoperative day were significantly lower than those in Group A ($t = 23.443$, $t = 28.784$, all $P = 0.000$); the total complication rate of patients in Group B (1; 2.33%) was significantly lower than that of Group A (9; 20.94%) ($\chi^2 = 7.242$, $P = 0.007$). *Conclusion:* In the surgical treatment of gastric cancer, laparoscopic-assisted total gastrectomy can promote patients' recovery, reduce patients' pain, and lower the probability of complications.

Keywords: Laparoscopy; Total gastrectomy; Gastric cancer; Open surgery

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1. Introduction

As one of the most common malignant tumors of the digestive system, gastric cancer has a high morbidity and mortality rate worldwide ^[1]. For a long time, traditional open total gastrectomy has been the main method of surgical treatment for gastric cancer. However, although this method can effectively resect the tumor and clear the lymph nodes, its significant surgical trauma, slow postoperative recovery, and high complication rate make the patients' quality of life seriously affected ^[2]. With the continuous progress of medical technology, laparoscopic technology has gradually been widely used in the field of surgery, especially in the surgical treatment of gastric cancer, laparoscopic-assisted total gastrectomy has become an emerging and minimally invasive treatment option. With the help of a high-definition camera and precise surgical instruments, this technique enables the surgeon to perform precise resection and lymph node dissection of the stomach under direct vision ^[3,4], without

the need to perform extensive open abdominal operations on the patient as in traditional surgery. For this reason, more and more studies have begun to focus on the effectiveness of its application in clinical practice. In this paper, the clinical application of laparoscopic-assisted total gastrectomy in the surgical treatment of gastric cancer is analyzed in depth, aiming to provide a more scientific and reasonable basis for the choice of surgical treatment for gastric cancer patients.

2. Materials and methods

2.1. General information

The clinical data of 86 cases of gastric cancer patients requiring total gastrectomy included in the study were collected and divided into 43 cases each in Groups A and B using the randomization method.

Inclusion criteria: (1) preoperative pathological diagnosis of gastric cancer and the need for total gastrectomy; (2) clinical stage I-III; (3) indications for surgery without contraindications such as coagulation dysfunction and severe cardiopulmonary insufficiency; (4) voluntary participation in this study and signing of informed consent.

Exclusion criteria: (1) those with combined heart, liver, kidney, and other important organ injury; (2) those with incomplete clinical data.

2.2. Methods

Group A underwent an open total gastrectomy. Patients need to be evaluated for systemic conditions before surgery, and patients with dehydration and electrolyte disorders need to be appropriately infused with fluids and electrolyte supplementation to correct water and electrolyte disorders before surgery. For patients with pyloric obstruction, preoperative fasting, gastrointestinal decompression, fluid infusion, and daily gastric lavage are required to reduce the inflammation and edema of the gastric mucosa. During surgery, the patient is placed in a lying position under general anesthesia. After the anesthesia took effect, the middle position of the upper abdomen was used as the incision for total gastrectomy, which included separating the greater omentum, cutting off the right gastric omental vein and the right gastric omental artery, and removing the subpyloric lymph nodes. After confirming where the right gastric vessel was located, the loose tissue around it was dissected and removed, and the duodenal stump was closed. The tissues of various groups of lymph nodes that are closely related to the metastasis of gastric cancer were removed. The gastrosplenic ligament was sequentially cut and ligated, and the posterior peritoneum of the tail of the pancreas and the lymph nodes anterior and superior to it were removed. Then anastomosis of the esophagus and jejunum or other ways of reconstruction of the digestive tract were performed according to the specific conditions of the patients.

Group B underwent laparoscopic-assisted total gastrectomy. Consistent with open total gastrectomy, patients were required to undergo a generalized assessment of their condition prior to surgery. Then, the patient was placed in the lying position for general anesthesia, and after the anesthesia took effect, 3–4 small holes were made in the patient's abdomen, and the abdominal cavity was accessed through the instruments of the laparoscope to establish the operating platform. A laparoscopic exploration of the abdominal cavity was performed to determine whether the tumor condition was suitable for laparoscopic-assisted total gastrectomy. After confirming the laparoscopic-assisted total gastrectomy, the perigastric tissues, greater omentum, ligaments, etc. were dissected, and the left and right gastric blood vessels and the left and right gastric omentum blood vessels were ligated; lymph nodes were cleared, and the scope of clearance needed to be cleared up to the second station of the gastric lymph nodes; most of the stomach was resected or the whole stomach was

resected according to the location of the tumor; and a small incision was made on the abdomen to anastomose the stomach and the duodenum or the jejunum after the gastric tumor has been dissected under the laparoscopic procedure. Anastomosis was performed.

2.3. Observation indexes

In this study, the general data of patients were counted, and the level of clinical indexes of patients was assessed by operation time, incision length, time of the first flatulence, and hospitalization time; the pain level of patients was assessed by numerical rating scale (NRS); and the occurrence of complications including incisional infection, duodenal stump fistula, lung infection, and incomplete adhesion intestinal obstruction was assessed.

2.4. Statistical analysis

Statistical processing was performed with SPSS 20.0, and the data were expressed as either mean \pm standard deviation (SD) or [n (%)], and the two-sample t -tests and χ^2 tests were used for comparison between groups, with $P < 0.05$ as the difference being statistically significant.

3. Results

3.1. Comparison of general information of patients in the two groups

As shown in **Table 1**, the baseline information of the two groups of patients was not statistically significant ($P > 0.05$).

Table 1. Comparison of general information of patients in the two groups

Groups	Gender (male/female)	Average age (years)	Tumor diameter (unit)
Group A ($n = 43$)	23/20	65.38 \pm 4.22	2.53 \pm 1.36
Group B ($n = 43$)	25/18	66.47 \pm 4.25	2.62 \pm 1.32
χ^2 / t	0.189	1.193	0.311
P	0.664	0.236	0.756

3.2. Comparison of the level of clinical indicators between the two groups of patients

As shown in **Table 2**, the operation time, incision length, first flatulence time, and hospitalization time of patients in Group B were significantly shorter than those in Group A (all $P = 0.000$).

Table 2. Comparison of the level of clinical indicators between the two groups of patients (mean \pm SD)

Groups	Operative time (min)	Incision length (cm)	Time to first anal evacuation (d)	Length of hospitalization (d)
Group A ($n = 43$)	175.32 \pm 12.87	6.20 \pm 0.82	3.31 \pm 0.97	21.21 \pm 6.67
Group B ($n = 43$)	35.36 \pm 9.97	3.05 \pm 0.32	1.45 \pm 0.88	12.10 \pm 4.21
t	56.375	23.467	9.313	7.574
P	0.000	0.000	0.000	0.000

3.3. Comparison of pain levels between the two groups

As shown in **Table 3**, the NRS scores of patients in Group B were significantly lower than those of Group A on postoperative day 1 and postoperative day 2 ($t = 23.443$, $t = 28.784$, both $P = 0.000$).

Table 3. Comparison of pain levels between the two groups

Groups	NRS score	
	Postoperative day 1	Postoperative day 2
Group A (<i>n</i> = 43)	5.21 ± 0.28	3.88 ± 0.22
Group B (<i>n</i> = 43)	4.02 ± 0.18	2.78 ± 0.12
<i>t</i>	23.443	28.784
<i>P</i>	0.000	0.000

3.4. Comparison of complication rates between the two groups of patients

As shown in **Table 4**, the total complication rate of patients in Group B (1; 2.33%) was significantly lower than that of Group A (9; 20.94%) ($\chi^2 = 7.242$, $P = 0.007$).

Table 4. Comparison of complication rates between the two groups of patients

Groups	Incision infection	Duodenal stump fistula	Lung infection	Incomplete adhesive bowel obstruction	Overall incidence
Group A (<i>n</i> = 43)	4 (9.30%)	1 (2.33%)	1 (2.33%)	3 (6.98%)	9 (20.94%)
Group B (<i>n</i> = 43)	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (2.33%)	1 (2.33%)
χ^2	-	-	-	-	7.242
<i>P</i>	-	-	-	-	0.007

4. Discussion

Gastric cancer is a malignant tumor originating from the epithelium of the gastric mucosa, and its incidence and mortality rates are high worldwide. The occurrence of gastric cancer is related to a variety of factors, including genetics, environment, diet, and *Helicobacter pylori* infection [5]. The clinical manifestations of gastric cancer are varied and commonly include stomach pain, decreased appetite, weight loss, nausea, and vomiting [6]. Since the early symptoms of gastric cancer are not obvious, many patients have entered the middle and late stages when diagnosed, and they need to be treated comprehensively by various therapeutic means, such as surgery, chemotherapy, radiotherapy, and so on. Surgery plays an important role in the treatment of gastric cancer, among which, total gastrectomy is a commonly used surgical method for treating gastric cancer, which is suitable for patients with a wide invasion of gastric cancer and inability to preserve gastric function. During the surgery, the surgeon removes the whole stomach tissue of the patient and may remove part of the esophagus, duodenum, and adjacent lymph nodes at the same time. After the surgery, the patient will need to undergo reconstruction of the digestive tract to restore eating function.

Among total gastrectomy surgeries, they are usually categorized into traditional open total gastrectomy and laparoscopic total gastrectomy. Among them, the former requires a larger abdominal incision, which makes the surgery traumatic and postoperative recovery slow. Due to the traumatic surgery, patients may face the risk of complications such as infection, bleeding, and intestinal obstruction after surgery. For this reason, the advantages of laparoscopic total gastrectomy are highlighted.

In this study, the operation time, incision length, first flatulence time, and hospitalization time of patients in Group B were shorter than those in Group A (all $P = 0.000$), indicating that laparoscopic total gastrectomy can promote the recovery of patients in the surgical treatment of gastric cancer. The main reasons are as follows: first, laparoscopic-assisted total gastrectomy can shorten the operation time. Its use of high-definition cameras

and specialized instruments provides surgeons with a clearer surgical field of view and more delicate operating ability. Compared with traditional open surgery, laparoscopic surgery is able to locate tumors and lymph nodes more quickly and accurately, reducing the intraoperative time for finding and confirming the target ^[7]. It can also effectively reduce unnecessary operating steps and errors, further improving surgical efficiency. Second, laparoscopic-assisted total gastrectomy can reduce the length of incision. It uses a small hole to enter the abdominal cavity, eliminating the need to incise large areas of skin and tissue as in conventional surgery. The significant reduction in the length of the incision reduces the trauma of the surgery to the body ^[8,9] and also facilitates the patient's postoperative recovery. Third, laparoscopic total gastrectomy reduces the time to first anal defecation. Because this approach has relatively little interference with the intra-abdominal cavity, it can reduce the risk of adhesion and obstruction of the intestinal tract. Compared with conventional surgery, patients' bowel motility function recovers faster after laparoscopic surgery, which helps to achieve anal emission earlier. Early anal emission not only indicates that the intestinal function has returned to normal but also helps the patient to start eating and nutritional supplementation earlier, which promotes the recovery of the body. Fourth, laparoscopic total gastrectomy can shorten hospitalization time. As laparoscopic surgery is less traumatic and quicker recovery, patients have relatively low postoperative pain and can get out of bed earlier for activities and self-care.

This study also pointed out that the NRS scores of patients in Group B were significantly lower than those of Group A on postoperative day 1 and postoperative day 2 ($t = 23.443$, $t = 28.784$, both $P = 0.000$), indicating that laparoscopic-assisted total gastrectomy is able to effectively reduce the level of patient's pain in the surgical treatment of gastric cancer, which is mainly due to its minimally invasive and precise nature. Laparoscopic total gastrectomy enters the abdominal cavity through a small hole, eliminating the need to incise large areas of skin and tissue as in traditional surgery, thus significantly reducing the trauma to the organism caused by the surgery. This minimally invasive nature results in less pulling and damage to the surrounding tissues during the procedure, which in turn reduces the level of post-operative pain. Plus, laparoscopic surgery uses a high-definition camera that provides the surgeon with a clear, magnified view of the surgery, which helps the surgeon locate tumors and lymph nodes more precisely and reduces unnecessary tissue damage. This precision further reduces the level of pain during surgery.

This study also proposed that the total complication rate of patients in Group B (1; 2.33%) was significantly lower than that of Group A (9; 20.94%) ($\chi^2 = 7.242$, $P = 0.007$), indicating that laparoscopic-assisted total gastrectomy can effectively reduce the occurrence of patients' complications in the surgical treatment of gastric cancer. There are several main reasons for this: firstly, minimally invasive operation avoids the extensive exposure and damage to the abdominal cavity of traditional open surgery, significantly reduces the surgical interference with the intra-abdominal organs, and reduces the tissue damage and inflammatory reaction ^[10]. Secondly, the clear surgical field can help the surgeon separate tissues and clear lymph nodes more finely, reducing the risk of accidental injury and bleeding. Meanwhile, laparoscopic-assisted total gastrectomy has fast postoperative recovery, which can reduce complications caused by prolonged bed rest and fasting, such as lung infection and deep vein thrombosis. This is consistent with the findings of Zhang Xiong *et al.* ^[11], who found that by comparing the occurrence of complications between laparoscopic and open total gastrectomy for the treatment of stage I gastric cancer, the complication rate of laparoscopic total gastrectomy for the treatment of patients with stage I gastric cancer was lower compared with that of open total gastrectomy.

In conclusion, laparoscopic-assisted total gastrectomy can promote the recovery of patients, reduce the pain of patients, and lower the probability of complications in the surgical treatment of gastric cancer.

Disclosure statement

The authors declare no conflict of interest.

References

- [1] Yuan P, Zheng K, 2018, Progress in the Epidemiologic Study of Gastric Cancer. *Journal of Chronic Disease*, 19(12): 1671–1675 + 1680.
- [2] Cao B, Dong Y, Chen Z, 2019, Analysis of Complications and Surgery-Related Indexes in Gastric Cancer Patients After Laparoscopic Total Gastrectomy and Open Total Gastrectomy. *Cancer Progress*, 17(23): 2796–2799.
- [3] Yan L, 2018, Application of Laparoscopic-Assisted Total Gastrectomy in the Surgical Treatment of Gastric Cancer. *China Medical Device Information*, 24(6): 126–127.
- [4] Wang Y, 2020, Observation on the Efficacy of Laparoscopic-Assisted Radical Surgery for Distal Gastric Cancer. *China Health Standard Management*, 11(22): 96–98.
- [5] Fang W, 2023, Analysis of Risk Factors for Pulmonary Complications After Laparoscopic Gastric Cancer Surgery. *China Modern Drug Application*, 17(12): 1–6.
- [6] Xia Y, 2019, Clinical Efficacy and Safety Evaluation of Laparoscopic Radical Gastrectomy for Progressive Gastric Cancer. *China Medical Device Information*, 25(19): 94–95.
- [7] Yang T, 2018, Effect of Laparoscopic-Assisted Total Gastrectomy on Postoperative Intestinal Function and Serum T-Cell Subsets and Carcinoembryonic Antigen Levels in Gastric Cancer Patients. *International Medicine and Health Guide*, 24(18): 2799–2803.
- [8] Ma J, 2017, Comparison of the Efficacy of Laparoscopic Radical Gastrectomy and Open Radical Gastrectomy for Progressive Gastric Cancer. *Clinical Medicine Research and Practice*, 2(29): 71–72.
- [9] Chen Y, Li Y, 2018, Effect of Laparoscopic Radical Gastric Cancer Surgery with Lymph Node Dissection on Postoperative Intestinal Barrier Function. *Clinical Medicine Research and Practice*, 3(25): 53–54.
- [10] Guo X, Yang Z, Fu Y, et al., 2022, Application Effect of Laparoscopic Total Gastrectomy in Gastric Cancer Patients. *Henan Medical Research*, 31(2): 278–281.
- [11] Zhang X, Zhang X, Li S, et al., 2023, Comparison of Recent Results and Complications Between Laparoscopic and Open Total Gastrectomy for Stage I Gastric Cancer. *Clinical Medicine Research and Practice*, 8(10): 52–55.

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Study on the Therapeutic Effects and Mechanisms of Human Mesenchymal Stem Cell-Derived Exosomes Carrying *NGF* Gene in Treating Ischemic Stroke in Rats

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Abstract: *Objective:* To investigate the therapeutic effects and mechanisms of human mesenchymal stem cell-derived exosomes (hMSCs-Exo) carrying the *NGF* gene in treating ischemic stroke in rats, aiming to provide new insights and treatment methods for ischemic stroke therapy. *Methods:* After successful construction of the cerebral ischemia model in 40 male SPF-grade SD rats aged 6–8 weeks, the model rats were randomly divided into 4 groups: Sham group, PBS group, hMSCs-Exo group, and NGF-hMSCs-Exo group, with 10 rats in each group. The rat MCAO model was prepared using the classic filament method, and NGF-hMSCs-Exo were injected via the tail vein into the MCAO model rats. The expression of the *NGF* gene in brain ischemic tissues, neuronal regeneration, and rat neurological function recovery were observed using TTC staining, memory function evaluation, Western blot, qRT-PCR, and other methods. *Results:* Compared with the Sham group, neurological deficits were significant in the PBS group ($P < 0.01$). Compared with the PBS group, neurological scores improved in the hMSCs-Exo group and NGF-hMSCs-Exo group ($P < 0.05$). Compared with the hMSCs-Exo group, the improvement in neurological deficits was more significant in the NGF-hMSCs-Exo group ($P < 0.05$). The infarct area after NGF-hMSCs-Exo intervention was significantly reduced ($P < 0.05$) compared with the Sham group. Compared with the PBS group, relative expression levels of *NGF* mRNA and protein decreased, while *Caspase-3* mRNA and protein expression significantly increased in the PBS group ($P < 0.01$). Compared with the PBS group and hMSCs-Exo group, there were differences in *NGF* and *Caspase-3* mRNA and protein expression in the NGF-hMSCs-Exo group rat brain tissues ($P < 0.05$). *Conclusion:* Treatment with human mesenchymal stem cell-derived exosomes carrying the *NGF* gene improves cognitive function and exerts protective effects on SD rats while inhibiting apoptotic levels in cells.

Keywords: *NGF* gene; Human mesenchymal stem cell-derived exosomes; Ischemic stroke in rats; Mechanism of action

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1. Introduction

Ischemic stroke leads to rapid necrosis of brain tissue, with limited clinical treatment efficacy and methods. An ideal treatment method aims to repair damaged brain tissue early and restore the neurovascular network^[1]. Due

to their low immunogenicity and ability to penetrate the blood-brain barrier, exosomes are considered potential resources for repairing injured brain tissue ^[2,3]. In this study, focusing on the NGF gene, we developed an efficient and safe NGF-hMSCs-Exo nanodrug system and injected it into MCAO model rats. Through TTC staining, memory function tests, Western blot, qRT-PCR, and other techniques, we monitored NGF gene expression, neuronal regeneration, and neurological function recovery to assess the potential of NGF therapy for ischemic stroke and explore new approaches for treating ischemic stroke ^[2,3].

2. Materials and methods

2.1. Identification of exosomes from human-derived mesenchymal stem cell sources

Human bone marrow mesenchymal stem cells were purchased from the Shanghai Cell Bank. Exosomes were extracted and identified from these cells: the supernatant from hMSC cultures was collected and subjected to ultracentrifugation or an exosome extraction kit as per the manufacturer's instructions. After collection, the cell supernatant was centrifuged at 300 g and 2,000 g for 10 minutes each to remove cells and debris, then filtered through a 0.22 µm filter to eliminate any residual debris or impurities. For ultracentrifugation, the supernatant was centrifuged at 100,000 g (Beckman) at 4°C for 70 minutes, resuspended in 1 mL of pre-cooled PBS, and centrifuged again at 100,000g at 4°C for 70 minutes. The exosomes were finally collected in 100 µL of PBS. The exosome extraction and purification kit was used according to the operational steps, and the exosomes were collected in 100 µL of PBS.

2.2. Construction and characterization study of the NGF-hMSCs-Exo complex

To construct the NGF-hMSCs-Exo complex, 50–100 µg of hMSCs-Exo was co-incubated with 10 µg of NGF expression plasmid for 4 hours. The NGF expression plasmid, hMSCs-Exo, and electroporation buffer (250 µL) were then mixed in an electroporation cuvette. Electroporation was performed at 400 V and 350 µF. After electroporation, the exosomes were incubated at 37°C for 30 minutes. If necessary, sucrose density gradient centrifugation (15%–60%) was used to separate excess free NGF from the exosomes.

2.3. Construction of the rat MCAO model and grouping

The rat MCAO model was constructed using the classical suture method. Rats were first anesthetized with intraperitoneal injection of chloral hydrate, followed by a midline neck incision to expose and ligate the right common carotid artery and external carotid artery. The common carotid artery was ligated near its bifurcation, leaving a suture in place at the distal end. A miniature artery clamp was used to occlude the distal common carotid artery, and a small incision was made at the proximal end. A monofilament nylon suture was inserted through the incision into the origin of the middle cerebral artery, with an insertion depth of approximately 1.8 cm to block the blood supply to the middle cerebral artery for 90 minutes. All procedures were performed at room temperature, and rectal temperature was monitored. After model construction, neurological function was evaluated in awake rats using the Zea-Longa score, which ranges from 0 to 4. Scores of 1, 2, or 3 were considered successful model construction.

Forty male SPF-grade SD rats aged 6–8 weeks were successfully modeled for cerebral ischemia and then randomly divided into four groups: Sham, PBS, hMSCs-Exo, and NGF-hMSCs-Exo, with 10 rats in each group. Twenty-four hours later, approximately 10¹¹ labeled exosomes were injected into the rats via the tail vein. An intraperitoneal injection regimen was implemented over three days before the rats' euthanasia. This included 5-bromo-2'-deoxyuridine (BrdU), administered at a standard dose of 50 mg/kg body weight, and given twice daily.

2.4. Neurological function deficit scoring

Neurological function deficits were assessed using the modified neurological severity score (mNSS) and rotarod test or grid walking test to evaluate the motor, sensory, reflex, and balance functions of ischemic rats. These tests were conducted at multiple time points: 1 day, 3 days, 7 days, 14 days, and 28 days post-modeling, to assess the improvement in neurological function following exosome transplantation treatment.

2.5. TTC staining for infarct volume percentage

TTC staining was used to determine changes in the infarct area, expressed as the infarct volume percentage. The infarct volume percentage was calculated as (infarct volume / total brain volume) \times 100%.

2.6. Quantitative PCR (RT-PCR) for NGF and Caspase-3 mRNA expression

For the experiments, SD rats treated for one month were euthanized humanely using cervical dislocation, and brain tissue samples were quickly collected. Total RNA was extracted using Trizol reagent, and RT-PCR was performed according to the fluorescence quantitative PCR kit's guidelines. The relative expression levels of the target gene mRNA were quantified using the $2^{-\Delta\Delta CT}$ method.

2.7. Western blot for NGF and Caspase-3 expression

Fresh brain tissue from the rats was added to 1 mL RIPA cell lysis buffer and incubated on ice for 45 minutes. The lysate was then transferred to a cooled PE tube and centrifuged at 12,000 rpm for 15 minutes at 4°C using a pre-cooled high-speed centrifuge to obtain total protein. A 10% separation gel and 4% stacking gel were prepared, with 50 ng of protein loaded per lane. The gel was submerged in 1 \times SDS loading buffer and electrophoresed at 40 V for 2 hours. The proteins were then transferred to a nitrocellulose membrane. After washing with TBST, the membrane was incubated overnight with primary antibodies for NGF and apoptosis-related factor Caspase-3. The membrane was then washed with TBST and incubated at room temperature with a mouse anti-goat HRP secondary antibody for 30 minutes. β -actin served as a loading control, and DAB was used for detection. Protein bands were analyzed using ImageJ software.

2.8. Statistical analysis

Statistical analyses were performed using SPSS 26.0 software. Results are presented as the mean \pm standard deviation (SD). Comparisons of measurement data were made using one-way analysis of variance (ANOVA) (F-test), and comparisons between groups were made using the *t*-test. A *P*-value of < 0.05 was considered statistically significant.

3. Results

3.1. Comparison of neurological function deficits in each group of rats

Rats in the Sham group showed no abnormal symptoms. Compared to the Sham group, the PBS group exhibited motor impairments such as the inability to fully extend the contralateral forepaw or turn to the opposite side, with significant neurological deficits ($P < 0.01$). Compared to the PBS group, the hMSCs-Exo group and NGF-hMSCs-Exo group showed improvements in neurological function scores ($P < 0.05$), with the NGF-hMSCs-Exo group showing more significant improvements compared to the hMSCs-Exo group ($P < 0.05$). See **Figure 1**.

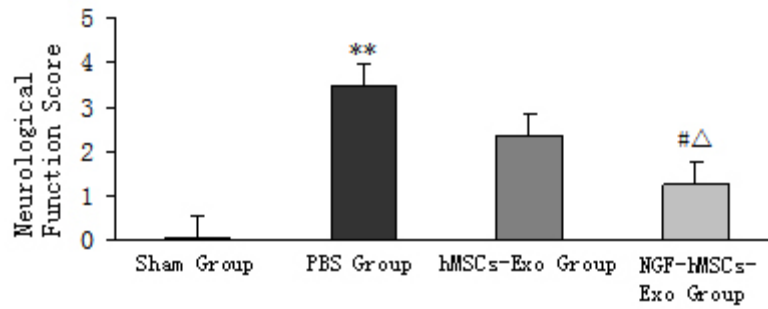


Figure 1. Effect of NGF-hMSCs-Exo injection on neurological function deficits. Compared to the Sham group, ** $P < 0.01$; compared to the PBS group, # $P < 0.05$; compared to the hMSCs-Exo group, $^{\Delta}P < 0.05$

3.2. Comparison of infarct area changes in each group of rats

MCAO modeling resulted in a 43.3% infarct area in the ipsilateral hemisphere of the rats, indicating successful preparation of the cerebral ischemia model. Compared to the PBS group, the hMSCs-Exo group and NGF-hMSCs-Exo group had significantly reduced infarct areas ($P < 0.01$), with the NGF-hMSCs-Exo group showing a more significant reduction in infarct area compared to the hMSCs-Exo group ($P < 0.05$). See **Figure 2**.

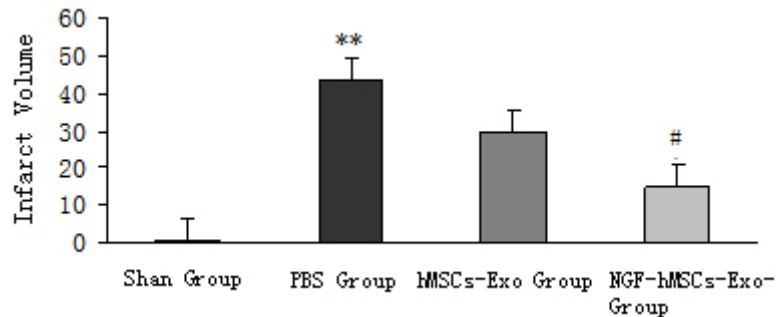


Figure 2. Effect of NGF-hMSCs-Exo injection on infarct area in rats. Compared to the Sham group, ** $P < 0.01$; compared to the PBS group, # $P < 0.05$; compared to the hMSCs-Exo group, $^{\Delta}P < 0.05$

3.3. Expression of NGF and apoptosis-related factor Caspase-3 mRNA in each group of rats

Using RT-PCR, it was observed that compared to the Sham group, the PBS group had decreased relative expression levels of NGF mRNA and significantly increased relative expression levels of Caspase-3 mRNA ($P < 0.01$). Compared to the PBS group, the NGF-hMSCs-Exo group had significantly increased NGF mRNA relative expression levels and significantly decreased Caspase-3 mRNA relative expression levels ($P < 0.05$). See **Table 1**.

Table 1. Comparison of relative expression levels of NGF and apoptosis-related factor Caspase-3 mRNA in each group of rats

Group	<i>n</i>	NGF mRNA expression	Caspase-3 mRNA expression
Sham group	10	1.00 ± 0.01	1.00 ± 0.01
PBS group	10	0.82 ± 0.02	2.14 ± 0.03
hMSCs-Exo group	10	2.07 ± 0.03	0.78 ± 0.02
NGF-hMSCs-Exo group	10	2.95 ± 0.04	0.43 ± 0.01
F-value		22.185	14.756
P-value		0.000	0.000

3.4. Western blot analysis of NGF and apoptosis-related factor Caspase-3 protein expression levels

Western blot analysis showed that compared to the Sham group, the PBS group had decreased NGF protein expression levels and significantly increased Caspase-3 protein expression levels ($P < 0.01$). Compared to the PBS group, the NGF-hMSCs-Exo group had significantly increased NGF protein expression levels and significantly decreased Caspase-3 protein expression levels ($P < 0.05$). See **Figures 3 and 4**.

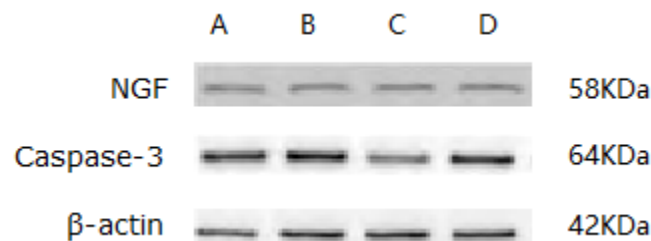


Figure 3. Western blot analysis of NGF and Caspase-3 protein expression. Note: A, Sham group; B, PBS group; C, hMSCs-Exo group; D, NGF-hMSCs-Exo group

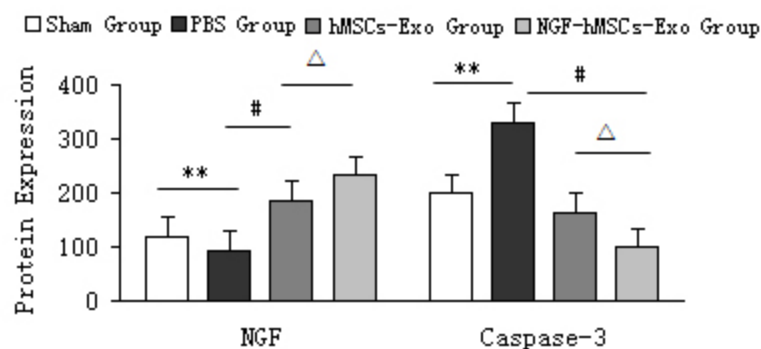


Figure 4. Protein expression levels of NGF and Caspase-3 in rat brain tissue for each group. Compared to the Sham group, $**P < 0.01$; compared to the PBS group, $^{\#}P < 0.05$; compared to the hMSCs-Exo group, $^{\Delta}P < 0.05$

4. Discussion

Ischemic stroke, commonly known as a stroke, along with coronary heart disease and cancer, ranks among the three major diseases affecting human health. Mesenchymal stem cells (MSCs), important members of the stem cell family, are multipotent stem cells that have garnered increasing attention due to their multi-lineage differentiation potential, immune modulation, and self-renewal capabilities ^[4,5]. Recent studies confirm that paracrine effects are the primary mechanism through which stem cells exert their effects after transplantation ^[6,7]. It is currently known that the survival efficiency of stem cells in vivo post-transplantation is very low, possibly less than 5% ^[8]. However, extracellular vesicles secreted by the cells, which mediate paracrine effects, are considered to be the main functional molecules, carrying the effective components and functional attributes of their source cells, and can be developed into a novel therapeutic method for clinical regenerative medicine ^[9]. Nerve growth factor (NGF) is a neurocellular growth regulator with dual biological functions of neuronal nourishment and axonal growth promotion. It plays a crucial neuroprotective role post-stroke by promoting nerve regeneration, axonal reconstruction, and long-lasting neurotrophic effects, thereby facilitating neurological recovery ^[10].

In the experimental results of this study, we observed that rats in the Sham group did not exhibit any abnormal behaviors or physiological symptoms. In stark contrast, rats in the PBS group exhibited significant

motor dysfunction, manifested as an inability to fully extend the contralateral forepaw and an unstable state of the body turning to the opposite side, indicating notable neurological deficits. Compared to the PBS group, the NGF-hMSCs-Exo group showed improved neurological function scores. MCAO modeling resulted in a 43.3% infarct area in the ipsilateral hemisphere of the rats, indicating successful preparation of the cerebral ischemia model. Compared to the PBS group, the hMSCs-Exo and NGF-hMSCs-Exo groups had significantly reduced infarct areas, with the NGF-hMSCs-Exo group showing a more substantial reduction in infarct area compared to the hMSCs-Exo group. This indicates that treatment with NGF gene-carrying human mesenchymal stem cell-derived exosomes can improve neurological function and reduce infarct area. Compared to the Sham group, the PBS group had decreased relative expression levels of NGF mRNA and protein, with significantly increased expression levels of Caspase-3 mRNA and protein. Compared to the PBS and hMSCs-Exo groups, the NGF-hMSCs-Exo group showed statistically significant differences in the expression of NGF and Caspase-3 mRNA and protein in rat brain tissue. This suggests that treatment with NGF gene-carrying human mesenchymal stem cell-derived exosomes has a protective effect on cognitive function in SD rats while inhibiting the level of apoptosis.

Therefore, by analyzing the effects and molecular mechanisms of NGF-hMSCs-Exo in treating ischemic stroke through neuroprotection and neurovascular regeneration, as well as assessing the potential of exosomes as endogenous carriers for treating ischemic stroke, this study provides a solid scientific basis for exploring clinical treatments for ischemic stroke.

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Disclosure statement

The authors declare no conflict of interest.

References

- [1] You T, Tan W, 2020, The Effect of Tanreqing Injection on Motor Function and Nerve Growth Factor Content in Acute Stroke Complicated with Pulmonary Infection. *Chinese Journal of Traditional Chinese Medicine*, 38(8): 187–190.
- [2] Chen N, Fan F, Li S, et al., 2022, The Role and Mechanism of Traditional Chinese Medicine in Regulating Mesenchymal Stem Cell-Derived Exosomes for the Treatment of Ischemic Stroke. *Chinese Journal of Tissue Engineering Research*, 26(13): 2081–2086.
- [3] Zhou G, Bai W, Shao J, 2023, Research Progress on the Effects of Exosomes and Their Carried microRNAs on Brain Ischemia-Reperfusion Injury. *Journal of Chongqing Medical University*, 48(7): 840–847.
- [4] Wang Y, Lai X, Wu D, et al., 2021, Umbilical Mesenchymal Stem Cell-Derived Exosomes Facilitate Spinal Cord Functional Recovery Through the miR-199a-3p/145-5p-Mediated NGF/TrkA Signaling Pathway in Rats. *Stem Cell Res Ther*, 12(1): 117. <https://doi.org/10.1186/s13287-021-02148-5>
- [5] Hao L, Lu L, Li Q, et al., 2024, The Effect of Bone Marrow Mesenchymal Stem Cell-Derived Exosomes Overexpressing miR-124-1 Gene on the M2 Polarization of Microglia in Stroke. *Journal of Army Medical University*, 46(5): 458–466.
- [6] Liu A, Chen Y, Zhang J, et al., 2021, Correlation Between Prognosis of Acute Ischemic Stroke and Serum Fibroblast Growth Factor 23 and Chemokine CXCL12. *Chinese Journal of Gerontology*, 41(20): 4350–4352.

- [7] He J, Chen L, Tang F, et al., 2023, Multidisciplinary Team Collaboration Impact on NGF, BDNF, Serum IGF-1, and Life Quality in Patients with Hemiplegia after Stroke. *Cell Mol Biol (Noisy-Le-Grand)*, 69(12): 57–64. <https://doi.org/10.14715/cmb/2023.69.12.10>
- [8] Chen S, Zhu J, Zhao X, et al., 2023, Effects of Neural Stem Cell-Derived Exosomes on Astrocyte TGF- β 1 Signaling and Inflammatory Factors in Rats with Cerebral Ischemia-Reperfusion Injury. *Practical Journal of Medicine*, 39(13): 1600–1605.
- [9] Tani A, Sakakima H, Otsuka S, et al., 2023, Stimulation of Functional Recovery Via Neurorepair Mechanisms by the Traditional Japanese Kampo Medicine, Ninjin'yoeito, and Physical Exercise in A Rat Ischemic Stroke Model. *J Ethnopharmacol*, 302(Pt B): 115927. <https://doi.org/10.1016/j.jep.2022.115927>
- [10] Povarnina PY, Antipova TA, Gudasheva TA, et al., 2022, Neuroprotective and Neuroregenerative Properties of Dimeric Dipeptide Mimetics of Individual NGF and BDNF Loops Under Conditions of an Experimental Ischemic Stroke Model. *Pharm Chem J*, 56: 1019–1023. <https://doi.org/10.1007/s11094-022-02745-5>

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Diagnostic Value of Endoscopic Ultrasound in Staging Rectal Cancer

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Abstract: *Objective:* To explore the diagnostic value of endoscopic ultrasound (EUS) in the staging of rectal cancer. *Methods:* Fifty patients with rectal cancer, treated at the Department of Endoscopy, Cancer Hospital Affiliated to Fudan University, from March 2023 to June 2024, were selected. All patients underwent EUS and computed tomography (CT) examination within two weeks before surgery. The postoperative pathological staging was used as the standard to compare the accuracy of tumor TN staging using EUS and/or CT. *Results:* The accuracy rates of T and N staging by abdominal spiral CT were 72.00% and 76.00%, respectively, while those by EUS were 88.00% and 74%, respectively. There was a significant difference in the accuracy of T staging between the two methods (both $P < 0.05$). *Conclusion:* EUS has high diagnostic value in the staging of rectal cancer, providing important reference information for clinicians, aiding in the development of personalized treatment plans, and assessing patient prognosis.

Keywords: Endoscopic ultrasound; Rectal cancer; Diagnostic value

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1. Introduction

Rectal cancer is a common malignant tumor, especially prevalent among middle-aged and elderly populations. Its incidence and mortality rates are second only to esophageal cancer, gastric cancer, and primary liver cancer, ranking fourth among digestive system malignancies^[1-3]. Early-stage rectal cancer often lacks obvious symptoms but may primarily manifest as changes in bowel habits, occult abdominal pain, anemia, and progressive weight loss^[4]. Like other malignant tumors, rectal cancer can metastasize to other organs and tissues through lymphatic, hematogenous, and direct spread, ultimately threatening the patient's life^[5,6]. Currently, surgical treatment is the main approach for rectal cancer patients. Although surgery can effectively remove extensive lymph nodes, unclear preoperative pathological staging diagnosis may reduce clinical treatment efficacy^[7]. Therefore, early clarification of pathological staging and formulation of individualized treatment plans are key to effectively controlling disease progression and improving patient prognosis^[8]. The staging of rectal cancer mainly relies on imaging examinations, endoscopic examinations, and pathological examinations. Among these, endoscopic ultrasound (EUS), as a non-invasive, high-resolution examination

method, has unique advantages in rectal cancer staging. EUS can visually display the size of the tumor, the depth of infiltration, and the status of extramural lymph node metastasis, aiding in the accurate determination of the lesion's staging. However, despite the potential importance of EUS in rectal cancer staging diagnosis, its accuracy and clinical application in practice remain controversial. This study aims to systematically explore the accuracy of EUS in the staging diagnosis of rectal cancer, analyze its consistency with pathological staging results, and further evaluate its clinical application prospects, providing more reliable diagnostic evidence for clinicians.

2. Materials and methods

2.1. General information

This study included 50 subjects from the Department of Endoscopy, Cancer Hospital Affiliated to Fudan University, comprising 26 male and 24 female patients, with an average age of 52.8 years (age range 30 to 70 years). Inclusion criteria: (1) Patients pathologically confirmed to have rectal cancer; (2) Consent to participate in the study and sign informed consent; (3) Underwent both rectal endoscopic ultrasound (EUS) and computed tomography (CT) examinations; (4) No significant advanced metastases or other major diseases. Exclusion criteria: (1) Presence of other significant advanced tumors; (2) Presence of significant cardiovascular, renal, or other major diseases; (3) Refusal to participate in the study or failure to sign informed consent; (4) Poor quality of rectal EUS or CT examinations; (5) Lack of complete clinical data.

2.2. Methods

2.2.1. Normal bowel wall ultrasound endoscopic layering

Displayed as a five-layer structure from inside to outside.

- (1) The innermost layer is a thin high-echo line representing the interface between the superficial mucosa and the intestinal lumen. It is very clear when the lumen contains fluid but can merge with the echo of gas when the lumen contains air.
- (2) The second layer is low echo, representing the deep mucosa. This layer shows edema first in cases of bowel inflammation.
- (3) The third layer is high echo, representing the submucosa, which becomes thinner when the bowel is dilated.
- (4) The fourth layer is low echo, representing the muscularis propria.
- (5) The fifth layer is a thin high-echo line, representing the serosa, generally indistinguishable from the surrounding mesentery and fat.

2.2.2. Rectal cancer staging

The TNM staging system (staging based on tumor size, lymph node involvement, and metastasis):

- (1) T (Tumor): Describes the size and depth of the primary tumor, classified from T0 to T4.
- (2) N (Node): Describes lymph node involvement, classified from N0 to N2.
- (3) M (Metastasis): Describes the presence of distant metastasis, with M0 indicating no distant metastasis and M1 indicating the presence of distant metastasis.

2.2.3. Abdominal spiral CT and EUS examination methods

2.2.3.1. Abdominal spiral CT examination

- (1) A Siemens 256-slice high-end dual-source CT (model Flash) was used.

- (2) Patients were required to fast for 6–8 hours before the examination to maintain an empty stomach.
- (3) Patients needed to drink 500–800 mL of water 30 minutes before the examination to help expand the abdominal organs and reduce artifacts.
- (4) Laxatives were not administered to ensure the clarity of the intestines was not affected.
- (5) The scan slice thickness was 3–4 mm, and the slice spacing was 3–4 mm, to obtain more detailed images.
- (6) The examination involved a full abdominal scan to locate abdominal organs, followed by intravenous injection of iodixanol (62.5 g/100 mL) to acquire arterial enhancement and venous phase images.

2.2.3.2. EUS examination

- (1) A Pentax ultrasound endoscope EG-3270UK was used for EUS.
- (2) Laxatives were orally administered before the procedure to clean the bowel, ensuring clarity and stability in the digestive tract.
- (3) The endoscopist inserted the Pentax ultrasound endoscope EG-3270UK into the rectum, removed air from the bowel, and injected degassed water while observing. The entire process involved positioning the endoscope centrally within the intestinal lumen, with the lesion at the 6 o'clock position on the ultrasound screen. The examination involved observing the lesion's infiltration into surrounding and deeper tissues, and checking for involvement of extramural lymph nodes, surrounding tissues, and organs to facilitate accurate assessment.

2.3. Observational indicators

At least two experienced radiologists and endoscopists independently assessed the images using a double-blind method to ensure consistent conclusions. The accuracy of different imaging techniques in staging rectal cancer was compared, using pathological evaluation as the reference standard. EUS T staging criteria:

- (1) T1: If the mucosal layer shows disruption between the first and second echo bands, and the submucosal strong echo band is either fuzzy or disrupted, with the muscularis propria low echo band remaining intact.
- (2) T2: If the lesion continues to invade, disrupting the muscularis propria low echo band without involving the serosa.
- (3) T3: If the tumor breaches the serosa, causing disruption of the high echo band of the serosa, potentially connecting with surrounding tissues.
- (4) T4: Presence of low echo masses in adjacent tissues.

EUS N staging: If lymph nodes change in size, with a diameter greater than 5 mm, abnormal internal echoes, and sharp edges, they are considered cancer-involved lymph nodes.

2.4. Statistical analysis

SPSS 23.0 software was used for statistical analysis of the data in this study. Data were expressed as either mean \pm standard deviation (SD) or [*n* (%)], and comparisons between the two groups were made using two-sample *t*-tests or chi-squared tests, with $P < 0.05$ considered statistically significant.

3. Results

3.1. Comparison of staging accuracy for rectal cancer between two examination methods

As shown in **Table 1**, the accuracy of T and N staging by abdominal spiral CT was 72.00% and 76.00%, respectively. In contrast, the accuracy of T and N staging for colorectal cancer diagnosed via endoscopic ultrasound was 88.00% and 74%, respectively. The difference in T staging accuracy between the two

examination methods was statistically significant ($P < 0.05$).

Table 1. Comparison of staging accuracy for rectal cancer between two examination methods [n (%)]

Examination methods	n	T	N
CT	50	36 (72.00%)	38 (76.00%)
EUS	50	44 (88.00%)	37 (74.00%)
χ^2	-	4.000	0.053
P	-	0.046	0.817

3.2. Image analysis

As shown in **Figure 1**, endoscopic ultrasound examination demonstrated higher accuracy and sensitivity in staging rectal cancer, clearly depicting tumor size, depth of invasion, and extramural lymph node metastasis.

Figure 1(c–f) are endoscopic ultrasound images showing tumor invasion into the serosa, with a sawtooth-like interruption in the fifth high-echo layer. Low-echo lymph nodes with sharp edges are visible outside the bowel wall.

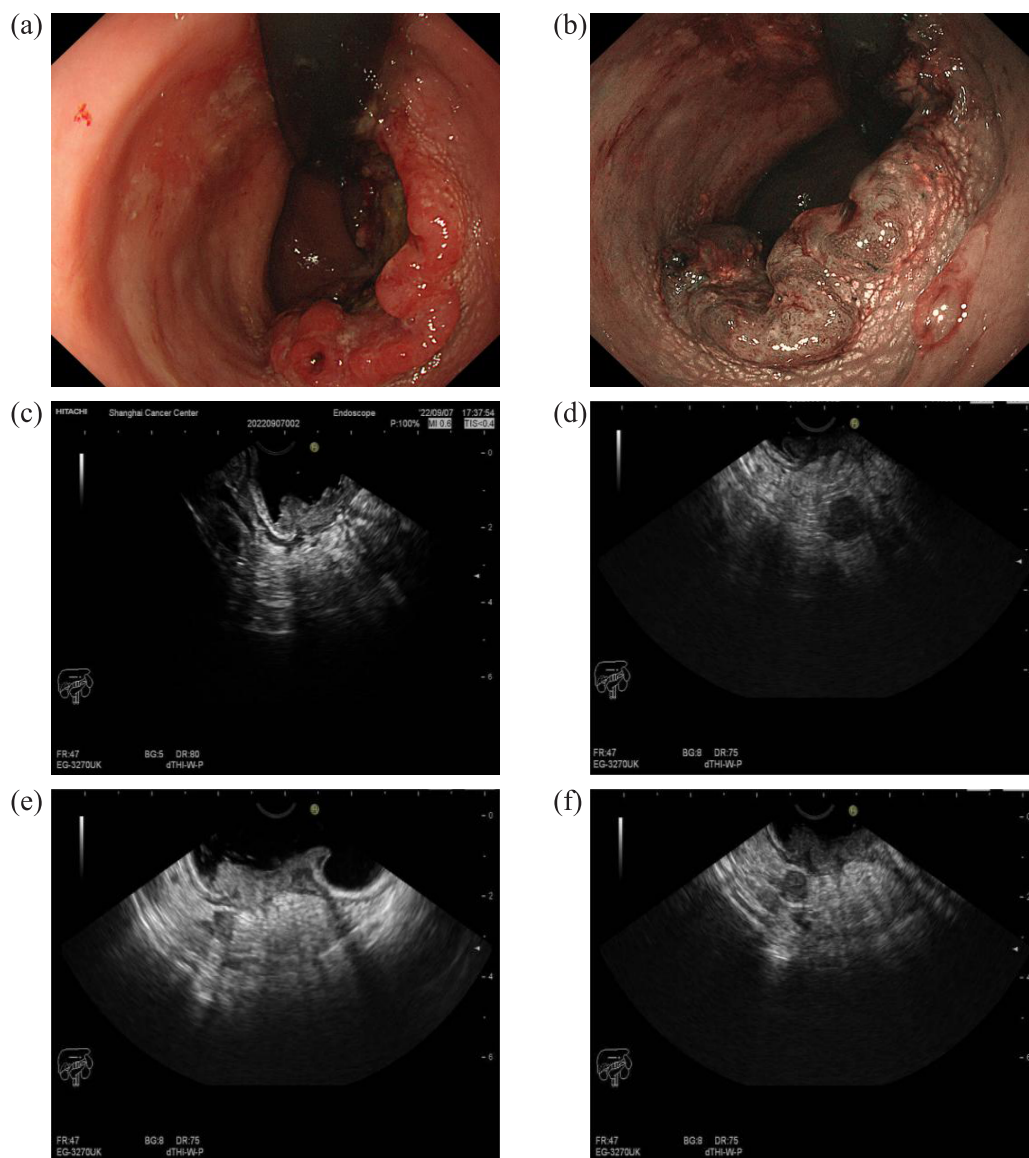


Figure 1. Images from endoscopic ultrasound examinations

4. Discussion

In rectal cancer, CT scans can provide valuable information regarding tumor size, depth of invasion, and distant metastasis, aiding in the accurate assessment of preoperative staging ^[9,10]. On the other hand, endoscopic ultrasound (EUS), as a method for precisely locating the depth of tumor invasion, has been widely used in this regard ^[11]. EUS can provide high-resolution images, clearly displaying the location of the rectal cancer lesion and the surrounding structures. Through EUS, doctors can evaluate characteristics such as the size, morphology, depth of invasion, and extramural lymph node status of the tumor, providing important evidence for TN staging of rectal cancer. Studies have shown that the accuracy of EUS in assessing the T stage of rectal cancer exceeds 80%, performing well in staging early rectal cancer. Although there is no statistical difference between the two methods in N stage, EUS can accurately evaluate lymph node metastasis in rectal cancer. EUS can assess whether surrounding lymph nodes are affected, determining the extent of lymph node involvement. Some studies indicate that EUS has a higher detection rate for extramural lymph node metastasis in rectal cancer, able to identify smaller lymph nodes and internal conditions that CT might miss, aiding in determining the extent of surgery and the selection of preoperative chemoradiotherapy regimens. However, due to the limited scanning depth of EUS, it is less accurate than CT in assessing distant organ metastasis and extramural lymph nodes. EUS can also guide precise needle biopsies to obtain tissue samples for definitive pathological diagnosis, further guiding treatment plan selection. Clinical manifestations of rectal cancer typically include weight loss, rectal bleeding, abdominal pain, and fatigue. If not treated promptly, the condition may progress, leading to serious complications such as rectal bleeding, abdominal masses, and even distant metastasis, which may ultimately result in patient death ^[12,13]. Related studies have shown that with changes in lifestyle and dietary structure, the incidence of colorectal cancer is rising annually, with a trend towards younger onset ^[14]. Therefore, early detection and accurate assessment of the disease can help develop more effective treatment plans, improving the survival rate and quality of life for rectal cancer patients.

This study suggests that the accuracy of EUS in the T staging of rectal cancer is significantly higher than that of abdominal spiral CT. This may be attributed to the fact that EUS, as a close-range endoscopic examination, avoids other confounding factors and provides more precise and clear details of the rectal wall and extramural lesions. It helps in accurately locating the lesion, assessing the depth of invasion, and detecting lymph node metastasis, with no statistical difference in N staging accuracy between the two methods. In contrast, abdominal spiral CT, as a systemic imaging method, can provide a comprehensive view of the lesion but is limited in displaying intracolonic structures and cannot distinguish the internal structure of lymph nodes. Therefore, it has a lower accuracy in determining the depth of invasion and lymph node involvement but can provide useful information on distant metastasis. Based on the study results, it can be concluded that for the T staging diagnosis of rectal cancer, EUS may be a more reliable and accurate choice, providing clinicians with more informative guidance for developing personalized treatment plans, improving treatment outcomes, and patient survival rates. Therefore, in clinical practice, EUS should be prioritized for staging diagnosis and evaluation of rectal cancer, supplemented by CT to achieve better clinical outcomes.

In summary, endoscopic ultrasound examination has significant diagnostic value in the staging of rectal cancer. Its accurate assessment of invasion depth and sensitivity in detecting extramural lymph nodes help clarify the staging and condition of rectal cancer, providing an important basis for developing individualized treatment plans for patients. In the clinical diagnosis and treatment of rectal cancer, EUS should be widely used to improve treatment outcomes and survival rates for rectal cancer patients.

Disclosure statement

The authors declare no conflict of interest.

References

- [1] Zhang L, 2022, Clinical Study of MRI/CT Imaging Technology Guiding Rectal Cancer Diagnosis. *Modern Medical Imaging*, 31(4): 656–658.
- [2] Liu X, Yan S, Sun S, 2023, Diagnostic Value and Imaging Characteristics Analysis of Dynamic Contrast-Enhanced CT Scan and MRI for Hepatocellular Carcinoma. *Practical Cancer Journal*, 38(1): 89–91.
- [3] Han Z, Liu S, 2022, Study on the Value of MRI Conventional Sequences and Diffusion-Weighted Imaging in the Preoperative Diagnosis of Rectal Cancer. *Imaging Research and Medical Applications*, 6(8): 89–91.
- [4] Yin Q, Sun H, Hu K, 2020, Analysis of the Value of Combined CT and MRI Imaging in the Clinical Preoperative Staging of Rectal Cancer and the Diagnosis Accuracy of Lymph Node Positivity. *Jilin Medical Journal*, 41(10): 2320–2322.
- [5] Yang Y, Wei H, Fu F, et al., 2023, Multimodal MRI Radiomics Combined with Clinical Risk Factors for Preoperative Prediction of Lymphovascular Invasion in Rectal Cancer Without Lymph Node Metastasis. *Magnetic Resonance Imaging*, 14(1): 94–99 + 110.
- [6] Liu S, Mao H, 2022, Value Analysis of Multi-Phase Enhanced MSCT in Preoperative TN Staging of Colorectal Cancer and Its Comparison with Pathological Staging. *International Journal of Medical and Health Guidance News*, 28(4): 464–467.
- [7] Zhang Y, Liu Y, 2022, Imaging Characteristics and Diagnostic Efficiency Analysis of MRI and CT in the Diagnosis of Liver Cirrhosis Complicated with Hepatocellular Carcinoma. *Clinical Medical Engineering*, 29(3): 307–308.
- [8] Guo C, Zhou J, Yu H, et al., 2019, Consistency Analysis of Imaging Findings of Mediastinal Lung Cancer with CT and MRI and Postoperative Pathological Diagnosis. *Chinese CT and MRI Journal*, 17(6): 49–51 + 68.
- [9] Klessen C, Rogalla P, Taupitz M, 2007, Local Staging of Rectal Cancer: The Current Role of MRI. *Eur Radiol*, 17(2): 379–389. <https://doi.org/10.1007/s00330-006-0388-x>
- [10] Rollvén E, Holm T, Glimelius B, et al., 2013, Potentials of High Resolution Magnetic Resonance Imaging Versus Computed Tomography for Preoperative Local Staging of Colon Cancer. *Acta Radiol*, 54(7): 722–730. <https://doi.org/10.1177/0284185113484018>
- [11] Puli SR, Reddy JB, Bechtold ML, et al., 2009, Accuracy of Endoscopic Ultrasound to Diagnose Nodal Invasion by Rectal Cancers: A Meta-Analysis and Systematic Review. *Ann Surg Oncol*, 16(5): 1255–1265. <https://doi.org/10.1245/s10434-009-0337-4>
- [12] Qu J, Yu C, He S, 2023, Value of Spectral CT Combined with MRI in the T Staging Diagnosis of Colorectal Cancer. *Chinese Journal of Oncology Surgery*, 15(1): 60–65.
- [13] Wang P, 2023, Clinical Value of CT Combined with High-Resolution Pelvic MRI in the Diagnosis and Staging of Colorectal Cancer. *Chinese Journal of Integrative Medicine Imaging*, 21(1): 44–47.
- [14] Zhang W, Huang X, Zhi S, et al., 2022, Clinical Value and Imaging Analysis of PET/CT and MRI Fusion Images in Diagnosing Lymph Node Metastasis of Rectal Cancer. *Journal of Qiqihar Medical College*, 43(7): 657–661.

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Yinqiao Sanhuang Paste Combined with Traditional Chinese Medicine Plaster for the Intervention of Drug Rash Induced by Targeted Therapy in Lung Cancer

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Abstract: *Objective:* To observe the efficacy and safety of Yinqiao Sanhuang Paste combined with traditional Chinese medicine plaster in treating drug rash caused by targeted therapy in lung cancer. *Methods:* A total of 100 lung cancer patients treated at our hospital from January 2021 to December 2023 were selected and randomly divided into an observation group and a control group, with 50 patients in each group. The control group received conventional medication, while the observation group was treated with Yinqiao Sanhuang Paste combined with traditional Chinese medicine plaster. The clinical symptom improvement and adverse reactions in both groups were observed. *Results:* The effective rate in the control group was 80.00%, while in the observation group, it was 96.00%, with a statistically significant difference between the two groups ($P < 0.05$). The onset time, duration, and significant effect time in the control group were (2.41 ± 0.29) days, (4.42 ± 1.21) days, and (5.45 ± 0.29) days, respectively; in the observation group, they were (2.44 ± 0.21) days, (4.28 ± 1.11) days, and (5.57 ± 1.01) days, respectively. There was no statistically significant difference in the total onset time and total duration between the two groups ($P > 0.05$). The incidence of adverse reactions in the control group was 28.00%, higher than the observation group's 10.00% ($P < 0.05$). *Conclusion:* Yinqiao Sanhuang Paste combined with traditional Chinese medicine plaster can effectively reduce the symptoms of drug rash induced by targeted therapy in lung cancer and lower the incidence of adverse reactions, indicating good clinical application value.

Keywords: Yinqiao Sanhuang Paste; Traditional Chinese medicine plaster; Drug rash; Efficacy

Online publication: August 12, 2024

1. Introduction

As medical technology continues to advance, targeted therapies have become an important means of treating lung cancer. However, these therapies often come with a range of adverse reactions, the most common of which is drug-induced rash (drug rash). Drug rash not only significantly affects the patient's quality of life but may also interfere with the continuation of targeted therapy, potentially impacting the treatment outcome and

survival time. The pathogenesis of drug rash is complex, involving immune responses and cytotoxic reactions, among other factors. Traditional treatments include the use of corticosteroids and antihistamines, but these often have limited efficacy and significant side effects. Therefore, finding a safer and more effective intervention for drug rash is of great clinical significance. In recent years, the role of traditional Chinese medicine in cancer treatment has gained increasing attention. Yinqiao Sanhuang Paste is a traditional Chinese medicine preparation with the effects of clearing heat, detoxifying, cooling the blood, and reducing swelling, and it has been widely used in the treatment of skin diseases. Traditional Chinese medicine plaster is an external treatment method in Chinese medicine, where the medicine is applied to the affected area, allowing the active ingredients to penetrate directly to the site of the lesion to achieve a therapeutic effect. This study aims to explore the intervention effects of Yinqiao Sanhuang Paste combined with traditional Chinese medicine plaster on drug rash caused by targeted therapy in lung cancer, providing new ideas and methods for the treatment of drug rash. This research helps further elucidate the mechanisms and treatment methods for drug rash and enriches the theoretical application of traditional Chinese medicine in cancer treatment.

2. Materials and methods

2.1. General information

This study involved 100 cases, including 54 males and 46 females, aged between 38 and 78 years with an average age of 63.21 ± 12.42 years. The duration of illness ranged from 1 to 9 years, with an average duration of 3.09 ± 0.58 years. Among them, 30 cases had stage III lung cancer, and 70 cases had stage IV lung cancer. Using a random number table method, the patients were divided into two groups: the control group with 50 patients and the observation group with 50 patients. The control group primarily exhibited symptoms such as rash, fever, joint pain, and headache, while the observation group primarily exhibited symptoms such as rash, fever, joint pain, cough, and chest tightness. Both groups met the diagnostic criteria for drug rash in Traditional Chinese Internal Medicine ^[1], and all patients received the same clinical medication regimen. All enrolled patients signed informed consent forms and fully understood the content of the study.

2.2. Methods

The control group received conventional medication, following standard treatment principles with anti-infection and sedative drugs to control infections, alleviate symptoms, and prevent disease progression. The observation group received the same treatment with the addition of Yinqiao Sanhuang Paste combined with traditional Chinese medicine plaster. The specific method was:

- (1) Composition of Yinqiao Sanhuang Paste: 10 g of *Lonicera japonica*, 10 g of *Forsythia suspensa*, 5 g of *Phellodendron chinense*, 6 g of dandelion, 10 g of *Rehmannia glutinosa*, 10 g of *Angelica dahurica*, 10 g of peony tree root bark, 10 g of *Artemisia annua*, 3 g of licorice, 1 slice of fresh ginger, and 3 g of mint.
- (2) Traditional Chinese medicine plaster procedure: Patients were positioned in a sitting or supine position. The above-mentioned herbs were mixed with water in a ratio of Rhubarb:*Scutellaria baicalensis*:*Coptis chinensis*:Chinese Peony:Licorice = 1:2:3:1:0.5, then heated until the herbs were completely dissolved and appeared semi-transparent. The mixture was applied to the affected area once daily for 1–2 hours each time. Patients were advised to rest, avoid fatigue, refrain from consuming spicy, greasy, or allergenic foods, maintain a clean living environment, and strengthen nutrition to promote recovery. Both groups received compound preparations containing corticosteroids for anti-inflammatory and anti-

allergic treatment, such as Methylprednisolone (Taiwan South Sun Chemical Pharmaceutical, National Medicine Standard HJ20170197) and Dexamethasone Sodium Phosphate Injection (Hainan Better Pharmaceutical Co., Ltd., National Medicine Standard H320215611).

2.3. Observation indicators

The study observed the clinical efficacy and adverse reactions in both groups.

- (1) Clinical efficacy was determined based on the “Guidelines for Clinical Research of New Chinese Medicines,” using a “three-step method”: (a) Significant effect: Complete resolution of rash and related symptoms; (b) Effective: Marked improvement in rash and related symptoms, but not complete resolution; (c) Ineffective: No change or worsening of rash and related symptoms.
- (2) Traditional Chinese medicine symptoms were evaluated using a TCM symptom scoring system ^[2]: (a) Mild (0–5 points): Only a few erythema or papules, generally not exceeding 1/3 of the body surface area; (b) Moderate (6–10 points): In addition to erythema and papules, there is also itching; (c) Severe (> 10 points): In addition to the above symptoms, there are blisters, vesicles, exudation, or erosion.
- (3) Adverse reactions were defined as adverse events related to the medication during the treatment process, including allergic reactions, nervous system reactions, hematologic system reactions, liver function damage, kidney function damage, myocardial infarction, vascular embolism, arrhythmia, gastrointestinal reactions, central nervous system damage, etc. The occurrence of any of the following adverse events was considered an adverse event ^[3]: (a) Anaphylactic shock; (b) Acute renal failure; (c) Hematologic system reactions (thrombocytopenic purpura, hemolytic anemia, neutropenia, aplastic anemia, leukopenia); (d) Nervous system reactions (dizziness, headache, ataxia, tremor, convulsions, seizures, coma).

2.4. Statistical analysis

Statistical analysis was performed using the SPSS19.0 software package. Measurement data conforming to a normal distribution were expressed as mean \pm standard deviation (SD), and non-normally distributed data were expressed as M (P25–P75). Between-group comparisons were conducted using the *t*-test; count data were expressed as the number of cases (%) and compared using the χ^2 test or Fisher’s exact test.

3. Results

3.1. Comparison of overall effective rate between the two groups

The effective rate was 80.00% in the control group and 96.00% in the observation group. The difference in effective rates between the two groups was statistically significant ($P < 0.05$), as shown in **Table 1**.

Table 1. Comparison of overall effective rate between the two groups [*n* (%)]

Group	Significant effect	Effective	Ineffective	Total effective rate
Control group (<i>n</i> = 50)	24 (48.00)	16 (32.00)	10 (20.00)	40 (80.00)
Observation group (<i>n</i> = 50)	29 (58.00)	19 (38.00)	2 (4.00)	48 (96.00)
χ^2				6.061
<i>P</i>				0.014

3.2. Comparison of traditional Chinese medicine symptoms between the two groups

The onset time, duration, and significant effect time in the control group were (2.41 ± 0.29) days, (4.42 ± 1.21)

days, and (5.45 ± 0.29) days, respectively. In the observation group, these times were (2.44 ± 0.21) days, (4.28 ± 1.11) days, and (5.57 ± 1.01) days, respectively. There was no statistically significant difference in the total onset duration and total duration between the two groups ($P > 0.05$), as shown in **Table 2**.

Table 2. Comparison of TCM symptoms between the two groups (mean \pm SD; days)

Groups	Onset of action	Duration	Significant effect time
Control group ($n = 50$)	2.41 ± 0.29	4.42 ± 1.21	5.45 ± 0.29
Observation group ($n = 50$)	2.44 ± 0.21	4.28 ± 1.11	5.57 ± 1.01
t	0.091	0.603	0.808
P	> 0.05	> 0.05	> 0.05

3.3. Incidence of adverse events

During the observation period, no serious adverse events occurred in either group. In the control group, 14 cases experienced rash itching with pain, while 5 cases in the observation group experienced similar symptoms. The incidence rate was 28.00% in the control group, higher than 10.00% in the observation group ($P < 0.05$), as shown in **Table 3**.

Table 3. Incidence of adverse events in the two groups

Groups	Number of adverse reactions	Incidence of adverse reactions
Control group ($n = 50$)	14	28.00%
Observation group ($n = 50$)	5	10.00%
χ^2		5.263
P		0.022

4. Discussion

Targeted therapies for lung cancer have shown significant efficacy, but the associated issue of drug-induced rash cannot be overlooked. Drug-induced rashes not only impact the quality of life of patients but may also negatively affect the continuity and effectiveness of targeted therapies. Therefore, finding effective interventions for drug-induced rashes is crucial.

Drug-induced rash is a common clinical adverse reaction with a relatively high incidence, most of which can resolve spontaneously. Studies have shown that lung cancer-targeted drugs, including paclitaxel, gefitinib, and erlotinib, pose certain risks of inducing or exacerbating drug-induced rashes ^[4,5]. This study conducted a treatment observation on 100 lung cancer patients, showing an overall effective rate of 67.9% in the control group and 85.1% in the observation group, with no statistically significant difference ($P > 0.05$).

As tumor-targeted therapy technology continues to advance and improve, the treatment outcomes for lung cancer have significantly improved, but the accompanying adverse reaction issues have also increased, with drug-induced rashes being the most common, accounting for over 50% of total adverse events ^[6]. Data indicate that the incidence of adverse reactions was 43.0% in the control group and 22.6% in the observation group, with a statistically significant difference ($P < 0.05$); mild and moderate itching constituted the primary adverse symptoms, accounting for about 80% of all adverse symptoms ^[7,8].

This study compared the efficacy of Yinqiao Sanhuang Paste combined with traditional Chinese medicine

plaster with conventional drug therapy in treating lung cancer-targeted therapy-induced rashes. The results showed that the effective rate was 80.00% in the control group and 96.00% in the observation group, with a statistically significant difference between the two groups ($P < 0.05$). The onset time, duration, and significant effect time were (2.41 ± 0.29) days, (4.42 ± 1.21) days, and (5.45 ± 0.29) days in the control group, respectively, compared to (2.44 ± 0.21) days, (4.28 ± 1.11) days, and (5.57 ± 1.01) days in the observation group, with no statistically significant difference in total onset duration and total duration between the two groups ($P > 0.05$). The incidence rate was 28.00% in the control group, higher than 10.00% in the observation group ($P < 0.05$), indicating that Yinqiao Sanhuang Paste combined with traditional Chinese medicine plaster has good efficacy and safety in treating lung cancer-targeted therapy-induced rashes. This study used a combination of Yinqiao Sanhuang Paste and traditional Chinese medicine plaster to intervene in lung cancer-targeted therapy-induced rashes. The results showed that this method significantly improved the rash symptoms in patients and alleviated their discomfort. This may be related to the heat-clearing, detoxifying, and swelling-reducing effects of Yinqiao Sanhuang Paste and the drug penetration effects of traditional Chinese medicine plaster. The combination of these methods can directly and effectively act on the affected area, promoting the resolution of the rash. TCM has unique advantages in treating drug-induced rashes^[9]. First, TCM emphasizes overall conditioning, which can improve the patient's constitution and enhance the body's tolerance to drugs, thereby reducing the occurrence of drug-induced rashes^[10]. Second, TCM has relatively few side effects, which do not impose an additional burden on the patient^[11]. Finally, the diverse treatment methods in TCM can be tailored to the patient's specific conditions, enhancing the treatment's effectiveness.

Although this study achieved certain results, there are still some limitations. First, the sample size is relatively small, which may affect the reliability of the results. Future research should expand the sample size to further verify the intervention effects of Yinqiao Sanhuang Paste combined with traditional Chinese medicine plaster on drug-induced rashes. Second, this study did not conduct stratified analysis based on different types and severities of rashes; future research could explore the applicability of this method under different circumstances. Finally, this study did not delve into the mechanisms of action; future research could integrate molecular biology and pharmacology approaches to further elucidate the molecular mechanisms underlying the effects of Yinqiao Sanhuang Paste combined with traditional Chinese medicine plaster on treating drug-induced rashes.

In conclusion, Yinqiao Sanhuang Paste combined with traditional Chinese medicine plaster can effectively alleviate symptoms of drug-induced rashes in lung cancer patients undergoing targeted therapy and reduce the incidence of adverse reactions, providing a new intervention measure for clinical practice. Future research could further explore the applicability and mechanisms of this method to provide more reliable evidence for clinical application.

Disclosure statement

The authors declare no conflict of interest.

References

- [1] Ma X, Huo Z, Bi J, et al., 2023, Clinical Observation of Shufeng Touzhen Decoction Combined with Yinqiao Sanhuang Paste in the Treatment of Lung Cancer-Targeted Drug-Related Rash. *Journal of Zhejiang University of Traditional Chinese Medicine*, 47(8): 897–902.
- [2] Zou H, Leng W, Guo S, et al., 2023, Effect of Xiaofeng Powder Bath on Rash Reaction after Lung Cancer Targeted

Therapy. *Guangming Journal of Chinese Medicine*, 38(10): 1897–1899.

- [3] Ma X, Huo Z, Bi J, et al., 2023, Clinical Study of Qingzao Jiufei Decoction Combined with Yinqiao Sanhuang Ointment in the Treatment of Lung Adenocarcinoma-Targeted Drug-Related Rash. *Evaluation and Analysis of Drug Use in Chinese Hospitals*, 23(4): 407–411.
- [4] Yu J, Zhang S, Yang A, 2023, Study on the Effect of Chinese Medicine Danggui Yinzi in Treating Rash Caused by Molecular Targeted Drugs for Lung Cancer. *China Health Standard Management*, 14(7): 182–186.
- [5] Gao Y, Sun T, 2023, Clinical Study on Adjuvant Treatment of Rash Caused by Lung Cancer Targeted Drugs with Chinese Medicine Anti-Itch Formula for Oral and External Washing. *Modern Journal of Integrated Traditional Chinese and Western Medicine*, 32(7): 970–974.
- [6] Wang Z, 2022, Effect of Yinqiao Qingdai Decoction Combined with Gefitinib on Locally Advanced Non-Small Cell Lung Cancer and Its Impact on Patients' Quality of Life. *Chinese Medical Innovation*, 19(5): 83–87.
- [7] Fang Z, Wang L, Li J, 2022, A Case of Psoriasis-Like Drug Eruption Caused by PD-1 Inhibitor in a Patient with Advanced Lung Cancer and Literature Review. *Chinese Journal of Leprosy and Skin Diseases*, 38(3): 161–163.
- [8] Ding R, Huo J, Xing H, 2019, Clinical Study of 30 Cases of “Yaoshen Formula” as the Main Prevention and Treatment of Erlotinib-Induced Skin Toxicity. *Jiangsu Journal of Traditional Chinese Medicine*, 51(12): 49–51.
- [9] Luo B, Liu H, Li H, et al., 2019, Efficacy of Biological Targeted Therapy for Lung Cancer and TCM's Herxheimer Reaction. *World Journal of Traditional Chinese Medicine*, 14(9): 2519–2523.
- [10] He J, Hua B, 2020, Experience of Hua Baojin in Treating Lung Cancer-Targeted Drug-Related Rash. *Liaoning Journal of Traditional Chinese Medicine*, 47(2): 51–53.
- [11] Zhou T, 2019, Clinical Observation of Yinqiao Xiaozhen Formula Combined with External Washing with Sihuang Water on Wind-Heat Type Rash Caused by Icotinib, thesis, Hunan University of Traditional Chinese Medicine.

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Research Progress of Traditional Chinese Medicine External Treatment for Cancer Pain

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Abstract: Cancer pain, as a common complication in patients with malignant tumors, is regarded as the fifth vital sign. The involvement of traditional Chinese medicine (TCM) in the treatment of malignant tumors has become a distinctive feature of oncology treatment in China. It is also an important component of cancer pain management. TCM analgesic treatments include various methods, such as internal medicine and external therapies. External analgesic therapies, in particular, are significant methods in TCM pain management and offer both local treatment and systemic regulation. These methods are simple, easy to perform, and non-invasive. They can enhance pain relief effects while reducing the difficulty of oral medication intake and avoiding adverse gastrointestinal reactions, providing new perspectives and approaches for cancer pain treatment with broad development prospects. This article provides a review of the external TCM therapies for cancer pain to share with peers in the field.

Keywords: Cancer pain; External treatment of traditional Chinese medicine; Research progress

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1. Introduction

Cancer pain primarily refers to pain caused by the direct invasion of surrounding tumor tissues or distant metastasis, as well as induced by anti-tumor treatments, such as surgery and radiotherapy. As one of the common complications in patients with malignant tumors, the main characteristics of cancer pain include persistent pain, often accompanied by sudden pain. The nature of the pain mainly includes pain that resembles burning, stabbing, tearing, and cutting. Data surveys show that the incidence of pain in newly diagnosed malignant tumor patients is about 25%. In comparison, the incidence in patients with advanced malignant tumors can reach 60% to 80%, with about one-third of the patients experiencing severe pain^[1]. Currently, the treatment for cancer pain mainly follows the “three-step analgesia” treatment principle advocated by the World Health Organization (WHO), while some of them still bear unsatisfactory analgesic effects^[2,3]. Moreover, the adverse reactions caused by opioid drugs, including constipation, nausea, and vomiting, impose a heavy burden

on cancer patients. Traditional Chinese Medicine (TCM) external therapy, as an adjunct analgesic method, is characterized by convenience, significant efficacy, and economic safety. This article summarizes the treatment of cancer pain with TCM external therapy, hoping to provide thoughts and methods for the clinical TCM treatment of cancer pain.

2. Dialectical thinking

Cancer pain is classified under the “pain” category based on its clinical manifestations in TCM. The main etiologies and pathogenesis include “pain due to obstruction” and “pain due to lack of nourishment.” Excess syndromes are often seen with conditions such as cold congealing, Qi stagnation, and blood stasis. In contrast, deficiency syndromes are often associated with depletion of fluids, Yang deficiency, and organ deficiency. The pathogenic factors are often intertwined, with both excess and deficiency coexisting, making the pathogenesis increasingly complex ^[4]. Professor Li Peiwen has a unique approach to the differentiation of cancer pain, comprehensively using methods such as warming Yang to disperse cold, activating blood to unblock the collaterals, promoting Qi to dissolve stagnation, resolving phlegm to regulate Qi, clearing heat to detoxify, and softening hardness to disperse knots. Based on these methods, he incorporates “site-specific” TCM herbs, such as Qianhu, Gualou, and *Houttuynia cordata* for lung cancer pain, and rose, August melon, and lychee core for breast cancer pain, the combination of herbs and treatment methods effectively alleviates cancer pain ^[5].

Professor Li Suling categorizes primary liver cancer pain into four types: excess pain, deficiency pain, cold pain, and heat pain ^[6]. Accordingly, he classifies liver cancer pain into seven syndromes: Qi and blood deficiency, liver Qi stagnation, phlegm-damp stagnation, Yin deficiency with fluid depletion, Qi stagnation and blood stasis, Yang deficiency with cold congealing, and heat toxin accumulation. He innovatively proposes the “One tonification and four attacks” method for treatment, in which “tonification” aims to support the body’s proper energy, and the “attack” involves clearing heat toxins, eliminating blood stasis, promoting bile discharge, and using toxins to attack toxins. He first emphasizes distinguishing between “deficiency in the right and excess in the evil,” then differentiating between cold and heat, achieving significant results.

Professor Hua Baojin believes that cancer pain often arises from “pain due to obstruction,” where the Yang Qi in malignant tumor patients becomes obstructed, leading to the formation of metabolic products such as Qi stagnation, water retention, and phlegm-dampness, which hinder the circulation of Yang Qi and cause pain ^[7]. Based on the etiology and pathogenesis, he proposes the method of unblocking yang to treat cancer pain, emphasizing “unblocking Yang” rather than “warming Yang.” He selects penetrating herbs to enable Yang Qi to circulate throughout the body, which can be divided into methods such as unblocking yang to dissolve nodules and unblocking Yang to promote water metabolism and resolve dampness. By enabling the circulation of Yang Qi, it is expected to warm and nourish the entire body, thereby alleviating cancer pain and other symptoms. In summary, although different TCM practitioners have various understandings of the etiology and pathogenesis of cancer pain, they all focus on the dual aspects of “excess and deficiency.” Based on syndrome differentiation, combined with the characteristics of the pathogenesis, they choose specific treatment methods such as activating blood, benefiting Qi, resolving phlegm, and dissolving nodules, achieving good therapeutic effects.

3. External treatment of TCM

TCM external therapy has a long history and the “Li Yue Pian Wen” records that: “The principles of external treatment are the same as those of internal treatment; the medicines used for external treatment are the same as those for internal treatment, differing only for their method.” Modern researchers believe that advanced tumor

patients often have undergone treatments such as radiotherapy, chemotherapy, and targeted therapy, leading to deficiency in their body's vital energy and weakness in the spleen and stomach. Oral medications at this period can burden the spleen and stomach, causing poor appetite and impaired digestion. External therapy can alleviate pain through skin, meridians, and acupoints^[8]. Current TCM external therapies for cancer pain treatment include acupuncture, moxibustion, and external application of medicinal plasters. The following content is a brief overview of the progress of these treatments.

3.1. Acupuncture

Acupuncture, as one of the effective TCM external therapies for cancer pain, is known for its convenience and high safety. Li D *et al.* (2020) conducted a study on 60 patients with moderate to severe cancer pain^[9]. The control group was treated with oral oxycodone hydrochloride sustained-release tablets, while the observation group combined this treatment with acupuncture. The results showed that the cancer pain relief rate in the observation group was 90.0%, significantly higher than that of 76.7% in the control group. Moreover, the combination of acupuncture reduced the dosage of analgesic drugs. He Y *et al.* (2021) reviewed databases and included clinical research data from 28 studies on acupuncture for cancer pain^[10]. They designed a Delphi survey questionnaire to analyze and summarize the acupuncture point selection patterns and high-frequency acupoints for cancer pain treatment. The results indicated that acupuncture point selection for cancer pain should follow a personalized approach, with primary points including Zusanli (ST36), Taichong (LR3), Hegu (LI4), Yanglingquan (GB34), Sanyinjiao (SP6), and Ashi points, providing evidence basis for clinical acupuncture treatment of cancer pain. In recent years, there also have been advancements in the research on the mechanisms of acupuncture for cancer pain relief^[11]. Studies showed that acupuncture could transmit impulses to the spinal cord, activate higher central releases of descending inhibitory impulses to achieve analgesia, and integrate pain signals in the cerebral cortex and spinal cord to exert a collective analgesic effect. Additionally, the analgesic mechanism of acupuncture is related to the synthesis and release of large amounts of serotonin (5-HT) in the brain^[12]. Thus, acupuncture can determine different treatment principles and methods through syndrome differentiation and point selection, adhering to individualized principles, and achieving good results in cancer pain management.

3.2. Moxibustion

Moxibustion for pain relief involves the use of moxa to heat specific acupuncture points, combining external moxibustion with acupoints to achieve analgesic effects. Chen J *et al.* (2020) conducted a randomized study on 120 cancer pain patients where both groups received oral oxycodone hydrochloride sustained-release tablets as the basic analgesic treatment^[13]. The treatment group additionally received moxibustion on the back-shu points, specifically bilateral Jueyinshu (BL14), Ganshu (BL18), Danshu (BL19), Shenshu (BL23) and Sanjiaoshu (BL22), each point for 5 minutes, ensuring that patients felt warm sensation without burning pain. They compared the NRS scores and pain improvement rates before/after treatment and measured the levels of IL-6 and TNF- α in peripheral blood. The results showed that the treatment group had better pain assessment and improvement rates than the control group ($P < 0.05$), and significantly inhibited the expression of IL-6 and TNF- α ($P < 0.05$). The preliminary evidence suggests that moxibustion on back-shu points can be analgesic by inhibiting the expression of inflammatory factors IL-6 and TNF- α . Ouyang J *et al.* (2018) randomly divided 120 cancer pain patients into a treatment group (60 patients) and a control group (60 patients)^[14]. Both groups received conventional three-step analgesia treatment, while the treatment group additionally received warming yang moxibustion. The basic acupoints were Zhongwan (RN12), Shenque (RN8) and Guanyuan (RN4), with

additional points for specific pain locations: Yangbai (GB14), Hegu (LI4) and Zusanli (ST36) for forehead pain; Taiyang (EX-HN5), Shuaigu (GB8), Yanglingquan (GB34) and Waiguan (SJ5) for lateral head pain. After 4 weeks, the NRS scores of the treatment group decreased significantly compared with the control group ($P < 0.05$). Furthermore, compared with using the three-step analgesia treatment alone, the treatment group showed significant improvement in quality of life (including sleep, mental state, and daily activities) after using warming yang moxibustion ($P < 0.05$), further confirming the effectiveness of moxibustion for cancer pain. Moxibustion can promote blood circulation and unblock meridians through its warming effect, relieving cancer patients' pain. However, moxibustion mainly targets cold syndrome cancer pain patients. Further exploration is needed to verify its analgesic effects on other types of cancer pain.

3.3. External application

The external application of medicinal plasters involves using herbal ointments or patches applied to the pain area for transdermal absorption to achieve analgesic effects. Li H *et al.* (2021) divided 60 cancer pain patients into a study group and a control group, each with 30 patients^[15]. The control group received treatments such as ibuprofen sustained-release capsules, tramadol hydrochloride sustained-release tablets, and oxycodone hydrochloride sustained-release tablets based on the severity of the pain. The study group received these treatments in combination with the external application of compound soapwort thorn ointment to evaluate its efficacy and safety in cancer pain. The results showed that the combination of oral analgesics with the external application of the compound soapwort thorn ointment significantly reduced the patients' NRS scores ($P < 0.05$), decreased medication dosage, and improved quality of life. Liang Y *et al.* (2021) believed that the occurrence of cancer pain is often due to "toxins and stasis" blocking the meridians^[16]. They used the Shuanghuang Sanjie Powder, which performed heat-clearing, detoxifying, and pain-relieving properties, for external application in combination with opioid medications. The results indicated that the combined use of Shuanghuang Sanjie Powder with opioids significantly improved patients' quality of life, shortened the time to pain relief, reduced the frequency of breakthrough pain, decreased the dosage of opioids, and reduced adverse reactions compared to using opioids alone ($P < 0.05$). Yu Y (2020) randomly divided 60 liver cancer patients with mild to moderate cancer pain of Qi stagnation and blood stasis type into the treatment group and control group, each with 30 patients^[17]. The control group received acetaminophen and dihydrocodeine tablets for pain relief, while the treatment group combined these with the external application of Aitong Powder. The results showed that the treatment group had significant improvement in pain relief, reduced pain duration, and improved TCM clinical symptoms compared to the control group ($P < 0.05$), confirming the effectiveness of Aitong Powder for patients with mild to moderate liver cancer pain of Qi stagnation and blood stasis type. In conclusion, the external application of medicinal plasters for cancer pain is relatively simple, easy to manage, does not interfere with the patient's daily activities, and can be used as an adjunct to standard analgesic treatments.

3.4. Acupoint injection

Acupoint injection involves using specific drugs injected into the body's designated acupoints to achieve analgesic effects. Guo H (2019) randomly divided 100 advanced cancer pain patients into two groups, the control group received oral morphine sustained-release tablets for pain relief, while the observation group received acupoint injections in addition^[18]. The specific method involved disinfecting bilateral Ququan (LR8), Xinshu (BL15), and Ganshu (BL18) points and then injecting 2 milliliters of compound Danshen injection every other day. After 4 weeks, the efficacy assessment showed that the total effective rate in the observation group was 88%, significantly higher than the control group's 70% ($P < 0.05$). Additionally, researchers have

explored the analgesic effects of auricular acupuncture. Bai T *et al.* (2019) conducted a study on 97 cancer pain patients and randomly divided them into groups ^[19]. The observation group received auricular acupoint injections in addition to oxycodone for pain relief and selected primary points corresponding to the disease and auxiliary points such as Jiaogan, Shenmen, and Sanjiao. The injection used was 0.2 to 0.4 mL of compound Danshen injection. After 12 days, the observation group's BPI pain scores were significantly lower than those of the control group ($P < 0.05$) and their depression and anxiety scores also improved significantly ($P < 0.05$). As to the choice of injection drugs, different explorations have been made. Luo J *et al.* (2018) randomly divided patients with severe cancer pain into groups ^[20]. The treatment group received morphine injections at the Zusanli (ST36) with a dose of 5 mg. After one month, compared to patients receiving intramuscular injections, those who received morphine injections at Zusanli (ST36) had significantly faster onset times, longer maintenance of analgesic effects, and fewer adverse reactions ($P < 0.05$). Additionally, this method improved the expression of CD3⁺, CD4⁺, and the CD4⁺/CD8⁺ ratio, while reducing NK cell expression, thus enhancing immune function. In summary, acupoint injection can be of analgesia for cancer pain patients. However, current research on the analgesic effects of acupoint injection mainly consists of small-scale clinical observations. There is no consensus on the drugs used and acupoints selected, often based on personal experience. Issues such as off-label drug use and inconsistent injection dosages require further exploration.

4. Discussion

Cancer pain, as one of the common complications in patients with malignant tumors, severely affects patients' quality of life and can even hinder the normal course of treatment ^[21]. Current researches on the mechanisms of cancer pain suggest that it results from multiple factors, including the influence of the tumor microenvironment ^[22], microglial activation ^[23], central and peripheral nerve sensitization ^[24,25], and the activation of multiple signaling pathways ^[26-28]. The treatment largely follows the “three-step ladder” approach proposed by the World Health Organization, which involves assessing the pain level based on the patient's specific conditions, such as complaints, medical history, symptoms, and signs. As the pain is categorized into three levels: mild, moderate, and severe, with medication administered accordingly. The treatment model is widely used for its effectiveness and convenience ^[29]. However, there are still gaps in the understanding and use of analgesics by some healthcare professionals ^[30,31]. Additionally, varying regulations on these medications across different regions can lead to inadequate pain control in some cancer patients ^[32]. Moreover, the main adverse reactions of opioids, such as constipation and nausea, increase the psychological burden on patients. As one of the main methods of TCM for treating cancer pain, TCM external therapy follows the principle of syndrome differentiation and treatment, addressing “pain due to obstruction” and “pain due to lack of nourishment” by using methods such as activating blood circulation, removing blood stasis, tonifying Qi, nourishing blood, clearing heat and detoxifying. Techniques include acupuncture, moxibustion, external application of medicinal plasters, and acupoint injection. These methods help reduce the burden of oral analgesics and provide synergistic pain relief. However, current studies on TCM external therapy for cancer pain mainly involve small sample clinical observations, with subjective patient experiences or scale scores as primary indicators. Point selection and medication often rely on individual practitioners' experience and lack unified standards and in-depth research on analgesic mechanisms. Therefore, further exploration and research are needed to enhance the application of TCM external therapy in cancer pain management, leveraging TCM's holistic approach and the advantages of local treatments to alleviate patients' pain and improve their quality of life.

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Reference

- [1] National Health Commission of the People's Republic of China, 2018, Standard Diagnosis and Treatment of Cancer Pain (2018). *Chinese Clinical Oncology*, 23(10): 937–944.
- [2] Siegel RL, Miller KD, Fuchs HE, et al., 2021, Cancer Statistics 2021. *CA: A Cancer Journal for Clinicians*, 71(1): 7–33.
- [3] Sung H, Ferlay J, Siegel RL, et al., 2021, Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*, 71(3): 209–249.
- [4] Liu ZD, Wang HM, Zhu XY, 2020, Research Progress of Applying Traditional Chinese Medicine in the Treatment of Cancer Pain. *Journal of Sichuan of Traditional Chinese Medicine*, 38(1): 211–213.
- [5] Wang YY, Li PW, Jia LQ, et al., 2021, Academic Thinking and Clinical Experience of Li Pei-wen in Treating Cancerous Pain. *China Journal of Traditional Chinese Medicine and Pharmacy*, 36(3): 1450–1452.
- [6] Ding YX, Li SL, 2021, Professor Li Su-ling's Experience in Differentiating and Treating Cancer Pain of Primary Liver Cancer. *Chinese Journal of Integrated Traditional and Western Medicine on Liver Diseases*, 31(1): 87–89.
- [7] Bao YJ, Tang YT, Jiang XC, et al., 2020, Hua Bao-jin's Experience in Treating Cancer Pain by Tongyang Method. *China Journal of Traditional Chinese Medicine and Pharmacy*, 35(11): 5582–5584.
- [8] Hou GJ, Bai ZP, Pan MQ, et al., 2018, Study on Medication Principles of External Treatment of Cancer Pain with Traditional Chinese Medicine Based on Cluster Analysis. *Journal of Hunan University of Chinese Medicine*, 3: 292–295.
- [9] Li D, Sun RR, Li QL, et al., 2020, Acupuncture Combined with Opioid Drugs on Moderate and Severe Cancer Pain: A Randomized Controlled Trial. *Chinese Acupuncture & Moxibustion*, 40(3): 257–261.
- [10] He YH, Xu NG, Zhang HB, et al., 2021, Acupoint Selection for Cancer Pain: Based on Current Evidence and Delphi Method. *Chinese Acupuncture & Moxibustion*, 41(10): 1161–1165.
- [11] Chen XX, Yao MH, Sai JT, et al., 2020, The Study of the Analgesic Effect of Acupuncture in Cancer Pain. *World Chinese Medicine*, 15(15): 2346–2353.
- [12] Yang J, Wahner-Roedler DL, Zhou X, et al., 2021, Acupuncture for Palliative Cancer Pain Management: Systematic Review. *BMJ Support Palliative Care*, 11(3): 264–270.
- [13] Chen J, Qiao HF, Li J, et al., 2020, The Clinic Research of Back-Shu Point Moxibustion Plus Oral Oxycodone in Cancer Pain. *Shaanxi Journal of Traditional Chinese Medicine*, 41(1): 105–107.
- [14] Ou JB, Zhou LQ, Liang QY, 2018, Effects of Wenyang Moxibustion Combined with Three-Step Analgesia on Pain and Quality of Life in Patients with Cancer Pain. *Modern Journal of Integrated Traditional Chinese and Western Medicine*, 27(21): 2319–2321.
- [15] Li H, Gong WJ, Zheng YF, 2021, Clinical Study on the Treatment of Cancer Pain with Compound Chinese Honeylocust Spine Ointment. *China Modern Doctor*, 59(34): 97–100.
- [16] Liang YH, Zhang C, Xie AQ, et al., 2021, Clinical Observation of External Application of Shuanghuang Sanjie Powder Combined with Opioid Drugs in the Treatment of Cancer Pain. *Hubei Journal of Traditional Chinese Medicine*, 43(3): 42–44.
- [17] Yu YY, 2020, Clinical Study on “Aitong Powder” in Treating Mild to Moderate Cancer Pain of Liver Cancer with Qi

Stagnation and Blood Stasis Type, thesis, Shandong University of Traditional Chinese Medicine.

- [18] Guo HL, 2019, Study on the Effect of Acupoint Injection on Analgesia in Patients with Liver Cancer Pain. *Electronic Journal of General Stomatology*, 6(26): 172–173.
- [19] Bai T, Zheng G, Hu YQ, 2019, Effects of Auricular Acupoint Injection on Pain, Anxiety, Depression and Quality of Life in Patients with Cancer Pain. *Modern Journal of Integrated Traditional Chinese and Western Medicine*, 28(24): 2697–2700.
- [20] Luo JH, Liu ZH, Li ZH, 2018, Clinical Effect of Zusanli Acupoint Injection of Morphine on Severe Cancer Pain. *Shaanxi Journal of Traditional Chinese Medicine*, 39(2): 253–255.
- [21] Jin XQ, Rao GL, 2018, Effect of Hydroxycodone Release Table Combined with Morphine in the Titration Therapy for Cancer Pain. *Chinese Journal of Cancer Prevention and Treatment*, 25(S1): 172–173.
- [22] Chen J, Cong X, Zhan X, et al., 2019, Effects of Parecoxib on Pain Threshold and Inflammatory Factors IL-1 β , IL-6 and TNF- α in Spinal Cord of Rats with Bone Cancer Pain. *Journal of the College of Physicians and Surgeons Pakistan*, 29(6): 528–531.
- [23] Zhou KX, He XT, Hu XF, et al., 2019, XPro1595 Ameliorates Bone Cancer Pain in Rats via Inhibiting p38-Mediated Glial Cell Activation and Neuroinflammation in the Spinal Dorsal Horn. *Brain Research Bulletin*, 2019(149): 137–147.
- [24] Ji RR, Donnelly CR, Nedergaard M, 2019, Astrocytes in Chronic Pain and Itch. *Nature Reviews Neuroscience*, 20(11): 667–668.
- [25] Medeiros P, Negrini-Ferrari SE, Palazzo E, et al., 2019, N-Methyl-D-Aspartate Receptors in the Prelimbic Cortex are Critical for the Maintenance of Neuropathic Pain. *Neurochemical Research*, 44(9): 2068–2080.
- [26] Tian J, Song T, Wang H, et al., 2019, Thalidomide Alleviates Bone Cancer Pain by Down-Regulating Expressions of NF- κ B and GFAP in Spinal Astrocytes in a Mouse Model. *International Journal of Neuroscience*, 129(9): 896–903.
- [27] Zhang XQ, Zhang XC, Qiu XZ, et al., 2020, Effect of Raw Bone Capsule on the Expression of OPG and RANKL in Tibia of Osteoporotic Rats. *Chinese Journal of Traditional Medical Traumatology & Orthopedics*, 28(8): 21–25.
- [28] Han L, Jiang J, Xue M, et al., 2020, Sonic Hedgehog Signaling Pathway Promotes Pancreatic Cancer Pain via Nerve Growth Factor. *Regional Anesthesia & Pain Medicine*, 45(2): 137–144.
- [29] Hui JR, Zhang N, Li M, et al., 2019, Clinical Observation on Acupuncture Combined with Three-Step Analgesic Therapy in the Treatment of 40 Cases of Cancer Pain. *Journal of Traditional Chinese Medicine*, 60(2): 146–149.
- [30] Xia Z, 2017, Cancer Pain Management in China: Current Status and Practice Implications Based on the ACHEON Survey. *Journal of Pain Research*, 2017(10): 1943–1952.
- [31] Fan LL, Tian JJ, Zhang XB, et al., 2020, Cognition on Cancer Pain Treatment of Doctors in Beichuan Area of Mianyang City. *Chinese Journal of Geriatric Care*, 18(2): 69–72.
- [32] Huang ZR, Su XW, Diao YF, et al., 2019, Utilization and Patient Affordability of Opioid Analgesics for Cancer Pain Treatment in Different Regions of China. *Chinese Journal of Pharmacoepidemiology*, 28(6): 389–394 + 399.

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Economic and Social Impact Assessment of Chronic Diseases Among the Low-Income Population in Southern Punjab, Pakistan

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Abstract: *Introduction:* Chronic diseases are becoming more prevalent worldwide. The effects of chronic illnesses are disastrous not only for the diagnosed person but also for their entire family. This study explores chronic diseases' social and economic impact on low-income families. The study aims to determine the economic and social implications of various chronic diseases and the loss of income due to these conditions among low-income individuals in Southern Punjab, Pakistan. *Methodology:* A sample of 424 patients was selected from different areas of Southern Punjab. Data were collected using a questionnaire that included questions about economic status, self-reported health status, social status, management strategies, and health insurance, among other factors. *Results:* The mean monthly income of the respondents was found to be 57,097.6 Pakistani rupee (PKR), and the mean monthly expenses for treatment were 8,256.1 PKR. The loss of income was calculated at 15%. Additionally, 62% of patients spent more than 10% of their monthly income on managing their disease. Approximately 85% of the respondents reported that chronic diseases affected their social life. Furthermore, 80% of patients lacked health insurance. *Conclusion:* Chronic diseases impose significant economic and social burdens on patients and their families in Southern Punjab. To reduce the burden of chronic diseases, the government should enhance healthcare services in this region and provide health insurance to low-income families.

Keywords: Chronic diseases; Low-income families; Economic impact; Punjab; Pakistan

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1. Introduction

Conditions that require ongoing medical care and impair daily activities for a year or longer are generally referred to as chronic diseases ^[1]. It is commonly known that chronic illnesses now “matter” in terms of public health in developing nations, where they impose a sizeable and increasing disease burden, according to the World Health Organization (WHO) in 2001 ^[2]. Diabetes, cardiovascular disease, and cancer are the main chronic diseases, collectively accounting for roughly 79.0% of all chronic disease-related deaths worldwide in 2018 ^[3]. In 1990, there were an estimated 26.0 million deaths worldwide attributed to chronic diseases. This figure rose to 34.5

million in 2010 and approximately 40.5 million in 2016 ^[4]. The burden of chronic diseases is particularly high in low- and middle-income countries due to rapid lifestyle changes and increasing urbanization ^[5]. In these countries, the chronic disease rate was 78.05%. The limited facilities and resources available for managing these diseases contribute to their high burden ^[6].

Nevertheless, the detrimental impact of chronic illnesses extends beyond early mortality. They also lead to other contentious consequences linked to the financial stability of individuals, families, and the community at large. The loss of independence, inability to carry out daily tasks and inability to socialize as freely as before are among the most devastating consequences faced by people with chronic illnesses. The burden of a chronic illness falls not only on the ill individual but also on their entire family. Thus, the whole household ultimately suffers as an indirect victim. This is because chronic conditions are extensive and long-lasting, requiring ongoing patient care and out-of-pocket medication costs. People who have multiple chronic illnesses and disabilities, which frequently call for additional medical care and attention to prevent the condition from worsening, bear a disproportionate share of this burden ^[1].

According to WHO 2002, chronic illness and poverty are linked in a vicious cycle that has significant negative and underappreciated economic effects on families, communities, and nations. The poorest people are most at risk of developing chronic diseases and passing away prematurely from them in almost all countries. For various reasons, including higher risk exposure and restricted access to healthcare services, the impoverished are more susceptible to chronic illnesses. Of the 36 million people who died from chronic diseases in 2008, 9 million were under the age of 60, and 90% of these early deaths occurred in low- and middle-income countries. Chronic illnesses such as heart disease, stroke, cancer, chronic respiratory diseases, and diabetes are by far the leading causes of mortality worldwide, accounting for 63% of all deaths according to WHO 2005 ^[6].

Pakistan ranks fifth in the world in terms of population, with 207.7 million people. Pakistan is facing almost double the burden of chronic diseases ^[7]. Changes in diet and the adoption of a sedentary lifestyle have led to an increase in chronic diseases ^[7]. A comprehensive survey carried out in Punjab in 2013–2014 to ascertain the prevalence of chronic diseases using the WHO STEPS tool found that the percentage of hypertension was 53%, which is higher than in other South Asian nations ^[8]. The prevalence of hypertension is 29.8% in India and 28% in Bangladesh ^[9]. The main causes of hypertension were inadequate patient safety measures, lack of appropriate healthcare guidelines, and limited facilities ^[10]. Even though the developing world is most likely to experience an epidemic of chronic diseases ^[11]. Research on chronic diseases and their impacts on people in developing countries like Pakistan has been limited.

2. Methodology

A total of 424 patients from different districts of Southern Punjab were sampled. Patients were enrolled in the study and included when:

- (1) They had a diagnosis of at least one or more chronic diseases,
- (2) They had been in treatment for their disease for at least four months,
- (3) They were 18 years or older.

The duration for which they had to bear the substantial expenses of managing their illness was specified. Patients who were unable to respond were not included in the study. Data were collected from the patients with the help of a questionnaire. The questionnaire asked the respondents about sociodemographic data, self-reported health status, economic status, affordability of healthcare, managing strategies, social status, etc. Patients were also asked about their monthly income and the cost of the treatment of their condition per month. Additionally, patients were asked about health insurance. After the information was collected, the entire dataset was analyzed.

3. Results

Among the individuals surveyed, 85.38% ($n = 362$) were aged 18–60, while 14.62% ($n = 62$) were aged 61 and older, as shown in **Table 1**. The sample included more females (54.72%; $n = 232$) than males (45.28%; $n = 192$). Regarding employment, 66.04% ($n = 280$) of respondents were employed, and 33.96% ($n = 144$) were unemployed. Most participants had hypertension (28.30%; $n = 120$), followed by diabetes (26.18%; $n = 111$). The percentage of respondents with asthma was 18%, as depicted in **Figure 1**.

Table 1. Prevalence of chronic diseases in different age groups

	Frequency ($n = 424$)	Percentage (%)
Age		
18–60 years	362	85.38
61 years and older	62	14.62
Gender		
Male	192	45.28
Female	232	54.72
Employment status		
Employed	280	66.04
Not employed	144	33.96
Hypertension	120	28.30
Diabetes	111	26.18
Cancer (all types)	58	13.68
Bone or joint problems / Arthritis	21	4.95
Chronic kidney disease	2	0.47
Asthma	85	20.05
Heart disease	15	3.54
Mental illness / Depression	12	2.83

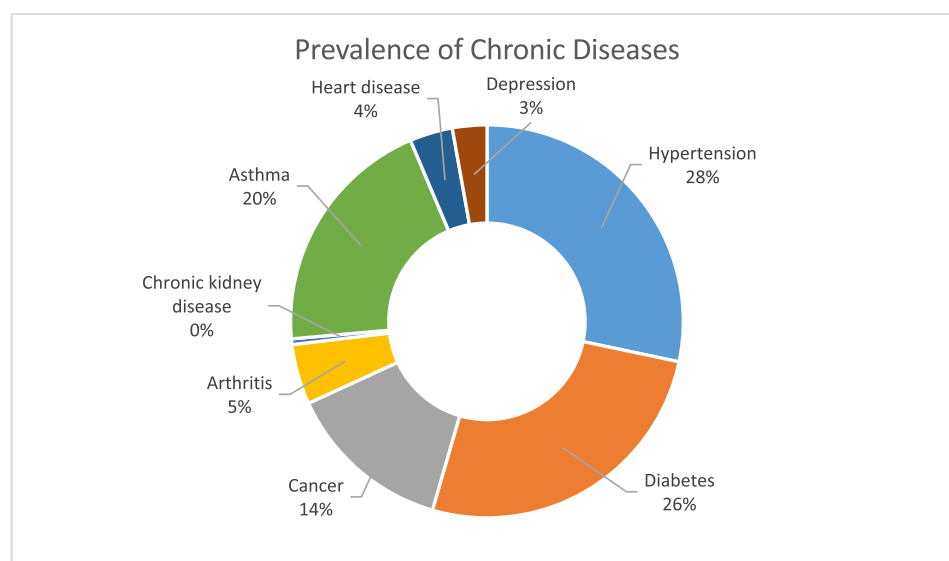


Figure 1. Prevalence of chronic diseases.

3.1. Monthly income and costs of chronic illness among respondents

Table 2 outlines the monthly income and costs associated with chronic illness treatment. Out of 424 respondents, most respondents had an income between 31,000 to 70,000 PKR, with 40% ($n = 171$) earning 31,000 and 50,000 PKR, while 30.6% ($n = 130$) earning 51,000 and 70,000 PKR every month. However, most respondents had chronic illness treatment costs ranged between 2,100 and 7,000 PKR, with 14.1% ($n = 60$) having treatment costs between 2,100 and 3,000 PKR, 25.47% ($n = 108$) between 3,100 and 5,000 PKR, and 13% ($n = 58$) between 5,100 and 7,000 PKR.

Table 2. Monthly income and treatment costs of the respondents

	Frequency ($n = 424$)	Percentage (%)
Monthly income (PKR)		
15,000–30,000	41	9.60
31,000–50,000	171	40.00
51,000–70,000	130	30.60
71,000–80,000	42	9.90
81,000–100,000	34	8.01
100,000 and more	6	1.41
Chronic illness cost (PKR)		
1,000–2,000	43	10.41
2,100–3,000	60	14.10
3,100–5,000	108	25.47
5,100–7,000	58	13.00
7,100–9,000	34	8.00
9,100–10,000	48	11.00
12,000–15,000	35	8.00
16,000–20,000	21	4.95
21,000–30,000	8	1.80

Table 3. Loss of monthly income due to treatment

Loss of income due to treatment	Frequency	Percentage (%)
1%–5%	51	12.00
6%–10%	119	28.00
11%–12 %	42	9.00
13%–15 %	36	2.12
16%–20 %	72	16.98
21%–30%	35	8.25
35% and above	13	3.06

Table 3 illustrates the loss of monthly income due to chronic disease treatment. 28% ($n = 119$) of the respondents experienced an income loss between 6% and 10%, while 16.28% ($n = 72$) experienced a loss between 16% and 20%, and 12.00% ($n = 51$) experienced a loss between 1% and 6%.

Table 4. Monthly average income of patients

Monthly income	Mean
Household monthly income	57,097.61 PKR
Loss in income percentage	15%

In Southern Punjab, the patients' average monthly income was 57,097.61 rupees, while the mean cost of chronic illness treatment was 8,256.10 rupees, representing 15% of their average monthly income. Thus, the income loss due to chronic disease amounted to nearly 15% of their total income, as detailed in **Table 4**.

3.2. Affordability of treatment

A large number of the participants were not able to afford treatment for their condition. About 34% of the respondents said that they could not afford the treatment (**Table 5**). These people stated that they had to sacrifice several things to manage these chronic diseases.

Table 5. Affordability of treatment

Criteria	Frequency	Percentage
Ability to finance all medications	278	66%
Unable to afford the treatment	141	34%

3.3. Health insurance

80% of people in Southern Punjab lack health insurance. Only 20% of the respondents stated that they have health insurance (**Figure 2**).

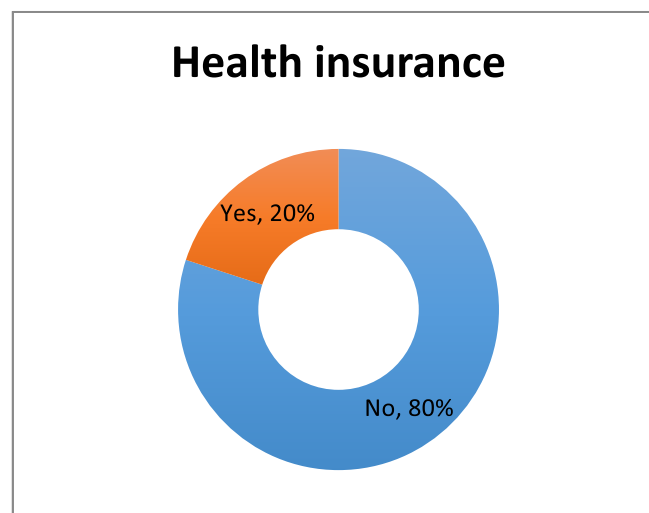


Figure 2. Percentage of health insurance among participants.

3.4. Effects on social life

85% of respondents stated that chronic diseases affect their social life badly (**Figure 3**). Out of 424 respondents, 29.49% said that chronic diseases reduce their social interactions 27.86 responded that these conditions had decreased their participation in activities. 28.53% of the total participants responded that these conditions have increased their dependence on others. 14.12% stated that they are emotionally distressed because of chronic diseases.

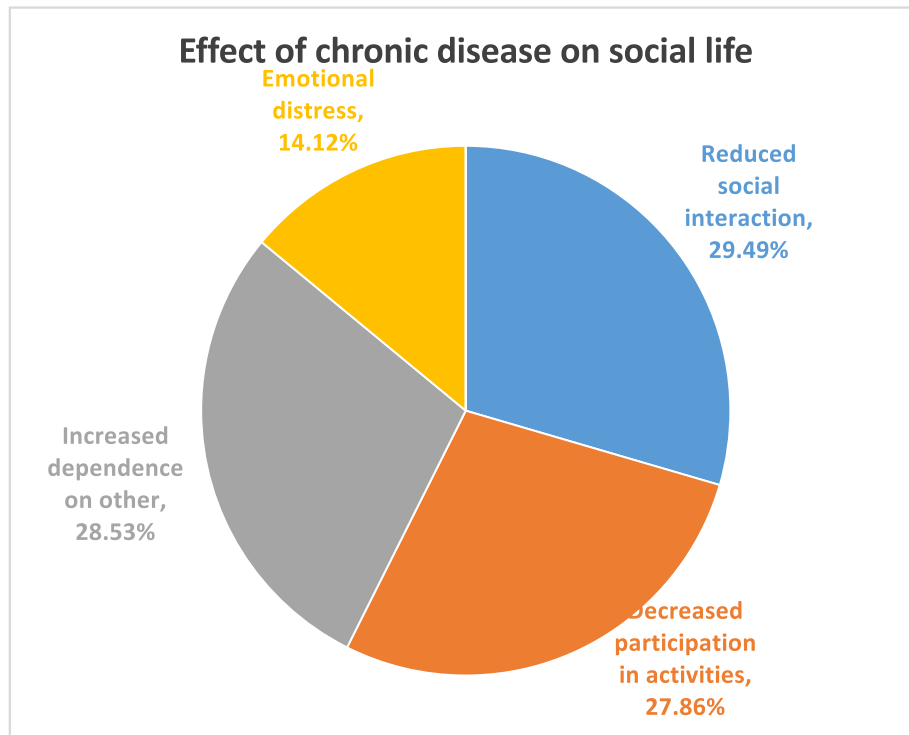


Figure 3. Social impact of chronic diseases in participants.

4. Discussion

Approximately 66% of the 424 respondents were employed, and the remaining were unemployed. Chronic diseases caused smaller changes in income for employed individuals compared to unemployed people. This is because employed individuals generally have higher monthly incomes and can better manage the burden of chronic diseases. Therefore, in developing countries like Pakistan, particularly in regions such as South Punjab, where the unemployment rate is high, managing chronic diseases becomes very difficult.

The effect of illness also varies with the type of disease. In the survey, hypertension was the most common disease among respondents, affecting 28% of them. In South Punjab, hypertension has a significant economic and social impact on a large scale. Diabetes was the second most prevalent disease among respondents at 26%, implying a substantial burden socially and economically. Cancer was also present among respondents, affecting 13%. Cancer is a particularly dangerous chronic disease due to its expensive treatment costs. Asthma and depression also had an impact, although they were present in a smaller percentage of respondents. Many respondents had more than one disease, such as hypertension and diabetes or diabetes and cancer, which further increased the overall burden of disease.

In this study, the average monthly income was found to be 57,097.61 PKR, and the average medical expenses were 8,256.10 PKR, representing 15% of the average monthly income. Using the resulting loss in monthly income as one of the economic effects of chronic illness is significant. It has been shown that an illness cost burden exceeding 10% can be disastrous. In the survey, 38% of respondents spent less than 10% of their monthly income on treatment, while 62% spent more than 10% of their income on disease management. Therefore, the treatment costs of chronic diseases lead to income loss for people in the Southern Punjab region. The burden of treating the disease affects not only the individual but also the entire family, as the loss of income that could be used for improving the lifestyle of other family members is diverted to disease management. Consequently, family members also experience emotional stress and anxiety.

This study explored the social impact of chronic diseases on respondents. Over 85% of participants reported that chronic diseases negatively affected their social life. In women, this impact may be exacerbated by hormonal imbalances and other conditions such as PCOS and anovulation. In contrast, 15% of respondents reported no social impact from their chronic diseases. Among those who were affected, 85% experienced changes in their ability to maintain relationships, leading to feelings of loneliness, guilt, anger, and frustration. Chronic illness also hindered their participation in volunteer activities. Additionally, family members of individuals with chronic diseases often suffer emotionally due to feelings of powerlessness and a lack of control^[12,13].

Respondents were also asked about their self-reported health status, with approximately 66% reporting fair health, 17% reporting good health, and 13% reporting poor health. Chronic diseases have a disastrous effect on their quality of life.

In addition, respondents were asked about their ability to afford treatments, with about 66% stating that they could afford them and 34% reporting that they could not afford the burden of diseases. Chronic diseases in low-income families lead to poverty^[6]. In the survey, about 20% of people stated that they sought health insurance like a Health Card for managing their diseases. Many developed nations provide their citizens with premium health insurance to cope with diseases.

Based on the data obtained and presented here, it is clear that chronic diseases can have disastrous social and financial effects on patients. Deaths from heart diseases and cancer are most common in low-income countries where resources for treatment are scarce. Unfortunately, a large number of people in Southern Punjab lack health insurance, forcing them to bear all treatment costs themselves. According to the study, 80% of people in Southern Punjab lack health insurance. Like some European countries, Pakistan should provide health insurance to low-income people nationwide. By providing health insurance, the burden of diseases can be efficiently reduced. The government should also improve healthcare facilities essential for managing diseases

5. Conclusion

From the information above, it is clear that the health and quality of life of patients are negatively impacted by one or more types of chronic diseases. In addition to their physical and emotional suffering, patients with chronic illnesses may also face significant medical costs. It is imperative to acknowledge the severe economic impact that chronic diseases have on the families of those who suffer from them in Southern Punjab. Moreover, most families manage these devastating financial burdens on their own. The Government of Pakistan should improve healthcare services and provide health insurance to low-income families in Southern Punjab.

Disclosure statement

The authors declare no conflict of interest.

References

- [1] Jayathilaka R, Joachim S, Mallikarachchi V, et al., 2020, Do Chronic Illnesses and Poverty Go Hand in Hand? PLoS One, 15(10): e0241232. <https://doi.org/10.1371/journal.pone.0241232>
- [2] Benkel I, Arnby M, Molander U, 2020, Living with a Chronic Disease: A Quantitative Study of the Views of Patients with a Chronic Disease on the Change in Their Life Situation. SAGE Open Med, 2020(8): 2050312120910350. <https://doi.org/10.1177/2050312120910350>
- [3] Kazmi T, Nagi M, Razzaq S, et al., 2022, Burden of Noncommunicable Diseases in Pakistan. Eastern Mediterr Health J,

28(11): 798–804. <https://doi.org/10.26719/emhj.22.083>

- [4] Momtazmanesh S, Moghaddam SS, Ghamari SH, et al., 2023, Global Burden of Chronic Respiratory Diseases and Risk Factors, 1990–2019: An Update from the Global Burden of Disease Study 2019, *eClinicalMedicine*, 2023(59): 101936. <https://doi.org/10.1016/j.eclinm.2023.101936>
- [5] Khorrami Z, Etemad K, Yarahmadi S, et al., 2017, Urbanization and Non-Communicable Disease (NCD) Risk Factors: WHO STEPwise Iranian NCD Risk Factors Surveillance in 2011. *East Mediterr Health J*, 23(7): 469–479. <https://doi.org/10.26719/2017.23.7.469>
- [6] Strong K, Mathers C, Leeder S, et al., 2005, Preventing Chronic Diseases: How Many Lives Can We Save? *Lancet*, 366(9496): 1578–1582. [https://doi.org/10.1016/S0140-6736\(05\)67341-2](https://doi.org/10.1016/S0140-6736(05)67341-2)
- [7] Roglic G, Unwin N, Bennett PH, et al., 2005, The Burden of Mortality Attributable to Diabetes: Realistic Estimates for the Year 2000. *Diabetes Care*, 28(9): 2130–2135. <https://doi.org/10.2337/diacare.28.9.2130>
- [8] Rafique I, Saqib MAN, Munir MA, et al., 2018, Prevalence of Risk Factors for Noncommunicable Diseases in Adults: Key Findings from the Pakistan STEPS Survey. *Eastern Mediterr Health J*, 24(1): 33–41.
- [9] Anchala R, Kannuri NK, Pant H, et al., 2014, Hypertension in India: A Systematic Review and Meta-Analysis of Prevalence, Awareness, and Control of Hypertension. *J Hypertens*, 32(6): 1170–1177. <https://doi.org/10.1097/HJH.0000000000000146>
- [10] Riaz M, Shah G, Asif M, et al., 2021, Factors Associated with Hypertension in Pakistan: A Systematic Review and Meta-Analysis. *PLoS One*, 16(1): e0246085. <https://doi.org/10.1371/journal.pone.0246085>
- [11] Kearney PM, Whelton M, Reynolds K, et al., 2005, Global Burden of Hypertension: Analysis of Worldwide Data. *The Lancet*, 365(9455): 217–223. [https://doi.org/10.1016/S0140-6736\(05\)17741-1](https://doi.org/10.1016/S0140-6736(05)17741-1)
- [12] Golics CJ, Azam Basra MK, Finlay AY, et al., 2013, The Impact of Disease on Family Members: A Critical Aspect of Medical Care. *J R Soc Med*, 106(10): 399–407. <https://doi.org/10.1177/0141076812472616>
- [13] Khan M, Iqbal S, Junaid R, et al., Evaluation of Knowledge and Assessment of Polycystic Ovarian Syndrome (PCOS) Among Healthcare Providers in Medical Institutes of Bahawalpur. *Advances in Obstetrics and Gynecology Research*, 2(3): 53–59. <https://doi.org/10.26689/aogr.v2i3.7652>

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PTEN as a Novel Diagnostic and Prognostic Biomarker of Head and Neck Squamous Cell Carcinoma

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Abstract: This review article explores phosphatase and tensin homolog (PTEN)'s role in head and neck squamous cell carcinoma (HNSCC) through comprehensive expression and methylation examinations, genetic mutation investigation, and prognostic evaluation. Using the UALCAN informational collection, PTEN expression examination uncovered a critical over-expression in HNSCC cells isolated from normal control samples, proposing its role in HNSCC multiplication. Further, analysis of PTEN expression across various clinical limits has shown critical up-regulation in different cancer development stages, racial groups, gender, and age classes within the context of HNSCC patients, suggesting its major role in cancer duplication. PTEN expression was validated by utilizing the GEPIA2.0 online tool, which showed PTEN expression was particularly significantly expressed in HNSCC cancer improvement when it appeared differently from normal control samples. Accordingly, examining PTEN validation across different phases of cancer advancement showed dysregulation in each of the four phases with the most raised expression in stage I and the least expression in stage IV. Thus, this study investigated the promoter methylation level of PTEN, figuring out a basic relationship between HNSCC samples and normal control samples. Analyzing promoter methylation across various clinical limits uncovered massive variations, with specific methylation patterns seen across malignant growth stages, race groups, gender, and age groups. Overall survival and disease-free survival (OS and DFS) utilizing the KM plotter tool showed a critical relationship between PTEN expression levels in HNSCC patients, showing high PTEN expression exhibited good overall survival when showed up distinctively comparable to low PTEN expression levels. In addition, in disease-free survival (DFS) evaluation HNSCC patients showing low PTEN expression experienced great DFS relative to HNSCC patients with high PTEN expression. Moreover, to validate PTEN expression against survival, the study examined the HNSCC patients into low and high-expression groups of PTEN. In HNSCC, low PTEN expression was connected with great overall survival (OS) when it appeared contrastingly relative to the high PTEN expression. In like manner, the study found that low PTEN expression level was connected with great DFS in HNSCC when it appeared contrastingly related to the high PTEN expression group. Genetic mutation analysis via cBioPortal identifies a minimal proportion of *PTEN* mutations in HNSCC, predominantly in-frame mutation, missense mutation, splice mutation, truncating mutation, and structural variant, indicating their basal significance in PTEN dysregulation within HNSCC. Further investigation of PTEN molecular components and their exchange inside the HNSCC microenvironment might disclose novel roads for designated treatment and accurate medication approaches in battling this harmful disease.

Keywords: Head and neck squamous cell carcinoma; Diagnosis; Treatment; Biomarker

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1. Introduction

Head and neck squamous cell carcinoma (HNSCC) comprises a group of cancers brought about by the squamous epithelium in the oral epithelium, oropharynx, larynx, and hypopharynx ^[1]. HNSCC is quite possibly the most well-known cancer and the 6th most common in the world ^[2]. Approximately 750,000 new cases and 360,000 deaths occur annually ^[3]. HNSCC represents roughly 3% of new malignant growth cases and 3% of deaths around the world ^[4]. Roughly 30%–40% of HNSCC patients have early-stage disease (stage I/II) at diagnosis and are typically cured by surgery or radiotherapy (RT) alone ^[5]. The US recorded around 50,000 new HNSCC cases and 10,000 deaths in 2017. Rates are increasing around 1% a year in whites and over two times as high in men as in women. Early diagnosis of the disease is possibly the main factor leading to less widespread and more effective treatment and better patient outcomes ^[6]. Tumorigenesis is a complex pathological process that includes multiple genetic changes, including over-expression of oncogenes or inactivation of tumor suppressor genes ^[7]. Notwithstanding surgery, radiation, and chemotherapy, about half of all patients die from the disease. The risk stratification of HNSCC relies upon the anatomical site, staging and histological attributes of the cancer. Notwithstanding the situation with HPV, numerous molecular and clinical risk factors have been concentrated that limit its clinical significance ^[8].

Phosphatase and tensin homolog deleted on chromosome ten (*PTEN*) was initially recognized as a tumor suppressor habitually lost from a region of chromosome 10q23 in different human cancers, including those of the brain, breast, and prostate ^[9,10]. Until now, the enormous malignant growth data set as of now records > 2700 mutations in *PTEN* from 28 distinct cancer types and the cBioPortal of The Cancer Genome Atlas (TCGA) records 1120 mutations in 27 cancer types ^[11,12]. *PTEN* is a double specific protein and lipid phosphatase, and its essential cell substrate is the second messenger phosphatidylinositol (3,4,5)-trisphosphate (PIP3), which it hydrolyzes to phosphatidylinositol (4,5)-bisphosphate (PIP2) ^[13,14]. Mounting evidence demonstrates that *PTEN* additionally has significant PIP3-independent functions. In particular, *PTEN* protein phosphatase activities are critical for *PTEN*-mediated inhibition of cellular migration ^[15]. In glioma cells, it has been shown that the protein phosphatase activity of *PTEN* is required to actuate *PTEN* phosphorylation and restrain cellular migration ^[16]. Besides, there is evidence that *PTEN* phosphatase activity might regulate glioma cell movement by suppressing Src family kinases ^[17]. *PTEN* additionally has nuclear capabilities, which are possibly independent of its ability to antagonize PI3K signaling. Different proteins have been displayed to influence *PTEN* nuclear localization, subsequently affecting *PTEN*'s ability to act in the nucleus and promote genomic stability ^[18,19]. In addition to protein regulation, different examinations have shown that *PTEN* is down-regulated by promoter methylation in thyroid, breast, lung, endometrial, ovarian, gastric, and brain cancers ^[18].

In the ongoing review, the study explores *PTEN* mutations, expression levels, prognostic outcomes on survival and utilitarian perspectives within the context of HNSCC through bioinformatics assessment. Moreover, the study analyzed the association between *PTEN* expression and promoter methylation levels. To accomplish this, the study utilized different databases, including The Cancer Genome Atlas (TCGA) data set, the UALCAN portal, the Kaplan-Meier tool, the Gene Expression Profiling and Interactive Analysis GEPIA2.0 and cBioPortal. The major point of this study was to evaluate the *PTEN* expression patterns in HNSCC and figure out its probable significance in malignant growth treatment and prognosis.

2. Materials and methods

2.1. Expression and promoter methylation analysis of *PTEN*

To analyze the expression of *PTEN*, the study used UALCAN online database. The UALCAN data set is a comprehensive, user-friendly, and attractive web resource for analyzing cancer genomics data ^[20]. It permits users to distinguish Biomarkers or act *in silico* validation of expected genes of interest, assess epigenetic

regulation of gene expression by promoter methylation and perform pan-cancer gene expression analysis. In the current review, this data set was utilized for the expression examination of PTEN across various phases of the predefined malignant growth, in which this gene shows significant dysregulation as well as a critical relationship with worse OS. To examine the promoter methylation level of PTEN in HNSCC, the study utilized a UCALAN data set. The study dissected promoter methylation data of PTEN in various clinical boundaries, such as the patient's age, gender, and race.

2.2. Validation analysis of PTEN

GEPIA2.0 is a widely used online tool for predicting expression and analyzing genomic data survival ^[21]. The GEPIA 2.0 database can be used to obtain the differences between PTEN expression and prognosis (OS and RFS) in HNSCC cancer patients.

2.3. Survival analysis of PTEN

The KM plotter is a well-known web-based survival analysis tool ^[22]. It permits scientists to evaluate the relationship between gene expression and survival in different malignant growth types, including breast malignant growth, lung cancer, ovarian cancer, and gastric disease. In this review, the KM plotter tool was utilized to analyze the impact of PTEN dysregulation on cancer patients' overall survival (OS).

2.4. Mutational analysis of PTEN

The cBioPortal for cancer genomics is a platform that permits scientists to access and dissect enormous datasets of cancer genomics information ^[12]. It provides an easy-to-understand point of interaction for investigating cancer mutations, gene expression and other genomic information across various cancer types. The platform intends to overcome any issues between complex genomic information and cancer specialists by intuitively admitting molecular profiles and clinical attributes. This study utilized this data to perform a mutational examination of PTEN across HNSCC cancer.

3. Results

3.1. Expression analysis of PTEN in HNSCC based on sample types

The study utilized the UALCAN database (**Figure 1**) to analyze the PTEN expression in HNSCC and normal control samples. Upon analysis, the study found critical up-regulation of PTEN expression in HNSCC cancer cells compared with normal control samples. This critical over-expression depicted a close association between PTEN expression and the proliferation of HNSCC cancerous cells.

3.2. Expression analysis of PTEN in HNSCC divided based on different clinical boundaries

Thus, the study facilitated an assessment of PTEN in HNSCC samples across different clinical parameters, including individual cancer stages, patient's race, gender and age (**Figure 2**). From the beginning, the study examined PTEN expression across various cancer development stages and noticed a basic over-expression of PTEN in HNSCC across all stages as contrasted with ordinary control samples (**Figure 2A**). Along these lines, the study assessed PTEN expression in HNSCC patients, revealing critical over-expression of PTEN in all three racial groups, such as Caucasian, African-American and Asian, when contrasted with normal control samples (**Figure 2B**). In addition, the study analyzed PTEN expression in HNSCC patients detached in gender, which showed notable up-regulation of PTEN in both male and female patients when separated from normal control samples (**Figure 2C**). Finally, the study researched the connection between PTEN expression and patient age in HNSCC. The investigation displayed over-expression of PTEN across different age packs among HNSCC patients (**Figure 2D**).

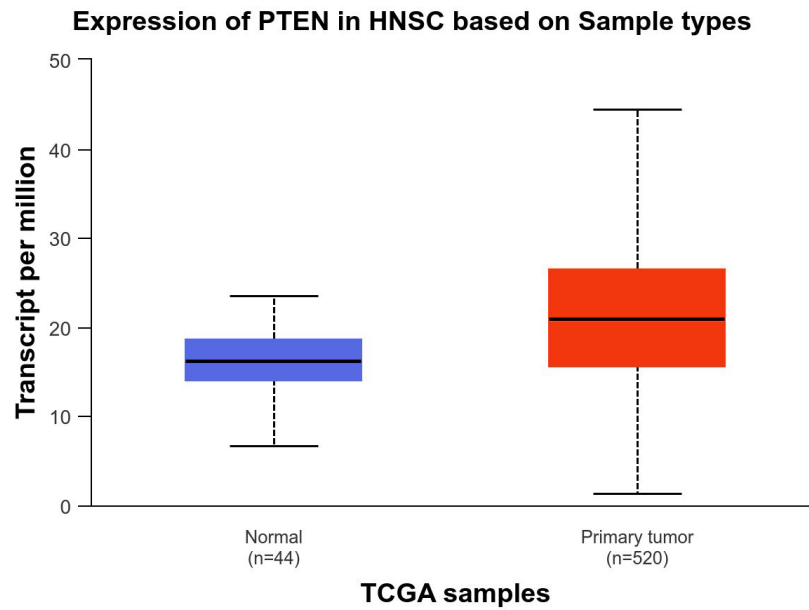


Figure 1. Expression profiling of PTEN in HNSCC and normal tissue samples.

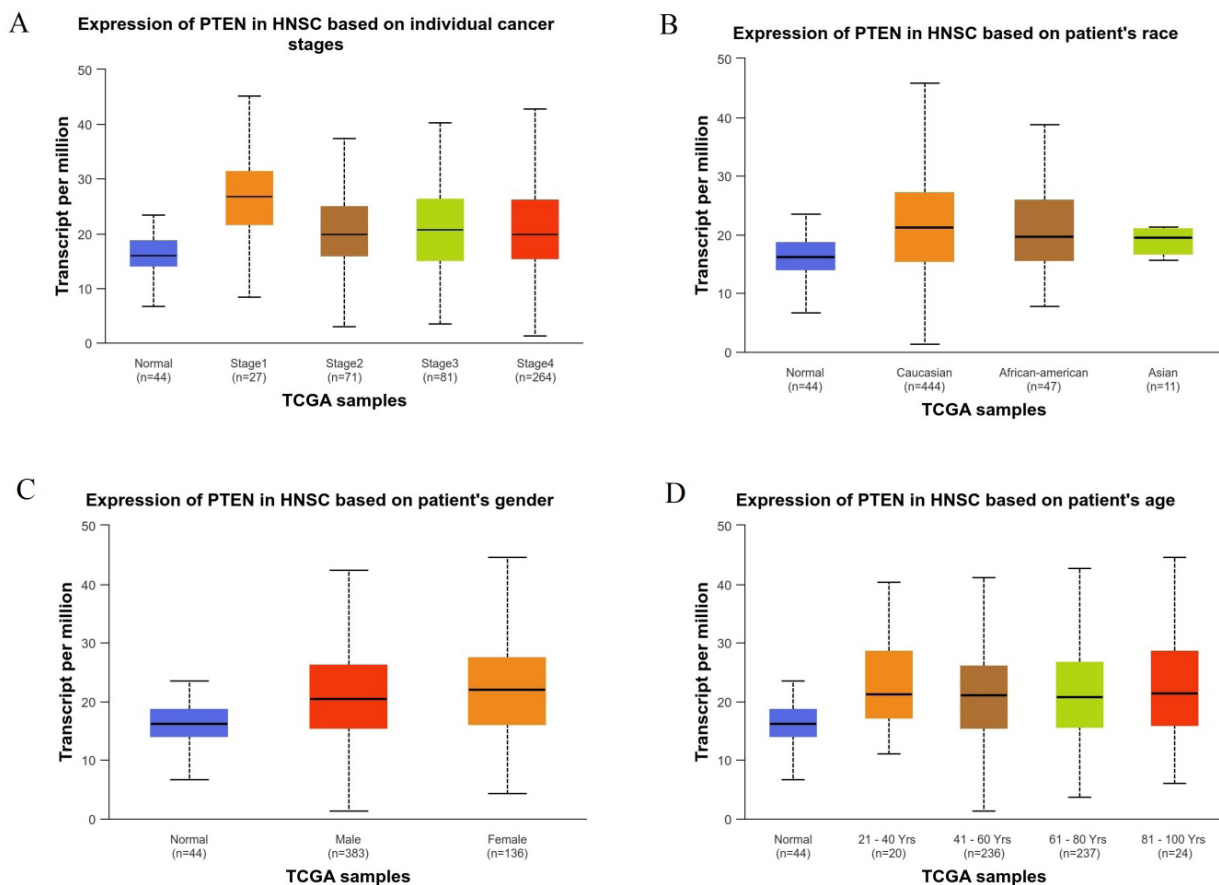


Figure 2. Expression of PTEN across different clinical boundaries.

3.3. Prognostic analysis of PTEN expression in HNSCC

The study used GEPIA2 to examine the PTEN expression between HNSCC cancer when it appeared differently in relation to normal tissues. The result showed that PTEN was especially highly expressed in head and neck squamous cell carcinoma HNSCC compared to normal control samples (**Figure 3A**). In addition, the study segregated the connection between PTEN expression and different cancer stages using the GEPIA2 database. The results showed that PTEN expression was determinedly associated with the phases of patients with HNSCC. In HNSCC, PTEN had the most raised expression in stage I and minimal expression in stage IV (**Figure 3B**).

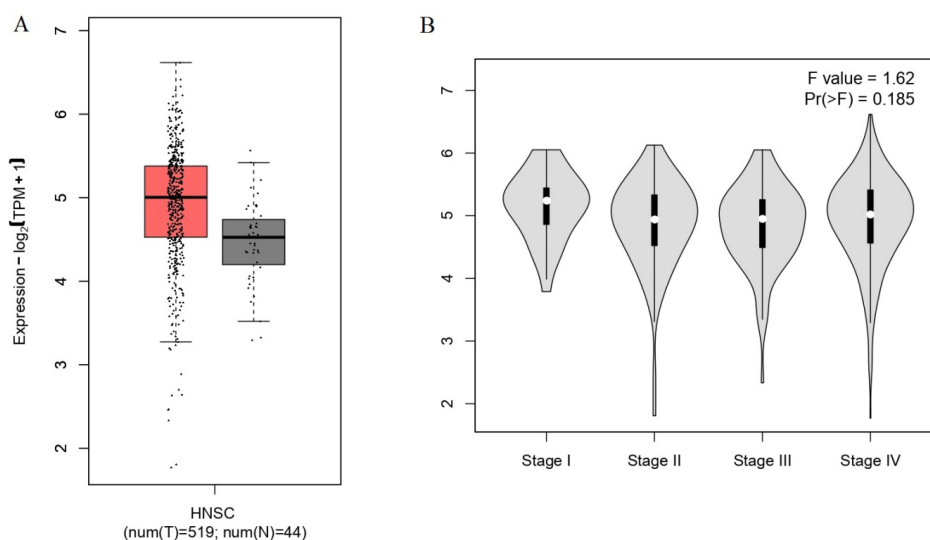


Figure 3. Validation of PTEN expression across different stages of HNSCC.

3.4. Promoter methylation of PTEN in HNSCC and normal control tissues

UALCAN database is utilized to differentiate the promoter methylation of PTEN in HNSCC and normal control samples (**Figure 4**). The study uncovered a significant assortment, expressly hypo-methylation, in the promoter methylation level of PTEN in HNSCC when it appeared differently from normal control samples. This nature proposed potential epigenetic dysregulation of PTEN, featuring its association with HNSCC pathogenesis. Such disclosures add to how the study could unravel the molecular role underlying HNSCC enhancement and idea experiences into the role of PTEN as a potential biomarker or therapeutic agent in HNSCC management.

Figure 4. Promoter methylation pattern of PTEN in HNSCC and normal control samples.

3.5. Promoter methylation of PTEN in HNSCC cancer divided based on different clinical parameters

The study researched different clinical parameters to look at the promoter methylation of PTEN in HNSCC (Figure 5). Fundamentally, the study inspected PTEN promoter methylation across various HNSCC cancer stages compared to normal control samples. Critical variations are displayed among stages. In which every one of four phases has shown significant hypo-methylation when contrasted with typical control samples (Figure 5A). Subsequently, the study investigated PTEN promoter methylation, considering the race of HNSCC patients. The study found information that hypo-methylation occurred in the PTEN promoter region across every one of the three racial groups, such as Caucasian, African-American and Asian as compared to normal control samples (Figure 5B). After this, an assessment of PTEN promoter methylation with females and males showed hypo-methylation (Figure 5C). Finally, the study researched PTEN promoter methylation with respect to patient age, uncovering changing methylation levels across various age packs (Figure 5D). These comprehensive evaluations highlight the astounding relationship between PTEN promoter methylation and different clinical parameters in HNSCC, investigating understanding the various structures critical in PTEN expression regulation in HNSCC pathogenesis.

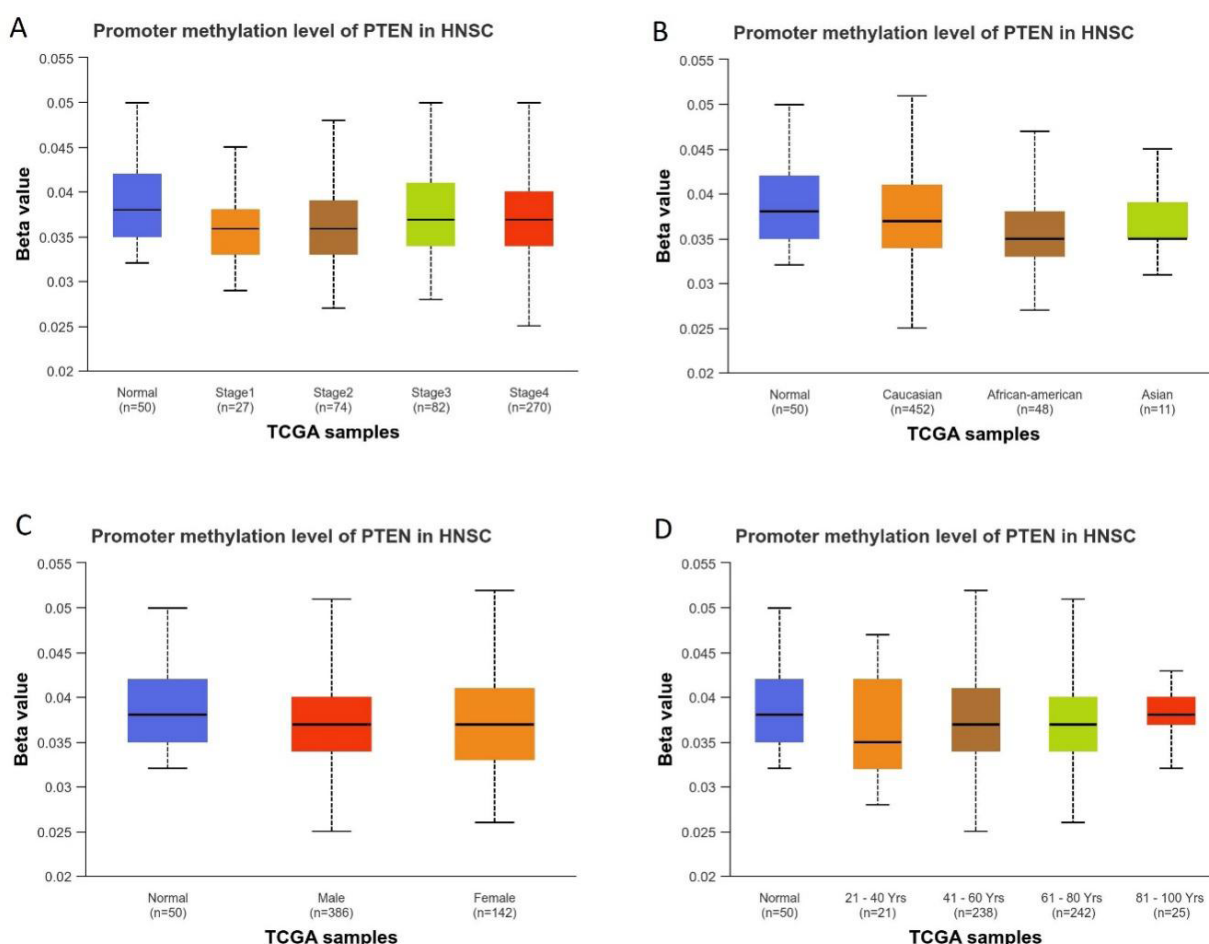


Figure 5. PTEN promoter methylation pattern across different clinical parameters.

3.6. Survival analysis of PTEN

To assess the PTEN gene expression in HNSCC, the study appraised overall survival (OS) and disease-free survival (DFS) using the KM plotter tool. A fundamental affiliation displayed between *PTEN* gene expression and patient survival results in the current review. In particular, HNSCC patients showing high PTEN expression

experienced favorable OS when was shown distinctively with low PTEN expression level (**Figure 6A**). Further, in disease-free survival (DFS) examination, HNSCC patients with low PTEN expression experienced good DFS compared with HNSCC those with high PTEN expression. These findings highlight the vital role of PTEN in influencing the survival results of HNSCC patients, underlying its potential clinical significance as a prognostic marker in HNSCC management.

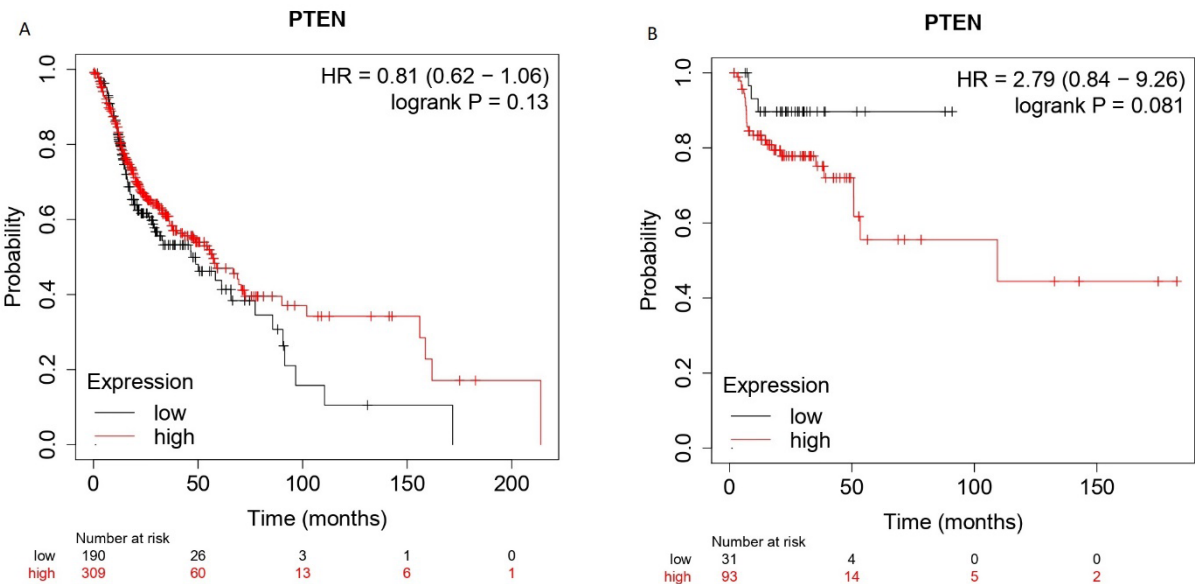


Figure 6. KM survival curve (OS, RFS) of PTEN in HNSCC patients.

3.7. Prognostic analysis of PTEN in HNSCC

The GEPIA2.0 informational index was used to focus on the prognostic worth of PTEN expression in HNSCC disease progression. The study isolated the HNSCC patients into low and high-expression groups of PTEN expression levels. In HNSCC, low PTEN expression was connected with good overall survival (OS) compared to high PTEN expression (**Figure 7A**). Likewise, the study found that a low PTEN expression level was connected with great DFS in HNSCC compared to a high PTEN expression pack (**Figure 7B**).

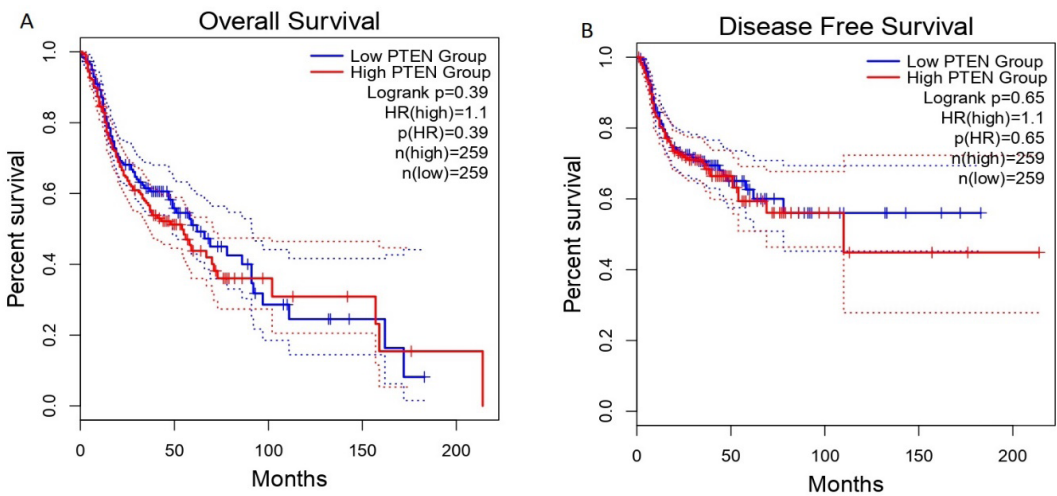


Figure 7. Survival curve (OS, RFS) of PTEN in HNSCC patients.

3.8. Mutational analysis of PTEN

The study further explored the genetic mutations of *PTEN* in HNSCC patients utilizing cBioPortal. The examination uncovered that just 7% of HNSCC samples showed genetic mutations in *PTEN*. The analyzed genetic mutations in HNSCC included in-frame mutation, missense mutation, splice mutation, truncating mutation and structural variant (**Figure 8**). These discoveries recommend that while genetic mutations in *PTEN* are generally uncommon in HNSCC, the noticed in-frame mutation, missense mutation, splice mutation, truncating mutation, and structural variant might assume a major part in the dysregulation of *PTEN* in HNSCC.

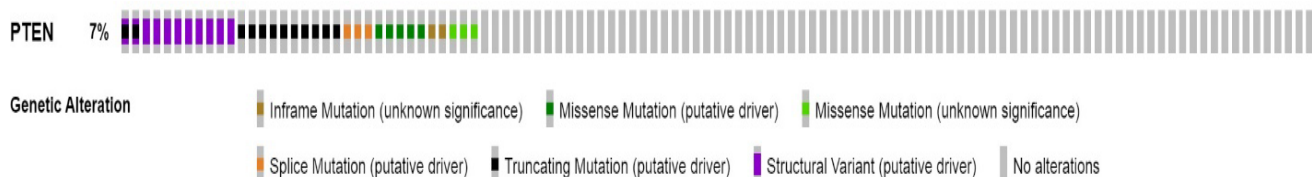


Figure 8. Oncoplot of *PTEN* in HNSCC cancer.

4. Discussion

This review article examined *PTEN* expression, prognosis, methylation, survival and mutations and drove an assessment in HNSCC using different bioinformatics online devices. In addition, overall survival and DFS were used to validate the differentially expressed significant head and neck squamous cell carcinoma. The outcomes portrayed that *PTEN* expression fundamentally influences the human body and reason a likely relationship between *PTEN* expression and HNSCC cancer expansion, proposing *PTEN* expression as a putative controller in HNSCC pathogenesis.

The incidence of HNSCC is expanding around the world ^[23]. The 5-year survival rate of patients with HNSCC is less than 50% ^[24], which is associated with a lack of reliable biomarkers ^[25]. Recent examinations have laid out a connection between molecular markers, for example, autophagy genes, immune genes, autophagy-related long noncoding RNAs (lncRNAs) and immune-related lncRNAs, and HNSCC prognosis ^[26,27], which might support in deciding clinical results. As ferroptosis is supposedly involved with both malignant growth progression and cancer suppression, it tends to be a novel therapeutic target for cancers ^[28]. In that capacity, prognostic ferroptosis-related signature genes have been laid out for different cancers, including hepatocellular carcinoma ^[29], glioma ^[30], uveal melanoma ^[31], and clear cell renal cancer ^[32]. SLC7A11 is a biomarker and therapeutic target for HPV-positive HNSCC ^[33]. Moreover, ferroptosis improves the clinical development inhibitory viability of PDT against oral tongue squamous cell carcinoma ^[34].

PTEN is a very strong and multi-layered cancer suppressor practically associated with a wide range of “hallmarks” of cancer. The principal system by which *PTEN* activity limits malignant growth development and progression remains its ability to down-modulate signaling through the PI3K pathway, in this manner in a roundabout way repressing AKT downstream targets, like GSK3, FOXO, B cell lymphoma 2 (BCL-2) antagonistic of cell death (BAD), the E3 ubiquitin-protein ligase MDM2 and p27, which control survival, cell multiplication, angiogenesis and cellular metabolism ^[35]. On the other hand, the mTORC1 arm of the PI3K/AKT/mTOR pathway is likewise actuated in light of the deficiency of *PTEN* inhibitory activity, bringing about the phosphorylation of p70 ribosomal protein S6 kinase (S6K; otherwise called RPS6K) and restraint of 4E-restricting protein 1 (4EBP1; otherwise called eIF4EBP1) to activate protein translation prompting to the enhanced translation of explicit mRNAs that are critical for cell development and proliferation ^[36,37]. Recently, 4EBP1 has, without a doubt, emerged as a crucial negative regulator of cell expansion downstream

of mTORC1, and its inactivation may straightforwardly advance the development of inconsistent malignant growths^[38,39]. Loss of *PTEN* plays an important role in the development of 30%–60% of melanomas. Evidence has shown that decreased *PTEN* transcript levels were associated with *PTEN* promoter methylation in melanoma^[40,41]. Decreased PTEN expression has been shown in pancreatic cancer cell lines, in spite of the fact that deletion or mutations that cause PTEN loss of activity have not been recognized with significant frequency in human pancreatic ductal adenocarcinoma (PDAC)^[42]. *PTEN* loss or inactivating mutations are found in a variable proportion (5%–30%) of sporadic colorectal cancers^[43–45]. *PTEN* mutations happen at a low frequency in NSCLC and in small cell lung cancer (SCLC), with the notable exemption of squamous cell carcinoma of the lung, in which *PTEN* is mutated in 6–9% of the cases and essentially modified in up to 15% of cases, considering the loss of expression as well^[46]. Germline *PTEN* mutation in Cowden syndrome has an inclination to breast malignant growth, where female CS patients have up to 85% LR of developing breast cancer^[47–49]. In sporadic breast carcinomas, the frequency of *PTEN* loss is 30%–40%^[50].

In the current assessment, the UALCAN database was applied to figure out the expression of PTEN in HNSCC. In the UALCAN assessment, the continuous analysis has shown that up-regulation of PTEN expression was seen in different cancer stages, individual cancer development types, age, gender and racial groups. As to advancement in tumors, the consequence of flow concentrates on portraying that the PTEN expression level was higher in HNSCC tissues than in normal control samples. Besides, in the KM plotter tool, the appraisal uncovered that HNSCC patients showing high PTEN expression experienced good overall survival. HNSCC patients showing low PTEN expression exhibited good DFS compared to low and high PTEN expression. In the examination, it was found that the PTEN expression level in tissue was a poor independent prognostic component. Further evaluations should explore the prognostic worth of PTEN expression in malignant growth development.

5. Conclusion

In conclusion, the analysis indicates that PTEN over-expression in HNSCC is closely associated with poor overall survival, promoter methylation levels, and genetic mutations. Through proper utilization of various public databases, including UALCAN, TCGA, cBioPortal, and KM plotter, the study has thrown light on the diagnostic, prognostic, and potentially therapeutic roles of PTEN in HNSCC. However, further research is required to validate and confirm these findings and explore the underlying mechanisms driving PTEN dysregulation in HNSCC. These findings may ultimately contribute to the development of improved diagnostic tools and therapeutic strategies for HNSCC patients.

Disclosure statement

The authors declare no conflict of interest.

References

- [1] Solomon B, Young RJ, Rischin D, 2018, Head and Neck Squamous Cell Carcinoma: Genomics and Emerging Biomarkers for Immunomodulatory Cancer Treatments. *Semin Cancer Biol*, 52(Pt 2): 228–240.
- [2] Vokes EE, Weichselbaum RR, Lippman SM, et al., 1993, Head and Neck Cancer. *The New England Journal of Medicine*, 328(3): 184–194.
- [3] Sung H, Ferlay J, Siegel RL, et al., 2021, Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and

Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*, 71(3): 209–249.

- [4] Siegel RL, Miller KD, Jemal A, 2020, Cancer Statistics, 2020. *CA: A Cancer Journal for Clinicians*, 70(1): 7–30.
- [5] Pfister DG, Spencer S, Brizel DM, et al., 2014, Head and Neck Cancers, Version 2. Clinical Practice Guidelines in Oncology. *Journal of National Comprehensive Cancer Network*, 12(10): 1454–1487.
- [6] Chai RC, Lambie D, Verma M, et al., 2015, Current Trends in the Etiology and Diagnosis of HPV-Related Head and Neck Cancers. *Cancer Med*, 4(4): 596–607.
- [7] Vogelstein B, Kinzler KW, 2004, Cancer Genes and the Pathways They Control. *Nature Medicine*, 10(8): 789–799.
- [8] The Cancer Genome Atlas Network, 2015, Comprehensive Genomic Characterization of Head and Neck Squamous Cell Carcinomas. *Nature*, 517(7536): 576–582.
- [9] Li J, Yen C, Liaw D, et al., 1997, PTEN, a Putative Protein Tyrosine Phosphatase Gene Mutated in Human Brain, Breast, and Prostate Cancer. *Science*, 275(5308): 1943–1947.
- [10] Steck PA, Pershouse MA, Jasser SA, et al., 1997, Identification of a Candidate Tumour Suppressor Gene, MMAC1, at Chromosome 10q23.3 That Is Mutated in Multiple Advanced Cancers. *Nature Genetics*, 15(4): 356–362.
- [11] Gao J, Aksoy BA, Dogrusoz U, et al., 2013, Integrative Analysis of Complex Cancer Genomics and Clinical Profiles Using the cBioPortal. *Science Signaling*, 6(269): 2004088.
- [12] Cerami E, Gao J, Dogrusoz U, et al., 2012, The cBio Cancer Genomics Portal: An Open Platform for Exploring Multidimensional Cancer Genomics Data. *Cancer Discovery*, 2(5): 401–404.
- [13] Li DM, Sun H, 1997, TEP1, encoded by a Candidate Tumor Suppressor Locus, is a Novel Protein Tyrosine Phosphatase Regulated by Transforming Growth Factor Beta. *Cancer Research*, 57(11): 2124–2129.
- [14] Stambolic V, Suzuki A, de la Pompa JL, et al., 1998, Negative Regulation of PKB/Akt-Dependent Cell Survival by the Tumor Suppressor PTEN. *Cell*, 95(1): 29–39.
- [15] Leslie NR, Yang X, Downes CP, et al., 2007, PtdIns(3,4,5)P(3)-Dependent and -Independent Roles for PTEN in the Control of Cell Migration. *Current Biology*, 17(2): 115–125.
- [16] Raftopoulou M, Etienne-Manneville S, Self A, et al., 2004, Regulation of Cell Migration by the C2 Domain of the Tumor Suppressor PTEN. *Science*, 303(5661): 1179–1181.
- [17] Dey N, Crosswell HE, De P, Parsons R, Peng Q, Su JD, et al., 2008, The Protein Phosphatase Activity of PTEN Regulates SRC Family Kinases and Controls Glioma Migration. *Cancer Res*, 68(6): 1862–1871.
- [18] Song MS, Salmena L, Pandolfi PP, 2012, The Functions and Regulation of the PTEN Tumour Suppressor. *Nature Reviews Molecular Cell Biology*, 13(5): 283–296.
- [19] Mayo LD, Donner DB, 2002, The PTEN, Mdm2, p53 Tumor Suppressor-Oncoprotein Network. *Trends Biochem Science*, 27(9): 462–467.
- [20] Chandrashekar DS, Bashel B, Balasubramanya SAH, et al., 2017, UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. *Neoplasia*, 19(8): 649–658.
- [21] Tang Z, Kang B, Li C, et al., 2019, GEPIA2: An Enhanced Web Server for Large-Scale Expression Profiling and Interactive Analysis. *Nucleic Acids Research*, 47(W1): W556–W560.
- [22] Maciejczyk A, Szelachowska J, Czapiga B, et al., 2013, Elevated BUBR1 Expression Is Associated with Poor Survival in Early Breast Cancer Patients: 15-Year Follow-Up Analysis. *J Histochem Cytochem*, 61(5): 330–339.
- [23] Bray F, Ferlay J, Soerjomataram I, et al., 2018, Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*, 68(6): 394–424.
- [24] Canning M, Guo G, Yu M, et al., 2019, Heterogeneity of the Head and Neck Squamous Cell Carcinoma Immune Landscape and Its Impact on Immunotherapy. *Frontier in Cell and Developmental Biology*, 2019(7): 52.
- [25] Chow LQM, 2020, Head and Neck Cancer. *The New England Journal of Medicine*, 382(1): 60–72.
- [26] Chen Y, Luo TQ, Xu SS, et al., 2021, An Immune-Related Seven-lncRNA Signature for Head and Neck Squamous Cell

Carcinoma. *Cancer Medicine*, 10(7): 2268–85.

- [27] Yang C, Mei H, Peng L, et al., 2020, Prognostic Correlation of an Autophagy-Related Gene Signature in Patients with Head and Neck Squamous Cell Carcinoma. *Computational and Mathematical Methods in Medicine*, 28:7397132.
- [28] Wang Y, Wei Z, Pan K, et al., 2020, The Function and Mechanism of Ferroptosis in Cancer. *Apoptosis*, 25(11–12): 786–798.
- [29] Liang JY, Wang DS, Lin HC, et al., 2020, A Novel Ferroptosis-Related Gene Signature for Overall Survival Prediction in Patients with Hepatocellular Carcinoma. *International Journal of Biological Sciences*, 16(13): 2430–2441.
- [30] Zhuo S, Chen Z, Yang Y, et al., 2020, Clinical and Biological Significances of a Ferroptosis-Related Gene Signature in Glioma. *Frontier of Oncology*, 10(590861).
- [31] Luo H, Ma C, 2021, A Novel Ferroptosis-Associated Gene Signature to Predict Prognosis in Patients with Uveal Melanoma. *Diagnostics*, 11(2).
- [32] Mou Y, Wu J, Zhang Y, et al., 2021, Low Expression of Ferritinophagy-Related NCOA4 Gene in Relation to Unfavorable Outcome and Defective Immune Cells Infiltration in Clear Cell Renal Carcinoma. *BMC Cancer*, 21(1): 020–07726.
- [33] Hémon A, Louandre C, Lailler C, et al., 2020, SLC7A11 as a Biomarker and Therapeutic Target in HPV-Positive Head and Neck Squamous Cell Carcinoma. *Biochemical and Biophysical Research Communications*, 533(4): 1083–1087.
- [34] Zhu T, Shi L, Yu C, et al., 2019, Ferroptosis Promotes Photodynamic Therapy: Supramolecular Photosensitizer-Inducer Nanodrug for Enhanced Cancer Treatment. *Theranostics*, 9(11): 3293–3307.
- [35] Manning BD, Cantley LC, 2007, AKT/PKB Signaling: Navigating Downstream. *Cell*, 129(7): 1261–1274.
- [36] Ciuffreda L, Di Sanza C, Incani UC, et al., 2010, The mTOR Pathway: A New Target in Cancer Therapy. *Current Cancer Drug Targets*, 10(5): 484–495.
- [37] Ma XM, Blenis J, 2009, Molecular Mechanisms of mTOR-Mediated Translational Control. *Nature Reviews Molecular Cell Biology*, 10(5): 307–318.
- [38] Dowling RJ, Topisirovic I, Alain T, et al., 2010, mTORC1-Mediated Cell Proliferation, but Not Cell Growth, Controlled by the 4E-BPs. *Science*, 328(5982): 1172–1176.
- [39] She QB, Halilovic E, Ye Q, et al., 2010, 4E-BP1 is a Key Effector of the Oncogenic Activation of the AKT and ERK Signaling Pathways that Integrates Their Function in Tumors. *Cancer Cell*, 18(1): 39–51.
- [40] Hollander MC, Blumenthal GM, Dennis PA, 2011, PTEN Loss in the Continuum of Common Cancers, Rare Syndromes and Mouse Models. *Nature Reviews Cancer*, 11(4): 289–301.
- [41] Mirmohammadsadegh A, Marini A, Nambiar S, et al., 2006, Epigenetic Silencing of the PTEN Gene in Melanoma. *Cancer Research*, 66(13): 6546–6552.
- [42] Aguirre AJ, Brennan C, Bailey G, et al., 2004, High-Resolution Characterization of the Pancreatic Adenocarcinoma Genome. *Proceedings of the National Academy of Sciences of the United States of America*, 101(24): 9067–9072.
- [43] Zhou XP, Loukola A, Salovaara R, et al., 2002, PTEN Mutational Spectra, Expression Levels, and Subcellular Localization in Microsatellite Stable and Unstable Colorectal Cancers. *The American Journal of Pathology*, 161(2): 439–447.
- [44] Frattini M, Signoroni S, Pilotti S, et al., 2005, Phosphatase Protein Homologue to Tensin Expression and Phosphatidylinositol-3 Phosphate Kinase Mutations in Colorectal Cancer. *Cancer Research*, 65(23): 11227.
- [45] Naguib A, Cooke JC, Happerfield L, et al., 2011, Alterations in PTEN and PIK3CA in Colorectal Cancers in the EPIC Norfolk Study: Associations with Clinicopathological and Dietary Factors. *BMC Cancer*, 11(123): 1471–2407.
- [46] Dearden S, Stevens J, Wu YL, et al., 2013, Mutation Incidence and Coincidence in Non-Small-Cell Lung Cancer: Meta-Analyses by Ethnicity and Histology (mutMap). *Annals of Oncology*, 24(9): 2371–2376.
- [47] Eng C, 2003, PTEN: One Gene, Many Syndromes. *Human Mutation*, 22(3): 183–198.

- [48] Mester JL, Moore RA, Eng C, 2013, PTEN Germline Mutations in Patients Initially Tested for Other Hereditary Cancer Syndromes: Would Use of Risk Assessment Tools Reduce Genetic Testing? *Oncologist*, 18(10): 1083–1090.
- [49] Marsh DJ, Coulon V, Lunetta KL, et al., 1998, Mutation Spectrum and Genotype-Phenotype Analyses in Cowden Disease and Bannayan-Zonana Syndrome, Two Hamartoma Syndromes with Germline PTEN Mutation. *Human Molecular Genetics*, 7(3): 507–515.
- [50] Zhang HY, Liang F, Jia ZL, et al., 2013, PTEN Mutation, Methylation and Expression in Breast Cancer Patients. *Oncology Letters*, 6(1): 161–168.

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A Bioinformatics Analysis of FAM3A to Identify its Potential Role as a Biomarker in Liver Hepatocellular Cancer

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Abstract: Liver hepatocellular cancer (LIHC) is positioned as the third cancer with the highest mortalities worldwide, and high mortalities are associated with late diagnosis and recurrence. This study advances bioinformatics analysis of FAM3A expression in LIHC to evaluate its potential as a prognostic, diagnostic and therapeutic biomarker. Bioinformatics tools such as UALCAN, GEPIA2, KM plotter, TIMER2 and cBioPortal are employed to conduct analysis. Initially, the expression analysis revealed up-regulation of FAM3A in LIHC based on various variables. Further, the study observed that FAM3A methylation regulates expression as variation in methylation level of FAM3A was assessed in LIHC. Moreover, this over-expression of FAM3A results in poor overall survival (OS) in LIHC patients. All of these proposed that FAM3A has a role in the progression and development of LIHC. While examined association of FAM3A expression and infiltration level of CD8⁺ T cells in LIHC patients using TIMER2 revealed that FAM3A has a positive correlation with purity in LIHC that highlights the molecular landscape. Analysis of genetic alteration revealed minute role of FAM3A in LIHC still provides valuable insight. Overall, our findings reveal that FAM3A has potential as diagnostic, therapeutic and prognostic biomarkers in LIHC.

Keywords: Liver hepatocellular cancer (LIHC); FAM3A expression; Bioinformatics analysis; Biomarker

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1. Introduction

Cancer is a fatal disease with millions of cases globally. In 2022, about 20 million cases and 9.7 million cancer-related mortalities were recorded worldwide. There is various type of cancer, identified by their origin and specific attributes ^[1-3]. Liver cancer is the sixth most common cancer and 3rd cancer with high mortality worldwide, with 757,948 deaths recorded in 2022 ^[2]. Liver hepatocellular carcinoma (LIHC) is the most prevalent type of liver cancer, accounting for 80–90% of cases ^[4,5]. Liver infection, hepatitis B virus (HBV) and hepatitis c virus (HCV) infection, alcohol consumption, obesity, diabetes and smoking are the leading determinants of LIHC ^[4,6,7]. Moreover, in Asia and Africa, LIHC cases are higher compared to Europe. However, LIHC cases are increasing in developed countries due to high rates of diabetes, obesity and hepatitis ^[7,8]. Surgery, radiation, therapy, target

therapy, hepatectomy, chemotherapy and liver transplantation are major therapies used for LIHC^[9,10]. However, the mortality rate in LIHC is quite high due to recurrence and diagnosis at higher stage. LIHC is diagnosed at advanced stages due to lack of symptoms. LIHC patients have poor survival rates at advanced stages, and at early stages, recurrence is high even after treatment^[11-14]. Therefore, it is a compelling necessity to identify efficient prognostic, therapeutic and diagnostic biomarkers for LIHC.

FAM3A (FAM3 metabolic regulating signaling molecule A) is a member of the FAM3 family and encodes cytokine-like proteins. ATP production is amplified in the liver and other tissues by FAM3A. It is a mitochondrial protein where mitochondrial respiration is enhanced in hepatocytes, neuronal cells and vascular smooth muscle cells by FAM3A. FAM3A plays a significant role in inhibiting gluconeogenesis and lipogenesis^[15-19]. FAM3A plays a crucial role during skeletal muscle repair by promoting mitochondrial respiration^[20]. FAM3A inhibits gluconeogenesis. Insulin resistance or insulin insufficiency is enhanced due to overexpression of gluconeogenesis genes in the liver, resulting in type 2 diabetes mellitus^[21-23]. FAM3A arouses adipogenesis, optimizes endoplasmic reticulum (ER) stress in various cell types and protects the liver against ischemia or injury^[24-26]. Previous studies stated that FAM3A is overexpressed in Lynch syndrome and results in poor prognosis^[27]. LICH increases the risk of colorectal cancer, endometrial cancer, ovarian cancer and stomach cancer.

Overall, these data suggest that FAM3A has an important function in the liver and that changes in the expression of FEM3A lead to liver abnormalities. The study intended to conduct a bioinformatics analysis of FAM3A in LICH. The study assessed the expression, mutation and survival analysis of FAM3A utilizing bioinformatics tools. There is no such analysis has been conducted to investigate the potential of FAM3A in LICH.

2. Material and Method

2.1. UALCAN

UALCAN is a web tool that is comprehensively used for expression profiling of specific genes in cancers^[28]. The study analyzed the expression and methylation level of FAM3A in LIHC using UALCAN. The analysis was run using the default setting of FAM3A. UALCAN is also used to evaluate the expression and methylation profiling of FAM3A in LIHC based on different parameters.

2.2. Kaplan-Meier Plotter

Kaplan-Meier (KM) Plotter is a web-based tool that is for evaluating the survival rate of the concerned gene in various subtypes of cancer^[29]. In the present study, the prognostic value of FAM3A was examined in LIHC. While Hazard ratio and 95% confidence intervals were displayed, P -value < 0.05 is set as significant.

2.3. GEPIA2

GEPIA2 is an online tool based on the Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx). It evaluates the expression profiling and prognostic value of desired genes in various cancer subtypes^[30]. The study employed GEPIA2 to conduct a comprehensive expression analysis and survival analysis of FAM3A in LIHC.

2.4. cBioPortal

cBioPortal is an easy-to-use online database that is employed to assess genetic alteration in different human cancers^[31]. In this study, cBioPortal is employed to investigate assess the genetic alteration of FAM3A in LIHC.

2.5. Timer2

Timer2 is a widely used database to investigate the immune infiltrate in various cancer subtypes thoroughly^[32]. The study harnessed TIMER2 to evaluate the Spearman correlation between the FAM3A expression and CD8⁺ T immune cells in LIHC.

3. Results

3.1. Analyzing the FAM3A expression in LIHC

The study investigated the expression of FAM3A in LIHC in contrast with a normal sample using UALCAN. The study assessed that the expression of FAM3A is significantly up-regulated in the LIHC samples as compared to normal samples (**Figure 1**). Previous studies have stated that gene overexpression is associated with the progression of cancer^[33,34]. Therefore, significant up-regulation illustrated that FAM3A plays a role in the progression of LIHC.

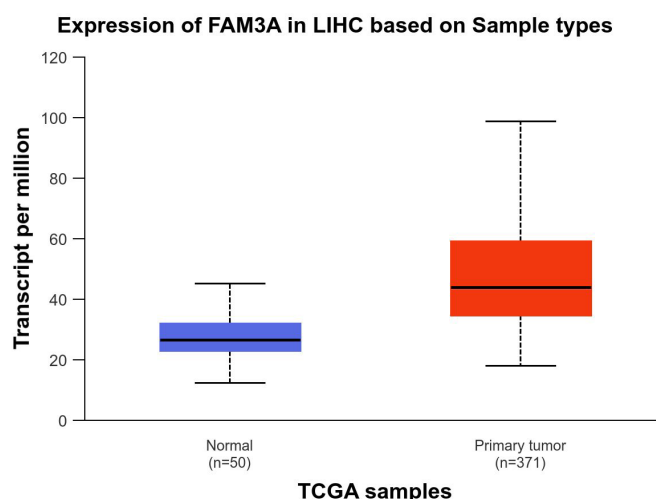


Figure 1. Sample-based expression analysis of FAM3A in LIHC using UALCAN.

3.2. Assessment of FAM3A expression in LIHC based on various parameters

The study harnessed the UALCAN database to assess the expression of FAM3A in LIHC and evaluated significant overexpression in observed parameters. In the pathological stage, the study observed up-regulation but variation in the expression of FAM3A in LIHC, as FAM3A was highly overexpressed in stage 2 as compared to other stages (**Figure 2A**). Next, an analysis in LIHC revealed up-regulation in FAM3A expression, but FAM3A was highly up-regulated in males as compared to females (**Figure 2B**). Similarly, analysis of LIHC patient's age and race also revealed significant overexpression of FAM3A (**Figure 2C–D**). All of this data suggested that up-regulation of FAM3A expression is associated with the progression of LIHC.

3.3. Investigating promoter methylation level of FAM3A in LIHC and normal samples

Previous studies have stated that methylation of the gene has a role in the regulation of gene expression^[35,36]. Therefore, the study analyzed the promoter methylation level of FAM3A in LIHC using UALCAN. FAM3A in LIHC samples was significantly hypomethylated in contrast with normal samples (**Figure 3**). This observed hypomethylation of FAM3A suggested up-regulation of the FAM3A of expression in LIHC. Altogether, these findings explain the role of FAM3A in the progression of LIHC.

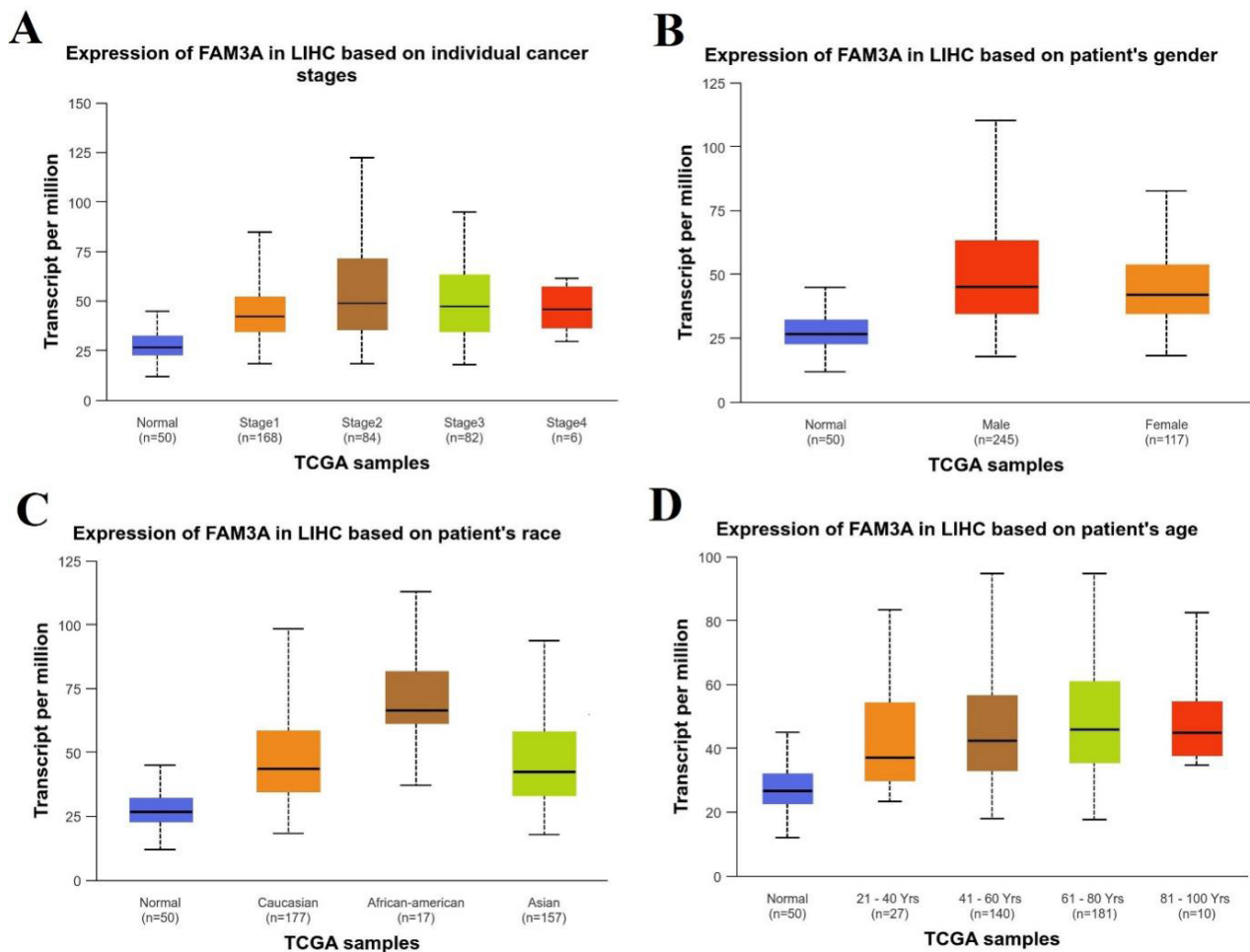


Figure 2. Analysis of FAM3A expression in various parameters. (A) Expression of FAM3A in pathological stages of LIHC. (B) Expression of FAM3A in the LIHC patient's gender. (C) Expression of FAM3A in the LIHC patient's race. (D) Expression of FAM3A in the LIHC patient's age.

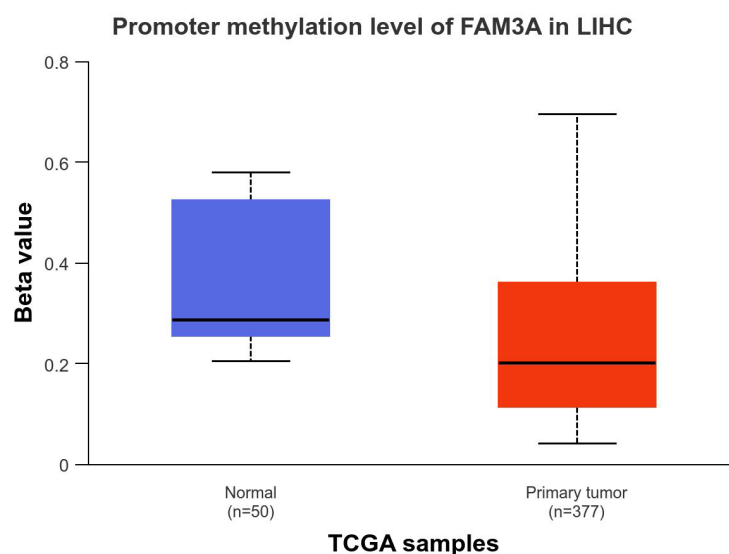


Figure 3. Analysis of promoter methylation level of FAM3A in LIHC and Normal control samples.

3.4. Analysis of the promoter methylation level of FAM3A in LIHC segmented by multiple variables

Simultaneously, the study evaluated promoter methylation level of FAM3A in LIHC segmented by multiple variables like patient's age, gender, race and cancer stages. At first, the study reviewed promoter methylation levels in LIHC cancer stages and evaluated that FAM3A was hypomethylated at these stages except stage 4 (**Figure 4A**). FAM3A was hypermethylated in LIHC stage 4, which explains the diversity in methylation level. Similarly, the study analyzed FAM3A promoter methylation levels in LIHC patients' gender samples and noted variations in methylation levels (**Figure 4B**). The study assessed that FAM3A was significantly hypomethylated in male samples and hypermethylated in female samples. However, investigation based on LIHC patient's age and race revealed that FAM3A is hypomethylated in both of these samples (**Figure 4C–D**). All of these findings highlight the multifaceted role of FAM3A methylation level in the regulation of FAM3A expression and in the progression of LIHC.

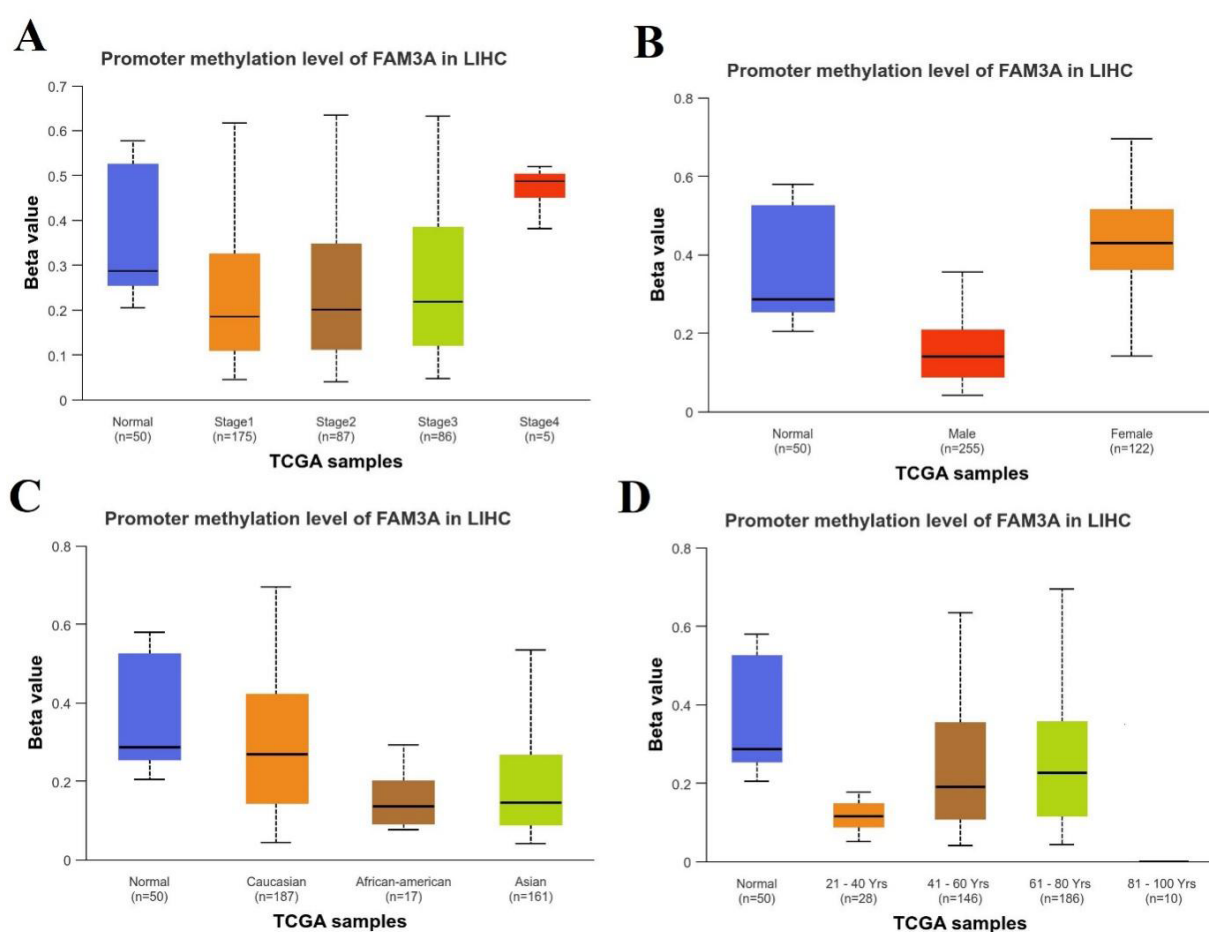


Figure 4. Investigation of FAM3A Promoter methylation level in different parameters. (A) Promoter methylation level of FAM3A in pathological stages of LIHC. (B) Promoter methylation level of FAM3A in LIHC patient's gender. (C) Promoter methylation level of FAM3A in LIHC patient's race. (D) Promoter methylation level of FAM3A in LIHC patient's age.

3.5. Prognostic analysis of FAM3A in LIHC

The study harnessed a Km plotter to conduct a prognostic analysis of FAM3A in LIHC. This curve demonstrated that overexpressed FAM3A in LIHC has a poor survival rate, while low-expressed FAM3A in LIHC has a better survival rate (**Figure 5**). The calculated hazard ratio is 1.49, which indicates that patients with overexpressed

FAM3A have 1.49 times more risk of death as compared to lower-expressed patients. In addition, both values have statistical differences, as the examined *P*-value is 0.025. This result illustrated that overexpressed FAM3A plays a role in the progression of LIHC.

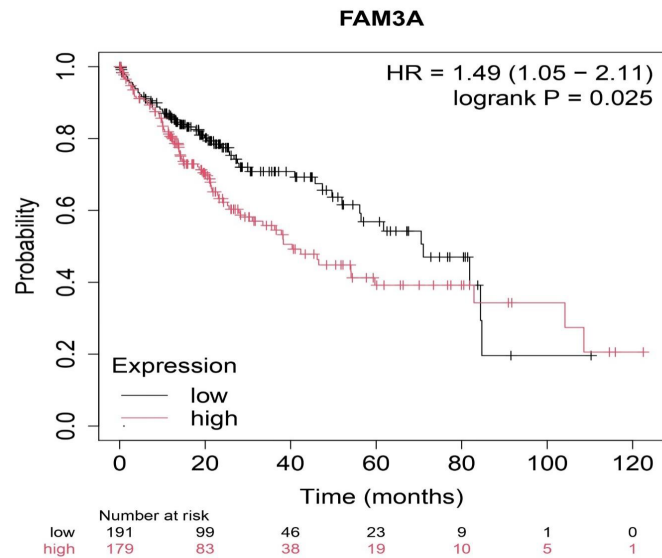


Figure 5. Prognostic analysis of FAM3A expression in LIHC employing KM plotter.

3.6. Expression and prognostic analysis of FAM3A in LIHC using GEPIA2

The study used GEPIA2 to conduct expression and prognostic analysis of FAM3A in LIHC to validate its findings. Initially, sample-based expression analysis of FAM3A was performed in LIHC using GEPIA2 and assessed that FAM3A was overexpressed in LIHC samples (**Figure 6A**). Next, the study utilized the stage plot module of GEPIA2 and examined the expression of FAM3A in different stages of LIHC. The study assessed that expression does not statistically differ in these stages, and the calculated *p*-value of 0.168 supports it (**Figure 6B**). These findings coincide with the previous result that FAM3A is overexpressed in LIHC and plays a role in the development and progression of LIHC.

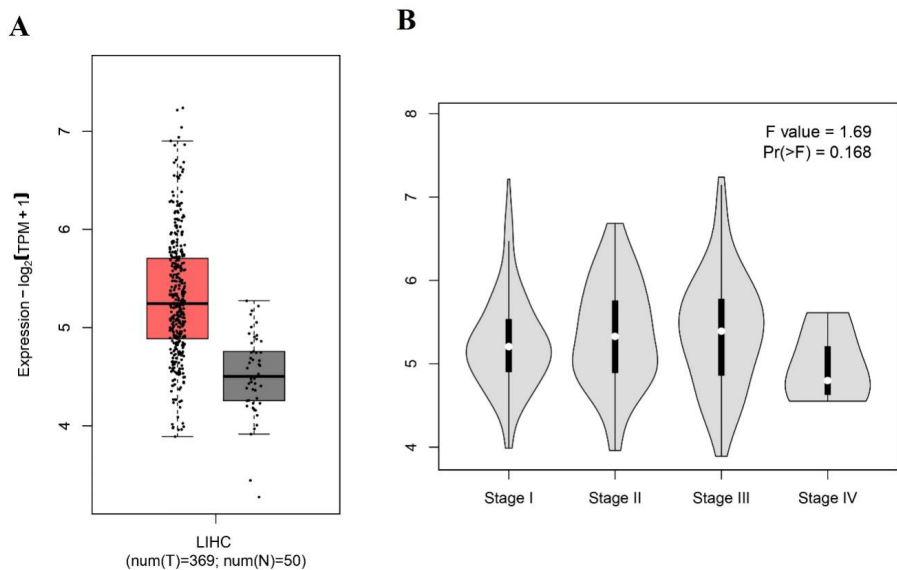


Figure 6. (A) Expression analysis of FAM3A in LIHC and normal control sample employing GEPIA2. (B) Analyzing FAM3A expression in individual cancer stages employing GEPIA2.

Following this, the study examined the impact of FAM3A expression on the overall survival (OS) of LIHC patients employing the survival module of GEPIA2. The result indicated varying survival results (**Figure 7**). It can be observed that elevated FAM3A expression has poor OS in LIHC in comparison with lower expressed FAM3A expression. However, the difference between the two values is not significant, as the calculated log-rank P -value is 0.2. These results suggest that FAM3A overexpression is linked with poor OS of LIHC patients and that FAM3A has a role in LIHC progression.

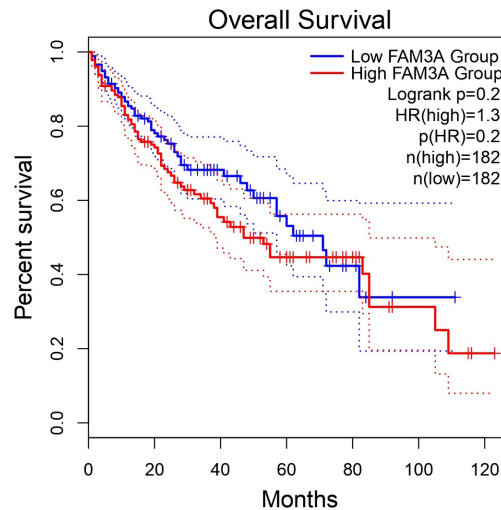


Figure 7. Prognostic analysis of FAM3A expression in LIHC using GEPIA2.

3.7. FAM3A expression and infiltration level of cd8+ t cells in LIHC patients

The study utilized the TIMER2 database to analyze the association of FAM3A expression level with purity and CD8⁺ T immune cells in LIHC. Previous studies discuss immunity and tumor development as strongly associated [37], while the proportion of cancer cells in tumor samples is called purity [38]. The left-scattered plot highlights the positive association between FAM3A expression level and purity (**Figure 8A**). The evaluated association is significant as the calculated p -value is 0.00334. Further, there is a noted weak association between FAM3A expression level and CD8⁺ T immune cells, as shown in the right scattered plot (**Figure 8B**). The calculated p -value is 0.127, indicating that the association is not statistically significant.

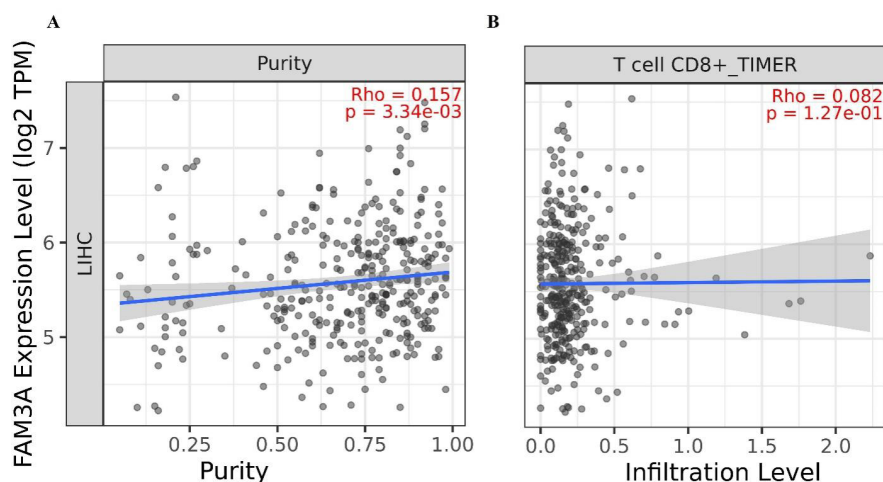


Figure 8. Assessment of FAM3A expression level association with purity and CD8⁺ T immune cells in LIHC using TIMER2.

3.8. Genetic alteration of FAM3A in LIHC

The study examined the genetic alteration of FAM3A in LIHC by employing cBioPortal. 2.3% of genetic alteration of FAM3A was observed in LIHC. Amplification, deep deletion and missense mutation are the examined mutation of FAM3A in LIHC (**Figure 9**). These findings suggest that genetic alteration in LIHC have minute contribution in regulation of FAM3A expression.

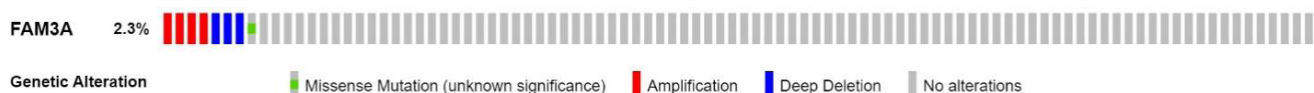


Figure 9. Genetic alteration of FAM3A in LIHC using cBioPortal.

4. Discussion

Cancer is described by poor prognosis and poor medical consequences worldwide ^[30]. Liver cancer is the sixth most common cancer, with thousands of deaths worldwide. While liver hepatocellular carcinoma (LIHC) accounts for 80–90% of cases, LIHC is diagnosed at a higher stage and recurrence rate, leading to high mortalities ^[40,41]. Therefore, it is crucial to diagnose new diagnostic, therapeutic and prognostic biomarkers. The study performed a bioinformatics analysis of FAM3A in LIHC to evaluate its potential as an efficient biomarker. FAM3A inhibits hepatic gluconeogenesis, protects cognitive functions, have a role in vascular pathology and metabolic processes ^[42–44].

First, the study analyzes the expression of FAM3A in LIHC and noted that FAM3A was significantly up-regulated in LIHC. P -value < 0.05 indicated significant overexpression than normal samples. The study examined the expression of FAM3A characterized by various variables such as LIHC patient's age, gender, race and pathological stages. The study evaluated significant up-regulation of FAM3A expression based on these variables. These results show that FAM3A has a role in the progression of LIHC. Following this, the study assessed promoter methylation levels of FAM3A in LIHC. The study examined hypomethylated FAM3A methylation levels in LIHC. This demonstrated up-regulation of expression as methylation and expression are reversely associated.

Moreover, to further evaluate the role of FAM3A methylation in LIHC, the study conducted analysis segmented on multiple variables like LIHC patient's age, gender, race and pathological stages. The study observed variations in methylation levels in LIHC pathological stages. FAM3A was hypomethylated in stages 1–3. However, in stage 4, FAM3A was hypermethylated. Subsequently, analysis based on gender revealed that FAM3A is significantly hypomethylated in male samples and hypermethylated in female samples. However, assessment based on age and race demonstrated hypomethylation in FAM3A promoter methylation level. These variations illustrated that FAM3A methylation level regulates FAM3A expression and results in the progression and development of LIHC.

Furthermore, the study investigated the impact of FAM3A expression on the OS of LIHC patients utilizing a KM plotter. The study identified that overexpressed FAM3A have poor OS and lower expressed FAM3A have better OS in LIHC patients. This highlights that FAM3A has a role in the progression of LIHC and high mortalities. After that, to validate the results of FAM3A expression analysis and survival analysis, the study employed GEPIA2. The outcomes from the expression and survival analysis conducted with GEPIA2 accord with earlier results. This illuminates the role of FAM3A in the development and progression of LIHC.

Simultaneously, the study analyzes the genetic alteration of FAM3A in LIHC using cBioPortal. The study evaluated 2.5% of mutation of FAM3A as amplification, deep deletion and missense mutation. This revealed the

minute role of FAM3A mutation in LIHC yet brings deep insight. Moreover, the study analyzed the association of FAM3A expression level with purity and CD8⁺ T immune cells in LIHC. This revealed that FAM3A is positively associated with purity and negatively associated with CD8⁺ T immune cells. This indicates that FAM3A has a role in tumor growth by altering immune cells in the microenvironment. Overall, these highlight the significance of FAM3A as a therapeutic, diagnostic and prognostic biomarker in LIHC.

5. Conclusion

In conclusion, the study performed bioinformatics analysis of FAM3A expression, methylation level, genetic alteration and prognostic rate in LIHC. It can be observed that up-regulated FAM3A contributes to the development and progression of LIHC. In addition, these findings suggested that FAM3A is a unique prognostic, therapeutic and diagnostic biomarker in LIHC. Nevertheless, extensive research is essential before practical implementation.

Disclosure statement

The author declares no conflict of interest.

References

- [1] Upadhyay A, 2021, Cancer: An Unknown Territory, Rethinking Before Going Ahead. *Genes & Diseases*, 8(5): 655–661.
- [2] Bray F, Laversanne M, Sung H, et al., 2024, Global Cancer Statistics 2022: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*, 74(3): 229–263.
- [3] Ma X, Yu H, 2006, Cancer Issue: Global Burden of Cancer. *The Yale Journal of Biology and Medicine*, 79(3–4): 85.
- [4] Kaur H, Bhalla S, Raghava GPS, 2019, Classification of Early and Late Stage Liver Hepatocellular Carcinoma Patients from Their Genomics and Epigenomics Profiles. *PLOS ONE*, 14(9).
- [5] Bray F, Ferlay J, Soerjomataram I, et al., 2018, Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*, 68(6): 394–424.
- [6] Herceg Z, Paliwal A, 2011, Epigenetic Mechanisms in Hepatocellular Carcinoma: How Environmental Factors Influence the Epigenome. *Mutation Research*, 727(3): 55–61.
- [7] Yang JD, Roberts LR, 2010, Hepatocellular Carcinoma: A Global View. *Nature Reviews Gastroenterology & Hepatology*, 7(8): 448–458.
- [8] Siegel RL, Miller KD, Jemal A, 2019, Cancer Statistics. *CA: A Cancer Journal for Clinicians*, 69(1): 7–34.
- [9] Wang S, Liu Y, Feng Y, et al., 2019, A Review on Curability of Cancers: More Efforts for Novel Therapeutic Options Are Needed. *Cancers*, 11(11): 1782.
- [10] Chen L, Yuan L, Wang Y, et al., 2017, Co-expression Network Analysis Identified FCER1G in Association with Progression and Prognosis in Human Clear Cell Renal Cell Carcinoma. *International Journal of Biological Sciences*, 13(11): 1361.
- [11] Sun J, Zhang Z, Cai J, et al., 2024, Identification of Hub Genes in Liver Hepatocellular Carcinoma Based on Weighted Gene Co-expression Network Analysis. *Biochemical Genetics*.
- [12] Poon D, Anderson BO, Chen LT, et al., 2009, Management of Hepatocellular Carcinoma in Asia: Consensus Statement from the Asian Oncology Summit 2009. *The Lancet Oncology*, 10(11): 1111–1118.
- [13] Balogh J, Victor III D, Asham EH, et al., 2016, Hepatocellular Carcinoma: A Review. *Journal of Hepatocellular Carcinoma*, 2016(3): 41–53.

- [14] Bruix J, da Fonseca LG, Reig M, 2019, Insights into the Success and Failure of Systemic Therapy for Hepatocellular Carcinoma. *Nature Reviews Gastroenterology & Hepatology*, 16(10): 617–630.
- [15] Yang W, Wang J, Chen Z, et al., 2017, NFE2 Induces miR-423-5p to Promote Gluconeogenesis and Hyperglycemia by Repressing the Hepatic FAM3A-ATP-Akt Pathway. *Diabetes*, 66(7): 1819–1832.
- [16] Wang C, Chi Y, Li J, et al., 2014, FAM3A Activates PI3K p110 α /Akt Signaling to Ameliorate Hepatic Gluconeogenesis and Lipogenesis. *Hepatology*, 59(5): 1779–1790.
- [17] Zhang X, Yang W, Wang J, et al., 2018, FAM3 Gene Family: A Promising Therapeutic Target for NAFLD and Type 2 Diabetes. *Metabolism*, 81: 71–82.
- [18] Jia S, Chen Z, Li J, et al., 2014, FAM3A Promotes Vascular Smooth Muscle Cell Proliferation and Migration and Exacerbates Neointima Formation in Rat Artery After Balloon Injury. *Journal of Molecular and Cellular Cardiology*, 74: 173–182.
- [19] Wang C, Chi Y, Li J, et al., 2014, FAM3A Activates PI3K p110 α /Akt Signaling to Ameliorate Hepatic Gluconeogenesis and Lipogenesis. *Hepatology*, 59(5): 1779–90.
- [20] Sala D, Cunningham TJ, Stec MJ, et al., 2019, The Stat3-Fam3a Axis Promotes Muscle Stem Cell Myogenic Lineage Progression by Inducing Mitochondrial Respiration. *Nature Communications*, 10(1): 1796.
- [21] Yang W, Wang J, Chen Z, et al., 2017, NFE2 Induces miR-423-5p to Promote Gluconeogenesis and Hyperglycemia by Repressing the Hepatic FAM3A-ATP-Akt Pathway. *Diabetes*, 66(7): 1819–32.
- [22] Yang J, Zhang LJ, Wang F, et al., 2019, Molecular Imaging of Diabetes and Diabetic Complications: Beyond Pancreatic β -cell Targeting. *Advanced Drug Delivery Reviews*, 139: 32–50.
- [23] Jin ES, Beddow SA, Malloy CR, et al., 2013, Hepatic Glucose Production Pathways After Three Days of a High-Fat Diet. *Metabolism*, 62(1): 152–162.
- [24] Chi Y, Li J, Li N, et al., 2017, FAM3A Enhances Adipogenesis of 3T3-L1 Preadipocytes via Activation of ATP-P2 Receptor-Akt Signaling Pathway. *Oncotarget*, 8(28): 45862.
- [25] Song Q, Gou WL, Zhang R, et al., 2016, FAM3A Attenuates ER Stress-Induced Mitochondrial Dysfunction and Apoptosis via CHOP-Wnt Pathway. *Neurochemistry International*, 94: 82–89.
- [26] Li J, Yan H, Xiang R, et al., 2022, ATP Secretion and Metabolism in Regulating Pancreatic Beta Cell Functions and Hepatic Glycolipid Metabolism. *Frontiers in Physiology*, 13.
- [27] Dong QT, Ma DD, Gong Q, et al., 2023, FAM3 Family Genes Are Associated with Prognostic Value of Human Cancer: A Pan-Cancer Analysis. *Sci Rep*, 13(1): 15144.
- [28] Chandrashekar DS, Bashel B, Balasubramanya SAH, et al., 2017, UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. *Neoplasia*, 19(8): 649–658.
- [29] Chen C, Wang X, Huang S, et al., 2017, Prognostic Roles of Notch Receptor mRNA Expression in Human Ovarian Cancer. *Oncotarget*, 8(20): 32731.
- [30] Tang Z, Li C, Kang B, et al., 2017, GEPIA: A Web Server for Cancer and Normal Gene Expression Profiling and Interactive Analyses. *Nucleic Acids Research*, 45(W1): W102.
- [31] Gao J, Aksoy BA, Dogrusoz U, et al., 2013, Integrative Analysis of Complex Cancer Genomics and Clinical Profiles Using the cBioPortal. *Science Signal*, 6(269): p11.
- [32] Li T, Fu J, Zeng Z, et al., 2020, TIMER2.0 for Analysis of Tumor-Infiltrating Immune Cells. *Nucleic Acids Research*, 48(W1): W514.
- [33] Zhou WH, Li YF, Al-Aroomi MA, et al., 2022, Overexpression of Fibronectin 1 Promotes Cancer Progression and Associated with M2 Macrophages Polarization in Head and Neck Squamous Cell Carcinoma Patients. *International Journal of General Medicine*, 15: 5027–5042.
- [34] Wan S, He Y, Zhang B, et al., 2022, Overexpression of CDCA8 Predicts Poor Prognosis and Promotes Tumor Cell

Growth in Prostate Cancer. *Frontiers in Oncology*, 12.

- [35] Moarii M, Boeva V, Vert JP, et al., 2015, Changes in Correlation Between Promoter Methylation and Gene Expression in Cancer. *BMC Genomics*, 16(1): 873.
- [36] Wang K, Dai R, Xia Y, et al., 2022, Spatiotemporal Specificity of Correlated DNA Methylation and Gene Expression Pairs Across Different Human Tissues and Stages of Brain Development. *Epigenetics*, 17(10): 1110–1127.
- [37] Pandya PH, Murray ME, Pollok KE, et al., 2016, The Immune System in Cancer Pathogenesis: Potential Therapeutic Approaches. *Journal of Immunology Research*, 2016(1): 4273943.
- [38] Aran D, Sirota M, Butte AJ, 2015, Systematic Pan-Cancer Analysis of Tumour Purity. *Nature Communications*, 6(1): 8971.
- [39] Sarkar S, Horn G, Moulton K, et al., 2013, Cancer Development, Progression, and Therapy: An Epigenetic Overview. *International Journal of Molecular Sciences*, 14(10): 21087–21113.
- [40] Su C, Yang JC, Rong Z, et al., 2023, Identification of CCDC115 as an Adverse Prognostic Biomarker in Liver Cancer Based on Bioinformatics and Experimental Analyses. *Heliyon*, 9(9): e19233.
- [41] Cancer Genome Atlas Research Network, 2017, Comprehensive and Integrative Genomic Characterization of Hepatocellular Carcinoma. *Cell*, 169(7): 1327–1341.
- [42] Xu W, Liang M, Zhang Y, et al., 2019, Endothelial FAM3A Positively Regulates Post-Ischaemic Angiogenesis. *EBioMedicine*, 43: 32–42.
- [43] Song Q, Gao Q, Chen T, et al., 2021, FAM3A Ameliorates Brain Impairment Induced by Hypoxia–Ischemia in Neonatal Rat. *Cellular and Molecular Neurobiology*, 43(1): 1–14.
- [44] Xiang R, Chen J, Li S, et al., 2020, VSMC-Specific Deletion of FAM3A Attenuated Ang II-Promoted Hypertension and Cardiovascular Hypertrophy. *Circulation Research*, 126(12):1746–59.

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Clinical Effect of Tislelizumab Combined with Chemotherapy in the Treatment of Stage IIIb–IV Non-Small Cell Lung Cancer

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Abstract: *Objective:* To analyze the therapeutic effect of tislelizumab combined with chemotherapy in patients with stage IIIb–IV non-small cell lung cancer (NSCLC). *Methods:* A total of 50 patients with stage IIIb–IV NSCLC admitted between January 2022 and January 2024 were randomly divided into two groups using a random number table. The observation group included 25 cases treated with tislelizumab combined with chemotherapy, while the reference group included 25 cases treated with conventional chemotherapy. The clinical control rate, adverse reaction rate, tumor markers, immune function indicators, and quality of life scores were compared between the two groups. *Results:* The observation group had a higher clinical control rate and a lower adverse reaction rate compared to the reference group ($P < 0.05$). Before treatment, there were no significant differences in tumor markers, immune function indicators, and quality of life scores between the two groups ($P > 0.05$). Three months after treatment, the tumor marker levels in the observation group were lower than those in the reference group. Except for $CD8^+$, all immune function indicators in the observation group were higher than those in the reference group, and the quality-of-life scores in the observation group were higher than those in the reference group ($P < 0.05$). *Conclusion:* Implementing tislelizumab combined with chemotherapy in patients with stage IIIb–IV NSCLC can improve the clinical control rate, reduce the adverse reaction rate, lower tumor marker levels, protect immune function, and improve quality of life.

Keywords: Tislelizumab; Chemotherapy; Stage IIIb–IV; Non-small cell lung cancer

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1. Introduction

Non-small cell lung cancer (NSCLC) is a common type of lung cancer with a high incidence rate. The disease often lacks typical symptoms in the early stages, leading to a high rate of missed or incorrect diagnoses. As the disease progresses, symptoms such as significant coughing, hemoptysis, and fever become evident, often indicating the disease has reached stages IIIb–IV, where treatment becomes more challenging^[1,2]. Chemotherapy, primarily using the TP regimen, is a common treatment for patients with stage IIIb–IV NSCLC. It can inhibit tumor growth and prolong survival, but prolonged chemotherapy can lead to adverse reactions and

suboptimal clinical control rates. Therefore, combining chemotherapy with immunotherapy, such as the use of new drugs like tislelizumab, may reduce the cytotoxic effects of T lymphocytes, accelerate tumor cell apoptosis, effectively combat cancer, and improve disease prognosis^[3]. Based on this, this study selected 50 patients with stage IIIb–IV NSCLC to evaluate the therapeutic efficacy of tislelizumab combined with chemotherapy.

2. Materials and methods

2.1. General information

The study period was from January 2022 to January 2024, involving 50 patients with stage IIIb–IV non-small cell lung cancer (NSCLC). After random division using a number table, the observation group included 25 cases (13 males and 12 females), aged 41 to 78 years, with an average age of 57.65 ± 2.19 years. The clinical staging included 11 cases in stage IIIb and 14 cases in stage IV. The reference group also included 25 cases (14 males and 11 females), aged 40 to 77 years, with an average age of 57.94 ± 2.28 years. The clinical staging included 12 cases in stage IIIb and 13 cases in stage IV. There was no significant difference in general data between the two groups ($P > 0.05$).

Inclusion criteria: Patients were diagnosed with NSCLC at stages IIIb–IV based on imaging, clinical symptoms, and signs; met the indications for chemotherapy and tislelizumab; had complete clinical data; and could fully cooperate with the study. Exclusion criteria: Patients with other malignant tumors, severe infections, allergies to the study drug, heart, liver, or kidney dysfunction, mental disorders, or those who withdrew midway through the study.

2.2. Methods

The conventional chemotherapy method for the reference group was the TP regimen: on days 1 and 8, 100 mg/m^2 of paclitaxel was administered intravenously; on day 1, carboplatin was administered at a dose of 300 mg/m^2 . One cycle lasted 21 days, with continuous administration until toxicity intolerance or disease progression. The observation group received the same chemotherapy regimen, combined with tislelizumab, administered intravenously at a dose of 200 mg every 21 days, for a total of two cycles.

2.3. Observation indicators

- (1) Adverse reaction rate: Including liver and kidney function damage, bone marrow suppression, neurotoxicity, and nausea/vomiting.
- (2) Tumor markers: Fasting venous blood (5 mL) was collected, serum was separated, and the levels of lung tumor antigen (LTA), cytokeratin 19 fragment antigen 21-1 (CYFRA21-1), neuron-specific enolase (NSE), and squamous cell carcinoma antigen (SCC) were assessed using radioimmunoassay.
- (3) Immune function indicators: Fasting venous blood (3 mL) was collected, and flow cytometry was used to assess CD3^+ , CD4^+ , CD8^+ , and $\text{CD4}^+/\text{CD8}^+$ indicators.
- (4) Quality of life score: The Functional Assessment of Cancer Therapy (FACT) questionnaire was used, including physical condition (28 points), emotional condition (24 points), social/family condition (28 points), and functional condition (28 points), with a total score of 108 points, scored positively.

2.4. Efficacy evaluation criteria

Complete response (CR) was defined as the complete disappearance of the tumor for more than 4 weeks; partial response (PR) was defined as a reduction in tumor size by at least 50%, sustained for more than 4 weeks; stable

disease (SD) was defined as a reduction in tumor size by 25%–50%, sustained for 4 weeks; and progressive disease (PD) was defined as no change in tumor size or an increase of more than 25%, or the discovery of new lesions. The clinical control rate was calculated as the sum of the percentages of CR, PR, and SD.

2.5. Statistical analysis

Data were processed using SPSS 28.0 software. Measurement values were compared using *t*-tests, and count values were compared using chi-squared tests. A *P*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Comparison of clinical control rates between the two groups

The clinical control rate in the observation group was higher than that in the reference group ($P < 0.05$), as shown in **Table 1**.

Table 1. Comparison of clinical control rates between the two groups [*n* (%)]

Group	CR	PR	SD	PD	Clinical control rate
Observation group (<i>n</i> = 25)	10 (40.0)	10 (40.0)	3 (12.0)	2 (8.0)	92.0 (23/25)
Reference group (<i>n</i> = 25)	5 (20.0)	8 (32.0)	4 (16.0)	8 (32.0)	68.0 (17/25)
χ^2	-	-	-	-	4.500
<i>P</i>	-	-	-	-	0.034

3.2. Comparison of adverse reaction rates between the two groups

The adverse reaction rate in the observation group was lower than that in the reference group ($P < 0.05$), as shown in **Table 2**.

Table 2. Comparison of adverse reaction rates between the two groups [*n* (%)]

Group	Hepatic and renal impairment	Bone marrow suppression	Neurotoxicity	Nausea and vomiting	Incidence
Observation group (<i>n</i> = 25)	0	0	0	1 (4.0)	1 (4.0)
Reference group (<i>n</i> = 25)	2 (8.0)	1 (4.0)	1 (4.0)	2 (8.0)	6 (24.0)
χ^2	-	-	-	-	4.153
<i>P</i>	-	-	-	-	0.042

3.3. Comparison of tumor marker levels between the two groups

Before treatment, there was no difference in tumor marker levels between the two groups ($P > 0.05$). Three months after treatment, the tumor marker levels in the observation group were lower than those in the reference group ($P < 0.05$), as shown in **Table 3**.

Table 3. Comparison of tumor marker levels between the two groups before and after treatment (mean \pm SD)

Group	LTA (U/L)		CYFRA21-1 (ng/mL)		NSE (ng/mL)		SCC (ng/mL)	
	Before	After	Before	After	Before	After	Before	After
Observation group ($n = 25$)	197.98 \pm 21.37	98.19 \pm 6.81	5.52 \pm 0.47	2.21 \pm 0.38	28.49 \pm 3.67	8.06 \pm 1.33	47.89 \pm 4.91	18.16 \pm 2.06
Reference group ($n = 25$)	198.02 \pm 20.84	112.08 \pm 6.97	5.54 \pm 0.49	3.68 \pm 0.42	28.51 \pm 3.65	14.09 \pm 1.38	47.86 \pm 4.86	28.17 \pm 2.35
<i>t</i>	0.007	7.127	0.147	12.977	0.019	15.731	0.022	16.016
<i>P</i>	0.995	< 0.001	0.884	< 0.001	0.985	< 0.001	0.983	< 0.001

3.4. Comparison of immune function indicators between the two groups

Before treatment, there was no difference in immune function indicators between the two groups ($P > 0.05$). Three months after treatment, except for CD8⁺, the immune function indicators in the observation group were higher than those in the reference group ($P < 0.05$), as shown in **Table 4**.

Table 4. Comparison of immune function indicators between the two groups before and after treatment (mean \pm SD)

Group	CD3+ (%)		CD4+ (%)		CD8+ (%)		CD4 ⁺ /CD8 ⁺	
	Before	After	Before	After	Before	After	Before	After
Observation group ($n = 25$)	41.83 \pm 4.95	60.03 \pm 6.91	28.81 \pm 2.59	40.81 \pm 4.26	29.69 \pm 3.42	20.42 \pm 3.11	0.99 \pm 0.17	2.04 \pm 0.32
Reference group ($n = 25$)	41.80 \pm 4.76	53.09 \pm 6.77	28.77 \pm 2.56	33.17 \pm 4.18	29.74 \pm 3.40	23.79 \pm 3.17	0.98 \pm 0.16	1.49 \pm 0.28
<i>t</i>	0.022	3.587	0.055	6.401	0.052	3.794	0.214	6.467
<i>P</i>	0.983	0.001	0.956	< 0.001	0.959	< 0.001	0.831	< 0.001

3.5. Comparison of quality-of-life scores between the two groups

Before treatment, there was no difference in the comparison of the quality-of-life scores of the two groups ($P > 0.05$). Three months after treatment, the quality-of-life scores in the observation group were higher than those in the reference group ($P < 0.05$), as shown in **Table 5**.

Table 5. Comparison of quality-of-life scores between the two groups before and after treatment (mean \pm SD; points)

Group	Physical status		Emotional status		Social and family status		Functional status	
	Before	After	Before	After	Before	After	Before	After
Observation group ($n = 25$)	16.75 \pm 2.19	23.95 \pm 2.71	15.99 \pm 2.27	20.19 \pm 2.57	17.91 \pm 2.43	22.37 \pm 2.61	18.39 \pm 2.08	23.94 \pm 2.27
Reference group ($n = 25$)	16.72 \pm 2.24	20.19 \pm 2.67	15.97 \pm 2.25	17.25 \pm 2.53	17.95 \pm 2.51	20.02 \pm 2.43	18.42 \pm 2.04	20.34 \pm 2.23
<i>t</i>	0.048	4.942	0.031	4.076	0.057	3.295	0.051	5.657
<i>P</i>	0.962	< 0.001	0.975	< 0.001	0.955	0.002	0.959	< 0.001

4. Discussion

Lung cancer typically affects the glands and bronchial mucosa and is one of the most common types of malignancies. Non-small cell lung cancer (NSCLC) accounts for approximately 80% of lung cancer cases and can be further divided into subtypes such as squamous cell carcinoma and adenocarcinoma. The typical symptoms include cough, chest pain, and hemoptysis ^[4]. Compared to small cell lung cancer, NSCLC has a longer metastasis timeline, with slower tumor cell growth. The early stages of the disease often lack specific symptoms, making early diagnosis challenging, and most cases are diagnosed at stages IIIb to IV. At this point, patients have often missed the optimal window for curative surgery and typically undergo chemotherapy and immunotherapy, with the aim of extending survival and improving quality of life ^[5,6].

The TP regimen is a foundational chemotherapy treatment for patients with this disease. Paclitaxel can bind to albumin receptors on the cell membrane, activating caveolin and thereby increasing the intracellular concentration of the drug in tumor cells, enhancing its anti-tumor mechanism ^[7]. Carboplatin binds to DNA, forming various complexes that inhibit cell division, thus exerting anti-tumor effects. However, the clinical control rates of these drugs are generally moderate, and they come with significant side effects that can increase patient discomfort, necessitating the combination with other therapeutic agents ^[8]. Tislelizumab is a common monoclonal antibody against programmed death protein-1 (PD-1), with strong binding affinity to the PD-1 receptor, thus showing a potent anti-tumor effect.

Tislelizumab is an immune checkpoint inhibitor that interferes with the PD-1 pathway, enhancing immune response capabilities and providing effective anti-tumor effects, thus improving treatment outcomes ^[9]. It also reduces neovascularization, preventing rapid tumor spread or growth. When combined with paclitaxel and carboplatin, it can inhibit tumor cell division, effectively kill tumor cells, and disrupt the normal structure of tumor DNA, slowing tumor growth. The combination of multiple drugs can enhance efficacy and reduce toxicity, targeting multiple mechanisms of action, which reduces the side effects of chemotherapy drugs. Consequently, patients have higher clinical control rates and lower rates of adverse reactions ^[10]. In this study, the clinical control rate in the observation group was higher than in the reference group, and the adverse reaction rate was lower ($P < 0.05$).

Tislelizumab can block multiple signaling pathways in the PD-1/programmed death-ligand 1 (PD-L1) axis, inhibiting the mediation of T lymphocytes by these pathways and enhancing anti-tumor immune effects ^[11]. Additionally, it can regulate immune function, activate the anti-tumor immune system, reduce immune escape, and reactivate immune response mechanisms, thereby lowering tumor marker levels. In this study, three months after treatment, the tumor marker levels in the observation group were lower than those in the reference group ($P < 0.05$).

Tislelizumab's activation of the PD-1 and PD-L1 signaling pathways can reduce the sustained proliferation of T cells and inhibit the reversal of the tumor immune microenvironment associated with these pathways, enhancing T cell biological functions and improving the body's immunity ^[12]. In this study, except for CD8⁺, the immune function indicators in the observation group were higher than those in the reference group ($P < 0.05$). Based on the aforementioned treatment mechanisms, this drug can achieve better therapeutic effects, thereby reducing patient discomfort and improving quality of life.

In summary, the combination of tislelizumab and chemotherapy for patients with stage IIIb to IV NSCLC can enhance therapeutic efficacy, improve tumor marker and immune function indicator levels, reduce adverse reactions, and enhance patients' quality of life.

Disclosure statement

The author declares no conflict of interest.

References

- [1] Li X, Zhang Y, Yao S, et al., 2024, Clinical Study on the Treatment of Stage IIIb–IV Non-Small Cell Lung Cancer with Tislelizumab Combined with Chemotherapy. *Chinese Journal of Clinical Pharmacology*, 40(3): 335–339.
- [2] Liu S, Liu Q, Pang Q, et al., 2024, The Effect of Tislelizumab Combined with Chemotherapy in the Treatment of Stage IIIb–IV Non-Small Cell Lung Cancer. *Journal of Practical Hospital Clinical*, 21(3): 112–116.
- [3] Chen X, Liu T, Yang M, et al., 2022, Efficacy of Different Immune Checkpoint Inhibitors Combined with Chemotherapy in the Treatment of Non-Small Cell Lung Cancer and Their Impact on Tumor Marker Levels. *Chinese Clinical and Rehabilitation Oncology*, 29(7): 774–778.
- [4] Zhao H, Guo C, Zhao W, 2024, Clinical Study of Tislelizumab Combined with Chemotherapy as First-Line Treatment for Advanced Non-Small Cell Lung Cancer. *System Medicine*, 9(7): 175–178.
- [5] Lu S, Yu X, Hu Y, et al., 2023, Characteristics of Tumor Response to First-Line Treatment with Tislelizumab Combined with Chemotherapy in Locally Advanced or Metastatic Non-Squamous Non-Small Cell Lung Cancer. *Chinese Journal of Oncology*, 45(4): 358–367.
- [6] Chen F, Liang H, Cheng G, et al., 2023, Study on the Short-Term Efficacy and Safety of Tislelizumab Combined with Platinum-Based Chemotherapy for Advanced Non-Small Cell Lung Cancer. *Journal of Clinical and Experimental Medicine*, 22(6): 583–587.
- [7] Fu H, Nan Y, Li C, et al., 2023, The Effect of Albumin-Bound Paclitaxel and Carboplatin Combined with Tislelizumab in the Treatment of Advanced Non-Small Cell Lung Cancer. *Northwest Pharmaceutical Journal*, 38(4): 164–168.
- [8] Zhang J, Lu Z, Zhou X, 2022, Efficacy and Impact on Immune Function of Tislelizumab Combined with GP Chemotherapy Regimen in the Treatment of Advanced NSCLC. *Chinese Journal of Health Care Medicine*, 24(3): 252–254.
- [9] Cheng Y, Liu L, 2023, Predictive Value of NLR and MLR in the Efficacy of Tislelizumab Combined with TP Regimen for NSCLC. *Anhui Medical Journal*, 44(12): 1472–1477.
- [10] Chen Z, 2023, Clinical Study of Tislelizumab Combined with Gemcitabine and Cisplatin in the Treatment of Advanced Lung Squamous Carcinoma. *Chinese Journal of Clinical Pharmacology*, 39(16): 2306–2310.
- [11] Ren G, Wu X, Song X, et al., 2023, Analysis of Diagnosis and Treatment Strategies and Pharmaceutical Care for Tislelizumab-Induced Immune-Related Pneumonia. *Chinese Journal of Hospital Pharmacy*, 43(11): 1291–1295.
- [12] Wang H, Liu Y, Li A, et al., 2024, The Effects of Tislelizumab Injection Combined with Albumin-Bound Paclitaxel and Carboplatin on Intestinal Flora, SII, and PNI in Patients with Advanced NSCLC. *Progress in Modern Biomedicine*, 24(2): 338–342.

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Etiology of Pancytopenia in Tabriz Shahid Ghazi Hospital: A Cross-sectional Study in Iran

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Abstract: *Objective:* Pancytopenia is characterized by a reduction in all three types of blood cells: erythrocytes, leukocytes, and platelets. Pancytopenia is caused by a wide range of diseases, leading to diagnostic conundrums. These causes can range from drug reactions to life-threatening diseases such as aplastic anemia and leukemia. This study aims to investigate the causes of pancytopenia, specifically focusing on age and gender differences among patients. *Methods:* This cross-sectional study includes patients of all ages diagnosed with pancytopenia, as indicated by a CBC/H1 showing a WBC count less than 4,000/ μ L, platelet count less than 150,000/ μ L, and hemoglobin levels below 12 g/dL in women and less than 13 g/dL in men. The study only included patients with pancytopenia who underwent bone marrow examination and were not subjected to chemotherapy or radiation therapy. *Results:* A total of 133 patients with pancytopenia were included in the study. The average age was 47.35 ± 17.62 years old, with 66% of the participants being male and 34% being female. Acute leukemia, specifically acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL), was identified as the primary cause of pancytopenia, accounting for 31.5% of cases. Megaloblastic anemia was the second most common cause, accounting for 30% of cases, followed by aplastic anemia at 7.5%. *Conclusion:* Pancytopenia, a condition marked by the decrease in both erythrocytes and leukocytes as well as thrombocytes, can arise from a myriad of causes. The main findings of this study revealed that megaloblastic anemia, acute myeloid leukemia (AML), and acute lymphoid leukemia (ALL) were the most common causes. Significantly, a considerable proportion of cases of pancytopenia can be attributed to acute leukemia. Hence, expeditious and accurate diagnosis is imperative and has the potential to save lives in such cases.

Keywords: Pancytopenia; Leukemia; Anemia; Bone marrow; Blood cells

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1. Introduction

Pancytopenia poses a diagnostic challenge in the field of clinical hematology. Pancytopenia refers to a decline in the number of all three types of blood cells, namely erythrocytes, leukocytes, and platelets. This condition

can have life-threatening consequences due to the development of anemia, increased susceptibility to infections, and a tendency to bleed excessively.

Pancytopenia can result from one or more of the following mechanisms ^[1]:

- (1) Bone marrow infiltration or replacement: This can occur in various disorders such as hematologic malignancies (such as leukemia, lymphoma, multiple myeloma, and myelodysplastic syndrome), metastatic cancer, myelofibrosis, and infectious diseases (such as miliary tuberculosis and fungal infections).
- (2) Bone marrow aplasia: The causes of marrow aplasia include nutritional disorders such as deficiencies of vitamin B12 or folate, aplastic anemia, infectious diseases like HIV, viral hepatitis, parvovirus B19, immune destruction, and medication.
- (3) Excessive destruction of blood cells can occur in conditions such as disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, and ineffective hematopoiesis, which includes myelodysplastic syndrome and megaloblastic disorders.
- (4) Excessive sequestration of blood cells may be caused by hypersplenism, which can result from liver cirrhosis, storage diseases, lymphoma, or autoimmune disorders.

The level of circulating blood cells is determined by a delicate equilibrium between blood cell production, distribution in other organs, and continuous cell destruction, such as white blood cells fighting infections, platelets used in blood clots, and cellular senescence ^[2-5].

The frequency of various disorders leading to pancytopenia is influenced by several factors, including geographical distribution, which impacts factors such as nutritional status, climate, and the prevalence of infections, as well as demographic factors such as age patterns and genetic variation. Pancytopenia can vary from a benign drug reaction to a severe medical condition, such as aplastic anemia and acute leukemia.

Despite extensive research on the causes of pancytopenia, there are still limitations in fully comprehending its range of causes. Different methods are used to diagnose the cause of pancytopenia in patients. Neumann and Bizzozero's research established the correlation between blood and bone marrow ^[6,7]. Neumann observed in 1868 that bone marrow played a crucial role in the production of red blood cells ^[6]. The cause of pancytopenia can often be determined from the examination of a peripheral blood smear (PBS) ^[8-10]. Key findings in this test include:

- (1) Alterations in the morphology and size of red blood cells;
- (2) The presence of immature and atypical leukocytes;
- (3) Platelet size variations, as evidenced by megaloblastic changes and blast cells.

Following this, bone marrow biopsy becomes crucial in further understanding the etiology of pancytopenia. While some experts consider marrow examination important for accurate diagnosis, there is ongoing debate about its necessity in all cases of pancytopenia ^[11,12]. Finally, based on bone marrow aspiration results, the causes of pancytopenia are generally categorized into two groups, namely hypocellular and cellular ^[13-16].

Given these factors and the paucity of research on pancytopenia in the area, the study has chosen to investigate the etiology of the condition at Tabriz Shaheed Ghazi Hospital, a referral center for hematology and oncology in northwest Iran.

2. Materials and methods

This cross-sectional study was conducted at Tabriz Shahid Ghazi Hospital, a referral center for patients from Iran's northwest area. The study aimed to discover the causes of pancytopenia and was carried out from December 2015 to December 2016, spanning a one-year period. All these patients suffering from pancytopenia were referred to Tabriz Shahid Ghazi Hospital for the intent of hospitalization and took part in the study. A comprehensive history, which included information about drug consumption, was recorded for all cases,

regardless of age and gender. All patients with pancytopenia underwent a physical examination, peripheral blood smear (PBS), and complete blood count (CBC/H1). Additional inquiries were undertaken to assess the underlying reason for pancytopenia. The study included patients who met specific criteria, which consisted of having a white blood cell count below 4,000/ μ L, a platelet count below 150,000/ μ L and hemoglobin levels below 12 g/dL in women and below 13 g/dL in men ^[1,17,18]. All these patients underwent bone marrow aspiration. Each patient was provided with a detailed explanation of the procedure in their own language. Before conducting bone marrow aspiration, a peripheral blood smear was prepared. The study excluded patients with pancytopenia caused by prior chemotherapy or radiotherapy and those who did not undergo bone marrow examination. The identification of these patients occurred during routine visits to Shahid Ghazi Hospital. The team analyzed the patients' information and final diagnosis after filling out a data-collecting form.

The data were examined using descriptive statistics (mean, standard deviation, and variance analysis) in SPSS version 16. Afterward, frequency and diagnostic-related traits were analyzed. In addition, descriptive statistics, such as percentages, frequencies, and mean \pm standard deviation (SD) were used to display the data.

3. Results

The study included 133 individuals, 87 (66%) males and 46 (34%) females (**Figure 1**). The mean age of the patients was 47.35 ± 17.62 years old, ranging from 16 to 80 years old (**Figure 2**).

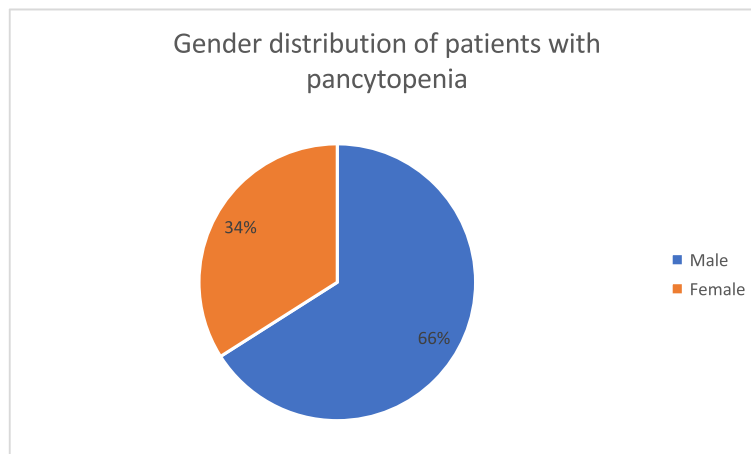


Figure 1. Gender distribution of cases of pancytopenia in the present study.

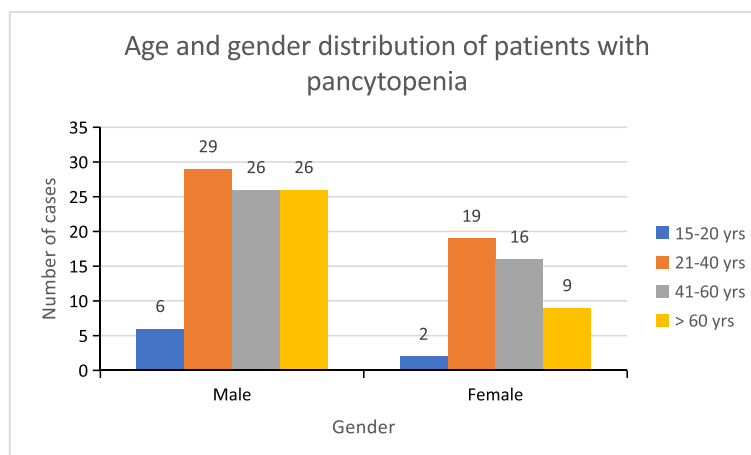


Figure 2. Age and gender distribution of patients with pancytopenia in the present study.

Table 1 demonstrates that in this study, the three most common factors contributing to pancytopenia were megaloblastic anemia, which accounted for 30% of cases, acute myelogenous leukemia (AML) at 21%, and acute lymphocytic leukemia (ALL) at 10.5%. The study indicates that the primary cause of pancytopenia was acute leukemia (AML and ALL), which affected a total of 42 patients, consisting of 26 males and 16 females. In addition, hairy cell leukemia (HCL) was diagnosed in two patients. In total, 44 individuals were diagnosed with leukemia, accounting for 33% of the cases of pancytopenia in this study. Among these cases, there were 28 males and 16 females.

Table 1. Etiologies of pancytopenia in the present study

Etiology	Number of cases	Percent (%)
Megaloblastic anemia (MA)	40	30
Acute myelogenous leukemia (AML)	28	21
Acute lymphocytic leukemia (ALL)	14	10.5
Aplastic anemia (AA)	10	7.5
Myelodysplastic syndrome (MDS)	8	6.1
Cirrhosis	6	4.5
Peripheral pancytopenia with normal bone marrow (non-cirrhosis)	10	7.5
Multiple myeloma (MM)	4	3
Sepsis	3	2.3
Viral infectious	3	2.3
Hairy cell leukemia (HCL)	2	1.5
Pharmaceutical causes (non-chemotherapy)	2	1.5
Myelofibrosis	1	0.75
Systemic lupus erythematosus (SLE)	1	0.75
Histiocytosis	1	0.75

Figures 3–6 demonstrate that the predominant cause of pancytopenia varied across different age cohorts in this study. ALL was the primary cause in the age group 15–20 years old. MA was found to be the predominant cause of anemia in the age groups of 21–40, 41–60, and over 60 years old.

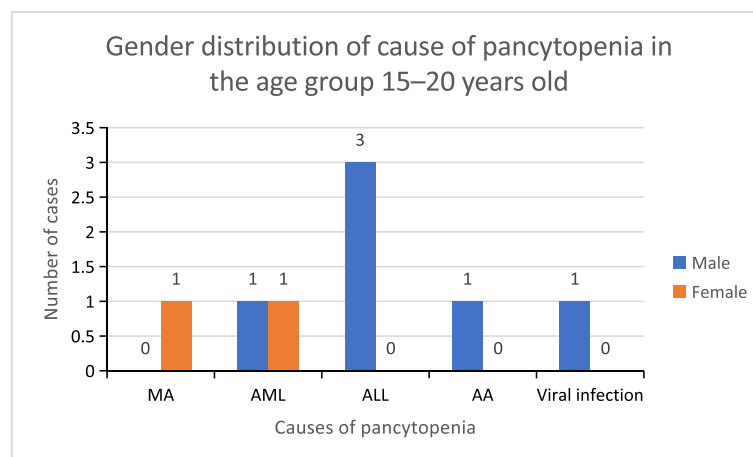


Figure 3. Gender distribution of cause of pancytopenia in the age group 15–20 years old. Abbreviation: MA, megaloblastic anemia; AML, acute myelogenous leukemia; ALL, acute lymphoid leukemia; AA, aplastic anemia

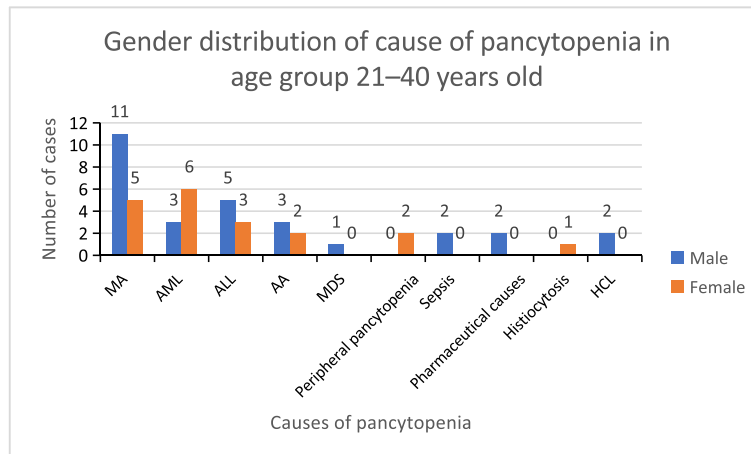


Figure 4. Gender distribution of cause of pancytopenia in the age group 21–40 years old. Abbreviation: MA, megaloblastic anemia; AML, acute myelogenous leukemia; ALL, acute lymphoid leukemia; AA, aplastic anemia; MDS, myelodysplastic syndrome; HCL, hairy cell leukemia

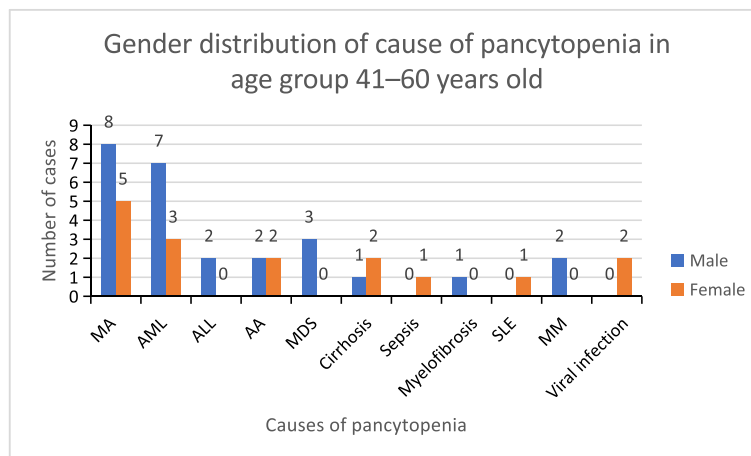


Figure 5. Distribution of causes of pancytopenia by gender in the age range of 41 to 60. Abbreviation: MA, megaloblastic anemia; AML, acute myelogenous leukemia; ALL, acute lymphoid leukemia; AA, aplastic anemia; MDS, myelodysplastic syndrome; SLE, systemic lupus erythematosus; MM, multiple myeloma

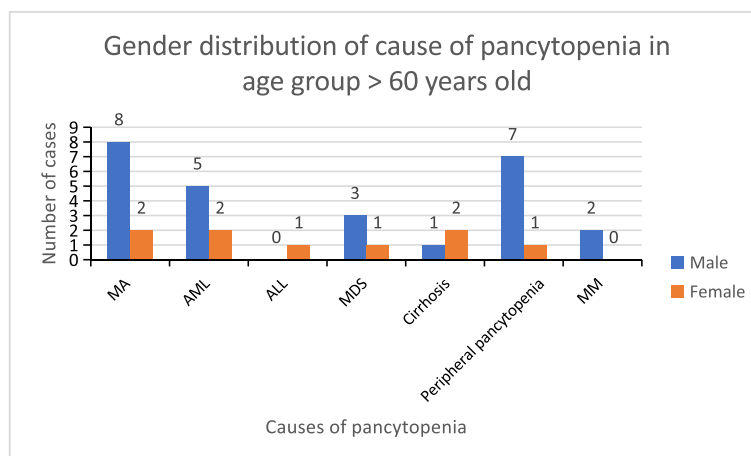


Figure 6. Distribution of causes of pancytopenia by gender in the age range of over 60. Abbreviation: MA, megaloblastic anemia; AML, acute myelogenous leukemia; ALL, acute lymphoid leukemia; MDS, myelodysplastic syndrome; MM, multiple myeloma

There were 56 cases of hematopoietic neoplasms, which made up 42% of all occurrences of pancytopenia. The distribution of these cases is as follows:

- (1) Acute leukemia (AML and ALL) accounted for 31.5% of cases.
- (2) Myelodysplastic syndrome (MDS) accounted for 6% of cases.
- (3) Multiple myeloma (MM) accounted for 3% of cases.
- (4) Hairy cell leukemia (HCL) accounted for 1.5% of cases.

Below is a list of the important hematological parameters that were examined in this study.

(1) Hemoglobin: The majority of patients showed hemoglobin levels ranging from 6 to 9 grams per deciliter (g/dL). Two cases revealed the lowest hemoglobin level of 3.5 g/dL. One of these instances was observed in a patient diagnosed with megaloblastic anemia, and the other was observed in a patient diagnosed with acute myeloid leukemia (AML), as described in **Table 2** and depicted in **Figure 7**.

Table 2. Values of hemoglobin in cases of pancytopenia in the present study

Hemoglobin (g/dL)	Number of cases	Percentage (%)
0–3	0	0
3–6	18	13.6
6–9	82	61.6
9–12	33	24.8

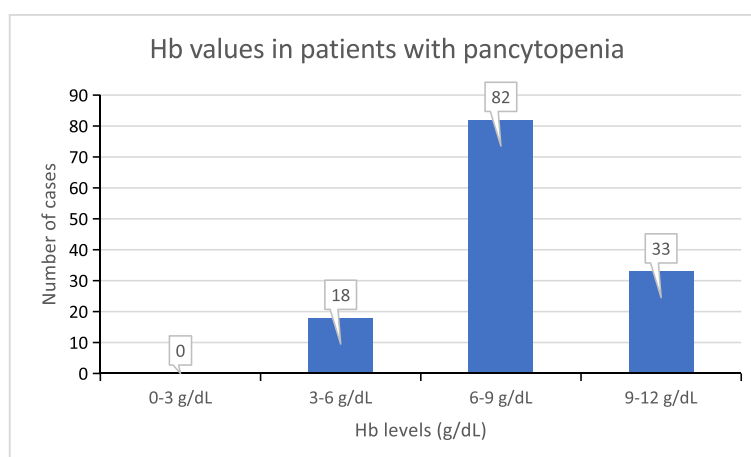


Figure 7. Values of hemoglobin in cases of pancytopenia in the present study

(2) White blood cells (WBC): The majority of patients showed a white blood cell count within the range of 1,000–2,000 cells/ μ L. The case of drug-induced pancytopenia (non-chemotherapy) displayed the lowest count of 370 cells/ μ L, as shown in **Table 3** and **Figure 8**.

Table 3. Values of WBC count in cases of pancytopenia in the present study

WBC (cells/ μ L)	Number of cases	Percentage (%)
0–1,000	4	3
1,000–2,000	55	41.5
2,000–3,000	46	34.5
3,000–4,000	28	21

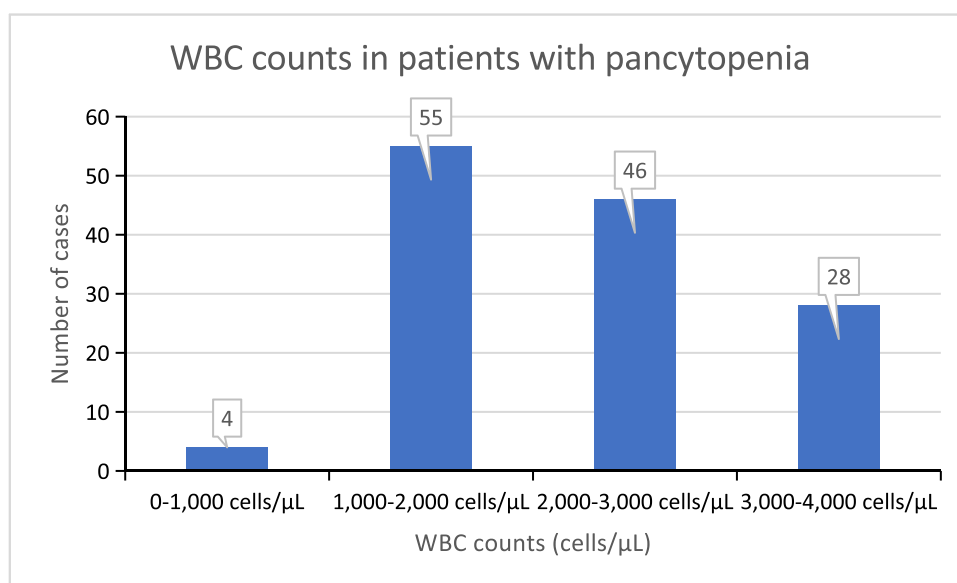


Figure 8. Values of WBC count in cases of pancytopenia in the present study

(3) Platelet: The majority of patients had a platelet count ranging from 50,000 to 100,000 per microliter. Two instances were seen where the platelet count reached a minimum of 6,000/μL. One case was associated with acute lymphoblastic leukemia (ALL), whereas the other case had peripheral pancytopenia with normal bone marrow, as shown in **Table 4** and **Figure 9**.

Table 4. Values of platelet count in cases of pancytopenia in the present study

Platelet count (/μL)	Number of cases	Percentage (%)
0–50,000	43	32.3
50,000–100,000	73	54.9
100,000–150,000	17	12.8

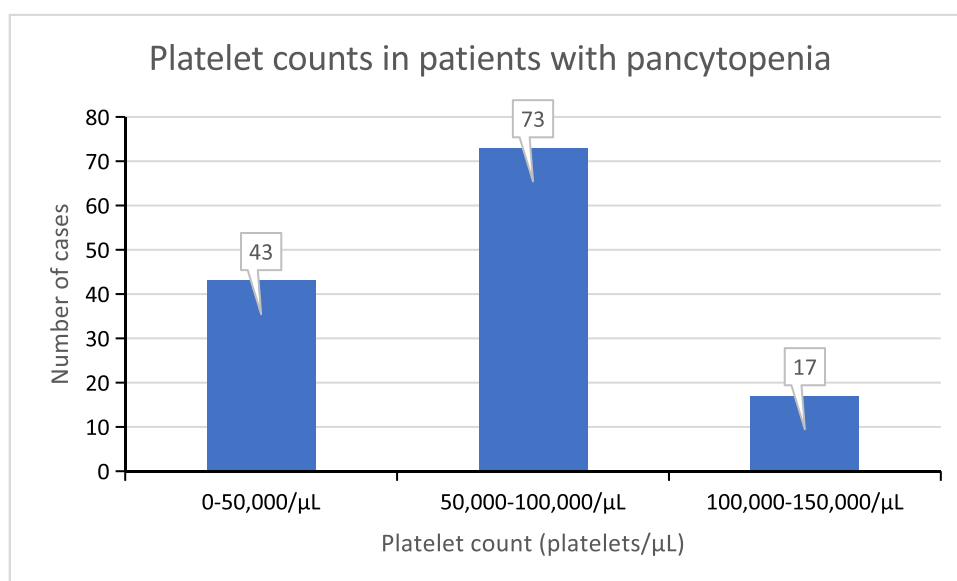


Figure 9. Values of platelet count in cases of pancytopenia in the present study

The hematological parameters in the most frequent causes of pancytopenia showed that the lowest hemoglobin value (3.5 g/dL) was observed in two cases: MA and AML. The lowest average white blood cell (WBC) count of 1,834/ μ L was seen in patients with AA, while the lowest average platelet count of 8,000/ μ L was found in patients with ALL (Table 5).

Table 5. Hematological parameters in more common causes of pancytopenia

Etiology	Hemoglobin (g/dL)			White blood cells (/ μ L)			Platelet (/ μ L)		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
MA	3.5	11.6	7.5	15,000	149,000	83,108	870	3,890	2,303
AA	7	10.9	8.4	8,000	110,000	44,800	970	3,000	1,834
Acute leukemia	3.5	11.4	7.83	10,000	149,000	55,021	970	3,920	2,020
AML	3.5	11.4	7.85	10,000	140,000	61,509	970	3,920	1,969
ALL	5.5	10.8	7.8	10,000	149,000	43,667	1,000	3,100	2,108

4. Discussion

Pancytopenia is a common hematological occurrence in our clinical practice and should be addressed when a patient presents with unexplained anemia, fever, and bleeding tendencies. There is a lack of research on the prevalence of the causes of pancytopenia in Iran. This study aimed to examine the etiology of pancytopenia in patients referred to Shaheed Ghazi Hospital, a specialized facility for Hematology and Oncology located in Tabriz City. An analysis was conducted to determine the causes of pancytopenia in these patients, taking into account their age and gender. A total of 133 patients with pancytopenia were included in the study conducted at Shaheed Ghazi Hospital. These patients were diagnosed with 15 distinct causes of pancytopenia.

The predominant etiologies of pancytopenia in the study were identified as follows: megaloblastic anemia (30%), acute myeloid leukemia (21%), acute lymphoid leukemia (10.5%), and aplastic anemia (7.5%). Furthermore, the mean age of the patients was 47.35 ± 17.62 years old. Of the patients, 66% were male and 34% were female, resulting in a male-to-female ratio of 1.94:1. Among the 133 patients, 8 were younger than 20 years old, accounting for just 6% of the cases. Patients aged 21 to 40 years old comprised 36% of the total, while those between 41 and 60 years old totaled 32%. The remaining 26% of patients were above 61 years old. Additionally, the current study found that hematological neoplasms (AML, ALL, MDS, and HCL) were the cause of pancytopenia in 56 instances, which constitutes 42% of the total. Leukemia was the cause of pancytopenia in 33% of the cases. The most common cause of pancytopenia in patients who were brought to the hospital was acute leukemia, comprising 31.5% of all cases.

There was an overlap between the main causes of cytopenia, and the routine hematological tests that were conducted lacked specificity. The peripheral blood smear was found to be effective in identifying the cause of the condition in patients with megaloblastic anemia and leukemia^[19]. Prior studies have emphasized the importance of conducting a bone marrow examination on patients diagnosed with pancytopenia syndrome^[20–22]. Within the realm of hematology, bone marrow aspiration is an essential diagnostic procedure used to assess various cases of cytopenia. Bone marrow aspiration is a crucial diagnostic technique in the field of hematology that aids in the evaluation of different instances of cytopenia. The advantages of bone marrow examination include its non-invasive nature, cost-effectiveness, and ability to yield prompt and dependable outcomes. Conducting a bone marrow aspiration is adequate for the diagnosis of nutritional anemia and for the initial identification of leukemia^[6]. Although bone marrow aspiration study is not frequently conducted for suspected

cases of megaloblastic anemia, it is recommended in situations where the diagnosis is uncertain or when immediate treatment is necessary, and hematological procedures are unavailable ^[6].

Table 6. Comparison with different studies

No.	Authors	Place	Age (years)	Cases no.	First common cause	Second common cause	Third common cause	Mean age or most commonly affected age group (years)	M:F
1	Present study	Iran	All ages	133	Acute leukemia (31.5%)	MA (30%)	AA (7.5%)	47.35 ± 17.62	1.99:1
2	Karsing <i>et al.</i> ^[23]	India	All ages	60	MA (66%)	AA (18.3%)	Malaria (5%)	41	1.3:1
3	Khunger <i>et al.</i> ^[24]	India	2–70	200	MA (72%)	AA (14%)	Acute leukemia (5%)	3 th decade	1.2:1
4	Khodke <i>et al.</i> ^[25]	India	3–69	50	MA (62%)	AA (20%)	MM (5.7%)	12–30	1.3:1
5	Kumar <i>et al.</i> ^[26]	India	All ages	166	AA (29.51%)	MA (22.28%)	Sub leukemic leukemia (12.04%)	-	-
6	Gayathri and Rao ^[27]	India	All ages	104	MA (74.04%)	AA (18.36%)	Sub leukemic leukemia (3.85%)	42	1.2:1
7	Varma and Dash ^[28]	India	Adults	202	AA (40.6%)	MA (23.26%)	AML (12.8%)	-	-
8	Khan <i>et al.</i> ^[11]	Pakistan	All ages	160	AA (37.5%)	MA (13.75%)	Acute leukemia (13.75%)	35	1.5:1
9	Kumar <i>et al.</i> ^[29]	Pakistan	All ages	62	MA (41.9%)	AML (27.4%)	AA (19.4%)	37.76 ± 16.38	1.38:1
10	Agarwal <i>et al.</i> ^[30]	India	All ages	70	Malaria (30%)	AA (14.28%)	Tuberculosis (12.86%)	1–30	1:1.2
11	Santra and Das ^[31]	India	13–65	111	AA (20.72%)	Cirrhosis (11.71%)	Kala-azar (9%)	36.9	1.47:1
12	Gupta <i>et al.</i> ^[19]	India	All ages	169	MA (37.78%)	Mixed nutritional deficiency anemia (15.98%)	AA (11.24%)	11–20	1.2:1
13	Jella <i>et al.</i> ^[10]	India	< 18	56	MA (42.9%)	AA (23.2%)	Malaria (7.1%)	34.9 ± 4.3	1.43:1
14	Patel <i>et al.</i> ^[8]	India	< 12	50	MA (58%)	AA (12%)	Cirrhosis (4%)	21–30	1.7:1
15	Tilak <i>et al.</i> ^[17]	India	5–70	77	MA (68%)	AA (7.7%)	Malaria	-	-
16	Jalaei-Khuu and Keihani ^[32]	Iran	All ages	188	Acute leukemia (35.6%)	AA (22.3%)	MA (14.9%)	2 th decade	1.38:1

Studies conducted in Pakistan and India have consistently shown that megaloblastic anemia is the primary underlying cause of pancytopenia, as indicated in **Table 6**. Acute leukemia was the predominant etiology of pancytopenia in the study, with megaloblastic anemia being the subsequent cause. Gayathri BN *et al.* (2015) documented a significantly elevated prevalence rate of 74.04% for megaloblastic anemia ^[25]. The variability in the occurrence of different diagnostic entities causing pancytopenia can be attributed to differences in methodology and strictness of diagnostic criteria, geographical location, duration of observation, genetic variations, and varying exposure to toxic substances in the bone marrow ^[27]. This study highlights the importance of pancytopenia in different hospital environments, with a particular emphasis on comprehending

the distinct causes associated with each type of hospital.

It is essential to acknowledge the study's limitations. While the sample size is small, the data collected are specific to the only oncology referral center in northwest Iran. To obtain more conclusive findings, it is advisable to undertake additional studies with a larger sample size and also to conduct systematic reviews in this field. Considering the therapeutic significance of the subject, it is vital to perform further studies in this sector to enhance early diagnosis.

5. Conclusion

Pancytopenia, a condition marked by the decrease in all types of blood cells, is a medical condition that often requires hospitalization. The etiology of this condition is multifactorial, and the reported cause is usually dependent on the specific healthcare center. The most common cause of pancytopenia in general hospitals is frequently claimed to be infections. Nevertheless, at the Tabriz Shaheed Ghazi Hospital, the most frequently seen causes of pancytopenia are acute leukemia and megaloblastic anemia. Significantly, acute myeloid leukemia (AML) is the dominant form of acute leukemia at this center, representing 21% of cases, which is approximately twice the rate of acute lymphoid leukemia (ALL), which stands at around 10.5%. This discrepancy underscores the diversity in the etiology of pancytopenia among various healthcare institutions. It is important to rapidly detect and diagnose pancytopenia, focusing on identifying its specific cause. Early intervention can result in early treatment of treatable conditions, which can significantly improve patient outcomes.

Ethics approval

The study received approval from the Medical Ethics Committee of Tabriz University of Medical Sciences and Health Services (IR.TBZMED.REC.94/3-7/23, 1394/08/30). Furthermore, all procedures were carried out in strict adherence to all relevant regulations and protocols.

Disclosure statement

The authors declare no conflict of interest.

Author contributions

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Reference

- [1] Chiravuri S, De Jesus O, 2023, Pancytopenia. StatPearls Publishing, Treasure Island.
- [2] Young NS, Abkowitz JL, Luzzatto L, et al., 2000, New Insights into the Pathophysiology of Acquired Cytopenias. ASH Education Program Book, 2000(1):18–38.
- [3] Pascutti MF, Erkelens MN, Nolte MA, et al., 2016, Impact of Viral Infections on Hematopoiesis: From Beneficial to

Detrimental Effects on Bone Marrow Output. *Frontiers in Immunology*, 7:364.

- [4] Marks PW, 2013, Hematologic Manifestations of Liver Disease. *Seminars in Hematology*, 50(3):216–221.
- [5] Risitano AM, Maciejewski JP, Selleri C, et al., 2007, Function and Malfunction of Hematopoietic Stem Cells in Primary Bone Marrow Failure Syndromes. *Current Stem Cell Research & Therapy*, 2(1):39–52.
- [6] Neumann E, 1868, Über die Bedeutung des Knochenmarks für die Blutbildung. *Zentralblatt für die medizinischen Wissenschaften*, 44: 122.
- [7] Bizzozero G, 1868, Sulla Funzione Ematopoetica del Midollo Delle Ossa. *Zentralbl Med Wissensch*, 6(1868): 885.
- [8] Patel F, Panjwani S, Lakhani K, et al., 2017, A Study of Clinical Profile of 50 Patients Having Pancytopenia in SIRT General Hospital, Bahvnagar. *European Journal of Pharmaceutical and Medical Research*, 4(66):349–377.
- [9] Ryan DH, Cohen HJ, 2002, Bone Marrow Aspiration and Morphology. *Hematology: Basic Principles and Practice*, 2460–8.
- [10] Jella R, Jella V, 2016, Clinico-Hematological Analysis of Pancytopenia. *International Journal of Advances in Medicine*, 3(2):176–9.
- [11] Khan TA, Khan IA, Mahmood K, et al., 2013, Clinicohaematological Spectrum of Pancytopenia in a Tertiary Care Hospital. *Journal of Postgraduate Medical Institute (Peshawar-Pakistan)*, 27(2):143–147.
- [12] Mudenge B, Savage DG, Allen RH, et al., 1999, Pancytopenia in Zimbabwe. *The American Journal of the Medical Sciences*, 317(1):22–32.
- [13] Valent P, 2012, Low Blood Counts: Immune-Mediated, Idiopathic, or Myelodysplasia. *ASH Education Program Book*, 2012(1):485–491.
- [14] Azaad MA, Li Y, Zhang Q, et al., 2015, Detection of Pancytopenia Associated with Clinical Manifestation and Their Final Diagnosis. *Open Journal of Blood Diseases*, 24(5):17–30.
- [15] Cashen AF, Van Tine B, 2012, Cause of Pancytopenia: The Washington Manual of Hematology and Oncology Subspecialty Consult. Lippincott Williams & Wilkins, 3rd ed, London, 81–90.
- [16] Kasper D, Fauci A, Hauser S, et al., 2018, Harrison's Principles of Internal Medicine. McGraw-Hill Professional Publishing. Chapter 98: Bone Marrow Failure Syndromes Including Aplastic Anemia and Myelodysplasia, 20th ed, New York, 3000P.
- [17] Tilak V, Jain R, 1999, Pancytopenia: A Clinico-Hematologic Analysis of 77 Cases. *Indian Journal of Pathology & Microbiology*, 42(4):399–404.
- [18] Heimpel H, 1987, Incidence of Aplastic Anemia: The Relevance of Diagnostic Criteria. *Blood*, 70(6):1718–1721.
- [19] Gupta M, Chandna A, Kumar S, et al., 2016, Clinicohematological Profile of Pancytopenia: A Study from a Tertiary Care Hospital. *Dicle Tıp Dergisi*, 43(1):5–11.
- [20] Bunch C, 1995, Bone Marrow Failure. *Medical International*, 10:495–499.
- [21] Imbert M, Scoazec JY, Mary JY, et al., 1989, Adult Patients Presenting with Pancytopenia: A Reappraisal of Underlying Pathology and Diagnostic Procedures in 213 Cases. *Hematologic Pathology*, 3(4):159–167.
- [22] Keisu M, Öst Å, 1990, Diagnoses in Patients with Severe Pancytopenia Suspected of Having Aplastic Anemia. *European Journal of Haematology*, 45(1):11–14.
- [23] Karsing P, Kh A, Singh D, et al., 2018, Clinicopathological Spectrum of Pancytopenia in a Tertiary Care Center in Northern India. *Recent Advances in Pathology & Laboratory Medicine*, 4(3):9–13.
- [24] Khunger JM, Arulselvi S, Sharma U, et al., 2002, Pancytopenia: A Clinico-Haematological Study of 200 Cases. *Indian Journal of Pathology & Microbiology*, 45(3):375–379.
- [25] Khodke K, Marwash S, Buxi G, et al., 2001, Bone Marrow Examination in Cases of Pancytopenia. *Journal of the Indian Academy of Clinical Medicine*, 2:55–59.
- [26] Kumar R, Kalra SP, Kumar H, et al., 2001, Pancytopenia: A Six-Year Study. *The Journal of the Association of*

Physicians of India, 49:1078–1081.

- [27] Gayathri BN, Rao KS, 2011, Pancytopenia: A Clinico-Hematological Study. *Journal of Laboratory Physicians*, 3(1):15–20.
- [28] Varma N, Dash S, 1992, A Reappraisal of Underlying Pathology in Adult Patients Presenting with Pancytopenia. *Tropical and Geographical Medicine*, 44(4):322–327.
- [29] Makheja KD, Maheshwari BK, Arain S, et al., 2013, The Common Causes Leading to Pancytopenia in Patients Presenting to Tertiary Care Hospital. *Pakistan Journal of Medical Sciences*, 29(5):1108–1111.
- [30] Agarwal R, Bharat V, Gupta BK, et al., 2015, Clinical and Hematological Profile of Pancytopenia. *International Journal of Clinical Biochemistry & Research*, 2(1):48–53.
- [31] Santra G, Das BK, 2010, A Cross-Sectional Study of the Clinical Profile and Aetiological Spectrum of Pancytopenia in a Tertiary Care Centre. *Singapore Medical Journal*, 51(10):806–812.
- [32] Jalaee-Khoo H, Keihani M, 2006, The Causes of Pancytopenia from 1373 to 1381 Who Were Admitted to Army 501 Hospital. *Tehran University of Medical Science Journal*, 64(2):91–94.

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Analysis of Osteoporosis Risk Factors in 148 Retired Employees Based on Physical Examination Results

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Abstract: *Objective:* To investigate and thoroughly understand the physical examination results of retired employees from a certain unit in Beijing, analyze their bone mineral density (BMD), and identify risk factors that may indicate osteoporosis. This provides a reference for the individualized prevention, identification, and control of osteoporosis among retired employees. *Methods:* The bone mineral density and potential factors of 148 retired employees from a unit in 2023 were analyzed and categorized into osteoporosis and non-osteoporosis groups. Key factors from the physical examinations of the two groups were compared. Spearman's correlation analysis was used to determine the correlation between key factors and osteoporosis. Significant key factors were included in a regression analysis. A multivariate binary logistics regression was employed to identify risk factors indicative of osteoporosis. *Results:* Correlation analysis revealed that gender, age, and ECG ST-segment length were significantly associated with osteoporosis. Regression analysis showed that for each additional year of age, the likelihood of developing osteoporosis increased by 1.058 times; females were 2.865 times more likely to develop osteoporosis compared to males; the longer the ECG ST-segment, the higher the likelihood of osteoporosis. *Conclusion:* Gender, age, and ECG ST-segment length are significantly associated with osteoporosis. These indicators can provide reference points for early identification, early intervention, and reducing the incidence of osteoporosis in clinical settings.

Keywords: Osteoporosis; Gender; Age; ECG ST-segment; Correlation analysis

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1. Introduction

Osteoporosis is a systemic disease characterized by reduced bone mass and decreased bone density, leading to increased bone fragility and a higher likelihood of fractures ^[1]. In elderly patients, the presence of multiple comorbidities means that fractures can exacerbate the risk of other systemic diseases, a concern that warrants significant attention. Large-scale epidemiological surveys in China have shown that the age-standardized

prevalence of osteoporosis in the population over 50 years old is 6.46% for men and 29.13% for women^[2]. This highlights the importance of addressing this disease due to its high prevalence. Osteoporotic fractures often involve decreased bone mass and poor bone stability, commonly occurring in weight-bearing areas such as the spine, hips, and wrists, and are associated with high disability and mortality rates, imposing a significant social and healthcare burden.

With the increasing aging population in China and the rising average lifespan of the elderly in Beijing, the health status of retired employees has become a growing concern. Clinical experience suggests that certain electrocardiogram (ECG) features may have indicative significance for osteoporosis. However, previous studies on osteoporosis risk factors have rarely mentioned blood indicators and ECG results from physical examinations. Therefore, this study commences from the health examination results of 148 retired workers, adhering to the guidelines^[3] and under the premise of common risk factors such as gender and age. To achieve a more comprehensive analysis, this study incorporates previously overlooked ECG characteristics and common blood indicators, in addition to other methods of reference. The goal is to provide a comprehensive analysis of osteoporosis risk factors based on physical examination results, categorize and classify patients, and offer a basis for clinical prevention education, identification of high-risk groups, and individualized patient management.

2. Materials and methods

2.1. Study subjects

This case-control study retrospectively selected 148 retired employees aged 55 and above (42 with normal bone mass, 47 with reduced bone mass, and 59 with osteoporosis) who underwent physical examinations at the Civil Aviation General Hospital in Chaoyang District, Beijing, from January to December 2023. Inclusion criteria: completed ultrasound bone density examinations. Exclusion criteria: patients with bone metastases from malignant tumors; those with secondary bone loss due to various causes and bone metabolism-related diseases; patients with major illnesses or bedridden for over 3 months in the past year; those requiring long-term corticosteroid use in the past six months; incomplete ultrasound bone density examination; incomplete blood indicator checks; no ECG examination; incomplete basic information such as age.

2.2. Detection methods and diagnostic criteria

2.2.1. Detection methods

Data for this study were collected from the medical examination management information system of the Civil Aviation General Hospital (T-PES2005 medical examination management information system by Beijing Tongfang Weikang Technology Co., Ltd.). All participants underwent relevant examinations at least 8 hours after fasting, starting the following morning. The following examinations were completed by trained nurses, technicians, and physicians in our department, with participants having the right to choose or decline specific examination items. The relevant items in this study include:

- (1) Laboratory tests: Biochemical tests (detected using Beckman Coulter AU5800 automatic biochemical analyzer);
- (2) Electrocardiogram (ECG): detected using GE MAC2000 electrocardiograph analyzer;
- (3) Bone density: detected using the BMD-9M3 ultrasound bone densitometer by Beijing Yueqi Chuangtong Technology Co., Ltd., which evaluates bone mineral density (BMD) and bone strength by measuring the speed of sound (SOS) and broadband ultrasound attenuation (BUA) in bones. The built-

in processor compares the measured BMD results with the reference database to generate a T-score. The bone density of both forearm radial and ulnar bones at the 1/3 distal end of the non-weight-bearing side was measured, obtaining the mean BMD and T-score, and comparing the measured bone density with the peak bone density of the same gender, noting the standard deviation ^[1].

2.2.2. Diagnostic criteria

- (1) Lipid levels: In biochemical tests, lipid levels are considered appropriate when total cholesterol (TC) < 5.2 mmol/L, triglycerides (TG) < 1.7 mmol/L, low-density lipoprotein cholesterol (LDL-C) < 3.4 mmol/L, and high-density lipoprotein cholesterol (HDL-C) ≥ 1.0 ; otherwise, they are considered dyslipidemia ^[4].
- (2) Blood glucose: According to the diagnostic criteria proposed by the WHO Expert Committee on Diabetes (1999), this study uses fasting venous blood glucose as the judgment standard, with hyperglycemia diagnosed when fasting blood glucose > 6.1 mmol/L.
- (3) Liver function: According to the reference values in the reagent instructions provided with the instruments, liver function is normal when alanine aminotransferase is 9–50 mmol/L and aspartate aminotransferase is 15–40 mmol/L; otherwise, liver function is abnormal.
- (4) Uric acid: According to the reference values in the reagent instructions provided with the instruments, hyperuricemia is diagnosed when UA > 422 $\mu\text{mol/L}$ in men and UA > 387 $\mu\text{mol/L}$ in women.
- (5) Heart rate: Adults' normal resting heart rate typically ranges between 60 and 100 beats per minute ^[5]; rates outside this range are considered abnormal.
- (6) ST-segment length ^[6]: The ST segment in an ECG is located between the end of the QRS complex (J point) and the beginning of the T wave, reflecting the early repolarization process of the ventricles. Measured using the DELIXI plastic digital caliper 20220325.
- (7) TP-segment length ^[7]: The TP segment in an ECG refers to the time interval from the end of the T wave of one heartbeat to the start of the P wave of the next heartbeat. Measured using the DELIXI plastic digital caliper 20220325.
- (8) Bone density: A T-score > -1 is considered normal; $-2.5 < \text{T-score} \leq -1$ indicates reduced bone mass; and a T-score ≤ -2.5 at one or more sites indicates osteoporosis. Normal bone mass is considered normal, while reduced bone mass and osteoporosis are considered abnormal ^[1].

2.4. Data processing and analysis

Data entry and processing were performed using EXCEL, and statistical analysis was conducted using SPSS 29.0 statistical software. Quantitative data not normally distributed were described as $M(Q_1 - Q_3)$. Differences in factors among the normal bone mass, reduced bone mass, and osteoporosis groups were analyzed using chi-squared tests for binary variables and Kruskal-Wallis rank-sum tests for continuous variables. Correlation analysis involved using Spearman correlation to analyze the relationship between key factors and the binary variable of osteoporosis. Multivariate binary logistic regression analysis was employed to examine the relationship between risk factors and osteoporosis. A *P*-value of < 0.05 was considered statistically significant.

3. Results

3.1. Basic information of examinees

Among the 148 samples, there were 43 males (29.1%) and 105 females (70.9%); the age range was 55–92 years, with a median age of 68 (62–72) years. Specifically, there were 15 people aged 55–59 (10.1%), 78

people aged 60–69 (52.7%), 41 people aged 70–79 (27.7%), and 14 people aged 80 and above (9.5%). For more details, see **Table 1**.

Table 1. Basic information of the examined population [*n* (%)]

Age group	Male		Female		Total	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
55–59	0	0.0%	15	14.3%	15	10.1%
60–69	25	58.1%	53	50.5%	78	52.7%
70–79	12	27.9%	29	27.6%	41	27.7%
80 years and over	6	14.0%	8	7.6%	14	9.5%

3.2. Overview of common blood indicators and ECG results in normal bone mass, reduced bone mass, and osteoporosis groups

Bone density was categorized into normal bone mass, reduced bone mass (31.08% total: 8.11% male, 22.97% female), and osteoporosis (40.54% total: 7.43% male, 33.11% female). The proportion of the examined population with abnormal bone mass (including reduced bone mass and osteoporosis) was 71.62% of the total. Differences in the risk factors analyzed among these three groups were tested, and the results are shown in **Table 2**. Among these, the differences in ST-segment length between the groups were significant, with $P < 0.001$. Other factors did not show significant differences.

Table 2. Analysis of blood indicators and ECG results in normal bone mass, reduced bone mass, and osteoporosis groups [*n* (%)]

Factor	Normal bone mass group	Reduced bone mass group	Osteoporosis group	χ^2 -value	<i>P</i> -value
Dyslipidemia	18 (32.73)	16 (29.09)	21 (38.18)	0.657	0.720
Hyperglycemia	31 (29.52)	34 (32.38)	40 (38.10)	0.495	0.781
Elevated liver transaminase levels	39 (27.86)	45 (32.14)	56 (40.00)	0.381	0.826
Increased uric acid	40 (31.01)	41 (31.78)	48 (37.21)	4.226	0.121
Abnormal heart rate	40 (27.97)	46 (32.17)	57 (39.86)	0.472	0.790
ST-segment length	52.63*	77.50*	87.68*	16.77	< 0.001
TP-segment length	67.50*	79.91*	74.02*	1.867	0.393

Note: * indicates the rank mean value. The ST-segment length and TP-segment length were tested using rank-sum tests, with the second-to-last column representing the H value.

3.3. Correlation analysis between various factors and osteoporosis

The normal bone mass and reduced bone mass groups were combined into a non-osteoporosis group, thus classifying the data into osteoporosis and non-osteoporosis. Factors potentially related to osteoporosis were then analyzed, with the results of the Spearman correlation analysis shown in **Table 3**. It can be seen that gender, age, and ST segment length are significantly correlated with osteoporosis, and these factors were included in the subsequent regression analysis.

Table 3. Correlation analysis between various factors and osteoporosis

Factor	<i>r</i>	<i>P</i>
Gender	-0.217	0.008
Age	0.164	0.047
Dyslipidemia	0.062	0.471
Hyperglycemia	0.056	0.495
Elevated liver transaminase levels	-0.012	0.889
Increased uric acid	0.141	0.087
Abnormal heart rate	0.001	0.995
ST-segment length	0.251	0.002
TP-segment length	0.000	0.997

3.4. Multivariate regression analysis of osteoporosis

The factors found to be correlated in the analysis (age, gender, ST segment length) were included in a multivariate binary logistic regression analysis, with the results shown in **Table 4**. As seen in **Table 4**, age is a significant risk factor for osteoporosis ($P = 0.013 < 0.05$), with a further impact coefficient of $0.585 > 0$, indicating that the likelihood of osteoporosis increases with age. For each age group increase, the likelihood of developing osteoporosis is 1.795 times the previous group. Gender is also a significant risk factor for osteoporosis ($P = 0.017 < 0.05$), with a further impact coefficient of $1.053 > 0$, indicating that women are more likely to develop osteoporosis than men. The likelihood of women developing osteoporosis is 2.865 times that of men. The ST-segment length is another significant risk factor for osteoporosis ($P = 0.026 < 0.05$), with a further impact coefficient of $0.472 > 0$, indicating that the likelihood of osteoporosis increases with the length of the ST segment. For every 1 mm increase in ST-segment length, the likelihood of developing osteoporosis is 1.604 times the previous value.

Table 4. Binary logistics regression analysis results for various factors and osteoporosis

	B	P	OR	95% confidence interval for OR	
				Lower limit	Upper limit
Age group	0.585	0.013	1.795	1.129	2.854
Gender	1.053	0.017	2.865	1.203	6.826
ST-segment length	0.472	0.026	1.604	1.057	2.434
Constant	-3.379	< 0.001	0.034		

Furthermore, if age was analyzed in finer detail, using each year as a unit rather than grouping by age ranges, further regression results shown in **Table 5** were obtained. It is evident that age is a significant risk factor for osteoporosis ($P = 0.019 < 0.05$), with a further impact coefficient of $0.056 > 0$, indicating that the likelihood of osteoporosis increases with age. For each additional year of age, the likelihood of developing osteoporosis increases by a factor of 1.058.

Table 5. Binary logistics regression analysis results for various factors and osteoporosis (detailed age analysis)

	B	P	OR	95% confidence interval for OR	
				Lower limit	Upper limit
Age	0.056	0.019	1.058	1.009	1.109
Gender	1.051	0.018	2.860	1.195	6.846
ST segment length	0.454	0.032	1.575	1.040	2.383
Constant	-6.367	< 0.001	0.002		

4. Discussion and conclusion

The prevention and treatment of osteoporosis in China face prominent issues, such as high prevalence and low awareness, diagnosis, and treatment rates, often referred to as the “one high and three lows” situation. This presents significant challenges for healthcare professionals involved in wellness and related care ^[8,9]. The prevalence of osteoporosis and reduced bone mass is increasing annually ^[10]. If pathological fractures occur, serious cases can lead to bedridden status, require home care, cause disability, or even result in death. The costs associated with treatment and care can impose a significant burden on families and society.

This study, based on the results measured by an ultrasonic bone density scanner, suggests that post-55-year-old females exhibit significantly higher levels of bone density abnormalities compared to males, consistent with domestic literature ^[1]. The prevalence of osteoporosis in women is significantly higher than the average level in Western countries but is similar to data from other Asian countries like Japan and Korea ^[11]. Previous studies have suggested that osteoporosis is an age-related skeletal disease, characterized by an increasing incidence with advancing age ^[12]. However, data from this study suggest that among retirees and elderly examinees in different age groups, there was no observed increase in the prevalence of reduced bone mass with age. This might be attributed to high medication compliance among retired workers in this particular unit, timely disease prevention, and effective treatment following annual physical examinations, which may have slowed the progression of bone loss. The data indicate that increased screening and education regarding bone density among retirees could enable timely intervention for those with abnormal bone density, effectively mitigating risks associated with further bone loss. This strategic approach could delay the progression from reduced bone mass to osteoporosis (including moderate and severe osteoporosis). The ultrasonic bone density scanner offers advantages such as being radiation-free, simple, and fast for screening high-risk populations for osteoporosis, although guideline recommendations ^[13] caution that measurement biases may impact results.

When pathological changes occur in blood electrolytes, especially a significant decrease in calcium ion concentration, the direct result can be a prolonged phase 2 action potential in cardiac electrophysiological activity, leading to an extended ST segment on an ECG. From the perspective of myocardial action potentials, the duration of phase 2 is determined by the time required for the action potential to transition into phase 3. The necessary condition for this transition is a significant change in intracellular electrolytes, particularly a sufficient decrease in potassium ions, which allows for the rapid outward flow of potassium ions, facilitating the transition to phase 3 ^[14]. However, during electrolyte disturbances, especially hypocalcemia, the reduced influx of calcium ions and corresponding changes in potassium ion outflow lead to decreased potassium content, extending the time for rapid potassium outflow. Consequently, the duration of phase 2 in the action potential is significantly prolonged, manifesting as an extended ST segment on the ECG. Therefore, a negative correlation

can be observed between serum calcium concentration and ST segment length during electrolyte disturbances.

Correspondingly, when the body lacks calcium, physiological changes occur to compensate for the deficiency, leading to decreased calcium ion levels in the blood. This can result in increased bone resorption and decreased bone density, triggering secondary hyperparathyroidism, which further releases calcium from bone tissue into the bloodstream, ultimately leading to osteoporosis^[15,16].

This study aims to explore the potential risk factors for osteoporosis through physical examination results. It innovatively conducted a multi-indicator correlation study, associating blood biochemical indicators, ECG indicators (ST segment length), and bone density data to explore their correlation with osteoporosis. This research provides valuable insights into the clinical prevention of osteoporosis, offering new perspectives and reference points for early detection. Monitoring these common and easily accessible examination indicators, utilizing multidimensional examination data, can help early identification of high-risk groups. Clinicians can then detect osteoporosis risk in middle-aged and elderly patients early, allowing for timely preventive measures, interventions, and treatment plans.

In summary, the analysis of the physical examination results of retirees from a particular unit suggests the need for increased education and active intervention regarding osteoporosis among retirees (middle-aged and elderly patients). This can improve the health status and quality of life of retirees.

Disclosure statement

The authors declare no conflict of interest.

References

- [1] Qiu M, Xie Y, Wang X, et al., 2020, Practice Guidelines for Patients with Osteoporosis. *Chinese Journal of Internal Medicine*, 59(12): 953–959.
- [2] Cheng X, Dong S, Wang L, et al., 2019, Investigation of Bone Density Levels and Osteoporosis Prevalence in the Chinese Population Using Dual-Energy X-ray Absorptiometry: A Large-Scale Multicenter Survey. *Chinese Journal of Health Management*, 13(1): 51–58.
- [3] Chinese Society of Osteoporosis and Bone Mineral Research, 2022, Guidelines for the Diagnosis and Treatment of Primary Osteoporosis (2022). *Chinese General Practice*, 26(14): 1671–1691.
- [4] Joint Expert Committee on the Revision of the Chinese Lipid Management Guidelines, 2023, Chinese Lipid Management Guidelines (2023). *Chinese Circulation Journal*, 38(3): 237–271.
- [5] Wu J, Wang D, Lu Z, et al., 2008, Survey on the Normal Range of Electrocardiograms in Healthy Adults. *Chinese Journal of Cardiac Arrhythmias*, 12(3): 189–194.
- [6] Expert Group on the Compilation of the Guidelines for ECG Measurement Technology, 2019, Guidelines for ECG Measurement Technology. *Practical Electrocardiology Journal*, 28(2): 77–86.
- [7] Zhang W, Li Y, 2012, Handbook of ECG Diagnosis (4th Edition). People's Military Medical Press, Beijing.
- [8] Chinese Center for Disease Control and Prevention, Chinese Society of Osteoporosis and Bone Mineral Research, 2021, China Osteoporosis Epidemiological Survey Report (2018). People's Medical Publishing House, Beijing.
- [9] Wang L, Yu W, Yin X, et al., 2021, Prevalence of Osteoporosis and Fracture in China: The China Osteoporosis Prevalence Study. *JAMA Netw Open*, 4(8): e2121106. <https://doi.org/10.1001/jamanetworkopen.2021.21106>
- [10] Chinese Society of Osteoporosis and Bone Mineral Research, 2019, Results of the China Osteoporosis Epidemiological Survey and the “Healthy Bones” Special Action. *Chinese Journal of Osteoporosis and Bone Mineral Research*, 12(4): 317–318.

- [11] Working Group on the “Guidelines for the Diagnosis and Treatment of Osteoporosis in the Elderly (2023)”, 2023, Guidelines for the Diagnosis and Treatment of Osteoporosis in the Elderly (2023). Chinese Journal of Bone and Joint Surgery, 16(10): 865–885.
- [12] Serio B, Paolino S, Casabella A, et al., 2013, Osteoporosis in the Elderly. Aging Clin Exp Res, 25 Suppl 1: S27–S29. <https://doi.org/10.1007/s40520-013-0107-9>
- [13] Cheng X, Yuan H, Cheng J, et al., 2020, Expert Consensus on Imaging and Bone Density Diagnosis of Osteoporosis. Chinese Journal of Bone and Joint, 9(9): 666–673.
- [14] Liu W, Zhao L, Bao S, 1994, Magnesium and Calcium in Biological Metabolism. Research on Trace Elements and Health, 1994(3): 52 + 33.
- [15] Sun S, 2007, Molecular Mechanisms of Osteoclast Formation and Local Regulation of Osteoclasts in Osteolytic Lesions, thesis, Fourth Military Medical University.
- [16] Wang W, 2015, Research on the Inhibition of Osteoclasts by Flavonoid Monomer Naringenin in Traditional Chinese Medicine, thesis, Shanghai Jiao Tong University.

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3D Collagen Gels: A Promising Platform for Dendritic Cell Culture in Biomaterials Research

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Abstract: The three-dimensional (3D) cell culture system has garnered significant attention in recent years as a means of studying cell behavior and tissue development, as opposed to traditional two-dimensional cultures. These systems can induce specific cell reactions, promote specific tissue functions, and serve as valuable tools for research in tissue engineering, regenerative medicine, and drug discovery. This paper discusses current developments in the field of three-dimensional cell culture and the potential applications of 3D type 1 collagen gels to enhance the growth and maturation of dendritic cells.

Keywords: Three-dimensional cell culture; Dendritic cells; Type 1 collagen gels; Bovine tendons and rat tails

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1. Introduction

Three-dimensional (3D) cell culture systems have gained significant attention in recent years as an alternative to traditional two-dimensional (2D) cultures for studying cellular behavior and tissue development ^[1]. These systems aim to mimic the complex cellular microenvironment found in living tissues, providing a more physiologically relevant model for studying cell function, disease progression, and drug responses ^[2]. In a 3D cell culture system, cells are grown in a matrix or scaffold that allows them to occupy a three-dimensional space. This can be achieved using various techniques, including hydrogels, scaffolds made from natural or synthetic polymers, and organoids. These matrices provide physical support and structural cues to the cells, enabling them to form complex cell-to-cell interactions and tissue-like structures ^[3].

One of the main advantages of 3D cell culture systems is their ability to recapitulate the organization and functions of specific tissues. By allowing cells to grow in a three-dimensional manner, these systems better represent the *in vivo* cellular microenvironment, resulting in more accurate physiological responses. This is particularly important when studying diseases that involve tissue architecture, such as cancer or organ dysfunction. Another advantage of 3D cell culture systems is that they can better simulate the diffusion of nutrients, oxygen, and waste products compared to traditional 2D cultures. This enhanced nutrient exchange allows cells to maintain their viability and metabolism over a longer period, closely resembling the conditions found in living tissues.

Furthermore, 3D cell culture systems provide a platform for studying cell-cell and cell-matrix interactions. These interactions play a crucial role in various cellular processes, including cell adhesion, migration, and differentiation. By incorporating different types of cells or extracellular matrix components into the culture system, researchers can investigate how these interactions influence cell behavior and tissue formation. In addition to basic research applications, 3D cell culture systems have gained attention in the field of drug discovery and development. Traditional 2D cultures often fail to accurately predict drug efficacy or toxicity due to the lack of physiological relevance. In contrast, 3D models can better mimic the complexity of human tissues, allowing for a more reliable assessment of drug responses.

In conclusion, three-dimensional cell culture systems offer significant advantages over traditional 2D cultures in terms of physiological relevance, tissue organization, and cell-cell interactions. These systems have revolutionized the field of cell biology and provided new opportunities for studying cellular behavior, disease mechanisms, and drug development. As technology continues to advance, 3D cell culture systems will undoubtedly play a crucial role in future research and therapeutic applications ^[4,5].

Dendritic cells are a type of immune cell that plays a crucial role in the adaptive immune response. They are named for their unique shape, resembling the branching dendrites of a neuron. Dendritic cells are primarily found in tissues that are in contact with the external environment, such as the skin, respiratory tract, and gastrointestinal tract. Their main function is to capture antigens, which are foreign substances or molecules that can trigger an immune response. When dendritic cells encounter an antigen, they take it up through a process called antigen capture ^[6]. They then process the antigen and present it on their cell surface using specialized molecules called major histocompatibility complex (MHC) molecules. This process is known as antigen presentation. The presentation of antigens by dendritic cells is crucial for initiating an immune response ^[7]. When dendritic cells present an antigen, they also express co-stimulatory molecules that help activate other immune cells, such as T cells. This interaction with T cells is essential for the adaptive immune response to mount a specific and targeted attack against the antigen.

Dendritic cells are often referred to as “sentinels” of the immune system because they are constantly scanning their surroundings for foreign invaders. They have specialized receptors that recognize pathogen-associated molecular patterns (PAMPs), which are specific molecules found on the surface of pathogens like bacteria or viruses. When these receptors detect PAMPs, they trigger an immune response against the invading pathogen. In addition to their role in antigen presentation, dendritic cells also secrete cytokines, which are small signaling molecules that help regulate the immune response. These cytokines can have different effects on other immune cells, such as promoting inflammation or modulating the activity of specific cell types. Overall, dendritic cells are crucial players in the immune system’s ability to detect and respond to foreign invaders. Their ability to capture, process, and present antigens, as well as their role in activating other immune cells, makes them essential for initiating and coordinating an effective immune response ^[7,8].

Type 1 collagen is a widely used substrate in cell culture due to its structural and functional properties. It is the most abundant protein in the extracellular matrix of connective tissues, providing essential support and flexibility to cells. Using type 1 collagen as a cell culture substrate offers several benefits. Firstly, it mimics the physiological conditions *in vivo*, providing cells with a more natural environment for growth and interaction. This helps in maintaining cell morphology and function. Collagen also possesses excellent biocompatibility and promotes cell adhesion, migration, and proliferation. It provides a three-dimensional scaffold for cells to attach and spread, aiding in the formation of multicellular structures and tissue-like organization. This promotes cell-cell interactions and enhances cellular responses to growth factors, cytokines, and other signaling molecules ^[9]. Another advantage of type 1 collagen in cell culture is its ability to support the differentiation of various cell types, including fibroblasts, epithelial cells, endothelial cells, and stem cells. Collagen substrates

can induce specific cellular responses and promote tissue-specific functions, making them valuable tools in regenerative medicine, tissue engineering, and drug discovery research. Furthermore, type 1 collagen can be modified and tailored to meet specific experimental requirements^[10]. It can be crosslinked to improve stability and mechanical properties, or functionalized with bioactive molecules, such as peptides or growth factors, to enhance cellular responses and specific tissue formation.

When using type 1 collagen for cell culture, it is important to ensure its purity and quality. Collagen should be sourced from reliable suppliers and should undergo rigorous testing to ensure its absence of contaminants. Additionally, proper handling and storage conditions are crucial to maintain its structural integrity and biological activity^[11]. In conclusion, type 1 collagen is a valuable substrate for cell culture due to its physiological relevance, promoting cell adhesion, migration, proliferation, and differentiation. Its versatility and ability to mimic the extracellular matrix make it an indispensable tool for various cellular and tissue engineering applications^[12].

2. Dendritic cell culture in biomaterials

Dendritic cells (DCs) are of increasing scientific and clinical interest due to their important role in cancer host responses, their potential use as biological adjuvants in tumor vaccines, and their involvement in the immunobiology of tolerance and autoimmunity. Biological materials are preferred for *ex vivo* or *in vivo* delivery of antigens because they are slightly immunogenic and stimulate DC activation. Biomaterial carriers are used for antigen delivery as they extend the lifetime of intact antigens, increase the uptake and processing capacity of DCs, and improve the controlled delivery of antigens^[13]. DCs used in immunotherapy protocols need to mature maximally and stably to promote T cell activation *in vivo*. Therefore, there is increasing interest in maturing human blood-derived DCs through a biologically applicable approach using type I collagen. The finding that culture on type I collagen induces DC maturation has been reported in both mouse and human systems. Mature dendritic cells have a characteristic cell shape, numerous processes (veils, dendrites), and are mobile^[14].

In vivo, DCs are in close proximity to extracellular matrix (ECM) proteins. ECM proteins influence DC morphology and function^[15]. Peptide-based biomaterial scaffolds, such as cell-seeded collagen, also hold promise for tissue repair and other medical applications. The 3D collagen lattice is a suitable environment for dendritic cell culture and migration^[16]. Within the 3D collagen matrix, DCs behave like highly mobile cells, and their migratory properties are strongly influenced by their origin, maturation state, and the structure of the surrounding collagen matrix^[17].

Analysis of cell behavior in various natural and synthetic three-dimensional contexts will help improve the study of cell-environment interactions and subsequent materials design. Collagen preparations are occasionally used in tissue culture, usually for the immediate purpose of growing particularly fastidious cells. This is primarily due to the role collagen plays in the maturation of cells in a three-dimensional environment. This microenvironment is specifically engineered to support T cell activation, localize T cells to relevant tissue sites (e.g., tumor biopsy sites), and create a “reservoir” of chemokines/cytokines that support T cell activation. The ability to design such a microenvironment would be an attractive feature of this approach^[18].

The specific structure of secondary lymphoid organs is thought to make the immune response highly efficient, providing a defined space and auxiliary signals for new lymphoid tissue formation. Scaffolds could support simple injection-based vaccination strategies, superior to purified disease-specific DCs. Collagen provides a suitable environment for cell culture maturation because the fiber distribution and biophysical structure of the collagen lattice closely resemble the reticular stroma of interstitial soft tissue, dermis, and lymph nodes^[19].

3. 3D culture systems and cell migration and maturation studies

Cell migration is a fundamental function of normal cellular processes, including cell proliferation and migration. Cell behavior *in vitro* is typically examined in a two-dimensional environment; however, in the body, cells exist in a three-dimensional extracellular matrix environment rich in type I collagen. In fact, cells cultured in 3D matrices better reflect *in vivo* cell physiology compared to traditional 2D systems. Culturing immature dendritic cells (iDCs) with polylactic-co-glycolic acid (PLGA) microparticles (MP) or film resulted in morphology similar to that of LPS-matured DCs and the association, or possible internalization, of PLGA MPs. Furthermore, biomaterial-treated iDCs demonstrated an increase in MHC class II and costimulatory molecule expression compared to iDCs, but to a lower level than that of LPS-matured DCs ^[20].

Granulocyte-macrophage colony-stimulating factor and IL-4-treated human monocytes were used as precursor cells to investigate the interaction of DCs at different maturation stages with extracellular matrix proteins like fibronectin, collagen type I, and collagen type IV. The binding of monocytes to collagen type I was less strong but induced the maturation of the precursor cells. These results indicate that proteins of the extracellular matrix play an important role in the development and function of human DCs ^[18].

Lymphocytes did not adhere to or migrate to 2D substrates such as glass coated with serum or fibronectin. They attached to the hydrated 3D collagen lattice and migrated within the lattice. When the collagen was dehydrated to form a two-dimensional surface, lymphocyte adhesion to the collagen was reduced ^[21].

4. Bio fabrication of 3D collagen gels

Collagen extraction was performed in two batches, yielding 8 grams of collagen from 60 grams of tendons in both batches. In batch II, the tendons were divided into two sets of 30 grams each, and the extraction protocol was followed. Collagen was not obtained from set 1 of batch II. **Figure 1** shows the extracted Type 1 collagen from bovine tendons. Type I collagen is the most abundant collagen type found in bovine tendons, known for its high tensile strength and role in structural support. Type I collagen extracted from bovine tendons is used in various applications, including biomedical devices (e.g., wound dressings, tissue engineering scaffolds), cosmetic products, food supplements, and research purposes (e.g., studying cell-matrix interactions). **Figure 2** shows the Type 1 collagen from rat tail. Extracting Type I collagen from rat tails follows a similar process to that of bovine tendons, but with some adjustments due to the smaller size and different tissue characteristics of rat tails.



Figure 1. Bovine tendon collagen extraction



Figure 2. The pellet of collagen obtained from rat tail

The 3D collagen gel consists of type 1 collagen, which forms a delicate gel with a difficult-to-control pH and does not adhere to the walls of the well plate. Adding agarose to collagen increases the gelation time. Good gelation occurred with a gel consisting of 1% agarose. A gel containing 0.5% agarose was also gelled. Collagen solution in acetic acid with 0.5% agarose in different proportions (2:1, 1:1) was tested with rat tail and bovine tendon collagen, resulting in gelation with a pH around 3-4. The gel and medium were kept overnight in a CO₂ incubator, causing the pH to change.

A solution of collagen in acetic acid with 0.5% agarose and 0.1 N NaOH showed that the pH rose to 5, but the collagen started to precipitate (since 0.1 N NaOH was used, the same volume as acetic acid was required to neutralize the acid). A solution of collagen in acetic acid with 0.5% agarose and 1 N NaOH, when the pH rose to 5, also caused collagen to begin precipitating. Using exactly the amount of NaOH needed to neutralize 1 ml of acetic acid, light precipitation was observed, but the gel remained intact with a pH of around 7. A solution of collagen in acetic acid with 0.5% agarose, which was allowed to dry for approximately 5 hours and thoroughly washed with PBS, provided a pH value of 7 for the medium added to this gel. The gel was viewed under an inverted microscope, revealing the porous nature of the scaffold (**Figure 3**).

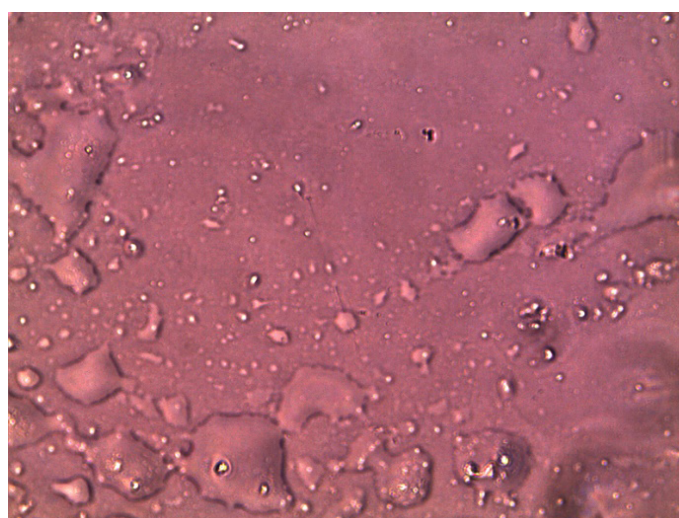


Figure 3. Porous collagen scaffold observed under the inverted microscope (40×)

5. Dendritic cell isolation and culture

From the peripheral blood, lymphocytes and monocytes (buffy coat) were separated (**Figure 4**). The monocytes from peripheral blood were cultured in RPMI medium. After a period of 8 days, the cell culture was observed under an inverted microscope, revealing the characteristic processes present on the surface of the dendritic cells (**Figure 5**).

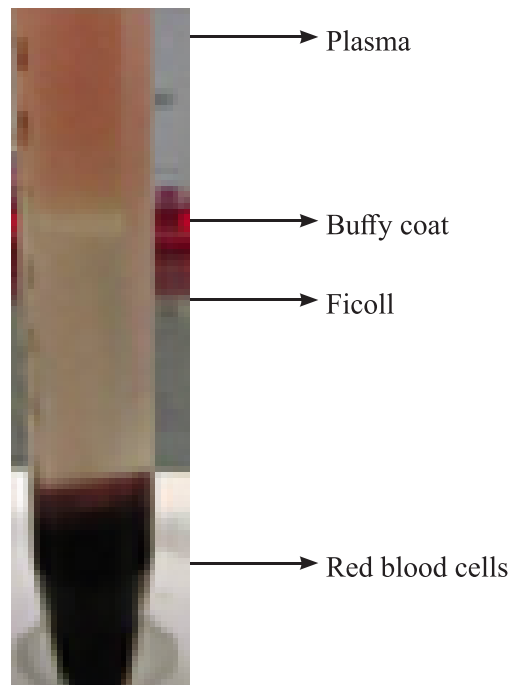


Figure 4. Buffy coat (second layer) isolated from PBMC

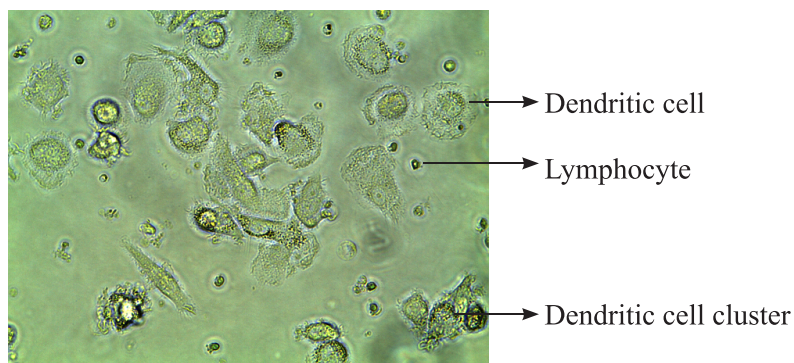


Figure 5. Observation of dendritic cells cultured without collagen after 8 days under the inverted microscope (40×)

6. Seeding of dendritic cells in the collagen scaffold and its maturation study

Monocytes from peripheral blood were observed after 24 hours of culture in the RPMI medium. The figure shows the immature dendritic cells (**Figure 6**). These cells were then injected into the collagen scaffold (various percentages of gel) and were observed after 7 days by removing the gel and placing it on a glass slide (**Figure 7**). The images of the collagen gel were observed under 40× magnification using an inverted microscope (**Figure 8**).

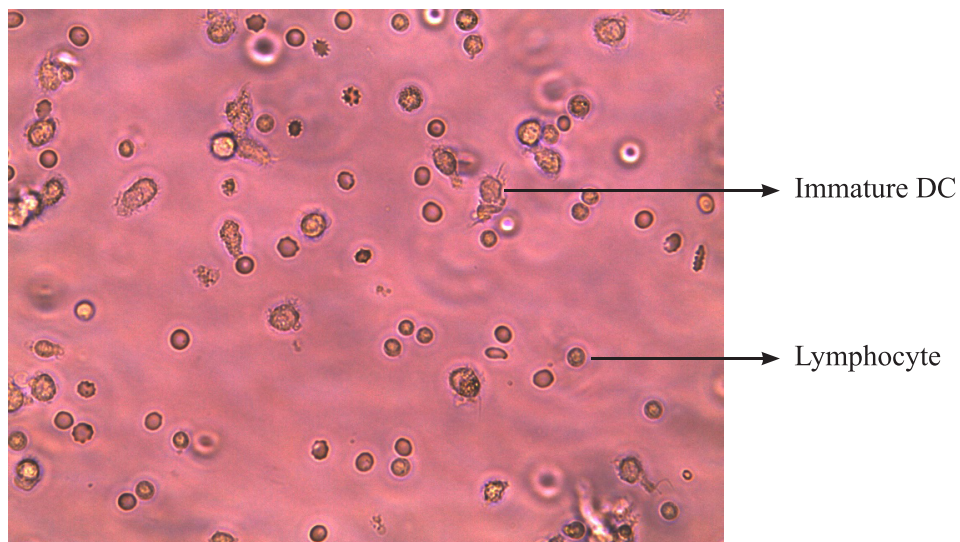


Figure 6. Observation of dendritic cells after 24 hours under the inverted microscope (40×)



Figure 7. The scaffold gel on a glass slide

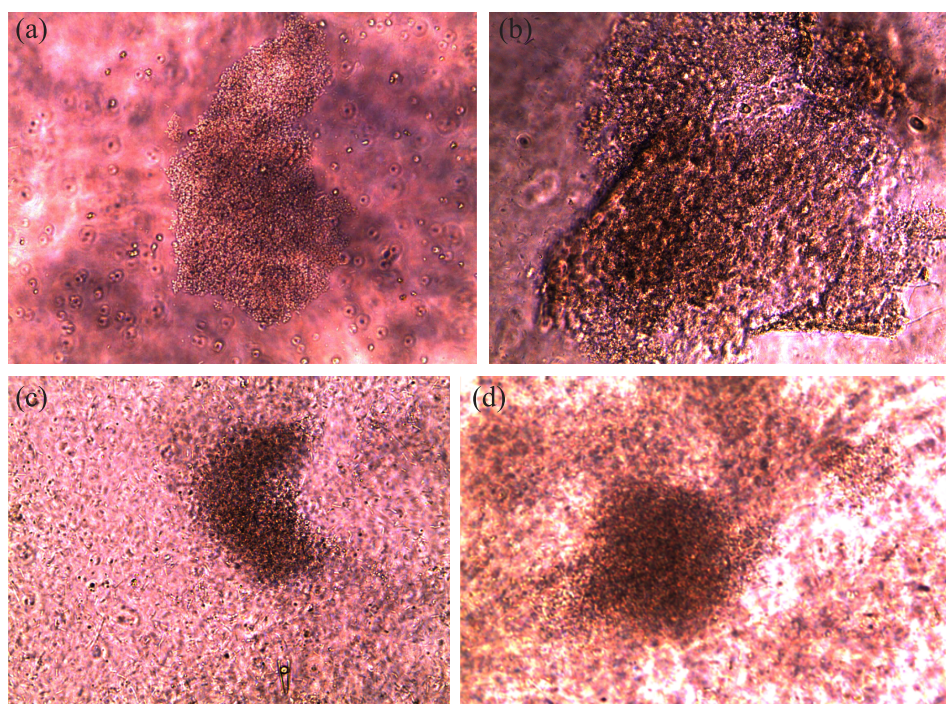


Figure 8. (a) Growth of dendritic cells into tissue in the 3D collagen scaffold 10% gel (rat tail collagen); (b) Growth of dendritic cells into tissue in the 3D collagen scaffold 15% gel (rat tail collagen); (c) Growth of dendritic cells into tissue in the 3D collagen scaffold 10% gel (bovine tendon collagen); (d) Growth of dendritic cells into tissue in the 3D collagen scaffold 15% gel (bovine tendon collagen)

7. Discussion

Type 1 collagen is extracted from bovine tendon, an inexpensive source, and from rat tail tendons, where collagen exists in pure form. Recent advances in medical technology have enabled the development of 3D DC culture systems that simulate the *in vivo* environment. Hydrated collagen gels are used in 3D cell culture systems because the structure of the collagen network resembles that of several tissues, including the dermis and the reticular stroma of lymph nodes ^[22,23].

In this study, a collagen-agarose scaffold was prepared and used for DC culture. Dendritic cell populations were isolated from monocytes in peripheral blood and seeded into the collagen-agarose scaffold. The results were consistent with previous findings ^[19]. DC maturation is a topic of current interest due to the wide array of potential applications for these cells in augmenting immune responses *in vivo*. Collagen, a ubiquitous and important component of the extracellular matrix, could mediate such maturation ^[24].

Dendritic cell maturation was studied in the presence and absence of collagen. In the absence of collagen, individual cells developed visible dendrites after 6–7 days of culture, and clusters of dead cells were seen after 10 days. Culturing follicular dendritic cells in a 3D collagen matrix allows physiological maturation to occur ^[17]. Exposure to type I collagen can promote the maturation of immature DCs ^[18]. Culturing peripheral blood mononuclear cells (PBMCs) with GM-CSF and agarose or polylactic acid results in the detachment of cells from the culture dish and the formation of cell clumps, characteristic of DC development. DCs cultured on agarose membranes showed visible cell detachment and cluster formation, possibly due to the hydrophilic nature of the agarose membrane ^[25].

The presence of collagen in the study led to tissue maturation and aggregation in collagen-agarose gels made from bovine and rat tail tendons. The triple helical nature of collagen and the three-dimensional structure of the scaffold promoted the growth of DCs in tissues. Without completely depleting all LPS or collagen in solution, a collagen-mediated effect during DC maturation cannot be ruled out ^[24]. Consistent with previous findings, this study observed that collagen supported DC maturation.

8. Biomedical application of dendritic cells

Dendritic cells (DCs) are a type of white blood cell that play a crucial role in the human body's immune system. DCs have many functions, including the ability to recognize pathogens, activate other immune cells, and present antigens to other immune cells. In recent years, they have become an important tool in biomedical research and have been used to treat a variety of diseases, including cancer, HIV/AIDS, and autoimmune disorders. In cancer therapy, DCs have been employed to stimulate the body's natural immune response to attack tumor cells. In HIV/AIDS therapy, DCs have been utilized to reduce the viral load and improve patients' quality of life. In autoimmune diseases, DCs have been used to reduce inflammation and modify the immune response.

DCs have also been instrumental in the development of vaccines. By introducing a specific antigen to DCs, they can induce an immune response to that particular antigen, leading to the development of vaccines for diseases such as influenza, hepatitis B, and HPV. Additionally, DCs have been used to deliver gene therapy. By introducing a gene into DCs, they can deliver it to other cells in the body. This approach has been applied to treat genetic diseases such as cystic fibrosis and to deliver gene therapies for cancer. Lastly, DCs have been used in regenerative medicine. By introducing stem cells into DCs, they can stimulate the growth of new cells and tissues, which have been used to regenerate damaged tissues, such as heart muscle after a heart attack ^[26].

Dendritic cells are highly specialized immune cells known for their unique ability to capture, process, and present antigens to other immune cells, thereby initiating and shaping immune responses. Due to these

remarkable properties, dendritic cells have been extensively studied for their biomedical applications. Notable applications include:

- (1) Cancer immunotherapy: Dendritic cell-based immunotherapies have shown promise in cancer treatment. DCs can be isolated from a patient's blood or generated in the laboratory, loaded with tumor-specific antigens, and re-introduced into the patient's body. These antigen-presenting dendritic cells help stimulate the patient's immune system to recognize and attack cancer cells more effectively.
- (2) Vaccines: Dendritic cells play a key role in vaccine development. They can be loaded with specific antigens, such as viral or bacterial antigens, to initiate a targeted immune response. By presenting these antigens to T cells, dendritic cells help activate and educate the immune system, leading to the production of pathogen-specific antibodies and memory T cells for long-term protection against infectious diseases.
- (3) Autoimmune diseases: Dendritic cells can be manipulated to regulate immune responses in autoimmune diseases. Through various strategies, such as targeting specific antigens or modifying their maturation and activation status, dendritic cells can be used to induce immune tolerance, preventing the immune system from attacking self-tissues in conditions like rheumatoid arthritis, multiple sclerosis, and type 1 diabetes.
- (4) Allergies and asthma: Dendritic cells also play a role in allergic reactions and asthma. By modulating dendritic cell function, researchers aim to develop therapies that can reduce allergic responses and promote immune tolerance to allergens, potentially offering relief to individuals suffering from these conditions.
- (5) Transplantation: Dendritic cell-based approaches are being explored to improve the success of organ and tissue transplantation. By manipulating dendritic cells, scientists aim to promote immune tolerance towards transplanted organs, reducing the need for lifelong immunosuppressive drugs and improving long-term graft survival.
- (6) Infectious diseases: Dendritic cells are key players in the immune response against pathogens. Research is ongoing to better understand the interactions between dendritic cells and various infectious agents, such as HIV, tuberculosis, and malaria. This knowledge can help in developing novel approaches to enhance immune responses and combat infectious diseases.

9. Conclusion

The extraction of type 1 collagen from bovine tendons and rat tails is more cost-effective compared to commercially available collagen from Recombinant DNA Technology and other 3D cell culture systems. This collagen can be used as a three-dimensional scaffold for cell culture systems, as culturing dendritic cells on this scaffold resulted in mature tissue aggregates of dendritic cells. The phenotypic changes and antigen-presenting abilities of these mature dendritic cells were investigated. These 3D collagen gels can serve as a platform for studying cell microenvironments and conducting various cancer immunology research.

Disclosure statement

The author declare no conflict of interest.

References

- [1] Edmondson R, Broglie JJ, Adcock AF, et al., 2014, Three-Dimensional Cell Culture Systems and Their Applications in Drug Discovery and Cell-Based Biosensors. *Assay Drug Dev Technol*, 12(4): 207–218. <https://doi.org/10.1089/adt.2014.573>
- [2] Ravi M, Paramesh V, Kaviya SR, et al., 2015, 3D Cell Culture Systems: Advantages and Applications. *J Cell Physiol*, 230(1): 16–26. <https://doi.org/10.1002/jcp.24683>
- [3] Chaicharoenaudomrung N, Kunhorm P, Noisa P, 2019, Three-Dimensional Cell Culture Systems as An In Vitro Platform for Cancer and Stem Cell Modeling. *World J Stem Cells*, 11(12): 1065–1083. <https://doi.org/10.4252/wjsc.v11.i12.1065>
- [4] Haycock JW, 2011, 3D Cell Culture: A Review of Current Approaches and Techniques. *Methods Mol Biol*, 695: 1–15. https://doi.org/10.1007/978-1-60761-984-0_1
- [5] Page H, Flood P, Reynaud EG, 2013, Three-Dimensional Tissue Cultures: Current Trends and Beyond. *Cell Tissue Res*, 352(1): 123–131. <https://doi.org/10.1007/s00441-012-1441-5>
- [6] Banchereau J, Briere F, Caux C, et al., 2000, Immunobiology of Dendritic Cells. *Annu Rev Immunol*, 18: 767–811. <https://doi.org/10.1146/annurev.immunol.18.1.767>
- [7] Liu K, Nussenzweig MC, 2010, Origin and Development of Dendritic Cells. *Immunol Rev*, 234(1): 45–54. <https://doi.org/10.1111/j.0105-2896.2009.00879.x>
- [8] Cabeza-Cabrerizo M, Cardoso A, Minutti CM, et al., 2021, Dendritic Cells Revisited. *Annu Rev Immunol*, 39: 131–166. <https://doi.org/10.1146/annurev-immunol-061020-053707>
- [9] Besseau L, Coulomb B, Lebreton-Decoster C, et al., 2002, Production of Ordered Collagen Matrices for Three-Dimensional Cell Culture. *Biomaterials*, 23(1): 27–36. [https://doi.org/10.1016/s0142-9612\(01\)00075-8](https://doi.org/10.1016/s0142-9612(01)00075-8)
- [10] Sheu MT, Huang JC, Yeh GC, et al., 2001, Characterization of Collagen Gel Solutions and Collagen Matrices for Cell Culture. *Biomaterials*, 22(13): 1713–1719. [https://doi.org/10.1016/s0142-9612\(00\)00315-x](https://doi.org/10.1016/s0142-9612(00)00315-x)
- [11] Rossert J, de Crombrughe B, 2002, Chapter 12 - Type I Collagen: Structure, Synthesis, and Regulation, in *Principles of Bone Biology* (Second Edition). Academic Press, 189–210. <https://doi.org/10.1016/B978-012098652-1/50114-1>
- [12] Ratanavaraporn J, Damrongsakkul S, Sanchavanakit N, et al, 2017, Comparison of Gelatin and Collagen Scaffolds for . *J Met Mater Miner*, 16(1).
- [13] Cruz LJ, Rueda F, Cordobilla B, et al., 2011, Targeting Nanosystems to Human DCs via Fc Receptor as An Effective Strategy to Deliver Antigen for Immunotherapy. *Mol Pharm*, 8(1): 104–116. <https://doi.org/10.1021/mp100178k>
- [14] Satthaporn S, Eremin O, 2001, Dendritic Cells (I): Biological Functions. *J R Coll Surg Edinb*, 46(1): 9–19.
- [15] Bhardwaj RS, Schwarz A, Becher E, et al., 1996, Pro-Opiomelanocortin-Derived Peptides Induce IL-10 Production in Human Monocytes. *J Immunol*, 156(7): 2517–2521.
- [16] Patente TA, Pinho MP, Oliveira AA, et al., 2019, Human Dendritic Cells: Their Heterogeneity and Clinical Application Potential in Cancer Immunotherapy. *Front Immunol*, 9: 3176. <https://doi.org/10.3389/fimmu.2018.03176>
- [17] Gunzer M, Schäfer A, Borgmann S, et al., 2000, Antigen Presentation in Extracellular Matrix: Interactions of T Cells with Dendritic Cells are Dynamic, Short Lived, and Sequential. *Immunity*, 13(3): 323–332. [https://doi.org/10.1016/s1074-7613\(00\)00032-7](https://doi.org/10.1016/s1074-7613(00)00032-7)
- [18] Brand U, Bellinghausen I, Enk AH, et al., 1998, Influence of Extracellular Matrix Proteins on the Development of Cultured Human Dendritic Cells. *Eur J Immunol*, 28(5): 1673–1680. [https://doi.org/10.1002/\(SICI\)1521-4141\(199805\)28:05<1673::AID-IMMU1673>3.0.CO;2-J](https://doi.org/10.1002/(SICI)1521-4141(199805)28:05<1673::AID-IMMU1673>3.0.CO;2-J)
- [19] Tasaki A, Yamanaka N, Kubo M, et al., 2004, Three-Dimensional Two-Layer Collagen Matrix Gel Culture Model for Evaluating Complex Biological Functions of Monocyte-Derived Dendritic Cells. *J Immunol Methods*, 287(1–2): 79–90. <https://doi.org/10.1016/j.jim.2004.01.014>
- [20] Yoshida M, Babensee JE, 2004, Poly(Lactic-Co-Glycolic Acid) Enhances Maturation of Human Monocyte-Derived

Dendritic Cells. *J Biomed Mater Res A*, 71(1): 45–54. <https://doi.org/10.1002/jbm.a.30131>

- [21] Haston WS, Shields JM, 1984, Contraction Waves in Lymphocyte Locomotion. *J Cell Sci*, 68: 227–241. <https://doi.org/10.1242/jcs.68.1.227>
- [22] Friedl P, Bröcker EB, 2000, The Biology of Cell Locomotion Within Three-Dimensional Extracellular Matrix. *Cell Mol Life Sci*, 57(1): 41–64. <https://doi.org/10.1007/s000180050498>
- [23] Gunzer M, Kämpgen E, Bröcker EB, et al., 1997, Migration of Dendritic Cells in 3D-Collagen Lattices. Visualisation of Dynamic Interactions with the Substratum and the Distribution of Surface Structures via A Novel Confocal Reflection Imaging Technique. *Adv Exp Med Biol*, 417: 97–103.
- [24] Lutz MB, Suri RM, Niimi M, et al., 2000, Immature Dendritic Cells Generated with Low Doses of GM-CSF in the Absence of IL-4 are Maturation Resistant and Prolong Allograft Survival In Vivo. *Eur J Immunol*, 30(7): 1813–1822. [https://doi.org/10.1002/1521-4141\(200007\)30:7<1813::AID-IMMU1813>3.0.CO;2-8](https://doi.org/10.1002/1521-4141(200007)30:7<1813::AID-IMMU1813>3.0.CO;2-8)
- [25] Yoshida M, Babensee JE, 2006, Differential Effects of Agarose and Poly(Lactic-Co-Glycolic Acid) on Dendritic Cell Maturation. *J Biomed Mater Res A*, 79(2): 393–408. <https://doi.org/10.1002/jbm.a.30798>
- [26] Stiriba SE, Frey H, Haag R, 2002, Dendritic Polymers in Biomedical Applications: From Potential to Clinical Use in Diagnostics and Therapy. *Angew Chem Int Ed Engl*, 41(8): 1329–1334. [https://doi.org/10.1002/1521-3773\(20020415\)41:8<1329::aid-anie1329>3.0.co;2-p](https://doi.org/10.1002/1521-3773(20020415)41:8<1329::aid-anie1329>3.0.co;2-p)

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Surveillance Report of the Prevalence and Risk Factors of Chronic Non-Communicable Diseases in Tinghu District, Yancheng City, 2021

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Abstract: *Objective:* To comprehensively understand the changes and prevalence of major chronic diseases among residents of Tinghu District, Yancheng City, in 2021, and to analyze the trends of the major risk factors for the onset of chronic diseases in the region. *Methods:* Chronic diseases and their risk factors in Tinghu District in 2021 were monitored among the resident population who had lived in the district for five years or more and were aged 18 years or older. The survey was conducted using random cluster sampling, with 7,130 questionnaires collected. After data processing, 7,012 valid questionnaires were obtained, resulting in a qualification rate of 98.35%. *Results:* Among the chronic diseases reported in the survey population, hypertension had the highest prevalence at 37.61%, followed by dyslipidemia at 37.19%. Other chronic diseases were ranked in order of prevalence from highest to lowest. Regardless of gender, the top three chronic diseases were hypertension, diabetes, and hyperlipidemia. Multifactorial regression analysis identified both non-preventable risk factors (such as family history, gender, and age) and preventable risk factors (such as smoking, sedentary behavior, overweight, and obesity) as significant contributors to the major chronic diseases in Tinghu District. *Conclusion:* Analyzing the trends in the main risk factors for chronic disease incidence in Tinghu District, Yancheng City, provides a basis for developing a new comprehensive chronic disease prevention and control plan to address chronic disease prevention and management.

Keywords: Chronic diseases; Non-communicable; Social factors; Monitoring report

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1. Introduction

With socio-economic development and accelerating urbanization, people are increasingly choosing refined foods and leading sedentary lifestyles, while psychological stress levels are rising ^[1]. Along with an aging population, China is facing a high burden of chronic diseases, posing significant challenges to healthcare services ^[2,3]. Currently, chronic non-communicable diseases (chronic diseases), primarily cardiovascular and cerebrovascular diseases, cancers, diabetes mellitus, and chronic respiratory diseases, are major health threats and leading causes of death ^[4]. Tinghu District, Yancheng City, conducted surveys on four chronic diseases

and their risk factors in 2011, 2012, 2016, and 2021, respectively, to understand the epidemiological status and trends of major chronic diseases and their risk factors. These surveys aimed to identify key intervention targets and priority health issues and to provide a scientific basis for developing chronic disease prevention and control plans across the district. With ongoing socio-economic advancements, improved living standards, and an aging population, new challenges continue to emerge in the prevention and control of chronic diseases in Tinghu District. To comprehensively assess the effectiveness of chronic disease prevention and control measures in the district and identify priority health issues, a new round of surveys on chronic diseases and their social factors was conducted from September 2021 to May 2022.

2. Purpose of the survey

The survey aimed to gain a comprehensive understanding of the changes and prevalence of major chronic diseases among residents of Tinghu District since 2017. It sought to assess the status of community environmental support, health services, and resources, the changes in residents' knowledge and skills related to chronic diseases, and the actual effectiveness of chronic disease prevention and control efforts. Furthermore, the survey aimed to analyze trends in major risk factors for chronic diseases in the district and identify priority health issues for residents. Additionally, it evaluated the current status of the support system for chronic disease prevention and treatment policies and assessed the effectiveness of comprehensive chronic disease prevention and control measures. This information will provide a basis for developing a new comprehensive chronic disease prevention and treatment plan for the region.

3. Materials and methods

3.1. Survey subjects

In 2021, the monitoring targets for chronic diseases and their risk factors in Tinghu District were permanent residents who had lived in the district for 5 years or more and were 18 years old or older (defined as those who had lived in the survey area for a total of 6 months or more).

3.2. Sampling method

To ensure that the monitoring samples were representative of the entire region, aligned with the socio-economic development of the area, and consistent with the age and sex ratio of the population, random whole-cluster sampling was used. This method also considered geographic distribution balance, cost-effectiveness, and the feasibility of the sampling scheme.

3.3. Content of the survey

The survey comprised two parts: a questionnaire and a physical examination.

- (1) Questionnaire content and method: (a) Family questionnaire on chronic diseases and their risk factors: This included basic family information, living environment, and family salt intake status; (b) Individual questionnaire on chronic diseases and their risk factors: This included basic personal information, history of major chronic diseases, family history, tobacco and alcohol use, dietary habits, physical activity, and residents' knowledge, behaviors, and attitudes related to chronic diseases.
- (2) Physical examination content and method: (a) Measurements included: height, weight, waist circumference, blood pressure, fasting blood glucose, and nutritional tests; (b) Blood pressure: Measurement was conducted according to the Chinese Guidelines for the Prevention and Control of

Hypertension (2018), with the average taken from three measurements; (c) Blood glucose monitoring: Fasting peripheral blood was used, with uniform specifications for blood glucose meters from the same manufacturer; (d) Nutritional monitoring: Nutritional status was assessed by drawing venous blood, testing blood lipid levels, and evaluating overall nutritional status.

3.4. Quality control

The specific quality control measures for this monitoring were as follows:

- (1) The District Health Committee organized supervisors to oversee the entire survey process.
- (2) The CDC sent staff for periodic or on-site supervision.
- (3) Strict implementation of operational procedures and norms was enforced, with all physical examination and monitoring equipment calibrated or debugged before and during use to ensure accurate measurements.
- (4) The survey response rate was required to be above 95%, with efforts to minimize the non-response rate.
- (5) The survey information was compiled into a database according to a unified standard, with data management services provided by a third-party software company.

The survey collected 7,130 questionnaires, of which 7,012 were valid after data processing, resulting in a response rate of 98.35%, meeting the sample size requirements.

3.5. Statistical analysis

In this report on the monitoring of chronic diseases and their risk factors, data were primarily analyzed using descriptive statistics, with a focus on stratification by gender and age. All monitoring questionnaires were double-entered using EpiData 3.1 software, which was uniformly prepared and distributed. After data cleaning, checking, and organization, statistical analysis was conducted using SPSS 26.0 and Excel 2010.

4. Survey results

4.1. Basic information of survey respondents

The survey collected 7,130 questionnaires, of which 7,012 were valid after data processing, resulting in a qualification rate of 98.35%. The sample size meets the required criteria. Among the respondents, 3,796 (54.14%) were male and 3,216 (45.86%) were female, with a male-to-female ratio of 1.18:1. In terms of age distribution, different age groups accounted for 7.94%, 46.79%, and 45.27% of the total valid questionnaires, respectively (**Table 1**).

Table 1. Age and gender distribution of the population with chronic diseases and their risk factors in Tinghu District in 2021

Age groups	Male		Female		Total	
	Number of people surveyed	Composition ratio (%)	Number of people surveyed	Composition ratio (%)	Number of people surveyed	Composition ratio (%)
18–34	295	7.77	262	8.15	557	7.94
35–59	1,768	46.58	1,513	47.05	3,281	46.79
≥ 60	1,733	45.65	1,441	44.80	3,174	45.27
Total	3,796	100.00	3,216	100.00	7,012	100.00

4.2. Prevalence of major chronic diseases

Among the chronic diseases reported in this survey population, hypertension had the highest prevalence at

37.61%, followed by dyslipidemia at 37.19%. The prevalence of other chronic diseases, in descending order, included diabetes mellitus (15.57%), malignant neoplasms (4.14%), chronic obstructive pulmonary disease (COPD; 2.21%), stroke (2.10%), coronary heart disease (1.11%), and osteoporosis (0.27%). Across all genders, the top three chronic diseases were hypertension, diabetes, and hyperlipidemia (**Table 2**).

Table 2. Prevalence of major chronic diseases among the population monitored for chronic diseases and their risk factors in Tinghu District, 2021

Chronic disease	Male		Female		Total	
	<i>n</i>	Rate (%)	<i>n</i>	Rate (%)	<i>n</i>	Rate (%)
Hypertension	1,461	38.49	1,172	36.44	2,633	37.55
Dyslipidemia	1,411	37.17	1,197	37.22	2,608	37.19
Diabetes	601	15.83	491	15.27	1,092	15.57
Stroke	77	2.03	70	4.57	147	2.10
Malignant neoplasms	175	4.61	115	3.58	290	4.14
Coronary heart disease	40	1.50	37	1.15	77	1.11
Chronic obstructive pulmonary disease	89	2.35	66	2.05	155	2.21
Osteoporosis	5	0.13	14	0.44	19	0.27

4.3. Monitoring and analysis of social factors

Using multifactorial unconditional logistic regression analysis, the study identified the following independent risk factors:

- (1) Hypertension: Family history of hypertension, advanced age, overweight/obesity, and smoking, with particularly prominent risks associated with overweight/obesity, advanced age, and smoking (**Table 3**).
- (2) Diabetes: Family history of diabetes, advanced age, dyslipidemia, and overweight/obesity, with particularly prominent risks associated with family history and dyslipidemia (**Table 4**).
- (3) COPD: Smoking was identified as an independent risk factor (**Table 5**).
- (4) Stroke: Advanced age, male gender, smoking, and family history of stroke were independent risk factors, with family history being particularly prominent (**Table 6**).
- (5) Coronary heart disease: Advanced age and smoking were identified as independent risk factors, with smoking being particularly prominent (**Table 7**).
- (6) Malignant neoplasms: Smoking, low literacy, and family history of malignant neoplasms were identified as independent risk factors, with family history being particularly prominent (**Table 8**).

Table 3. Multifactorial unconditional logistic regression analysis for hypertension

Variable	β -value	<i>OR</i>	95% CI	<i>P</i>
Age	0.030	1.031	1.026–1.035	0.000
Degree of education	0.407	1.502	1.129–1.996	0.005
Smoking	0.232	1.261	1.093–1.455	0.001
Alcohol consumption	0.052	1.054	0.909–1.222	0.489
Overweight and obesity	0.402	1.494	1.353–1.651	0.000
Dyslipidemia	0.114	1.121	1.011–1.244	0.031
Family history	0.158	1.171	1.053–1.302	0.004

Table 4. Multifactorial unconditional logistic regression analysis for diabetes mellitus

Variable	β -value	OR	95% CI	P
Age	0.014	1.014	1.009–1.020	0.000
Degree of education	-1.083	0.339	0.102–1.125	0.077
Smoking	0.153	1.165	0.989–1.372	0.068
Dyslipidemia	0.427	1.533	1.348–1.743	0.000
Overweight and obesity	0.360	1.434	1.258–1.633	0.000
family history	0.511	1.668	1.355–2.053	0.000

Table 5. Multifactorial unconditional logistic regression analysis for COPD

Variable	β -value	OR	95% CI	P
Age	0.000	1.000	0.983–1.018	0.961
Degree of education	16.898	0.750	0.636–0.885	0.999
Overweight and obesity	0.219	1.245	0.826–1.876	0.296
Dyslipidemia	-0.450	0.956	0.625–1.460	0.833
Smoking	0.895	2.447	1.599–3.745	0.000

Table 6. Multifactorial unconditional logistic regression analysis for stroke

Variable	β -value	OR	95% CI	P
Age	0.030	1.031	1.015–1.047	0.000
Dyslipidemia	0.265	1.303	0.917–1.852	0.139
Family history	0.835	2.306	1.252–4.247	0.000
Smoking	0.703	0.019	1.314–3.102	0.001

Table 7. Multifactorial unconditional logistic regression analysis for coronary heart disease

Variable	β -value	OR	95% CI	P
Age	0.082	1.085	1.063–1.108	0.000
Smoking	0.654	1.923	1.142–3.239	0.004
Family history	0.045	1.047	0.325–3.374	0.939

Table 8. Multifactorial unconditional logistic regression analysis for malignant neoplasms

Variable	β -value	OR	95% CI	P
Age	0.013	1.013	1.001–1.026	0.039
Degree of education	-2.007	0.134	—	0.001
Family history	0.651	1.918	1.295–2.839	0.001
Smoking	0.383	1.466	1.183–2.186	0.002

5. Main findings

- (1) The prevalence of chronic diseases is high, with an overall increase in the crude incidence rate compared to the 2016 survey. Among the chronic diseases reported by the survey population, hypertension had the highest prevalence at 37.55%, followed by dyslipidemia at 37.19%, diabetes mellitus at 15.57%, malignant tumors at 4.14%, chronic obstructive pulmonary disease at 2.21%, stroke at 2.10%, coronary heart disease at 1.10%, and osteoporosis at 0.27%. Except for osteoporosis, the prevalence rates for these conditions are all greater than 1%.
- (2) A significant number of cases of hypertension, diabetes mellitus, and dyslipidemia remain undiagnosed, with poor overall control rates for blood pressure, blood glucose, and lipids. The comprehensive dyslipidemia rate in this survey was 37.19%, the awareness rate of dyslipidemia among those affected was 19.56%, and the treatment rate was 13.08%.
- (3) Multifactorial regression analysis identified the following risk factors for major chronic diseases in Tinghu District: non-preventable factors such as family history, gender, and age; and preventable factors such as smoking, sedentary lifestyle, overweight, obesity, and abdominal obesity.

6. Recommendations

- (1) In light of the aging population, it is recommended that the government further promote policies that integrate elderly care with medical services to achieve comprehensive coverage^[5,6].
- (2) Continually promote knowledge of tumor prevention and treatment, encourage tumor screening, and other early diagnosis and treatment techniques, and gradually meet the public's needs for regular physical examinations^[7,8].
- (3) Enhance the screening of chronic patients and high-risk groups, such as those with hypertension, diabetes, and hyperlipidemia, to achieve integrated management of these conditions^[9].
- (4) Enrich the scope of healthy living initiatives in Tinghu District to reduce and control the prevalence of chronic disease risk factors^[10].

7. Summary

The 2021 survey of chronic non-communicable diseases and social factors in Tinghu District, Yancheng City, employed a standardized design, strict organization, rigorous quality control, and scientific analysis. The survey effectively captured changes in major chronic disease prevalence and knowledge and skills related to chronic diseases among residents from 2017 to 2021. The findings reflect some success in the prevention and treatment of chronic diseases. The understanding and analysis of the main risk factor trends in chronic diseases in Tinghu District provide a basis for developing a new comprehensive chronic disease prevention and treatment plan.

Disclosure statement

The authors declare no conflict of interest.

References

- [1] Xu Y, Guo YF, Liu Z, et al., 2022, Analysis of Cardiovascular-Metabolic Risk Factor Aggregation and Relationship with Related Demographic and Economic Factors Among Adults in Shenzhen. *Modern Preventive Medicine*, 49(21): 3998–

4002.

- [2] Zhang Y, Kelsang P, Qiu H, et al., 2022, Correlation Study of Chronic Diseases and Influencing Factors in Tibetan Population in the Western Region of Tibet's Ali District. *Modern Preventive Medicine*, 49(23): 4246–4252.
- [3] Zhu H, Huang X, Zhou Y, et al., 2022, Analysis of Prevalence Characteristics and Influencing Factors of Common Chronic Diseases Among Urban and Rural Elderly in Hebei Province in 2018. *Modern Preventive Medicine*, 49(24): 4417–4422.
- [4] Ding X, Tang W, Chen L, et al., 2023, Co-Occurrence of Hypertension, Hyperlipidemia and Hyperglycemia and Related Influencing Factors Among Chongqing Residents Aged 30–79 Years. *China Chronic Disease Prevention and Control*, 31(1): 31–34.
- [5] Tan M, Liang Y, Chen Y, et al., 2023, Distribution of Chronic Disease Risk Factors and Their Aggregation Analysis Among Jiangmen Residents. *Modern Preventive Medicine*, 50(14): 2519–2525 + 2546.
- [6] Li L, Xiao L, Zhang D, 2024, Study on Factors Influencing the Number of Chronic Diseases Among Elderly Co-Morbid Patients in Guangdong Province Based on Health Ecology Model. *Chinese Family Medicine*, 27(2): 208–216.
- [7] Shen T, Wang Y, Jin W, et al., 2023, Risk Factor Analysis and Risk Modeling of Cognitive Decline in Elderly Patients with Chronic Diseases. *Journal of Jilin University (Medical Edition)*, 49(5): 1304–1309.
- [8] Liu Y, Liu Q, Liu H, et al., 2024, Analysis of Chronic Disease Co-Morbidity and Influencing Factors Among Hunan Residents. *China Chronic Disease Prevention and Control*, 32(2): 126–129.
- [9] Liu X, Yang Q, Liu D, et al., 2024, Mining the Association Situation of Chronic Disease-Related Behavioral Risk Factors Among Residents Over 35 Years Old in Shanghai. *China Health Statistics*, 41(1): 68–71.
- [10] Zhang R, Yi Z, Xu T, et al., 2024, Analysis of Chronic Disease Prevention and Control Resource Allocation in National Chronic Disease Comprehensive Prevention and Control Demonstration and Non-Demonstration Areas in 2020. *China Chronic Disease Prevention and Control*, 32(3): 193–197.

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Clinical Efficacy of Laparoscopic Radical Colorectal Cancer Treatment for Colorectal Cancer and Its Effect on Immune Function

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Abstract: *Objective:* To explore the therapeutic effect of laparoscopic radical colorectal cancer treatment in colorectal cancer patients. *Methods:* A total of 50 colorectal cancer patients treated between August 2018 and August 2023 were randomly divided into two groups: Group A underwent laparoscopic radical colorectal cancer surgery, while Group B received open surgery. Clinical indicators, inflammatory factors, immune function indicators, and complications were compared between the two groups. *Results:* Group A showed significantly shorter operation times, faster recovery times, and reduced hospital stays compared to Group B. Additionally, Group A had less abdominal drainage and intraoperative bleeding ($P < 0.05$). Levels of interleukin (IL)-4, IL-6, ultrasensitive C-reactive protein (hs-CRP), and tumor necrosis factor-alpha (TNF- α) were lower in Group A compared to Group B ($P < 0.05$). Furthermore, immune function indicators, including CD3⁺, CD4⁺, CD8⁺, and CD4⁺/CD8⁺ ratios, were better in Group A ($P < 0.05$). The complication rate in Group A was also lower than in Group B ($P < 0.05$). *Conclusion:* Laparoscopic radical treatment for colorectal cancer is efficient and feasible, causing minimal immune function impairment and inflammatory response. It also shortens postoperative recovery time.

Keywords: Colorectal cancer; Laparoscopic radical colorectal cancer surgery; Immune function; Efficacy

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1. Introduction

Colorectal cancer is a common monocarcinoma type and accounts for a high proportion of malignant gastrointestinal diseases, the causative agent of which is unclear and may be related to diet and gastrointestinal pathology ^[1]. In the context of changing dietary and lifestyle habits, the risk of colorectal cancer is elevated and progresses at a younger age ^[2]. Currently, colorectal cancer is mostly treated with surgical protocols in clinical practice. Conventional open surgery can completely remove the lesions in the colorectal region, but it is traumatic and can increase the degree of damage to the abdominal tissues, which is not conducive to the recovery of colorectal cancer patients ^[3]. Laparoscopic surgery is a commonly used minimally invasive type of surgery, which can shorten the operation time and reduce the surgical incision, which is conducive to patient

recovery ^[4]. This paper discusses the effect of laparoscopic colorectal cancer radical surgery on 50 cases of colorectal cancer patients admitted from August 2018 to August 2023.

2. Materials and methods

2.1. General information

Fifty cases of colorectal cancer patients admitted to the hospital during August 2018–2023 were grouped using a random number table. The colorectal cancer patients’ data were compared between the two groups and were found to be comparable ($P > 0.05$; **Table 1**).

Table 1. Analysis of colorectal cancer patients’ data

Groups	Sex (n)		Age (years)		Duration of illness (months)		Body mass index (kg/m ²)	
	Male	Female	Interval	Mean	Interval	Mean	Interval	Mean
Group A ($n = 25$)	14 (56.00)	11 (44.00)	40–76	61.84 ± 1.88	7–12	10.15 ± 1.42	19–24	22.84 ± 1.42
Group B ($n = 25$)	15 (60.00)	10 (40.00)	40–77	61.82 ± 1.92	7–13	10.13 ± 1.41	19–25	22.91 ± 1.36
χ^2 / t	0.0821		0.0372		0.0500		0.1780	
P	0.7745		0.9705		0.9604		0.8595	

2.2. Inclusion and exclusion criteria

Inclusion criteria: (1) pathologically confirmed diagnosis of colorectal cancer, expected to survive for more than 1 year; (2) informed consent; (3) imaging suggests that the disease is located in the sigmoid colon, rectum, proximal colon, and other regions.

Exclusion criteria: (1) hematological system pathology; (2) history of colorectal surgery; (3) complete intestinal obstruction; (4) intussusception.

2.3. Methods

- (1) Group A – Laparoscopic radical surgery for colorectal cancer: Patients received general anesthesia and were positioned in the lithotomy position. A CO₂ pneumoperitoneum was created with an abdominal pressure of 10–15 mmHg. The main operation port, measuring 12 mm in diameter, was placed 5 cm to the left of the umbilicus, while secondary ports, each 5 mm in diameter, were positioned in the right and left epigastric midclavicular regions and the right lower abdominal region. A laparoscope was inserted through the main port to observe the abdominal organs and assess for metastasis. The procedure included local blood vessel separation and ligation, mesenteric vessel processing, lymph node dissection, and mobilization of the hepatic flexure of the colon. The lesion, along with a 5 cm margin of the affected intestinal segment, was resected. The intestinal ends were then anastomosed, the abdominal cavity was irrigated with saline and drained, and the incisions were closed.
- (2) Group B – Open laparotomy: Patients also received general anesthesia and were positioned in the lithotomy position. A catheter was placed, and a 20 cm incision was made at the superior edge of the umbilicus. The abdominal wall was opened layer by layer, and the mesentery and retroperitoneal tissues were dissected. The mesenteric arteries and veins were ligated, lymph nodes were cleared, and the cancerous tissue was excised. Corresponding blood vessels were tied off, the abdominal cavity was rinsed, and a drainage tube was left in place.

2.4. Observation indexes

- (1) Clinical indicators: Recorded data included surgical operation time, time to first flatus, duration of hospital stay, volume of abdominal drainage, and intraoperative blood loss.
- (2) Inflammatory factors: A 3 mL sample of fasting venous blood was collected, and the supernatant was centrifuged. Interleukin-4 (IL-4), IL-6, and tumor necrosis factor-alpha (TNF- α) levels were measured using enzyme-linked immunosorbent assay (ELISA), and C-reactive protein (CRP) was monitored using an automated biochemical analyzer.
- (3) Immune function: Flow cytometry was used to detect CD3⁺, CD4⁺, and CD8⁺ cells, and to calculate the CD4⁺/CD8⁺ ratio.
- (4) Complications: Recorded complications included urinary tract infections, incision infections, intestinal obstruction, and others.

2.5. Statistical analysis

Data were processed using SPSS 21.0 software. Categorical data were described using percentages and analyzed with the chi-squared (χ^2) test, while continuous data were presented as mean \pm standard deviation (SD) and analyzed with the *t*-test. A *P*-value < 0.05 was considered statistically significant.

3. Results

3.1. Clinical indicators

Table 2 shows that the surgical operation time, exhaustion time, and hospitalization time of Group A were shorter than that of Group B. Abdominal drainage and intraoperative bleeding were also lesser in Group A ($P < 0.05$).

Table 2. Comparison of clinical indicators (mean \pm SD)

Groups	Operation time (min)	Exhaustion time (d)	Hospitalization time (d)	Abdominal drainage (mL)	Intraoperative bleeding (mL)
Group A (<i>n</i> = 25)	151.25 \pm 6.84	3.61 \pm 0.48	8.51 \pm 1.25	101.84 \pm 7.25	142.84 \pm 8.43
Group B (<i>n</i> = 25)	184.25 \pm 8.44	5.48 \pm 0.69	15.44 \pm 2.43	126.36 \pm 8.49	215.36 \pm 9.68
<i>t</i>	15.1882	11.1239	12.6800	10.9814	28.2483
<i>P</i>	0.0000	0.0000	0.0000	0.0000	0.0000

3.2. Inflammatory factor indicators

After surgery, IL-4, IL-6, hs-CRP, and TNF- α were significantly lower in Group A than in Group B ($P < 0.05$), as shown in **Table 3**.

Table 3. Comparison of inflammatory factor indicators before and after operation (mean \pm SD)

Groups	IL-4 (pg/mL)		IL-6 (pg/mL)		hs-CRP (mg/L)		TNF- α (pg/mL)	
	Before	After	Before	After	Before	After	Before	After
Group A (<i>n</i> = 25)	87.11 \pm 2.87	62.11 \pm 1.43	32.72 \pm 2.42	12.65 \pm 1.25	3.68 \pm 1.21	2.21 \pm 0.84	82.38 \pm 2.48	50.61 \pm 1.74
Group B (<i>n</i> = 25)	87.16 \pm 2.89	75.36 \pm 1.89	32.69 \pm 2.41	24.21 \pm 1.68	3.69 \pm 1.18	4.39 \pm 0.96	82.39 \pm 2.51	65.75 \pm 1.96
<i>t</i>	0.0614	27.9533	0.0439	27.6025	0.0296	8.5449	0.0142	28.8830
<i>P</i>	0.9513	0.0000	0.9652	0.0000	0.9765	0.0000	0.9888	0.0000

3.3. Immune function indexes

After operation, CD3⁺, CD4⁺, CD8⁺, and CD4⁺/CD8⁺ indexes of Group A were better than that of Group B ($P < 0.05$; Table 4).

Table 4. Comparison of immune function indexes before and after operation (mean \pm SD)

Groups	CD3+ (%)		CD4+ (%)		CD8+ (%)		CD4 ⁺ /CD8 ⁺	
	Before	After	Before	After	Before	After	Before	After
Group A (n = 25)	32.84 \pm 2.14	60.75 \pm 2.84	30.58 \pm 2.74	45.42 \pm 3.18	33.52 \pm 3.84	21.68 \pm 2.42	0.82 \pm 0.12	2.31 \pm 0.32
Group B (n = 25)	32.89 \pm 2.12	46.11 \pm 2.76	30.61 \pm 2.75	38.06 \pm 3.07	33.54 \pm 3.82	28.44 \pm 2.68	0.84 \pm 0.14	1.06 \pm 0.25
<i>t</i>	0.0830	18.4839	0.0386	8.3256	0.0185	9.3605	0.5423	15.3911
<i>P</i>	0.9342	0.0000	0.9693	0.0000	0.9853	0.0000	0.5901	0.0000

3.4. Complication indicators

Table 5 shows that the rate of postoperative complications in Group A was lower than in Group B ($P < 0.05$).

Table 5. Analysis of postoperative complication indicators of colorectal cancer [*n* (%)]

Groups	Urinary tract infections	Incisional infections	Bowel obstruction	Incidence rate
Group A (n = 25)	1 (4.00)	0 (0.00)	0 (0.00)	1 (4.00)
Group B (n = 25)	3 (12.00)	2 (8.00)	1 (4.00)	6 (24.00)
χ^2	-	-	-	4.1528
<i>P</i>	-	-	-	0.0416

4. Discussion

Colorectal cancer has a complex pathogenesis related to diet, environment, digestive system lesions, heredity, and other factors. It often presents no specific signs at the initial stage, leading some patients to delay treatment until the disease is advanced. Therefore, it is crucial to diagnose and treat colorectal cancer as early as possible [5,6]. Laparotomy for colorectal cancer can completely remove the tumor and lymph nodes, with a low risk of tumor recurrence after surgery. However, laparotomy involves a large incision, which can trigger physiological and psychological stress reactions, increase patients' pain, and prolong postoperative recovery time [7,8]. Additionally, some frail colorectal cancer patients are bedridden for a long time after surgery, increasing the risk of deep vein thrombosis and lung infection, which is unfavorable for prognosis [9,10].

In recent years, laparoscopic radical surgery for colorectal cancer has been increasingly used in clinical treatment. This minimally invasive surgery involves inserting laparoscopic instruments into the abdominal cavity to complete the necessary procedures, achieving similar efficacy to open surgery. Compared to open surgery, the advantages of laparoscopic surgery include the following [11,12]:

- (1) Reduced trauma: In laparoscopic surgery, 3–4 small incisions are made to perform the operation, reducing abdominal trauma and postoperative pain and promoting quicker recovery.
- (2) Wide applicability: This procedure can be used to treat colorectal cancer patients who are obese, elderly, or have other underlying conditions. It can be safely performed by skilled surgeons.
- (3) Lower complication rate: Large open abdominal incisions increase the amount of bleeding and expose

the abdominal cavity to air for extended periods, raising the risk of infection. Laparoscopic surgery minimizes these risks.

- (4) High accuracy: The laparoscope provides an expanded view of the surgical field, helping surgeons accurately locate lymph nodes and improve surgical precision.
- (5) Improved aesthetics: Open laparotomies often leave noticeable scars, while laparoscopic surgery results in small incisions and minimal scarring, preserving the abdomen's appearance.

Furthermore, laparoscopic radical surgery has become well-developed and is recognized for its efficacy by many colorectal cancer patients. However, it is essential to note that laparoscopic surgery requires high technical skill from the surgeon. Additionally, the procedure may affect gastrointestinal function, so patients should be advised to eat correctly and avoid stimulating and greasy foods postoperatively to shorten recovery time^[13,14].

Based on the data analysis in this paper, the surgical outcomes for colorectal cancer patients in Group A were better than those in Group B, with $P < 0.05$. This can be attributed to laparoscopy's ability to expand the surgical field and enhance surgical precision, allowing for complete lesion resection and prolonged patient survival. The smaller incisions in laparoscopic surgery also reduce damage to adjacent tissues, decrease intraoperative bleeding, and facilitate easier operation for the physician, which can shorten hospital stays for colorectal cancer patients^[15,16].

Another set of data showed that levels of IL-4, IL-6, hs-CRP, and TNF- α in Group A were lower than in Group B, with $P < 0.05$. This finding suggests that complete resection of cancerous tissue using laparoscopy can minimize mechanical stimuli and stress reactions, reduce immunosuppression, and enhance immune function^[17,18]. Laparoscopic surgery does not expose the abdominal cavity, reducing the impact of external stimuli on abdominal tissues, leading to a milder inflammatory response and better maintenance of body function stability.

Additional data showed that the immune markers CD3⁺, CD4⁺, CD8⁺, and CD4⁺/CD8⁺ were better in Group A compared to Group B, with $P < 0.05$. The precision of laparoscopic surgery, which expands the surgeon's operating field and reduces surgical trauma, supports gastrointestinal recovery, and has a smaller impact on immune function, resulting in better immune indices than open surgery^[19,20].

The final set of data indicated that the postoperative complication rate was lower in Group A than in Group B, with $P < 0.05$. This is because laparoscopic surgery reduces the exposure of the abdominal cavity and avoids large-scale mechanical operations, preventing infections, gastrointestinal injuries, and other adverse events, thus ensuring high overall safety.

In conclusion, laparoscopic radical colorectal surgery for colorectal cancer patients enhances the body's immune function, reduces inflammatory responses, and is safe and efficient, making it a valuable treatment option.

Disclosure statement

The author declares no conflict of interest.

References

- [1] Hu B, Chu C, Hu S, et al., 2022, Efficacy and Safety Analysis of Single-Port Plus One-Port Laparoscopic Radical Resection for Colorectal Cancer. *Journal of Laparoscopic Surgery*, 27(2): 124–129.
- [2] Mao Z, Du B, Sun H, et al., 2023, Analysis of Near-Term Efficacy and Long-Term Survival Outcomes of Laparoscopic Versus Open Surgery for Colorectal Cancer Patients Over 80 Years of Age. *Cancer Prevention and Control Research*, 50(11): 1121–1126.
- [3] Wang Y, Peng M, Xie W, et al., 2021, Analysis of Recent Efficacy of Two-Hole Laparoscopic Radical Resection for

Colorectal Cancer. *Chinese Journal of Gastrointestinal Surgery*, 24(1): 48–53.

- [4] Shu T, Wen H, Zhou H, et al., 2020, Study on the Effect and Safety of Laparoscopic Radical Colorectal Cancer Treatment for Elderly Colorectal Cancer. *Chongqing Medicine*, 49(2): 75–77.
- [5] Wei S, Guan S, Yang X, et al., 2022, Publication of Colorectal Cancer: Effect of Laparoscopic Radical Colorectal Cancer Treatment for Elderly Patients with Colorectal Cancer and the Effect on Gastrointestinal Function. *Journal of Interventional Radiology*, 31(5): 1.
- [6] Lu X, Zhao W, Huang P, 2023, Effect of Laparoscopic Radical Surgery for Colorectal Cancer in the Treatment of Obese Colorectal Cancer Patients and Its Effect on Gastrointestinal Hormones and Circulatory Function. *Laboratory Medicine and Clinics*, 20(19): 2872–2875.
- [7] Lou A, Niu G, Xu C, 2023, Effects of Laparoscopic Radical Rectal Cancer Surgery with Specimens Taken Through the Natural Cavity on Patients' Postoperative Recovery, Gastrointestinal Hormones, Body Fluids and Cellular Immunity. *Journal of Practical Cancer*, 38(8): 1308–1312.
- [8] Tang Y, 2023, Effect of Laparoscopic Radical Surgery on Gastrointestinal Function in Elderly Colorectal Cancer Patients. *Chinese and Foreign Medical Treatment*, 42(8): 49–53.
- [9] Shen L, Zhou S, Shi L, et al., 2023, Effects of Laparoscopic Radical Surgery on Stress Response, Immune Function Indexes and Complications in Patients with Rectal Cancer. *Zhejiang Trauma Surgery*, 28(1): 103–105.
- [10] Liu Y, Meng X, Zhang X, et al., 2020, Effect of Laparoscopic Radical Surgery on Stress Reaction and Gastrointestinal Hormone-Related Indexes in Patients with Rectal Cancer. *Journal of Clinical Military Medicine*, 2020(12): 1477–1478.
- [11] Lu J, Zhao S, Xie S, 2023, Clinical Study on Preservation of Left Colonic Artery in Laparoscopic Radical Rectal Cancer Surgery. *Chinese and Foreign Medicine*, 42(23): 60–63.
- [12] Jin J, Li X, Li S, et al., 2020, Clinical Study on the Preservation of Left Colonic Artery in Laparoscopic Radical Rectal Cancer Surgery. *Journal of Laparoscopic Surgery*, 25(5): 363–367.
- [13] Fu Y, Xu Q, 2020, Observation on the Effect of Laparoscopic and Open Radical Colorectal Cancer Surgery for Early Colorectal Cancer. *China Modern General Surgery Progress*, 23(7): 552–553.
- [14] Gao W, Tong Y, Meng Q, 2023, Effect of Laparoscopic Radical Surgery for Colorectal Cancer on Gastrointestinal Function and Trauma Recovery in Elderly Colorectal Cancer Patients. *Contemporary Medicine*, 29(3): 105–107.
- [15] Fan P, Xu H, Wang K, et al., 2021, Clinical Effect of Laparoscopic Radical Colorectal Cancer Surgery for Colorectal Cancer and Its Effect on Gastrointestinal Function. *Chinese Medical Science*, 11(12): 170–173.
- [16] Yu M, Zhang Y, Zhang W, 2021, Efficacy of Laparoscopic Radical Surgery for Colorectal Cancer in Treating Elderly Patients with Colorectal Cancer and Its Effect on Gastrointestinal Function. *Guizhou Medicine*, 45(11): 1747–1748.
- [17] Sun L, 2023, Effect of Laparoscopic Radical Surgery for Colorectal Cancer and Its Impact on Gastrointestinal Function of Patients. *Contemporary Medicine*, 29(12): 161–163.
- [18] Zhang H, Rong W, Wang Y, 2023, Effect of Pneumoperitoneum-Free Single-Port Laparoscopic Radical Colorectal Cancer Surgery on Stress Response and Complications in Colorectal Cancer Patients. *Cancer Progress*, 21(8): 874–876.
- [19] Zhang W, 2023, Analysis of the Effect of Laparoscopic Radical Surgery for Colorectal Cancer in the Treatment of Elderly Patients with Colorectal Cancer and Its Impact on Gastrointestinal Function. *China Practical Medicine*, 18(6): 10–14.
- [20] Jiang X, 2023, Clinical Efficacy Analysis of Laparoscopic Radical Surgery for Colorectal Cancer and Acute Intestinal Obstruction in Elderly Patients with Colorectal Cancer. *Chinese and Foreign Medical Treatment*, 42(8): 67–71.

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Optimization of Polysaccharides Extraction from *Physalis alkekengi* L. Peel and Its Effect on the Expression of Inflammation-Related Proteins in SW620 Cells

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Abstract: *Objective:* To establish an optimized aqueous extraction process for polysaccharides from *Physalis alkekengi* L. peel and to preliminarily explore its *in vitro* anti-inflammatory activity against colorectal cancer SW620 cells. *Methods:* A single-factor test combined with orthogonal test analysis was used to evaluate the effects of the material-to-liquid ratio, extraction temperature, and extraction time on the yield of polysaccharides from *Physalis alkekengi* L. peel. The antioxidant activity of the polysaccharides was assessed by analyzing their free radical scavenging ability *in vitro*, and the anti-inflammatory effect was evaluated using SW620 cells. *Results:* The optimal extraction conditions were a material-to-liquid ratio of m(g):V(mL) = 1:30, an extraction temperature of 100°C, and an extraction time of 40 minutes, with a predicted polysaccharide yield of 25.7%. The polysaccharides from *Physalis peruviana* peel effectively scavenged DPPH, superoxide anion, and hydroxyl radicals. After treatment with *Physalis peruviana* polysaccharides, the levels of IL-1 β , IL-18, and TNF- α in the cell culture medium were significantly reduced, and the phosphorylation level of P65 protein in SW620 cells was decreased. *Conclusion:* This extraction method is stable and reliable, and the prepared *Physalis alkekengi* L. polysaccharides exhibit significant *in vitro* antioxidant and anti-inflammatory activities. This study provides a theoretical basis for developing drugs for the prevention and treatment of colorectal cancer.

Keywords: *Physalis alkekengi* L. polysaccharide; Antioxidant; IL-1 β ; Extraction process; Colorectal cancer

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1. Introduction

Colorectal cancer (CRC) is the third most prevalent cancer worldwide. Despite a decline in the incidence of colon cancer over the past few decades, it remains one of the leading causes of cancer mortality and morbidity globally, posing a significant public health challenge ^[1]. Polysaccharides are polymers composed of aldose or ketose linked by glycosidic bonds and form a fundamental component of all living organisms, being

involved in various physiological functions ^[2]. Polysaccharides from traditional Chinese medicinal herbs exhibit pharmacological effects such as antioxidant, antitumor, antiviral, lipid-lowering, and blood glucose-lowering activities. In recent years, researchers have conducted antioxidant studies on hundreds of herbal monomers, active sites, and compound preparations, with polysaccharides showing strong antioxidant activity as biomacromolecules ^[3].

Physalis alkekengi L. belongs to the *Physalis* genus, and some secondary metabolites in this plant give it commercial value. Many compounds in *Physalis* have antibacterial, anti-inflammatory, and anticancer properties, making them valuable in the medical field. The whole plant of *Physalis* is used in traditional medicine to treat fever, diabetes, pharyngitis, boils, coughs, and mastitis. The main active components are saponins, polysaccharides, flavonoids, and others ^[4], among which polysaccharides from *Physalis alkekengi* L. possess anti-inflammatory, antitumor, and antioxidant effects ^[5]. It is hypothesized that the polysaccharides in *Physalis alkekengi* L. peel may have antioxidant activity, potentially offering therapeutic benefits for CRC treatment.

Inflammatory responses are involved in every stage of CRC development. This study uses single-factor and orthogonal test designs to optimize the extraction process and evaluate the free radical scavenging ability of *Physalis alkekengi* L. polysaccharides. The study also investigates the mechanism of action of these polysaccharides on inflammation in SW620 cells, providing a theoretical basis for developing targeted anti-inflammatory therapeutic strategies for CRC.

2. Materials and methods

2.1. Main materials

Physalis alkekengi L. peels were purchased by students from a *Physalis* production base in their hometown; Coomassie Brilliant Blue and ascorbic acid were purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd.; dihydroxybenzene was obtained from Shanghai Yuan Ye Biological Technology Co., Ltd.; colorectal cancer SW620 cells were procured from Wuhan Procell Life Technology Co., Ltd.; fetal bovine serum and DMEM medium were from Gibco, USA; P65, p-P65, and β -actin antibodies were from Cell Signaling Technology, USA; IL-1 β , IL-18, and TNF- α ELISA kits were from Wuhan Boster Biological Technology Co., Ltd.

2.2. Experimental methods

2.2.1. Single-factor extraction of polysaccharides from *Physalis alkekengi* L. peel

The *Physalis alkekengi* L. peel was washed, dried, and crushed through an 80-mesh sieve to obtain a fine powder. The powder was subjected to aqueous extraction under different conditions (material-to-liquid ratio, extraction temperature, and extraction time). The extraction solution was centrifuged, rotary evaporated, and concentrated to a certain volume, then precipitated with four times the volume of 95% ethanol at 4°C overnight and centrifuged at 5000 r/min for 20 minutes. Each sample was prepared and measured. Different extraction factors were set, and each experiment was repeated three times.

2.2.2. Orthogonal experiment

Based on the results of the single-factor experiments, an orthogonal experiment was designed, selecting A (material-to-liquid ratio [m(g):V(mL)]), B (extraction temperature [°C]), and C (extraction time [h]) for three-factor, three-level response conditions.

2.2.3. Process verification

Based on the optimized conditions obtained from the orthogonal experiment, three parallel experiments were conducted. The extracted polysaccharides were concentrated and freeze-dried to obtain refined *Physalis alkekengi* L. polysaccharides. Polysaccharide yield was calculated as (mass of refined polysaccharides/mass of raw material) \times 100%. This determined the optimal extraction process for *Physalis alkekengi* L. polysaccharides.

2.2.4. In vitro antioxidant activity of *Physalis alkekengi* L. polysaccharides

A stock solution of *Physalis alkekengi* L. polysaccharides was prepared at a concentration of 1 mg/mL. Aliquots of 0.2, 0.5, 1, 2, 4, and 6 mL of the stock solution were taken into test tubes, and distilled water was used to adjust each group to a final volume of 1 mL. VC served as a positive control. The DPPH radical scavenging rate, superoxide anion radical scavenging rate, and hydroxyl radical elimination rate of the polysaccharides were measured using a UV spectrophotometer.

2.2.5. Effect of *Physalis alkekengi* L. polysaccharide on SW620 cell viability

SW620 and 293 cells were seeded at a density of 2×10^5 /mL in 96-well plates, with 100 μ L per well, and cultured overnight. Cells were divided into a normal control group, an *Physalis alkekengi* L. polysaccharide control group, and groups with varying concentrations of *Physalis alkekengi* L. polysaccharides plus SW620/293 cells. After 24 hours of culture, 10 μ L/well of CCK8 reagent was added, and absorbance was measured at 450 nm. Cell viability was calculated as (mean value of experimental wells/mean value of control wells) \times 100%.

2.2.6. Western blot experiment

Total protein was extracted from each group of cells, and protein concentration was measured. SDS-PAGE electrophoresis was performed using 50 μ g/well, followed by membrane transfer, blocking, primary antibody incubation, secondary antibody incubation, and ECL chemiluminescence detection.

2.2.7. ELISA experiment

Cell culture supernatants from each group were collected to measure the expression levels of IL-1 β , IL-18, and TNF- α .

2.3. Statistical analysis

Data were processed and analyzed using SPSS 25.0. Results were expressed as mean \pm standard deviation (SD); variance analysis was performed for multiple groups, and Fisher's LSD test was conducted for pairwise comparisons between groups. Differences were considered statistically significant at $P < 0.05$.

3. Results and analyses

3.1. Single-factor experiment results for polysaccharide extraction from *Physalis alkekengi* L. peel

The results of this study showed that in experiments with different influencing factors, the extraction rate of polysaccharides from *Physalis alkekengi* L. peels initially increased and then decreased. When the material-to-liquid ratio m(g):V(mL) was increased to 1:30, the polysaccharide yield began to slowly decrease. The polysaccharide yield decreased as the extraction temperature increased beyond 90°C, so 90°C was selected as

the optimal extraction temperature. The polysaccharide yield reached its maximum at an extraction time of 30 minutes, after which it decreased, so 30 minutes was chosen as the optimal extraction time, as shown in **Figure 1**.

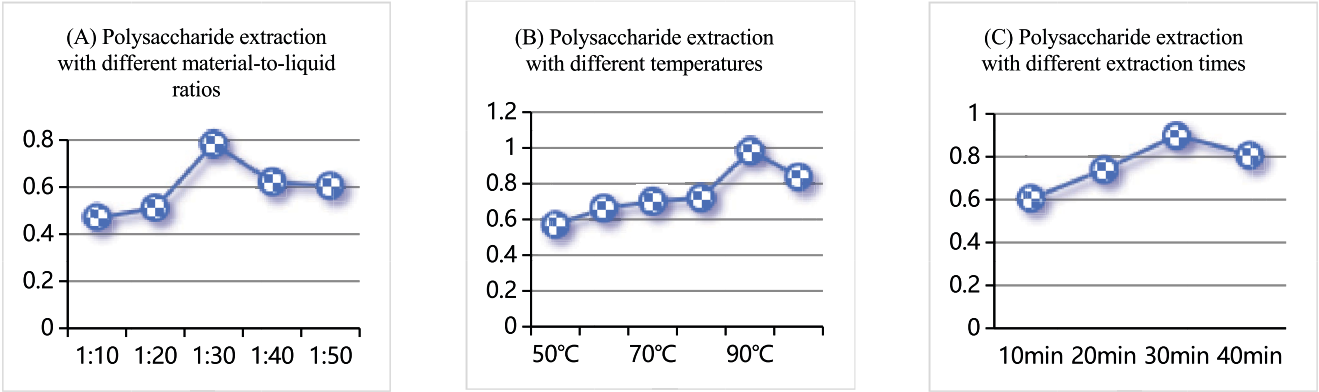


Figure 1. Single-factor extraction of polysaccharides from *Physalis alkekengi L.* peels with different (A) material-to-liquid ratios, (B) temperatures, and (C) extraction times.

3.2. Orthogonal experiment results for polysaccharide extraction from *Physalis alkekengi L.* peels

The orthogonal experiment, conducted under 3-factor and 3-level response conditions, determined the optimal extraction process for polysaccharides from *Physalis alkekengi L.* peels: a material-to-liquid ratio of $m(g):V(mL) = 1:30$, an extraction temperature of $100^{\circ}C$, and an extraction time of 40 minutes, with a predicted polysaccharide yield of 25.7%, as shown in **Table 1**. Under these conditions, the extraction was repeated three times.

Table 1. Orthogonal test of polysaccharides of *Physalis alkekengi L.* peel

No.	Material-to-liquid ratio [m(g):V(mL)]	Extraction temperature (°C)	Extraction time (min)	Yield (mg/g)
1	1:20	80	20	0.172
2	1:20	90	30	0.236
3	1:20	100	40	0.241
4	1:30	80	30	0.217
5	1:30	90	40	0.251
6	1:30	100	20	0.232
7	1:40	80	40	0.231
8	1:40	90	20	0.174
9	1:40	100	30	0.249
K1	0.216	0.207	0.193	
K2	0.233	0.219	0.235	
K3	0.218	0.241	0.239	
Polar deviation	0.017	0.034	0.046	

Optimal extraction process: 1:30, $100^{\circ}C$, 40 min, extraction influencing factors $C > B > A$.

3.3. Verification of optimal extraction conditions for *Physalis alkekengi* L. polysaccharides

The optimal extraction process for *Physalis alkekengi* L. polysaccharides obtained from the orthogonal experiment was verified by repeating the extraction three times, confirming that this extraction process yields stable *Physalis peruviana* polysaccharides, as shown in **Figure 2**.

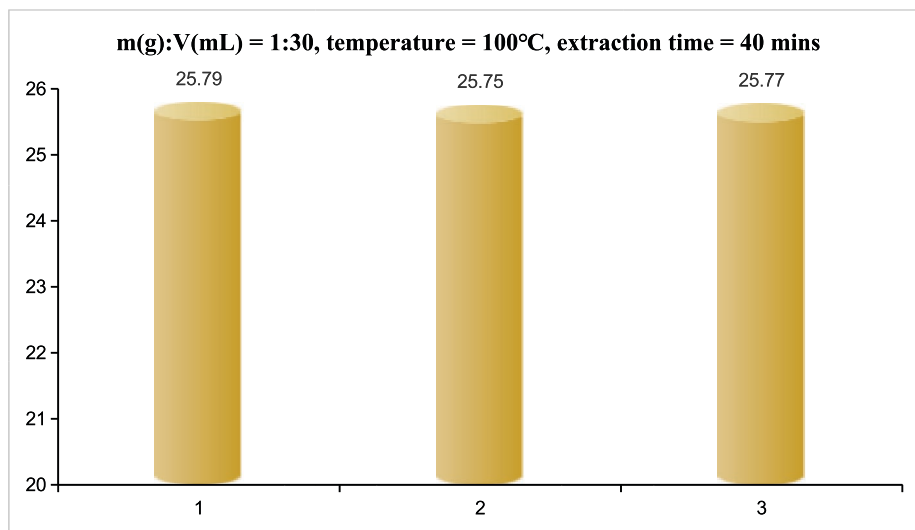


Figure 2. Verification of optimal extraction conditions for *Physalis alkekengi* L. polysaccharides

3.4. Antioxidant activity of *Physalis alkekengi* L. polysaccharides

The results of this study showed that the antioxidant activity of *Physalis alkekengi* L. polysaccharides was lower than that of ascorbic acid. However, as the concentration increased, the ability to scavenge DPPH, superoxide anion, and hydroxyl radicals also gradually increased, with scavenging rates reaching 68.67%, 61.46%, and 53.92%, respectively, as shown in **Figure 3**.

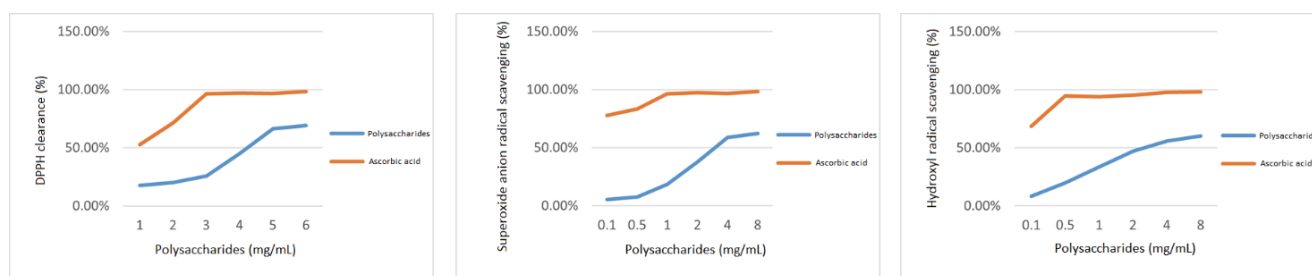


Figure 3. Antioxidant capacity of polysaccharides of *Physalis alkekengi* L.. (left) DPPH clearance (%); (middle) Superoxide anion radical scavenging (%); (right) Hydroxyl radical scavenging (%)

3.5. In vitro cytotoxicity experiment of *Physalis alkekengi* L. polysaccharides

The CCK8 assay was used to evaluate the growth inhibition and cytotoxicity of *Physalis alkekengi* L. polysaccharides on SW620 and HEK293 cells. The results showed that the cell viability of the SW620 group treated with 50 μ g/mL, 100 μ g/mL, and 200 μ g/mL *Physalis peruviana* polysaccharides was significantly reduced ($P < 0.01$), while the HEK293 group treated with the same concentrations showed no cytotoxicity ($P > 0.05$), as shown in **Figure 4**. The IC_{50} value for *Physalis alkekengi* L. polysaccharides on SW620 cells after 24 hours was determined to be 150 μ g/mL.

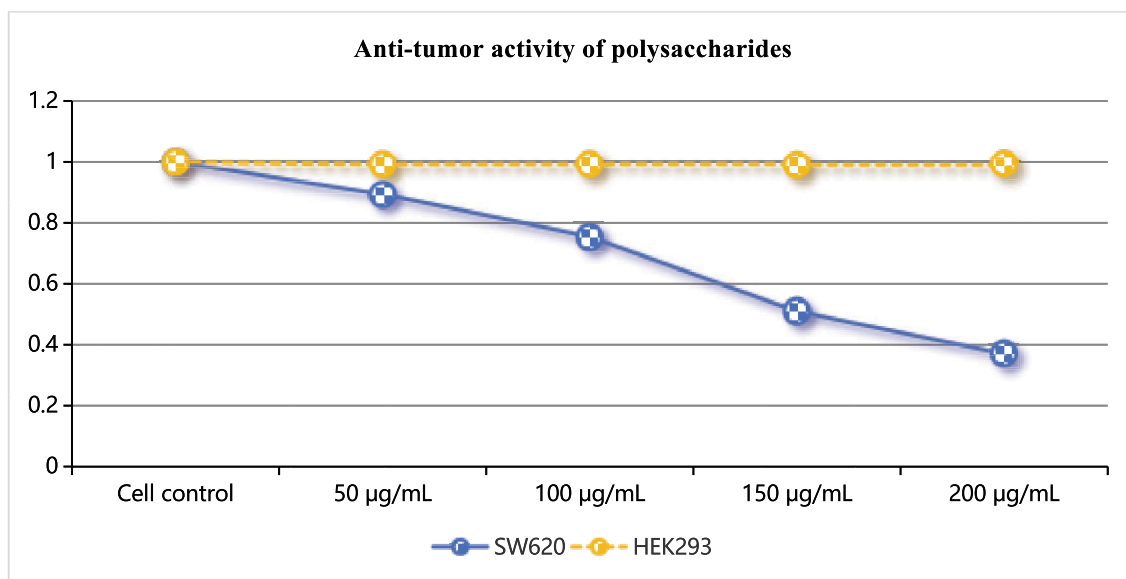


Figure 4. Cytotoxicity experiment of *Physalis alkekengi L.* polysaccharides on HEK293 cells and colorectal cancer cells SW620 showing growth inhibition of SW620 cells

3.6. Effect of *Physalis alkekengi L.* polysaccharides on the expression of inflammatory factors in SW620 cells

Compared with the SW620 control group, the relative expression level of p-NF-κB protein in the *Physalis alkekengi L.* polysaccharide-treated group was significantly reduced ($P < 0.01$), and the levels of IL-1β, IL-18, and TNF-α in the cell culture medium were also significantly decreased ($P < 0.01$), as shown in **Figure 5**, **Table 2**, and **Table 3**.

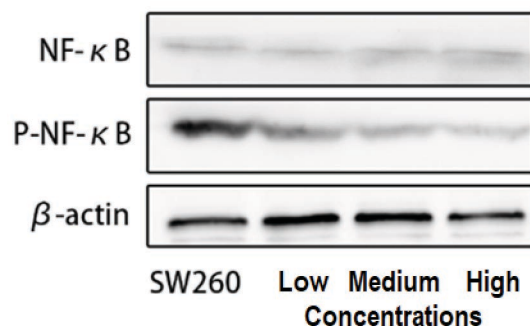


Figure 5. Western blot detection of NF-κB, p-NF-κB, and β-actin proteins

Table 2. Comparison of p-NF-κB protein expression levels in cells of each group

Groups	Relative expression of p-NF-κB protein
SW620	0.894 ± 0.09
Low concentration	0.334 ± 0.04
Medium concentration	0.256 ± 0.03
High concentration	0.126 ± 0.03*

*Comparison with the control group, $P < 0.01$.

Table 3. Comparison of levels of IL-1 β , IL-18, and TNF- α in cell cultures of each group (pg/mL)

Groups	IL-1 β	IL-18	TNF- α
SW620	126.305 \pm 13.02	88.253 \pm 9.12	283.259 \pm 30.82
Low concentration	72.286 \pm 4.52*	65.785 \pm 2.76*	174.311 \pm 16.23*
Medium concentration	41.412 \pm 19.26	34.481 \pm 1.55	95.571 \pm 8.95
High concentration	15.131 \pm 0.27 [†]	10.026 \pm 1.07 [†]	62.506 \pm 6.18 [†]

*Comparison with the control group, $P < 0.01$; [†]Comparison with the low concentration group, $P < 0.01$.

4. Discussion

Colorectal cancer is one of the three most prevalent malignant tumors worldwide. The currently recommended primary treatments are surgery, radiotherapy, and chemotherapy, all of which are often accompanied by poor prognosis and high recurrence rates. The 5-year relative survival rate for colorectal cancer is approximately 65% [2]. The incidence and mortality rates of colorectal cancer are increasing every year, especially in large and medium-sized cities, making it a significant public health issue in China. However, one of the major challenges in treating colorectal cancer is the development of drug resistance. Cancer cells can develop resistance to chemotherapeutic drugs, thereby reducing treatment efficacy. Researchers are focusing on exploring efficient and non-toxic natural drugs. Based on research reports from 89 related literature sources, from 2018 to 2021, experts extracted 48 different types of polysaccharides with CRC inhibitory effects from various plants, including *Dendrobium officinale*, *Nostoc flagelliforme*, and *Ganoderma lucidum*, among others [5-7]. In this study, the extraction process of *Physalis alkekengi* L. peel polysaccharides was optimized using an orthogonal experiment based on a single-factor experiment. The final optimal aqueous extraction and alcohol precipitation process for *Physalis alkekengi* L. peel polysaccharides was determined as a material-to-liquid ratio of m(g):V(mL) = 1:30, extraction temperature of 100°C, and extraction time of 40 minutes, yielding a polysaccharide extraction rate of 25.7%.

The development of CRC may be due to the combined effects of inflammation and immune regulation. As a driver of intestinal inflammation, intestinal barrier dysfunction plays a critical role in the inflammatory mechanisms of CRC pathogenesis. Impaired intestinal barrier integrity allows bacteria-derived molecules and other antigens to cross the intestinal barrier to sustain this intestinal inflammation. Within the intestine, it can aid the inflammatory response by activating the NF- κ B pathway through TLR4 receptors [8]. This major inflammatory pathway not only exacerbates intestinal barrier dysfunction but may also lead to the occurrence and progression of CRC [9].

Tumor necrosis factor- α (TNF- α) is a pleiotropic cytokine that primarily acts through the type 1 tumor necrosis factor receptor (TNFR1). After TNF binding, TNFR1 recruits TRADD (TNF receptor type 1-associated death domain). This interaction triggers the formation of signaling complexes claimed to induce apoptosis (through downstream caspase activation), inflammation (via NF- κ B), and stress pathways (JNK/p38) [10].

It is important to emphasize that IL-1 β , IL-18, and TNF- α are directly involved in cancer development [11]. For example, TNF- α stimulates tumor progression by activating NF- κ B, thereby regulating processes such as invasion, migration, cell proliferation, inhibition of apoptosis, and tumor angiogenesis [12]. IL-18 can initiate tumorigenesis and/or tumor progression by inhibiting dendritic cell differentiation, inducing immune tolerance early in tumor development, and promoting metastasis [13]. Additionally, saliva samples from oral cancer patients have higher levels of IL-18 compared to healthy individuals or patients with precancerous lesions [14,15].

In conclusion, this study used an orthogonal experiment to determine the extraction process of *Physalis peruviana* peel polysaccharides and verified their antioxidant activity through *in vitro* experiments. Based on the research results, it can be preliminarily determined that *Physalis alkekengi* L. peel polysaccharides have anti-inflammatory effects, providing a reference for future CRC drug research.

Disclosure statement

The authors declare no conflict of interest.

References

- [1] Li JJ, Li L, Su SS, et al., 2024, Anti-Inflammatory Properties and Characterization of Water Extracts Obtained from *Callicarpa kwangtungensis* Chun using In Vitro and In Vivo Rat Models. *Sci Rep*, 14(1): 11047. <https://doi.org/10.1038/s41598-024-61892-9>
- [2] Lee YM, Kim DS, 2024, Analgesic, Anti-Inflammatory, and Chondroprotective Activities of *Siraitia grosvenorii* Residual Extract. *Int J Mol Sci*, 25(8): 4268. <https://doi.org/10.3390/ijms25084268>
- [3] Lu J, Luo M, Wang L, et al., 2021, The *Physalis floridana* Genome Provides Insights into the Biochemical and Morphological Evolution of *Physalis* Fruits. *Hortic Res*, 8(1): 244. <https://doi.org/10.1038/s41438-021-00705-w>
- [4] El-Emam MMA, El-Demerdash AS, Abdo SA, et al., 2024, The Ameliorative Role of Aloe vera-Loaded Chitosan Nanoparticles on *Staphylococcus aureus* Induced Acute Lung Injury: Targeting TLR/NF- κ B Signaling Pathways. *Open Vet J*, 14(1): 416–427. <https://doi.org/10.5455/OVJ.2024.v14.i1.38>
- [5] Harasym J, Dziendzikowska K, Kopiasz Ł, et al., 2024, Consumption of Feed Supplemented with Oat Beta-Glucan as a Chemopreventive Agent against Colon Cancerogenesis in Rats. *Nutrients*, 16(8): 1125. <https://doi.org/10.3390/nu16081125>
- [6] Li J, Song C, He C, 2019, Chinese Lantern in *Physalis* is An Advantageous Morphological Novelty and Improves Plant Fitness. *Sci Rep*, 9(1): 596. <https://doi.org/10.1038/s41598-018-36436-7>
- [7] Ruan J, Zhang P, Zhang Q, et al., 2023, Colorectal Cancer Inhibitory Properties of Polysaccharides and Their Molecular Mechanisms: A Review. *Int J Biol Macromol*, 238: 124165. <https://doi.org/10.1016/j.ijbiomac.2023.124165>
- [8] Pan H, Wang Y, Na K, et al., 2019, Autophagic Flux Disruption Contributes to *Ganoderma lucidum* Polysaccharide-Induced Apoptosis in Human Colorectal Cancer Cells via MAPK/ERK Activation. *Cell Death Dis*, 10(6): 456. <https://doi.org/10.1038/s41419-019-1653-7>
- [9] Li YH, Niu YB, Sun Y, et al., 2015, Role of Phytochemicals in Colorectal Cancer Prevention. *World J Gastroenterol*, 21(31): 9262–9272. <https://doi.org/10.3748/wjg.v21.i31.9262>
- [10] Elshami M, Dwikat MF, Al-Slaibi I, et al., 2024, Understanding the Interplay of Colorectal Cancer Awareness and Attitudes among Palestinians: A National Cross-Sectional Study. *BMC Cancer*, 24(1): 590. <https://doi.org/10.1186/s12885-024-12357-9>
- [11] Shang Z, Xi S, Lai Y, et al., 2024, Single-Cell Transcriptomics and Mendelian Randomization Reveal LUCAT1's Role in Right-Sided Colorectal Cancer Risk. *Front Genet*, 15: 1357704. <https://doi.org/10.3389/fgene.2024.1357704>
- [12] Ren J, Han B, Feng P, et al., 2024, Mechanism of miR-7 Mediating TLR4/TRAF6/NF- κ B Inflammatory Pathway in Colorectal Cancer. *Funct Integr Genomics*, 24(1): 24. <https://doi.org/10.1007/s10142-024-01307-0>
- [13] Li Q, von Ehrlich-Treuenstätt V, Schardey J, et al., 2023, Gut Barrier Dysfunction and Bacterial Lipopolysaccharides in Colorectal Cancer. *J Gastrointest Surg*, 27(7): 1466–1472. <https://doi.org/10.1007/s11605-023-05654-4>
- [14] Tuysuz EC, Mourati E, Rosberg R, et al., 2024, Tumor Suppressor Role of the Complement Inhibitor CSMD1 and Its Role in TNF-Induced Neuroinflammation in Gliomas. *J Exp Clin Cancer Res*, 43(1): 98. <https://doi.org/10.1186/s13046->

024-03019-6

- [15] Hapil Zevkliler FZ, Çopuroğlu FE, Ertosun MG, et al., 2023, TNFR1 Signaling is Positively Regulated by Jak-2 and c-Src via Tyrosine Phosphorylation. Turk J Biol, 48(1): 1–12. <https://doi.org/10.55730/1300-0152.2677>

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Clinical Outcomes of Complete Mesocolic Excision for Right-Sided Colon Cancer Using 3D Laparoscopy versus 2D Laparoscopy

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Abstract: *Objective:* To study the clinical outcomes of complete mesocolic excision (CME) for right-sided colon cancer using 3D (three-dimensional) laparoscopy compared to 2D (two-dimensional) laparoscopy. *Methods:* From January 2022 to December 2023, 58 patients with right-sided colon cancer treated at the Affiliated Hospital of Hebei Engineering University were randomly divided into a 3D laparoscopy group (observation group) and a 2D laparoscopy group (control group), with 29 patients in each group. Intraoperative blood loss, postoperative time to first flatulence, length of hospital stay, and incidence of complications in both groups were recorded. *Results:* There was a statistically significant difference in intraoperative blood loss between the two groups ($P < 0.05$). There was no statistically significant difference in the time to first flatulence between the groups ($P > 0.05$). However, there was a statistically significant difference in the length of hospital stay ($P < 0.05$) and the incidence of complications ($P < 0.05$) between the two groups. *Conclusion:* 3D laparoscopy for CME can reduce intraoperative blood loss, shorten hospital stay, and decrease postoperative complications, showing significant clinical advantages over traditional 2D laparoscopy.

Keywords: 3D laparoscopy; 2D laparoscopy; Complete mesocolic excision; Colon cancer

Online publication: August 12, 2024

1. Introduction

With the continuous advancement of modern medical technology, laparoscopic techniques have been widely applied in surgical procedures worldwide, offering significant advantages such as reduced surgical trauma, shorter recovery time, and improved postoperative quality of life for patients. Complete mesocolic excision (CME) is particularly important in the treatment of right-sided colon cancer, as it involves the thorough removal of the tumor and its mesocolon, effectively reducing the risk of local recurrence. However, traditional 2D laparoscopy has certain limitations due to its two-dimensional imaging, making it challenging for surgeons to perceive depth and spatial relationships during surgery^[1]. This limitation can increase the difficulty of the procedure and the risk of intraoperative injuries. To overcome these limitations, 3D laparoscopy was developed,

providing surgeons with a more realistic, three-dimensional surgical view through stereoscopic vision. This significantly enhances surgical precision and safety [2].

In recent years, 3D laparoscopy has been increasingly used in clinical practice, demonstrating superior performance in various surgical procedures. It has gained widespread recognition and application, particularly in gastrointestinal tumor surgeries such as colorectal cancer. However, comparative studies on the clinical outcomes of 3D versus 2D laparoscopy for CME in the treatment of right-sided colon cancer are still relatively scarce [3]. Therefore, this study aims to further explore the advantages and disadvantages of these two surgical approaches by comparing their clinical outcomes in CME for right-sided colon cancer. Specifically, the study will collect and analyze data on perioperative indicators, surgical complications, and postoperative recovery of patients treated with these two methods, providing scientific evidence for clinical surgical decision-making and offering safer and more effective treatment options for patients.

2. Materials and methods

2.1. General information

From January 2022 to December 2023, 58 cases of right-sided colon cancer patients underwent surgical treatment at the Affiliated Hospital of Hebei Engineering University. Among them, 40 were male (67.0%) and 18 were female (33.0%). The age range was 35 to 81 years old. The longest disease course was 11 months, and the shortest was 5 months. All patients met the diagnostic criteria outlined in the “Guidelines for Diagnosis and Treatment of Surgical Oncology” [4]. Patients were randomly divided into two groups: the 3D laparoscopy group and the 2D laparoscopy group, with 29 patients in each group. There were no statistically significant differences between the two groups in terms of gender, age, disease duration, lesion size, and pathological type ($P > 0.05$; Table 1).

Table 1. Comparison of general information

General information	Control group ($n = 29$)	Observation group ($n = 29$)	t / χ^2 -value	P -value
Age (years)	55.37 ± 5.12	55.40 ± 5.10	0.022	0.979
Gender (cases)	Male 20 (65.79)	20 (67.50)	0.000	1.000
	Female 9 (34.21)	9 (32.50)	0.000	1.000
Tumor size (cm)	3.21 ± 0.80	3.22 ± 0.79	0.048	0.956
Duration of disease (months)	6.98 ± 0.58	6.87 ± 0.54	0.748	0.458

2.2. Methods

All patients received general anesthesia before surgery, using either endotracheal intubation or laryngeal mask ventilation. A pneumoperitoneum was established in the upper right abdomen with a diameter of approximately 10 mm. For patients with severe and extensive abdominal adhesions, partial intestinal ligation was performed before laparoscopic surgery. Surgical instruments were inserted through the pneumoperitoneum and inferior vena cava, and the common iliac artery and its branches were severed using an electric knife or plasma knife. The tissue spaces were opened fully using a mixture of povidocanol and heparin saline for irrigation. After separating the adhesions, a complete mesocolic excision (CME) was performed, including lymph node dissection as needed.

For the control group, 2D laparoscopy was used, following the conventional laparoscopic procedure:

- (1) Establishment of pneumoperitoneum;

- (2) Expansion, observation, and localization of the tumor tissue;
- (3) Mobilization of the lower ascending colon, transverse colon, and upper descending colon, with transection 10 cm from the ascending colon end, and jejunostomy tube placement through the right inguinal lymph nodes into the jejunum;
- (4) Mobilization of the left hemicolon, sigmoid colon, and rectum, with incision and widening of the seromuscular layer 1.5 cm from the left side ligation line;
- (5) Mobilization of the mesocolon and splenorenal artery, with hemostasis along the mesocolon and splenorenal artery;
- (6) Fixation of the intestinal loop to the operating table;
- (7) Removal of the remaining colon specimen, ensuring the preservation of the left mesocolon and spleen.

The observation group underwent the same procedure as the control group but with the added use of 3D laparoscopic equipment. This enhanced the surgical view, clearly displaying anatomical structures and the operative space, thus aiding in preoperative planning and increasing the safety of the surgery.

2.3. Observation indicators

The following were recorded for both groups: intraoperative blood loss, postoperative time to first flatulence, length of hospital stay, and incidence of complications.

- (1) Intraoperative blood loss: The blood loss for each group was recorded immediately post-surgery and on the first postoperative day. The groups were compared using the chi-squared test.
- (2) Postoperative time to first flatulence: Postoperative flatulence was evaluated on days 1, 3, 7, and 14. Each evaluation was independently assessed by two observers, and the average time was taken. The groups were compared using paired *t*-tests.
- (3) Length of hospital stay: The average discharge time for patients in both groups was recorded and compared using paired *t*-tests.
- (4) Postoperative complications: All patients underwent either a colostomy or ligation. The follow-up period was at least 6 months. Complications were categorized into grades I to IV based on recurrence frequency, and the appropriate treatment plan was developed accordingly. The groups were compared using the chi-square test, with $P < 0.05$ indicating statistical significance.

2.4. Statistical analysis

Data were analyzed using SPSS 19.0. Quantitative data were described using mean \pm standard deviation (SD), with between-group comparisons performed using independent sample *t*-tests and within-group comparisons using paired *t*-tests. Categorical data were expressed as percentages and analyzed using the chi-squared test, with $P < 0.05$ considered statistically significant.

3. Results

There was a statistically significant difference in intraoperative blood loss between the two groups ($P < 0.05$). There was no statistically significant difference in the postoperative time to first flatulence between the groups ($P > 0.05$). However, there was a statistically significant difference in the length of hospital stay ($P < 0.05$) and the incidence of complications ($P < 0.05$) between the two groups. The results are shown in **Table 2**.

Table 2. Comparison of intraoperative blood loss, postoperative time to first flatulence, length of hospital stay, and complication rates between the two groups

Groups	Intraoperative blood loss (mL)	Postoperative time to first flatulence (h)	Length of hospital stay (d)	Complication rates [n (%)]
Control group (<i>n</i> = 29)	254.60 ± 49.30	5.96 ± 1.61	10.36 ± 2.21	8 (27.59)
Observation group (<i>n</i> = 29)	213.58 ± 48.24	5.41 ± 1.56	8.13 ± 2.11	2 (6.90)
<i>t</i> / χ^2 -value	3.195	1.321	3.930	4.350
<i>P</i> -value	0.002	0.191	0.000	0.037

4. Discussion

Currently, China's medical technology has reached a world-leading level. With the continuous updates and development of surgical methods and equipment, minimally invasive surgery is becoming increasingly widespread in clinical applications. Laparoscopic surgery, a rapidly developing minimally invasive technique, has become a key direction in modern surgery due to its advantages such as minimal trauma and rapid recovery ^[5]. In recent years, 3D imaging systems have been introduced into laparoscopic minimally invasive surgery, transforming 2D images into 3D images. This not only allows doctors to observe intra-abdominal conditions more intuitively but also magnifies certain organs, such as the gastrointestinal tract, making it easier for surgeons to distinguish and remove lesions.

The results of this study show that there is a statistically significant difference in intraoperative blood loss between the two groups ($P < 0.05$); there is no statistically significant difference in the postoperative time to first flatulence ($P > 0.05$); there is a statistically significant difference in hospital stay ($P < 0.05$); and there is a statistically significant difference in the incidence of complications ($P < 0.05$). This indicates that 3D laparoscopy is significantly effective in reducing intraoperative blood loss, shortening hospital stay, and reducing postoperative complications, but it has no significant effect on shortening the postoperative time to the first flatulence ($P > 0.05$). Thus, compared with conventional 2D laparoscopy, 3D laparoscopy in complete mesocolic excision (CME) shows no significant difference in intraoperative blood loss, time to first flatulence, hospital stay, and complication rate. A study showed that compared to preoperative fiber colonoscopy, hospital stay was shortened by 3.2 days in the 3D laparoscopy group ^[6]. Overall, 3D laparoscopy offers more clinical advantages for patients, such as a wider field of view, higher clarity, and stronger stereoscopic perception. These advantages help improve surgical safety and promote patient recovery. The comparison reveals that 3D laparoscopy has significant advantages over 2D laparoscopy in CME, including intraoperative blood loss, time to first flatulence, hospital stay, and complication incidence. However, its advantages do not lie in postoperative functional recovery or tumor eradication, but in reducing the operational difficulty for surgeons and increasing the resolution of tissue structures.

2D laparoscopy, as a representative of traditional laparoscopic technology, has provided significant convenience in clinical surgery, but it still has certain limitations. For example, during surgery, the lack of stereoscopic perception and depth in 2D images may cause deviations in the surgeon's grasp of the surgical area, leading to less precise surgical operations ^[7]. In contrast, 3D laparoscopy provides a more realistic, three-dimensional surgical view through special imaging technology, making surgical operations more precise and safe ^[8].

From a clinical effectiveness perspective, 3D laparoscopy in CME for right-sided colon cancer shows

clear advantages. First, in terms of surgery time, the clearer and more three-dimensional surgical view provided by 3D laparoscopy allows doctors to complete surgical operations more quickly and accurately, resulting in significantly shorter surgery times compared to the 2D laparoscopy group^[9]. Second, in terms of intraoperative blood loss, the more precise surgical operations with 3D laparoscopy result in significantly less blood loss compared to the 2D laparoscopy group. Additionally, in postoperative recovery, patients in the 3D laparoscopy group have shorter times to first flatulence and shorter hospital stays, indicating better postoperative recovery outcomes.

In terms of safety, although 3D laparoscopy offers higher precision and safety for surgery, it also requires surgeons to have the corresponding operational skills and experience. Moreover, due to the unique nature of 3D imaging technology, patients may experience some discomfort during surgery. However, existing clinical studies suggest that these discomforts do not significantly affect surgical outcomes and patient recovery^[10]. Regarding feasibility, as 3D laparoscopy technology continues to develop and improve, its surgical indications are expanding. Currently, for most patients with right-sided colon cancer, 3D laparoscopic CME has become a safe and feasible surgical option.

In summary, 3D laparoscopic CME shows significant advantages in the treatment of right-sided colon cancer. Compared to traditional 2D laparoscopic technology, 3D laparoscopy offers a clearer, more three-dimensional surgical view, making surgical operations more precise and safe. Additionally, 3D laparoscopy can shorten surgery times, reduce intraoperative blood loss, and promote postoperative recovery. Therefore, for surgeons with the appropriate skills and experience, 3D laparoscopic CME is a worthwhile surgical technique to promote. However, it should also be recognized that 3D laparoscopy still has certain challenges and issues, such as how to further improve the clarity and stereoscopic perception of 3D imaging and how to reduce patient discomfort. These areas require further research and exploration. Furthermore, with the continuous advancement and development of medical technology, more advanced and efficient surgical techniques and methods may emerge in the future. Therefore, continuous learning and exploration of new knowledge and technical methods are necessary to provide patients with more scientific and rational treatment options.

Disclosure statement

The authors declare no conflict of interest.

References

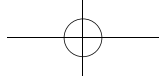
- [1] Chen Y, Cheng X, Tang G, 2023, Analysis of the Effects of Different Approaches for Laparoscopic Complete Mesocolic Excision in the Treatment of Right-sided Colon Cancer. *Medical Theory and Practice*, 36(20): 3483–3485.
- [2] Qin M, Li J, 2023, Clinical Effects of Laparoscopic Complete Mesocolic Excision Using the Left Border of the Mesenteric Artery as the Medial Boundary in the Treatment of Right-Sided Colon Cancer. *Clinical Medical Engineering*, 30(7): 895–896.
- [3] Du X, Liu J, Zhou W, et al., 2021, Clinical Effects of Laparoscopic Complete Mesocolic Excision Combined with Mesenteric Vessel Root Ligation in the Treatment of Right-Sided Colon Cancer. *Journal of Clinical and Experimental Medicine*, 20(23): 2544–2548.
- [4] Lan N, Tang J, Wang W, et al., 2021, Clinical Effects of Laparoscopic Right Hemicolectomy Guided by the Superior Mesenteric Artery in the Treatment of Right-Sided Colon Cancer. *Clinical Medicine Research and Practice*, 6(15): 17–19.
- [5] Lin W, Cui J, Huang Q, et al., 2020, Observation of the Efficacy of Laparoscopic D3 Resection and Complete Mesocolic

Excision in the Treatment of Right-Sided Colon Cancer. *Health Medicine Research and Practice*, 17(2): 64–68.

- [6] Tian Q, 2020, Clinical Effects of Laparoscopic Complete Mesocolic Excision in the Treatment of Right-sided Colon Cancer. *Henan Medical Research*, 29(2): 259–260.
- [7] Zhang J, Tian J, Ji E, et al., 2019, Comparative Study on the Application of 3D and 2D Laparoscopy in Complete Mesocolic Excision for Right-sided Colon Cancer. *Anhui Medical Journal*, 40(11): 1256–1258.
- [8] Jin D, Chen G, 2019, Observation of the Efficacy of Laparoscopic Complete Mesocolic Excision in the Treatment of Right-sided Colon Cancer. *Zhejiang Medicine*, 41(12): 1312–1315.
- [9] Zhang F, 2019, Comparative Study of 3D and 2D Laparoscopy in Complete Mesocolic Excision for Right-sided Colon Cancer, thesis, China Medical University.
- [10] Li Y, Yang B, 2019, Effects of 3D Laparoscopic Complete Mesocolic Excision on Postoperative Changes in MicroRNA-101 and CD4~+ Levels and Local Recurrence Rates in Patients with Right-Sided Colon Cancer. *Chinese Journal of Endoscopy*, 25(4): 24–31.

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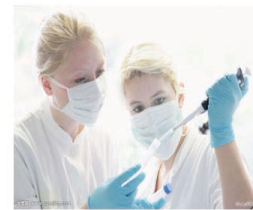
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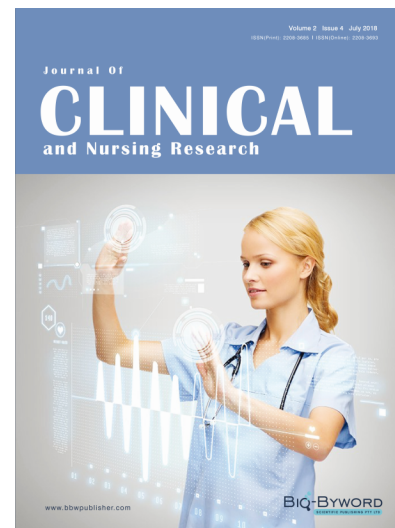
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