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A Proposed Solution to the Interference of IgG Kappa-Type M Protein on LDL-C Detection

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Abstract: *Objective:* To explore the interference of monoclonal immunoglobulin (M protein) on the detection of serum LDL-C in patients with multiple myeloma, improve the understanding of this matter, determine and establish the correct method, and provide more accurate clinical results through this case. *Methods:* A case was selected for analysis by the direct method. *Results:* The interference of IgG kappa-type M protein on LDL-C detection could not be completely eliminated by the enzymatic method. *Conclusion:* IgG-type type M protein affects the detection of LDL-C by the enzymatic method; thus, light reagents can be used with the direct method for detection.

Keywords: M protein; Interference; Biochemical detection

Online publication: March 29, 2023

1. Introduction

Monoclonal immunoglobulin (M protein) is an immunoglobulin molecule or its fragment with the same amino acid sequence and protein structure produced by the proliferation of monoclonal B lymphocytes or plasma cells [1]. It is commonly seen in multiple myeloma, macroglobulinemia, *etc*., all of which start with M; therefore, it is called "M protein." M protein can be IgG, IgM, IgA, IgE, or IgD, or any of the kappa (κ) or lambda (λ) light chains. In recent years, there have been many reports on the interference of IgM-type M protein on biochemical detection indicators, but there are only a few reports on the interference of IgG-type M protein on low-density lipoprotein (LDL) detection. Therefore, a case of IgG-type M protein interfering with the detection of LDL via the direct method and a solution that can eliminate the interference is reported in this paper.

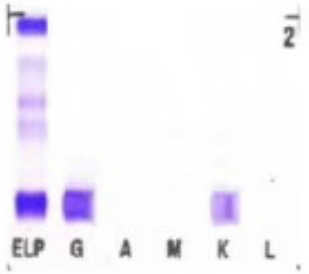
2. Materials and methods

2.1. Information

A 74-year-old male patient presented with lower back pain without any obvious inducement or radiation. The pain was dull and tolerable and was not aggravated by postural changes. He was not on any oral analgesics. He was admitted to Xushui District People's Hospital. His lower back pain improved after lumbar vertebrae surgery on May 27. On postoperative day one, the lower back pain recurred with the same nature as before; however, the pain was disregarded. On June 30, he visited Xushui District People's Hospital again, and a magnetic resonance imaging (MRI) was done over his lumbar spine; his MRI examination was similar with the original film, showing lumbar degeneration, hyperosteoplasia, and intervertebral disc deformation and contact. No further treatment was given. On July 4, during his third visit to Xushui District People's Hospital, he complaint of shortness of breath after exercise 4 days ago,

which was relieved with rest. It was accompanied by fatigue, loss of appetite, and loss of weight that developed one month ago. Otherwise, he did not experience any discomfort over his anterior chest. He was admitted for further investigations. His blood investigations were as follows: (routine blood examination) red blood cell (RBC) 2.09×10^{12}/L; hemoglobin (HGB) 72 g/L; (renal function) blood urea nitrogen (BUN) 14.26 mmol/L, creatinine (CR) 154 μmol/L, uric acid (UA) 606 μmol/L; immunoglobulin G (IgG) 72.6 g/L; (liver function) total protein (TP) 109 g/L, globulin (GLB) 81 g/L. Serum and urine immunofixation electrophoresis showed IgGκ type. In consideration of his clinical manifestations and investigation results, the patient was diagnosed with multiple myeloma (**Figures 1** and **2**).

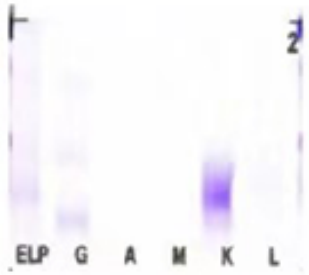
Inspection item		Result	Unit	Reference interval
IgG	IgG immunofixed electrophoresis	Positive *		Negative
IgA	IgA immunostationary electrophoresis	Negative		Negative
IgM	IgMimmunofixed electrophoresis	Negative		Negative
Kappa	K light chain immunofixed electrophoresis	Positive *		Negative
Lambda	λ light chain immunofixed electrophoresis	Negative		Negative



Notes: 1. There were abnormal zones in the ELP lane, and immunoprecipitation zones in the same position in the IgG and KAPPA lanes, which were IgG KAPPA type.

Figure 1. Serum immunofixation electrophoresis result

Inspection item		Result	Unit	Reference interval
IgG	IgG immunofixed electrophoresis	Positive *		Negative
IgA	IgA immunostationary electrophoresis	Negative		Negative
IgM	IgMimmunofixed electrophoresis	Negative		Negative
Kappa	K light chain immunofixed electrophoresis	Positive *		Negative
Lambda	λ light chain immunofixed electrophoresis	Negative		Negative



Notes: 1. ELP has abnormal zones, while IgG and KAPPA have immunoprecipitation zones at the same location. The suspected free light chain is IgG KAPPA with KAPPA light chain type.

Figure 2. Urine immunofixation electrophoresis result

2.2. Instruments and reagents

Beckman Coulter's AU5821 automatic biochemical analyzer and LDL-C original supporting reagents were used to detect LDL-C.

2.3. Methods

The direct method was used. The protective agent in Reagent 1 protected LDL from participating in the enzymatic reaction, while the addition of Reagent 2 released the protective agent from the LDL and inhibited hydroperoxide ester through sodium azide. LDL was quantitatively determined by an enzymatic chromogenic system (CHOPAP system).

3. Discovery of interference

3.1. Negative value for very-low-density lipoprotein (VLDL)

The results showed a negative value for VLDL (**Figure 3**).

Standard coding	Project name	Result		Unit	Reference interval
1 TCH	★ Total cholesterol	2.64	↓	mmol/L	3.00-5.18
2 TG	★ Triglyceride	0.70		mmol/L	<1.70
3 HDL	★ HDL cholesterol	1.25		mmol/L	1.04-1.55
4 LDL	★ Low density lipoprotein cholesterol	2.03		mmol/L	1.89-3.37
5 VLDL	Very low density lipoprotein cholesterol	-0.64	↓	mmol/L	0.00-0.76
6 APO-A1	Apolipoprotein A1	0.80	↓	g/L	1.00-1.60
7 APO-B100	Apolipoprotein B100	0.34	↓	g/L	0.60-1.20
8 A1/B100	A1/B100	2.35			0.90-2.67

Figure 3. Results showing negative very-low-density lipoprotein (VLDL)

3.2. Serum quality control

The serum appeared clear and bright without any visible clots, blood, *etc*. (**Figure 4**).

Figure 4. Serum after the test

3.3. Response curves

The response curves are shown in **Figures 5**, **6**, and **7**.

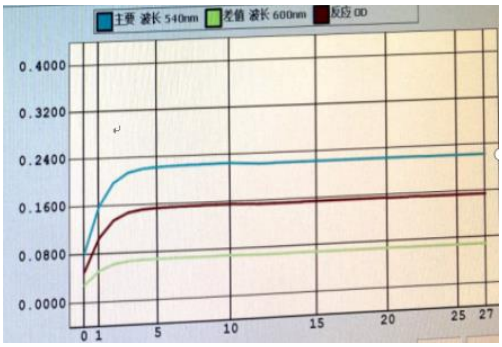


Figure 5. Normal response curve (total cholesterol). Blue: main wavelength; green: difference wavelength; red: reaction.

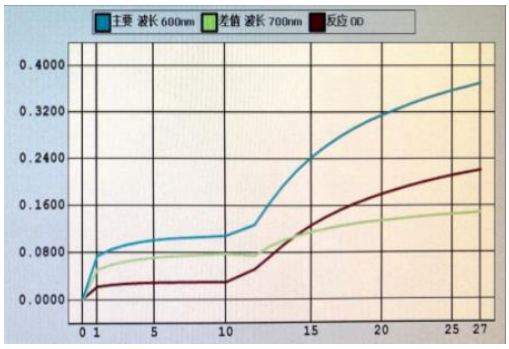


Figure 6. Normal response curve (high-density lipoprotein cholesterol). Blue: main wavelength; green: difference wavelength; red: reaction.

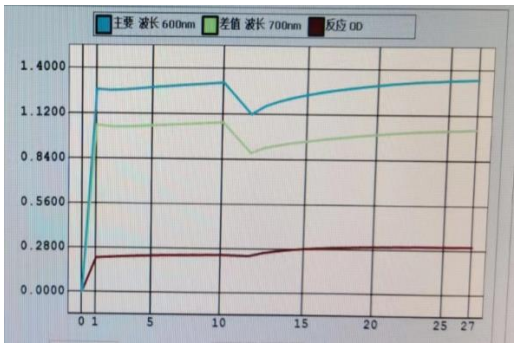


Figure 7. Abnormal response curve (low-density lipoprotein cholesterol). Blue: main wavelength; green: difference wavelength; red: reaction.

3.4. Reasons for interference

The response curves for total cholesterol (CHOL) and high-density lipoprotein cholesterol (HDL-C) were both normal, but the response curve for LDL-C was abnormal. As shown in **Figure 7**, at the 0–1 point, the increase in M protein causes hyperviscosity syndrome, and its specific binding with certain substances in the body (forming giant enzymes) can interfere with the experiment, thus affecting the detection results. After adding the sample, the absorbance increased abnormally, thus displaying an abnormal curve.

3.5. Solution

After changing the reagent, the curve became normal, as shown in **Figure 8**.

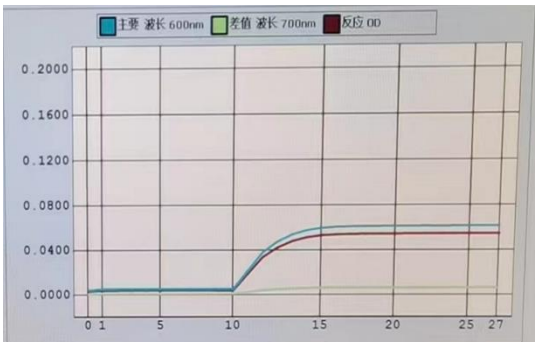


Figure 8. Response curve after changing reagent. Blue: main wavelength; green: difference wavelength; red: reaction.

According to Friedewald formula, when serum TG < 4.52 mmol/L, LDL-C = TC – HDL-C – TG/2.2. After calculation, LDL = 1.03, which was close to the detection result as shown in **Figure 3**.

4. Discussion

In clinical biochemical tests, hemolysis and lipemia are the most common influencing and interference factors [2] . In recent years, the interference of M protein on biochemical indicators has been reported from time to time, such as the interference of IgM protein on serum prealbumin detected by immune transmission turbidimetry [3] and IgG-κM protein in multiple myeloma patients on the analysis of bilirubin detected by the enzymatic method [4]. The present study found that IgG κM protein interferes with LDL-C and showed that using another reagent to detect LDL-C can solve the interference of IgG-type M protein on LDL-C. With the increasing number of case reports, the interference of M protein on many biochemical indicators is recognized. At the same time, reagent manufacturers have also begun to pay attention to this issue and taken corresponding measures, such as using reagent blanks to replace water blanks and dual wavelength and rate analysis methods to replace one-point end-point or two-point end-point methods; adjusting the pH value and ionic strength of the reagents to prevent protein precipitation; and adding surfactants to the reagents to promote protein dissolution [5,6]. However, as of now, there is no reagent that can eliminate the interference of M protein on all detection indicators. Therefore, there is still a need to investigate the interference of M protein on biochemical detection indicators and find a solution for it in order to provide more accurate test results in clinical practice settings.

Disclosure statement

The author declares no conflict of interest.

References

[1] Zhang Y, Liu F, Tian H, et al., 2021, Clinical Significance of M Protein Concentration Detection in Patients with Monoclonal Immunoglobulinemia. Journal of Nephrology and Dialysis Kidney Transplantation, 30(2): 130–35.

[2] Zhuang Y, 2020, Observation on the Effect of Hemolysis and Lipidemia in Serum Samples on the Results of Biochemical Tests. Clinical Research, 18(2): 112–113.

[3] He J, Wang Z, Hu Y, 2013, Interference of IgM M Protein on Clinical Biochemical Detection. Chinese Medicine Guide, 11(15): 432–434.

[4] Li J, Wang L, Zhao X, et al., 2011, Interference and Analysis of Rare IgA M Protein to Clinical Chemical Detection. Marker Immunoassay and Clinical Analysis, 18(6): 398–402.

[5] King RI, Florkowski CM, 2010, How Paraproteins Can Affect Laboratory Assays: Spurious Results and Biological Effects. Pathology, 42: 397–401.

[6] Yang Y, Howanitz PJ, Howanitz JH, et al., 2008, Paraproteins Are a Common Cause of Interferences with Automated Chemistry Methods. Archives of Pathology & Laboratory Medicine, 132(2): 217–223.

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Correlation between Hypovitaminosis D Status and Hyperactivation of IL-6/STAT3 Signaling in Clear Cell Renal Cell Carcinoma

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Abstract: *Objective:* To analyze serum vitamin D levels in patients with clear cell renal cell carcinoma (ccRCC) by flow cytometry and to investigate the relationship between hypovitaminosis D status and hyperactivation of IL-6/STAT3 signaling in ccRCC. *Methods:* Eighty patients diagnosed with ccRCC by our oncology department from January 2019 to December 2021 were selected as study subjects, and the control subjects were selected from patients who were receiving health check-up from our hospital (matched according to case group:control group, 1:2), with 160 healthy patients. All serum samples collected from the case-control subjects were allowed to stand for 1–2 hours, centrifuged at 3000 rpm for 10 minutes, and stored in a -80°C refrigerator, from which they were removed and thawed to measure 25-hydroxyvitamin D (25(OH)D) and interleukin 6 (IL-6) levels. *Results:* The blood calcium level of patients in the cancer group was significantly lower than that of patients in the non-cancer group, and the difference was statistically significant ($P < 0.05$). The IL-6 level of the cancer group was significantly higher than that of the non-cancer group. In high vitamin D state, the IL-6 level of the non-cancer group was higher than that of the cancer group, and the average concentration of IL-6 in both the cancer group and the non-cancer group was significantly higher in low vitamin D state compared with high vitamin D state ($P < 0.05$); the correlation between hypovitaminosis D status and renal Ki-67 was found to be positive. *Conclusion:* The results showed that serum IL-6 levels were elevated in the cancer group and circulating serum 25(OH)D levels were negatively correlated with IL-6 levels. In addition, signal transducer and activator of transcription 3 (STAT3) signaling in RCC tissues was activated in ccRCC patients and in those with low vitamin D status among the cancer group and was higher than that in those with high vitamin D status. These results suggest that hypovitaminosis D status in ccRCC patients is associated with activated IL-6/STAT3 signaling and the activation of tumor proliferation markers proliferating cell nuclear antigen (PCNA), cyclin D1, and Ki-67.

Keywords: Clear cell renal cell carcinoma; Vitamin D; Interleukin 6; STAT3; Hyperactivation

Online publication: March 30, 2023

1. Introduction

Clear cell renal cell carcinoma is a highly morbid and lethal malignancy with a high degree of clinical heterogeneity. Renal cell carcinoma (RCC) is one of the most common malignancies of the urinary system, accounting for 2%–3% of all adult malignancies [1]. In recent decades, the incidence of RCC has been increasing in both developed and developing countries [2]. Extensive epidemiological data have suggested

that chronic sodium intake, smoking, hypertension, obesity, and diabetes are major risk factors for the development of RCC [3-6]. Among them, clear cell renal cell carcinoma (ccRCC) is the most common pathological subtype of RCC, which is of wide concern due to its high malignancy and susceptibility to metastasis, accounting for approximately 70% of all RCCs [7].

2. Materials and methods

2.1. Participants

Eighty patients, comprising 34 male and 46 female patients, diagnosed with ccRCC by the Department of Urology of our hospital from January 2019 to December 2022 were selected as the study subjects. The control subjects were selected from patients receiving health check-up from our hospital (matched according to case group:control group, 1:2); 160 healthy patients, inclusive of 82 male and 78 female patients, were included. The control subjects were informed about the study, and consent was taken from the subjects and approved by the hospital ethics committee. All serum samples collected from the case-control subjects were allowed to stand for 1–2 hours, centrifuged at 3000 rpm for 10 minutes, and then stored in a -80°C refrigerator, from which 25-hydroxyvitamin D (25(OH)D) and interleukin 6 (IL-6) levels were measured after removal and thawing.

2.2. Study design

2.2.1. Reagents

Antibodies to phosphorylated signal transducer and activator of transcription 3 (pSTAT3), cyclin D1, and Ki-67 were purchased from Cell Signaling Technology (Beverley, MA). Antibodies to STAT3, proliferating cell nuclear antigen (PCNA), lamin A/C, and β-actin were purchased from Santa Cruz Biotechnologies (Santa Cruz, CA). Chemiluminescence assay kits were purchased from Pierce Biotechnology (Rockford, IL). All other reagents were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2.2. Measurement of active vitamin D levels in the serum of renal cell carcinoma cases

(1) Determination of serum 25(OH)D concentration
Continuous venous plasma assay was used, *i.e.*, whole blood or whole lipid plasma was extracted through venous whole blood or plasma, and the suspension was collected after centrifugation and separation; then, it was mixed with 10 μL of Standard, and its fluorescence value was detected by ultraviolet (UV) spectrophotometer.

(2) Laboratory standard for serum 25(OH)D test
The test was conducted in accordance with the Code of Practice for Hospital Clinical Test Items and Indicators, issued by the State Ministry of Health.

(3) Calculation of serum 25(OH)D concentration
The concentration of 25(OH)D in serum was determined by enzyme-linked immunosorbent assay, and concentration conversion was carried out by co-element analysis.

(4) Measurement time
6 h, 24 h, and 96 h after the hemagglutination inhibition test, the concentration of 25(OH)D in serum was detected by semi-quantitative fluorescent immunoassay analyzer.

2.2.3. Measurement of serum interleukin 6 levels by enzyme-linked immunosorbent assay (ELISA)

Serum IL-6 levels were measured using a commercial ELISA kit (R&D Systems, Abingdon, Oxon, UK) according to the assay protocol provided in the manufacturer's instructions.

2.3. Statistical analysis

Statistical analyses were performed using SPSS 16.0. The differences in continuous variables between two independent groups were compared with t-test or Mann-Whitney U test by using two independent sampling lines; comparative analysis of categorical variables was performed by chi-square test, and the association between 25(OH)D and IL-6 was analyzed using scatter plots and linear correlation. *P* values of less than 0.05 were considered to be statistically significant.

3. Results

3.1. Determination of hypovitaminosis D status

Since the blood calcium level of patients in the cancer group was low and the patients did not have abnormal bone metabolism, low vitamin D status could be determined by blood calcium concentration. The normal range of blood calcium is 3.52 ± 0.49 ng/mL–3.64 ± 0.58 ng/mL. In the present study, the blood calcium level of patients in the cancer group was significantly lower than that of patients in the non-cancer group, and the difference was statistically significant ($P < 0.05$). In addition, for the judgement standard of low vitamin D status, we adopted a more universal standard – 25(OH)D, that is, 5.65 ng/mL ≤ 25(OH)D < 7.12 ng/mL as low vitamin D-state standard; 7.12 ng/mL was defined as mean blood calcium level of 6.16 ng/mL.

3.2. Hypovitaminosis D status and hyperactivation of IL-6/STAT3 signaling

In the present study, we used IL-6/STAT3 signaling pathway inhibitors to investigate the correlation between hypovitaminosis D status and hyperactivation of IL-6/STAT3 signaling. We found that the cancer group had significantly higher IL-6 levels than the non-cancer group. The IL-6 level of the non-cancer group was higher than that of the cancer group in high vitamin D state, and the average concentration of IL-6 in both the cancer group and the non-cancer group was significantly higher in low vitamin D state compared with high vitamin D state ($P < 0.05$). In the present study, two different types of cancer cells (U87MG and HGCC) expressed IL-6/STAT3 signaling pathway. In hypovitaminosis D state, the cancer group had significantly lower cell viability than the non-cancer group, while in high vitamin D state, the cancer group had higher cell viability than the non-cancer group. Moreover, IL-6/STAT3 signaling pathway was observed in both groups of cells with hypovitaminosis D status. This suggests that low serum vitamin D levels may affect the viability and activity of cancer cells through two different pathways: first, by stimulating IL-6 expression, and second, by promoting STAT3 signaling pathway to produce more effector molecules. This leads to the activation of IL-6 and/or STAT3 signaling, thereby enhancing cancer cell viability. Cancer cell activity is affected when the concentration of these effector molecules increases.

3.3. Higher vitamin D levels result in more Ki-67 in the kidney and hyperactivation of IL-6/STAT3 signaling

In the present study, we further explored the correlation between hypovitaminosis D status and renal Ki-67 and found that vitamin D levels were positively correlated with renal Ki-67. We analyzed and examined the data and found that (1) in the cancer group, serum 25(OH)D levels were elevated, while IL-6 levels and Ki-67 signaling hyperactivation were significantly reduced ($P < 0.05$); (2) when serum 25(OH)D was less than 200 ng/mL, renal Ki-67 and IL-6 levels were 1.83 ± 0.35 ng/mL and 3.17 ± 1.24 ng/mL, respectively; when serum 25(OH)D was more than 200 ng/mL, renal IL-6 and STAT3 signaling hyperactivation were 7.12 ± 1.65 ng/mL and 4.21 ± 0.43 ng/mL, respectively; when serum 25(OH)D was 0–200 ng/mL, renal IL-6 and STAT3 signaling hyperactivation were significantly reduced; (3) there was no significant difference in Ki-67 levels within the non-cancer group and among the treatment regimens in the cancer group.

4. Discussion

Renal tumors are common malignant tumors of the urinary system with high morbidity and mortality rates. Some studies have shown that up to 10%–20% of patients with tumors are already in advanced stage when diagnosed, and their 5-year survival rate is less than 5%. Therefore, early diagnosis and reasonable treatment are keys to improving patients' quality of survival. A common phenomenon in clinical practice is elevated urinary calcium, but the amount of calcium in urine is often not evaluated following the detection of elevated blood calcium. Clinicians tend to overlook calcium abnormalities in the urine when examining patients. We have reported a case of a patient with bilateral ccRCC who was found to have elevated blood calcium and significantly increased serum 25(OH)D level. A diagnosis of bilateral multiple ccRCC was made after pathological examination, and further testing revealed a lymph node positive rate of more than 100%. However, we have not conducted any systematic study to verify whether elevated serum 25(OH)D levels lead to hyperactivation of IL-6/STAT3 signaling in the kidney, which is an important link in the pathological process. In the present study, we analyzed the correlation between serum 25(OH)D level and hyperactivation of IL-6/STAT3 signaling in ccRCC patients with abnormally elevated blood calcium based on the correlation between urinary calcium levels and serum 25(OH)D concentrations from a clinical perspective. The likely mechanism is that the abnormally elevated calcium may promote the hyperactivation of IL-6/STAT3 signaling, and the hyperactivation of this signaling pathway in renal cancer cells promotes tumor progression. The hypothesis that there is an association between serum 25(OH)D level and cancer needs to be further verified.

The following points should be taken into account in clinical work: (1) we should be aware that increased blood calcium is not necessarily an important marker of tumor development; (2) further studies are needed to confirm the association between serum 25(OH)D level and prognosis; (3) although no correlation was observed between blood calcium level and clinical and imaging indices in the present study, we suggest the inclusion of urinary 25(OH)D concentration in investigations. In the present study, we found no significant correlation between serum 25(OH)D levels and urinary calcium levels in patients with ccRCC, suggesting that hypovitaminosis D status cannot be an independent predictor of increased blood calcium in renal cancer patients. This study suggests that serum vitamin D level is one of the prognostic indicators rather than an independent risk factor for renal cancer. Further clinical studies are needed to verify its use as an important indicator for determining the severity of renal cancer under treatment. At present, there are very few studies relevant to this area; thus, further case data collection and prospective clinical analysis are needed to explore and verify the specific mechanism and related risk factors. In addition, we observed that hypovitaminosis D status cannot be used as a prognostic indicator for renal cancer patients; and the need for additional calcium or vitamin D supplementation for those with elevated blood calcium still requires further exploration and verification [8]. The present study also suggested that higher serum vitamin D levels are more likely to lead to increased blood calcium in patients with renal cancer. The correlation between serum vitamin D level and ccRCC also requires further exploration and verification [9]. Several related studies have been reported in China and abroad [10-12]. One of the studies on the relationship between vitamin D receptor gene polymorphism and the risk of breast cancer has shown that it could be used as a prognostic indicator for breast cancer.

Since patients with vitamin D deficiency are prone to cardiovascular disease, we hypothesize that vitamin D deficiency is associated with poor prognosis in patients with ccRCC. However, it is unclear whether or not low levels of blood calcium affect the prognosis of patients with renal cancer. Therefore, we propose the following conclusions: (1) low levels of vitamin D regulate renal cancer cells by regulating IL-6/STAT3 signaling; the regulation of this signaling pathway may control tumor cell growth [13]; (2) vitamin D deficiency or insufficiency is associated with elevated calcium and inflammatory response in renal carcinoma; this relationship may be due to the fact that increased renal carcinogenic calcium acts

through the regulation of key molecules in calcium-dependent signaling pathways, such as C-reactive protein (CRP) and monocyte chemoattractant protein-1 (MCP1) [14]; (3) vitamin D deficiency may increase the risk of tumor recurrence, metastasis, and death in patients with ccRCC [15]. Although we have strong evidence that vitamin D deficiency is associated with poor prognosis in patients with ccRCC, more studies are needed to further confirm this hypothesis.

In short, we found elevated serum IL-6 levels in the cancer group compared to the non-cancer group and a negative correlation between circulating serum 25(OH)D levels and IL-6 levels. In addition, we found that STAT3 signaling in RCC tissues was activated in those with low vitamin D status among the ccRCC patients and was higher than that in those with high vitamin D status. These results suggest that hypovitaminosis D status in ccRCC patients is associated with activated IL-6/STAT3 signaling and the activation of tumor proliferation markers PCNA, cyclin D1, and Ki-67.

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Disclosure statement

The authors declare no conflict of interest.

References

[1] Li F, Huo D, Luan S, et al., 2021, Role of IL-6/JAK2/STAT3 Signaling Pathway in Tumors. Chemistry of Life, 41(03): 535–540.

[2] Han X, Xi Z, Wang K, et al., 2020, In Vitro Experiments on the Regulation of IL-6/STAT3 Signaling Pathway by Hairy Crotonine to Inhibit Proliferation, Migration and Induce Apoptosis in Colon Cancer Cells. Chinese Journal of Gerontology, 40(18): 3955–3959.

[3] Xu S, 2020, Partial Mechanism of Vitamin D3 Inhibition of Epithelial-Mesenchymal Transition and Migration Invasion in Renal Cell Carcinoma, thesis, Anhui Medical University.

[4] Di W, 2019, Study on the Function and Mechanism of E3 Ubiquitin Ligase HECTD3 in Renal Clear Cell Carcinoma, thesis, Kunming University of Science and Technology.

[5] Song J, 2019, Correlation Between Serum Hypovitaminosis D Status and Excessive Activation of Renal IL-6/STAT3 Signaling in Patients with Renal Clear Cell Carcinoma, thesis, Anhui Medical University.

[6] Liu B, 2015, Expression and Correlation of Carbonic Anhydrase IX and PTEN Protein in Renal Clear Cell Carcinoma, thesis, Anhui Medical University.

[7] Gao Y, 2015, Study on the Mechanism of CREPT in Renal Clear Cell Carcinoma, thesis, Ningxia Medical University.

[8] Lian J, 2014, Expression of microRNA-122 in Renal Clear Cell Carcinoma Tissues and the Study of Molecular Regulatory Mechanism in Renal Clear Cell Carcinoma Invasion and Metastasis, thesis, Jilin University.

[9] Li R, 2013, Preliminary Study on the Relationship Between SCIN and Proliferation of Renal Clear Cell Carcinoma, thesis, Kunming Medical University.

[10] Yang Y, 2013, Expression of miR-145 in Kidney and Bladder Cancers and Its Preliminary Functional Study, thesis, Huazhong University of Science and Technology.

[11] Cai Y, Huang Y, 2012, CT and MRI Manifestations of Renal Clear Cell Carcinoma. Chinese and Foreign Medical Science, 31(07): 181 + 183.

[12] Fitch N, Becker AB, 2016, Vitamin D [1,25(OH)2D3] Differentially Regulates Human Innate Cytokine Responses to Bacterial Versus Viral Pattern Recognition Receptor Stimuli. Journal of Immunology, 196(7): 2965–2972.

[13] Colotta F, Jansson B, Bonelli F, 2017, Modulation of Inflammatory and Immune Responses by Vitamin D. J Autoimmun, 85: 78–97.

[14] Powe CE, Evans MK, Wenger J, et al., 2013, Vitamin D-Binding Protein and Vitamin D Status of Black Americans and White Americans. N Engl J Med, 369(21): 1991–2000.

[15] Chen YH, Fu L, Hao JH, et al., 2015, Maternal Vitamin D Deficiency During Pregnancy Elevates the Risks of Small for Gestational Age and Low Birth Weight Infants in Chinese Population. J Clin Endocrinol Metab, 100(5): 1912–1919.

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Relationship Between Serum microRNA-372-3p and Glucose Transporter 4 Levels and Insulin Resistance in Gestational Diabetes Mellitus

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Abstract: *Objective:* To observe the changes in insulin resistance in patients with gestational diabetes mellitus (GDM) based on the detection of serum microRNA-372-3p and glucose transporter protein 4 (GLUT4) levels. *Methods:* We conducted a retrospective cohort study of 42 patients who were diagnosed with GDM and hospitalized in our hospital during the period from January 2017 to December 2021 and another 42 patients who had normal pregnancy during the same period by collecting their clinical data. We analyzed their serum microRNA expression profiles and miR-372-3p levels to study the relationship between GDM and insulin resistance. *Results:* The relative expression of miR-372-3p in the serum of patients in the GDM group was significantly higher than that of patients in the control group, but the GLUT 4 level of the GDM group was significantly lower than that of the control group ($P < 0.05$). Compared with the control group, the GDM group had significantly higher levels of fasting blood glucose (FBG), fasting insulin (FINS), 2-hour postprandial blood glucose (2h-BG), total cholesterol (TC), triglyceride (TG), and homeostatic model assessment for insulin resistance (HOMA-IR) index but significantly lower homeostasis model assessment of β-cell function (HOMA-β) index ($P < 0.05$). The relative expression of miR-372-3p in serum was independently and positively correlated with HOMA-IR, while the level of GLUT4 was independently and negatively correlated with HOMA-IR ($P < 0.05$). *Conclusion:* Glycosylated hemoglobin test in the early stages of pregnancy (12–13 weeks of gestation) is important to ensure the health of pregnant women and fetuses. The screening and intervention for elevated glucose in pregnant women act as a guideline for the treatment of GDM. Patients with insulin resistance and related complications such as hyperinsulinemia and hypoglycemia should be given priority.

Keywords: Gestational diabetes mellitus; microRNA-372-3p; Glucose transporter; Insulin resistance

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1. Introduction

Gestational diabetes mellitus (GDM) is a disease state in which diabetes occurs during pregnancy. The prevalence of GDM ranges from 7.4% to 16.6%. For women of childbearing age, having GDM increases the risk of type 2 diabetes mellitus, preterm births, miscarriages, babies with low birth weight and congenital malformations, and neonatal mortality; moreover, the risk of perinatal mortality also increases with GDM. Therefore, the prevention and treatment of GDM is of great importance to women's health. The prevalence of GDM in the Chinese population is about 12%–18% [1-6]. In recent years, with the increased incidence of glucose metabolism abnormalities in pregnancy, GDM has become a major factor affecting the health of both mother and child. Elevated glucose in pregnancy is closely associated with insulin resistance, which, in turn, leads to metabolic abnormalities in the body and subsequently affects placental

function. The relationship between insulin resistance and the development of GDM is still inconclusive, and no clear mechanism has been proposed in the study of the pathogenesis of GDM. Serum glucose transporter 4 (GLUT4), as a novel glucose transporter, plays an important role in the regulation of glucose homeostasis, and studies have demonstrated its pivotal role in obesity and metabolic syndrome [7-10]. In recent years, an increasing number of studies have shown that abnormally elevated serum GLUT4 levels ($\geqslant$ 10 ng/mL) and significantly increased insulin resistance in patients with GDM correlate with obesity, hypertension, and other metabolic syndromes. The aim of this study was to investigate the relationship between elevated glucose levels and insulin resistance during pregnancy and its associated mechanisms.

2. Data and methods

2.1. General information

We conducted a retrospective cohort study of 42 patients who were diagnosed with GDM and hospitalized in our hospital during the period from January 2017 to December 2021 and another 42 patients who had normal pregnancy during the same period. Information on gender, age, body mass index (BMI), height, waist circumference, body mineral density (BMD), and microRNA (miRNA) expression results was collected. Inclusion criteria: (i) patients with a confirmed diagnosis of GDM; (ii) patients with BMI $\geqslant$ 30 kg/m^2; (iii) patients with previous GDM before 28 weeks of gestation; (iv) patients with type 1 diabetes, other autoimmune diseases, or a family history of diabetes. A pre-pregnancy fasting glucose test was done for all the patients, and their blood was collected at the 3rd, 4th, and 6th week of gestation. Their plasma calcitonin gene-related peptide (CGRP), S100 calcium binding protein A4 (S100A4), and miR-372-3p levels were measured by real-time quantitative polymerase chain reaction (RT-PCR). Their serum microRNA expression profiles and miR-372-3p levels were analyzed to investigate the relationship between GDM and insulin resistance.

2.2. Design

2.2.1. Detection of metabolism-related indexes

6mL of venous blood was collected from patients in the GDM group on the day after admission and from the control subjects at physical examination; the blood was centrifuged at 3000 RPM for 10 min (centrifugation radius, 8 cm), and the supernatant was taken and stored at -80°C. Serum total cholesterol (TC) and triglyceride (TG) levels were measured using BS-450 automatic biochemistry analyzer (Shenzhen Myriad Biomedical Electronics Co., Ltd.). The glucose oxidase method was used to detect fasting plasma glucose (FPG) levels, while radioimmunoassay was used to detect fasting insulin (FINS) levels; glucose tolerance test was performed to obtain their 2-h postprandial blood glucose (2h-BG) levels.

2.2.2. Detection of the relative expression of serum miR-372-3p and GLUT4 levels

Total serum RNA was extracted using TRIzol Total RNA Extraction Kit (Shanghai Rongwida Industrial Co., Ltd.), the concentration and purity were detected by ultraviolet (UV) spectrophotometer, and the integrity was verified by agarose gel electrophoresis. The RNA was reverse-transcribed to cDNA using TaKaRa Reverse Transcription Kit (Shanghai Yanhui Biotechnology Co., Ltd.) and stored in -20°C refrigerator.

2.3. Statistical analysis

SPSS 24.0 was used for data analysis. Measurement data were expressed in mean ± standard deviation and tested by t-test, while count data were expressed in percentage and tested by chi-square test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Relative expression of miR-372-3p in serum and GLUT4 level

The relative expression of miR-372-3p in the serum of patients in the GDM group was significantly higher than that of patients in the control group, and the level of GLUT4 of the GDM group was significantly lower than that of the control group ($P < 0.05$). The results are shown in **Table 1**.

Table 1. Comparison of the relative expression of miR-372-3p and GLUT4 levels in the serum of patients in both groups

Group	miR-372-3p	GLUT4 (μg/L)
Control group (n = 42)	1.24 ± 0.33	5.26 ± 1.62
GDM group (n = 42)	2.38 ± 0.69	2.47 ± 0.51
t	8.961	3.694
P	< 0.05	< 0.05

3.2. Metabolism-related indexes

The levels of FBG, FINS, 2h-BG, TC, TG, and HO-MA-IR of the GDM group were significantly higher than those of the control group; however, HOMA-β was significantly lower in the GDM group compared to the control group ($P < 0.05$), as shown in **Table 2**.

Table 2. Comparison of metabolism-related indexes between the two groups

Indicator	Control group (n = 42)	GDM group (n = 42)	t	*P*
FBG	4.52 ± 0.50	6.34 ± 0.89	6.398	< 0.05
FINS	7.87 ± 1.64	13.41 ± 2.37	4.256	< 0.05
2h-BG	6.03 ± 1.35	8.33 ± 1.09	5.362	< 0.05
TC	4.31 ± 1.02	5.94 ± 1.71	4.369	< 0.05
TG	1.00 ± 0.32	2.21 ± 0.60	3.568	< 0.05
HOMA-IR	2.52 ± 0.87	3.99 ± 0.96	6.982	< 0.05
HOMA-β	1.91 ± 0.60	1.13 ± 0.22	4.699	< 0.05

Abbreviations: 2h-BG, 2-hour postprandial blood glucose; FBG, fasting blood glucose; FINS, fasting insulin; HOMA-IR homeostatic model assessment for insulin resistance; HOMA-β, homeostasis model assessment of β-cell function; TC, total cholesterol; TG, triglyceride.

3.3. Multiple linear regression analysis of the relative expression of miR-372-3p in serum and the relationship between GLUT4 level and HOMA-IR in patients with gestational diabetes mellitus

Multiple linear regression analysis was conducted with the relative expression of serum miR-372-3p and GLUT4 level as independent variables and HOMA-IR as dependent variable. The results showed that the relative expression of serum miR-372-3p was positively and independently correlated with HOMA-IR. GLUT4 level, on the other hand, was found to be negatively correlated with HOMA-IR ($P < 0.05$), as shown in **Table 3**.

Table 3. Multiple linear regression analysis of the relative expression of miR-372-3p in serum and the relationship between GLUT4 and HOMA-IR in patients with gestational diabetes mellitus

Indicator	β	Standard error	t	*P*
miR-372-3p	0.612	0.201	2.987	< 0.05
GLUT4	-0.246	0.265	2.698	< 0.05

4. Discussion

Insulin resistance exists in patients with GDM and is one of the main risk factors for GDM. Insulin resistance refers to decreased sensitivity of the body to insulin and is often manifested as disorders of glucose and lipid metabolisms. In this study, 81.9% of pregnant women with normal gestation had reduced insulin sensitivity (fasting glucose ≤ 5.1 mmol/L), while only 15.8% of GDM patients had high fasting glucose levels. In addition, there is a special group of pregnant women with normal gestation who have decreased insulin sensitivity but do not have high blood glucose levels [11-13]. Studies have shown that, the incidence of glucose metabolism disorders, dyslipidemia, lipoprotein metabolism disorders, and vascular inflammation was higher in patients with GDM compared with controls. This may be due to the inaccuracy of conventional detection methods used in determining serum glucose and lipid levels in patients with GDM, which may lead to false positive results.

With lifestyle changes and the further aging trend of the society, the number of people suffering from diabetes mellitus is increasing. Studies have shown a close correlation between GDM and insulin resistance and the importance of early diagnosis of GDM during pregnancy or postpartum for the prevention and treatment of diabetes. In this study, the relationship between elevated blood glucose and insulin resistance in pregnant women was explored by comparing the serum microRNA and GLUT4 levels in healthy pregnant women and those with GDM. The results of this study showed the presence of gestational prediabetes status in pregnant women with normal fasting glucose and insulin resistance in patients with gestational diabetes. In addition, studies have found markedly increased glucose challenge test, insulin receptor substrate 1 (IRS-1), and GLUT1 levels in GDM patients, indicating the presence of insulin resistance in GDM patients [14,15]. However, the present study only analyzed the serum microRNA and GLUT4 levels in patients with GDM combined with IR. Therefore, more studies are needed to prove the relationship between these two indicators and GDM combined with IR. We believe that the relationship between these two indicators and GDM combined with IR will become clearer with subsequent studies.

HOMA-IR is an independent risk factor to predict the risk of diabetes. In the present study, this index was detected in all pregnant women during fasting glucose examination in late pregnancy. Hemoglobin A1c (HbA1c) was significantly higher in the GDM group compared to the non-diabetic group. The study also found that pregnant women in the first trimester of pregnancy were more likely to have abnormal insulin resistance and HOMA-IR indices. In addition, both HbA1c and HOMA-IR index were associated with glucose intake, obesity, or overweight. The correlation between serum GLUT4 levels during pregnancy and blood glucose levels in late pregnancy was not significant, probably because of its low concentration during pregnancy and little effect on disease in pregnancy. By adjusting the factor analysis, a significant correlation ($P < 0.05$) was observed between late pregnancy glucose level, BMI, fasting glucose level, and blood GLUT4 concentration; fasting insulin resistance index during pregnancy was also positively correlated with serum GLUT4 concentration. Therefore, there is still a need to further investigate the mechanism of action of GLUT4 in the regulation of glucose homeostasis in the blood of pregnant women with GDM and its use as a biomarker for predicting the risk of developing pregnancy-related diseases.

In the present study, the patients were not subjected to glucose loading test as there were insufficient data to verify whether or not it has an effect on glycemic changes; moreover, the serum samples used were obtained from women only during pregnancy. In assessing pregnant women with GDM using the GDM risk score, clinical examination indicators were not used to determine whether or not a pregnant woman has GDM. However, because the present study used non-clinical indicators to assess the differences between GDM patients and healthy pregnant women during pregnancy and 3 months after delivery, it was not possible to make a judgment about the relationship between it and insulin resistance. In addition, although clinical tests were used to assess the relationship between glucose elevation and insulin resistance in pregnant women during pregnancy, these clinical tests showed no correlation in the normal population. Therefore, there is a need for more in-depth studies on different populations in the future.

In conclusion, glycosylated hemoglobin examination in early gestation (12–13 weeks of gestation) is important to ensure the health of pregnant women and fetuses. The screening and intervention for elevated glucose in pregnant women act as a guideline for the treatment of GDM. Patients with insulin resistance and related complications such as hyperinsulinemia and hypoglycemia should be given priority.

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Disclosure statement

The authors declare no conflict of interest.

References

[1] Zhu S, Gu L, 2022, Correlation Between miRNA-372-3p, miRNA-149 and miRNA-7 and Insulin Resistance and Pregnancy Outcome in Patients with Gestational Diabetes Mellitus. China's Maternity and Child Care, 5(20): 3841–3845.

[2] Xing HM, Liang J, Tian M, et al., 2022, Relationship Between Serum Ferritin, CAT, MCP-1 and Insulin Resistance in Gestational Diabetes Mellitus and the Influencing Factors of Pregnancy Outcome. Modern Biomedical Progress, 22(18): 3534–3538.

[3] Chen L, Relationship Between Serum miR-146a Expression and Insulin Resistance in Patients with Gestational Diabetes Mellitus. Journal of Practical Medical Technology, 29(03): 279–282 + 337.

[4] Wang Y, Mao N, Chen S, 2021, Serum FABP-4, ADP, RBP4, Nesfatin-1 and Insulin Resistance and Diagnosis in Patients with Gestational Diabetes Mellitus. Chinese Journal of Family Planning, 29(08):1718–1721 + 1726.

[5] Wang K, 2021, Changes in Serum Ferritin and Lipocalin and Their Relationship with Insulin Resistance in Patients with Gestational Diabetes Mellitus. Chinese Community Physician, 37(15): 54–55.

[6] Huang H, Jia H, Wang X, et al., 2021, Expression Levels of miRNA-508-3p and HGF in Placental Tissues of Patients with Gestational Diabetes Mellitus and Their Effects on Trophoblast Insulin Resistance. Journal of Jilin University (Medical Edition), 47(01): 187–195.

[7] Bao J, 2020, Effect of Integrated Diet and Exercise Intervention on Glycemic Control and Insulin Resistance in Patients with Gestational Diabetes Mellitus. Journal of Aerospace Medicine, 31(12):

1514–1516.

[8] Shi F, Si H, Huang J, 2020, Relationship Between Serum miR-126 Expression and Insulin Resistance in Patients with Gestational Diabetes Mellitus. Chinese Journal of Diabetes, 28(06): 423–427.

[9] Bu C, Huang X, 2020, Relationship Between Insulin Resistance Levels and Sex Hormone Levels in Patients with Gestational Diabetes Mellitus. Chinese Journal of Family Planning, 28(06): 946–949.

[10] Dong C, Wu G, Zhang Y, et al., 2020, Relationship Between Serum miR-149 Levels and Insulin Resistance in Patients with Gestational Diabetes Mellitus. Western Medicine, 32(05): 700–703.

[11] Li R, Ouyang C, Zhang Z, et al., 2020, Changes in Serum Chemerin in Patients with Gestational Diabetes Mellitus and the Relationship with Insulin Resistance. China Maternal and Child Health Research, 31(01): 109–112.

[12] Zuo X, Kong F, Su T, 2018, Study on the Expression of Serum tOC and ucOC and Their Relationship with Insulin Resistance in Patients with Gestational Diabetes Mellitus. China Maternal and Child Health Care, 33(24): 5764–5766.

[13] Yang J, Wang X, Yang JN, 2018, Relationship Between Serum Lipocalin, Chemokine, Retinol-Binding Protein 4 and Insulin Resistance in Gestational Diabetes Mellitus Patients. China Family Planning and Obstetrics and Gynecology, 10(07): 74–77.

[14] Li D, Liu Y, Su D, et al., 2018, Exploring the Relationship Between Placental Tissue Lipoproteinase Gene Polymorphisms and Insulin Resistance in Patients with Gestational Diabetes Mellitus. Modern Preventive Medicine, 45(12): 2154–2157 + 2194.

[15] Ke H, Guo L, Ding H, et al., 2014, Relationship Between Serum Resistin and Insulin Resistance in Patients with Gestational Diabetes Mellitus in Mid-Pregnancy. Laboratory Medicine, 29(09): 921–924.

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Application Effect of Medium-Length Peripheral Catheter in Critically Ill Patients Undergoing Hepatobiliary Surgery

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Abstract: *Objective:* To investigate the effect of using peripheral medium-length catheters in critically ill patients undergoing hepatobiliary surgery. *Methods:* A retrospective analysis of the nursing experience and effect of using medium-length catheters for infusion in 102 critically ill patients undergoing hepatobiliary surgery from March 2021 to April 2022 was conducted. *Results:* All 102 patients had successful catheter placement with no catheter-associated infections, blockage, decannulation, or breakage. However, four cases had blood oozing from the puncture site, but it resolved after changing the dressing. *Conclusion:* Medium-length catheters are superior to traditional infusion tools in terms of benefit; thus, they deserve to be widely promoted in clinical practice.

Keywords: Medium-length peripheral catheter in critically ill patients; Application effect

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1. Introduction

Critically ill patients undergoing hepatobiliary surgery require many different types and large amounts of infusion. The infusion pathways commonly used in clinical practice include peripheral venous catheter, central venous catheter (CVC), peripherally inserted central catheter (PICC), *etc*. Repeatedly puncturing the blood vessels with needles not only causes pain to patients, but also leads to various adverse events, such as fluid infiltration and catheter-related infection. CVC, PICC, and so on are not only difficult to operate, but also associated with high cost, complications, and high qualification requirements for operators; thus, they are unable to meet the needs of critically ill patients for rapid and safe infusion. A key concern among medical staff is in the selection of infusion access, which must be both easy to operate and suitable for critically ill patients, so as to reduce infusion risks and complications [1]. The medium-length catheter is a peripheral venous access device, usually inserted through the basilic vein, cephalic vein, or median cubital vein, with a length of 7.5–20.0 cm [2], and its tip reaching the subclavian vein, but not exceeding the distal axillary vein [3]; its flow rate is 900 mL/min, and drug dilution is rapid, allowing continuous infusion of stimulating, highly osmolar, and strongly acidic and basic drugs. The recommended retention time is 1–6 weeks [4]; thus, it can meet the needs of patients for infusion of complex medications and medium- to long-term rehydration. From March 2021 to April 2022, 102 critically ill patients under our department received infusion therapy with medium-length catheters.

2. Data and methods

2.1. General information

A total of 102 critically ill patients who received infusion therapy with medium-length catheters under the Department of Hepatobiliary Surgery of Shaanxi Provincial People's Hospital from March 2021 to April 2022 were selected as the observation subjects. Inclusion criteria: (i) expected infusion treatment time of more than 7 days; (ii) normal routine blood and blood coagulation time; (iii) informed consent given by the patients and their families. Among the 102 patients who met the inclusion criteria, 56 were male and 46 were female; their mean age was 40.8 ± 13.5 years (31–73 years); there were 35 cases of acute severe suppurative cholangitis, 12 cases of pancreatoduodenectomy, 31 cases of liver cancer, 13 cases of acute severe pancreatitis, 7 cases of gallbladder cancer, and 4 cases of cholangiocarcinoma.

2.2. Design

2.2.1. Operator qualification

Puncture operator: all puncture operators were members of the intravenous therapy team who had obtained relevant qualifications in our department. Maintainer qualification: the maintenance was carried out by nurses who had obtained the Registered Nurse certificate, in which their skills for performing such procedures were approved by their instructor, and they performed under the supervision of their instructor; student nurses were not allowed to perform the procedure.

2.2.2. Catheter selection

Medium-length catheters made of medical silicone material, with integrated connection, three valves, and specifications of 4Fr, 3Fr, were selected.

2.2.3. Inform consent

Before catheter placement, the nurses explained the purpose, risk, *etc*., to the patients and their families, and the patients signed the informed consent [5].

2.2.4. Procedural approach

Patient health education was done before catheterization. The catheter was placed in a sterile environment, and the circumference of both arms and the length of the preset tube were measured. The patient was required to extend his/her arm 45°–90° forward from the trunk. The skin was wiped and disinfected with 2% chlorhexidine gluconate ethanol solution at the puncture point and left to dry in air. Maximum sterile barrier was established, the catheter was pre-flushed with normal saline, and the integrity of the catheter was inspected. B ultrasound was used to check the blood vessels in the upper arm; brachial or cephalic vein is routinely selected, and arteries and veins are strictly distinguished to prevent accidental arterial injury. The puncture point was located at one third of the upper condyle of the humerus to the apex of the armpit. The skin was disinfected the second time at the puncture point and left to dry in air. The modified Seldinger technique under ultrasonic guidance was used for catheterization. The guidewire was reserved at least 15 cm outside the body to prevent it from sliding into the body. The skin was stretched in the direction of the guidewire to prevent damage to the guidewire and blood vessels. The guidewire was removed gently to prevent damage to the integrity of the catheter and the guidewire.

2.2.5. Catheter care

At each shift, the skin at the puncture site was examined for redness, swelling, tenderness, and bleeding; the film was examined to determine if it was wet, contaminated, or fringed; and the catheter was observed for any obstruction, damage, protrusion, displacement, *etc*. The tube was flushed with 15–20 mL of normal

saline before and after infusion, especially after infusing drugs with high viscosity, irritants, and blood products, as well as between infusions of incompatible drugs. When flushing the tube, there should be no blood return or resistance. The tube was sealed by positive pressure sealing. A special maintenance kit was used twice a week. A wet, contaminated, or fringed film was replaced immediately. During maintenance, there was strict adherence to aseptic techniques.

2.2.6. Health education

The patients and their families were educated so that they understood the precautions for catheter maintenance and the preventive measures for complications. The patients were instructed to drink more water and alternatively squeeze a grip ball to the maximum and relax after 5 s using the limb of the catheterization side 24 hours after catheterization. They were also encouraged to do elbow flexion and extension exercises, along with internal and external wrist rotation exercises, 10 min each time.

2.2.7. Catheter removal

The catheter was removed by qualified members of the static therapy team. Strict aseptic technique was adhered to. The catheter was not removed by force if there were difficulties in removal. After removal of the catheter, the puncture site was covered for protection, and the film was removed 24 hours later; the length of the catheter was measured, the integrity of the catheter was assessed, and the fibrin sheath adhesion of the tube wall was carefully observed. The tip of the catheter, with a length of 2–3 cm, was taken for bacterial culture. The length and removal time were recorded.

3. Results

The placement of catheter was successful for all 102 patients. The retention time of the catheter was 10–31 days. After the completion of the treatment plan, the catheter was removed. During the indwelling period, high osmotic pressure fluid, intravenous vasoactive drugs, blood products, and parenteral nutrition were administered to the patient via the catheter. The patients did not experience any adverse reaction, and no tubes were blocked, shed, or broken. However, four patients showed different degrees of bleeding at the puncture points, which was stopped within 1–2 days after changing the dressing. One patient died due to disease progression.

4. Discussion

In clinical work, it is important for nursing staff to make a thorough assessment of critically ill patients and make reasonable selections of infusion tools. Medium-length catheters can reduce the number of phlebotomies in a patient, alleviate pain, protect blood vessels, and reduce the workload among nurses. The cost of placement and maintenance of medium-length catheters and the incidence of complications are significantly lower than those of CVC and PICC [6], thereby reducing treatment costs and the economic burden on patients. The retention time of medium-length catheters meets the treatment and hospitalization needs of critically ill patients undergoing hepatobiliary surgery. In a comprehensive evaluation, medium-length catheters are significantly better than traditional infusion tools in terms of benefit; thus, they should be given priority in the selection of intravenous access for critically ill patients.

Disclosure statement

The authors declare no conflict of interest.

References

[1] Fan X, 2016, Study on the Selection of Infusion Tools and Its Influencing Factors During Intravenous Infusion Therapy, thesis, Shanxi Medical University.

[2] Wang J, 2009, Transfusion Therapy Nursing Practice Guidelines and Implementation Rules, People's Military Medical Press, Beijing, 16–22.

[3] Sharp R, Esterman A, McCutcheon H, et al., 2014, The Safety and Efficacy of Midlines Compared to Peripherally Inserted Central Catheters for Adult Cystic Fibrosis Patients: A Retrospective, Observational Study. Int Nurs Stud, 51(5): 694–702.

[4] Hu M, Shen X, Gu P, et al., 2015, Clinical Application of Medium Length Catheter in Peripheral Vein. Nursing Research, 11(29): 3045–3048.

[5] Adams DZ, Little A, Vinsant C, et al., 2016, The Midline Catheter: A Clinical Review. The Journal of Emergency Medicine, 51(3): 252–258.

[6] Zhu Y, Zhao Y, Zhou Y, et al., 2021, Application of Peripheral Medium Length Catheter in Neurosurgery Patients. Journal of Practical Clinical Medicine, 25(2): 40–42.

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Value of N-Terminal Pro B-Type Natriuretic Peptide, High-Sensitivity C-Reactive Protein, and Homocysteine Levels in Predicting Cardiovascular Events in Chronic Heart Failure Patients After Discharge

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Abstract: *Objective:* To investigate the value of N-terminal pro B-type natriuretic peptide (NT-proBNP), high-sensitivity C-reactive protein (hs-CRP), and homocysteine (Hcy) levels in predicting cardiovascular events (CV) in patients with chronic heart failure (CHF). *Methods:* A total of 63 patients with CHF admitted to our hospital between June 2019 and July 2021 were selected. Their NT-proBNP, hs-CRP, and Hcy levels were detected at discharge, and a 12-month follow-up was done after their discharge to collect clinical data. The collected data were inclusive of data from 21 CHF patients with cardiovascular disease and 42 CHF patients without cardiovascular disease. The effect of NT-proBNP, hs-CRP, and Hcy levels on the occurrence of CV was analyzed. *Results:* The levels of NT-proBNP, hs-CRP, and Hcy in the group with cardiovascular disease were significantly higher than those in the group without cardiovascular disease ($P < 0.05$); the levels of serum NT-proBNP, hs-CRP, and Hcy at discharge had certain value in predicting short-term CV in CHF patients ($P < 0.05$). *Conclusion:* NT-proBNP, hs-CRP, and Hcy levels can be used to predict CV in CHF patients, thus having clinical application value.

Keywords: Chronic heart failure; N-terminal pro B-type natriuretic peptide; Homocysteine; High-sensitivity C-reactive protein

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1. Introduction

Chronic heart failure (CHF) is an important component of cardiovascular disease, and various cardiac markers can be used to predict the occurrence of cardiovascular events (CV) in CHF patients. China has a high prevalence of heart failure, and studies have found that the levels of N-terminal pro B-type natriuretic peptide (NT-proBNP), high-sensitivity C-reactive protein (hs-CRP), and homocysteine (Hcy) can be used to predict the occurrence of cardiac events. Although these three markers have some predictive value in terms of prognosis, their predictive value is limited in patients with CHF [1-4]. In a study published in the Chinese Journal of Cardiovascular Diseases on July 24, 2020, a retrospective analysis of the serum levels of NT-proBNP, hs-CRP, and Hcy in patients with CHF at discharge was carried out to evaluate the value of these three markers in predicting CV in CHF patients and discuss the effects of NT-proBNP, hs-CRP, and Hcy levels on the occurrence of cardiovascular disease in patients. By observing the correlation between NT-proBNP, hs-CRP, and Hcy levels at discharge and patient outcomes during follow-up, it was

found that NT-proBNP and Hcy levels were independently associated with the risk of CV in patients with CHF; their predictive values were as follows: NT-proBNP > 0.86 pg/mL, hs-CRP > 0.58 pg/mL, and Hcy > 0.32 mg/dL. This paper focuses on the effect of NT-proBNP, hs-CRP, and Hcy levels on the occurrence of CV in patients with CHF.

2. Data and methods

2.1. General data

Sixty-three patients with CHF admitted to our hospital between June 2019 and July 2021 were selected, including 36 male and 27 female patients, age ranging from 14 to 71 years, with a mean age of 39.23 ± 3.69 years. Their NT-proBNP, hs-CRP, and Hcy levels were measured at the time of discharge, and a 12-month follow-up was done after discharge to collect clinical data. The collected data were inclusive of data from 21 CHF patients who developed cardiovascular disease and 42 CHF patients who did not develop cardiovascular disease.

2.2. Methods

According to the Chinese Guidelines for the Diagnosis and Treatment of Heart Failure 2018 [5], for hypertension, diabetes mellitus, and other comorbidities, the conventional therapy includes bed rest, vasodilatation, diuresis, and cardiac contractility strengthening. All patients had 5 mL of fasting venous blood collected at discharge. Plasma NT-proBNP concentration was determined by fluorometry, human plasma hs-CRP content was determined by immunoturbidimetry, and Hcy content was determined by circulating enzyme-linked immunoassay.

2.3. Statistical analysis

SPSS 22.0 was used for data processing. Measurement data were expressed in mean ± standard deviation and tested by t-test, whereas counting data were expressed in rate (%) and tested by chi-squared test.

3. Results

3.1. Comparison of serum N-terminal pro B-type natriuretic peptide, high-sensitivity C-reactive protein, and homocysteine levels between the two groups

The levels of NT-probNP, hs-CRP, and Hcy in the group with cardiovascular disease were significantly higher than those in the group without cardiovascular disease ($P < 0.05$), as shown in **Table 1**.

Table 1. Comparison of serum NT-proBNP, hs-CRP, and Hcy levels between the two groups

Group	Group without cardiovascular disease (n = 42)	Group with cardiovascular disease (n = 21)
NT-proBNP (ng/L)	424.88 ± 170.94	712.16 ± 321.12[a]
hs-CRP (mg/L)	2.38 ± 1.26	3.44 ± 1.16[a]
Hcy (μmol/L)	19.16 ± 4.75	26.73 ± 5.89[a]

Abbreviations: Hcy, homocysteine; hs-CRP, high-sensitivity C-reactive protein; NT-proBNP, N-terminal pro B-type natriuretic peptide. [a]$P < 0.05$, comparing the cardiovascular event group with the group without cardiovascular event.

3.2. Predictive value of serum N-terminal pro B-type natriuretic peptide, high-sensitivity C-reactive protein, and homocysteine levels for cardiovascular events

Receiver operating characteristic (ROC) analysis showed that serum NT-probNP, hs-CRP, and Hcy levels of CHF patients at discharge had certain value in predicting short-term CV ($P < 0.05$), as shown in **Table 2**.

Table 2. Predictive value of serum NT-proBNP, hs-CRP, and Hcy levels for cardiovascular events

Variable	Sensitivity	Specificity	95% CI	Cut-off	Acreage
NT-pro BNP	0.75	0.81	0.678–0.874	672.61 ng/L	0.774
Hcy	0.71	0.77	0.647–0.852	25.61 μmol/L	0.776
hs-CRP	0.74	0.68	0.647–0.852	3.44 mg/L	0.772

Abbreviations: CI, confidence index; Hcy, homocysteine; hs-CRP, high-sensitivity C-reactive protein; NT-proBNP, N-terminal pro B-type natriuretic peptide.

4. Discussion

CHF is a serious cardiovascular disease and has a long and complex treatment process. The pathophysiological basis for heart failure is ventricular remodeling, including ventricular systolic and diastolic dysfunction as well as structural abnormalities, which ultimately lead to the development of heart failure. Patients with early-onset heart failure usually have structural and functional impairments due to a decrease in left ventricular ejection fraction, whereas those with late-onset heart failure often have structural and functional impairments due to increased left ventricular end-diastolic internal diameter. At present, the main methods for assessing myocardial systolic function are echocardiography and magnetic resonance imaging (MRI). Between them, echocardiography is the gold standard for diagnosing CHF, but its specificity is poor, and it cannot be used to predict the time at which heart failure may occur. Therefore, early detection of myocardial systolic function is crucial. MRI, on the other hand, has several advantages; it is noninvasive, rapid, highly specific, and highly sensitive. It is uniquely valuable in myocardial biopsy. Treatment of heart failure includes medications and heart transplantation. Although the available medications are effective in reducing the risk of mortality in patients with heart failure, recurrence or progression is likely to occur in 30%–40% of these patients. In patients with CHF, approximately 50% are still in heart failure upon discharge, and approximately 30% progress to heart failure within two years of discharge. Since cardiac events at discharge are different from those during hospitalization, early prediction may help patients make better decisions and reduce the length of hospital stay. Serum NT-proBNP, hs-CRP, and Hcy levels have been found to be strongly associated with the risk of CV in patients with CHF.

4.1. Correlation between N-terminal pro B-type natriuretic peptide, high-sensitivity C-reactive protein, and homocysteine levels and cardiovascular events in chronic heart failure patients after discharge

At present, there are only a few studies available about the risk of CV after discharge in CHF patients. Studies have shown that the occurrence of CV after discharge is associated with treatment during hospitalization. Since patients with CHF are often hospitalized, there is evidence that the occurrence of CV after discharge is strongly associated with elevated levels of NT-proBNP, hs-CRP, and Hcy at the time of admission. A study has shown 2.5-fold higher serum NT-proBNP levels in CHF patients at the time of discharge than at the time of admission. In another study, the NT-proBNP levels were 1.1-fold higher at discharge than at admission. There have been suggestions that NT-proBNP levels are associated with adverse events, such as acute myocardial infarction, stroke, and death. Besides NT-proBNP, a recent study has found significantly higher serum hs-CRP levels in patients with CHF at discharge than at admission, suggesting that serum hs-CRP levels are positively correlated with the occurrence of cardiac events during hospitalization. Another study has found a positive correlation between hs-CRP and left ventricular ejection fraction, diastolic pressure, and right ventricular ejection fraction. Serum hs-CRP levels have also been found to be significantly associated with mortality during hospitalization. A study of the relationship between serum NT-proBNP, hs-CRP, and Hcy levels at discharge and the risk of CV in CHF patients has

demonstrated a significant association between NT-proBNP levels at discharge and Hcy levels during hospitalization. Moreover, the Hcy levels at admission were found to be higher in the group with higher NT-proBNP levels than in the group with lower NT-proBNP levels at 1 month after discharge. Another study has also pointed out that serum NT-proBNP, hs-CRP, and Hcy levels can be used as auxiliary diagnostic indicators in patients with heart failure at discharge. Serum NT-proBNP levels have also been found to be correlated with the prognosis of heart failure, with higher serum NT-proBNP levels being better. These results are important to deepen our understanding of the development of CV in CHF patients after hospital discharge and guide us in making better clinical decisions.

Currently, most studies are retrospective and tend to focus on the characteristics of the study population (*e.g*., age, gender, and family history). As the population continues to age and the treatment and prognosis of CHF patients face greater challenges, researchers need to focus on the risk of CV in CHF patients at hospital discharge. NT-proBNP, a member of the BNP family, is a non-specific BNP enzyme produced *in vivo* by B cells and later by lymphocytes. Numerous studies have shown that NT-proBNP may be the most sensitive and reliable marker for predicting CV in patients with CHF. Hcy is a monoclonal antibody mainly produced by B lymphocytes and is associated with various diseases. Hcy levels are closely associated with diabetes, chronic kidney disease, and metabolic syndrome. In a retrospective analysis of 1,073 patients with CHF, Yang *et al*. [7] found a significant association between serum NT-proBNP, hs-CRP, and Hcy levels and CV at discharge and that these indicators elevated by 1.14 (95% CI 1.10–1.26), 0.96 (95% CI 0.92–0.99), and 1.04 (95% CI 1.03–1.12), respectively, within 2 years after discharge. A study by Li *et al*. [8] showed the same results and concluded that serum NT-proBNP, hs-CRP, and Hcy levels have good predictive value for CV in CHF patients. A study has found that when NT-proBNP, hs-CRP, and Hcy levels were used as baseline, the risk of CV in CHF patients at admission was 30%, 18%, and 24%, respectively. Wang *et al*. conducted a case-control study, which included 566 CHF patients, and divided the patients into two groups (92.5% were CHF patients): group 1 (NT-proBNP + hs-CRP) and group 2 (NT-proBNP + hs-CRP). They found that among the CHF patients, the first group was more likely to have CV (HR = 1.78, 95% CI: 1.23–1.95), risk of death (HR = 1.89, 95% CI: 1.15–2.03), and all-cause mortality (HR = 2.07, 95% CI: 2.00–3.05) [9]. In addition, several studies have shown that death may occur when hs-CRP levels exceed the threshold [10-13]. The correlation between hs-CRP levels and mortality is also supported by the findings of Li *et al*.

4.3. Prognosis of cardiac events at discharge

An analysis of the prognosis of cardiac events at hospital discharge, including acute myocardial infarction, stroke, left ear occlusion, and intracardiac thrombosis, was carried out, and the differences in NT-proBNP, hs-CRP, and Hcy levels at admission, 1 month, and 2 months after admission were compared [14]. According to the results, NT-proBNP and hs-CRP serve as independent predictors of the risk of cardiac events at hospital discharge [15]; NT-proBNP levels were 0.41 ng/mL (95% CI: 0.26–0.54) during hospitalization and 0.61 ng/mL (95% CI: 0.37–0.94) at discharge.

A cardiac event is defined as death due to a risk factor for cardiovascular disease during a patient's hospitalization or within 1 month after discharge. Risk factors have now become major predictors of cardiovascular disease. The American College of Cardiology (ACC) and European Society of Cardiology (ESC) guidelines have recommended that aggressive lifestyle improvement, blood pressure control, cholesterol reduction, smoking cessation, and glycemic improvement should be initiated within 2 weeks or less following myocardial infarction. However, there are some variations in the results of studies on CHF patients. There is a lack of uniform criteria for risk factors associated with cardiac events in CHF patients at discharge. In addition, the baseline blood pressure levels in patients with heart failure are also strongly associated with the risk of cardiovascular events, such as higher baseline blood pressure in patients without

heart failure.

The results of the present study showed that NT-proBNP, hs-CRP, and Hcy levels were significantly higher in patients with early-onset heart failure than in patients with late-onset heart failure at discharge, suggesting that they have certain predictive value for cardiovascular events. This provides an important basis for the formulation of prevention and control measures. As an independent risk factor for early prediction of cardiac events, hs-CRP can be used as a new indicator for predicting risk of cardiac events. Other than that, Hcy can be used to detect risk factors, such as hyperglycemia, hyperlipidemia, hypertension, obesity, *etc*. In clinical practice, treatment plans can be adjusted according to the changes in these indicators to improve the prognosis.

In conclusion, NT-probNP, Hs-CRP, and Hcy have clinical application value as they can be used to predict cardiovascular events in patients with CHF.

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References

[1] Wan C, 2022, Association of Serum UA, D-D, and Peptide, HGF with NYHA Cardiac Function Class and Cardiovascular Events in CHF Patients. Chinese Health Engineering, 21(05): 799–802.

[2] Ren S, Tian Z, Xu D, 2022, Serum hsTn Ⅰ, MMP-9, sST2 Expression Levels and Their Clinical Significance in Elderly Patients with Chronic Heart Failure. Henan Medical Research, 31(13): 2373–2376.

[3] Zhao D, Zhao Y, Yu M, et al., 2022, Value of Serum NGAL Levels in the Evaluation of Cardiac Function and Prediction of Major Cardiovascular Adverse Events in Patients with Chronic Heart Failure. Chinese Experimental Diagnostics, 26(03): 374–378.

[4] Yu Q, 2022, Study on the Value of Heart Failure Biomarkers in the Application of Chronic Heart Failure in the Elderly, thesis, Anhui Medical University.

[5] Meng J, He T, Li H, et al., 2021, Study on the Relationship Between Thyroid Hormone Levels and Cognitive Function, Cardiac Function and Cardiovascular Events in Elderly Patients with Chronic Heart Failure. Modern Biomedical Progress, 21(23): 4531–4535.

[6] Yuan Y, Qi S, Wang Y, et al., 2021, Impact of Iron Deficiency on Quality of Life and Predictive Value of Recent Major Adverse Cardiovascular Events in Patients with Chronic Heart Failure. Lingnan Journal of Cardiovascular Diseases, 27(06): 688–692 + 718.

[7] Yang Y, Li M, 2021, Predictive Value of Serum Periostin Protein and Hepatocyte Growth Factor on Cardiovascular Events in Patients with Chronic Heart Failure. Journal of Heart, 33(02): 156–159 + 164.

[8] Li X, Zhu W, Jiang F, 2017, Correlation Between Echocardiographic Function Indices and Serum Hcy in Patients with Chronic Heart Failure. Journal of Practical Medicine, 33(04): 579–582.

[9] Wang Z, 2020, Study on Prognostic Assessment of Traditional Risk Factors Combined with sST2 in Patients with Chronic Heart Failure, thesis, Jilin University.

[10] Chen W, Wu Z, Zhang Y, et al., 2020, Association of Elevated Serum S100B Levels with Clinical Severity, Impaired Renal Function and Cardiovascular Primary Endpoint Events in Patients with Chronic Heart Failure. Chinese Journal of Gerontology, 40(08): 1569–1572.

[11] Chen Y, Wang D, 2019, The relationship Between Serum Apoa-1 and the Severity of Chronic Heart Failure and Its Prognosis. Zhejiang Medicine, 41(04): 332–336.

[12] Wang Y, Yu H, 2020, The Role of Stepwise Exercise with Continuous Weight Intervention in Strengthening Cardiac Function and Reducing the Incidence of Adverse Cardiovascular Events in Patients with Chronic Heart Failure. Nursing Practice and Research, 17(24): 57–59.

[13] He J, 2014, Situational Analysis of the Effects of Renal Anemia and Ultrasensitive C-Reactive Protein on Cardiovascular Complications in Patients with Chronic Kidney Disease, thesis, Guangxi Medical University.

[14] Zou R, He L, Xiao F, et al., 2012, Comparison of the Prevalence of Cardiovascular Disease in Patients on Peritoneal Dialysis and Hemodialysis and Analysis of Influencing Factors. Chinese Journal of Clinical Physicians (Electronic Edition), 6(18): 5515–5519.

[15] Hou F, Ma Z, Mei C, et al., 2005, Investigation of Risk Factors for Cardiovascular Disease in Patients with Chronic Kidney Disease in Five Provinces, Cities and Autonomous Regions of China. Chinese Medical Journal, 2005(11): 753–759.

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History and Development of Zhuang Medicine Shallow Needling from Bone Needle, Pottery Needle, and Bronze Needle to Electroacupuncture

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Abstract: Zhuang medicine shallow needling has a long history and plays a significant role in Chinese medicine. With the development of medical treatment in China, there is a constant improvement in the use of needles. Bone needles have been modified to pottery needles, bronze needles, and electroacupuncture. From this long development history, there are some improvements in terms of material and technology. By analyzing and comparing the primitive and current acupuncture instruments used in China and their therapeutic effects, it can be seen that China's medical treatment is constantly improving and will continue to improve with technology.

Keywords: Shallow needling of Zhuang medicine; Bone needle; Bronze needle

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1. Introduction

In China, the shallow needling method of Zhuang medicine plays a significant role in the medical industry. The earliest acupuncture performed was with bone needles, which have been improved in terms of material in the later period. Bone needles were the earliest needles manufactured and used in China, dating back to 50,000 years ago. Bone was the main material, and they were the main acupuncture tool in primitive medical treatment. Following bone needles, bronze needles were manufactured, in which the main material is bronze. Pottery needles, on the other hand, with a long history in Zhuang medicine have shown significant clinical effects. The shallow needling method of Zhuang medicine, originating in the Guangxi region of China, has a significant role in Chinese medicine. With the continuous development of medical treatment in China, bone needles, bronze needles, pottery needles, and electric needles have been used successively [1]. In this article, we mainly analyze the medical instruments used for shallow needling in Zhuang medicine and expound the evolvement of these instruments.

2. Origin and development of the shallow needling method of Zhuang medicine

2.1. Origin of the shallow needling method of Zhuang medicine

The shallow needling method of Zhuang medicine originated from the Guangxi Zhuang Autonomous Region during the primitive period thousands of years ago in the era of old utensils. With the continuous development of human wisdom, the shallow needling method of Zhuang medicine continuously improved. However, the role of the acupuncture method of Zhuang medicine could not be fully utilized in treatment due to the lack of materials for acupuncture instruments.

In shallow needling, needles are used to stimulate relevant acupoints to achieve the purpose of treatment. Since Zhuang doctors themselves have mastered various curing methods and considering the feedback of patients who received shallow acupuncture, it has certain significance in medicine [2]. It can be divided into the skin theory and the medical theory. The skin theory is mainly based on skin reactions. Since the body's outer barrier is, in fact, the outer layer of the human body, it can reflect the manifestations of internal organ lesions, and since the outer barrier of the body is closely related to the human body, it is possible to determine if there is any disease based on the color and changes to the skin. The medical theory, on the other hand, is mainly based on relevant medical books and subcutaneous nerve tissue to understand diseases and prescribe relative treatments. Since there are many nerves around the spine, limbs, and bladder of the human body, the presence of nerve sensation is based on the gasification of certain tissue fluids and the movement of defensive Qi and a disease is judged based on nerve sensation. The main theory of Zhuang medicine is the synchronization of the three Qis: heaven, earth, and human. The shallow needling method of Zhuang medicine stimulates the skin of the human body. The needles are shallow, corresponding to the heaven part of the human body (the skin), and play an important role in the recovery and adjustment of Qi and blood.

2.2. Development of the shallow needling method of Zhuang medicine

The shallow needling method of Zhuang medicine has improved owing to the improvement in instruments. In the primitive society, the instruments used were mainly bone needles, and they were mainly used for treating mild conditions. In the later period, with the continuous improvement in instruments, the needles were used to effectively assist shallow needling, and the scope of treatment expanded from mild conditions to serious diseases. Patients became more appreciative of the shallow needling method of Zhuang medicine. There were many loopholes in the shallow needling method during the Paleolithic Age and other ancient times, and the materials used for the needles were limited, which led to its dependence on medical-related pathologies. The needling instrument has continuously improved, changing from an auxiliary function to an indispensable step in the shallow needling method. In modern-day medicine, the shallow needling method is widely recognized by patients as it has shown to be effective [3]. However, due to the particularity of the Guangxi region, its speed of development has been hindered to some extent, thus having a certain influence on the shallow needling method of Zhuang medicine. In order to prevent this influence, Zhuang medicine has made corresponding remedial plans. There has not been any incidence of disease misdiagnosis through the shallow needling method due to its crudeness, and the method has improved in the later stage and gradually become an important part of treatment.

3. Origin and development of Zhuang medicine acupuncture instruments

3.1. Origin of acupuncture instruments

Shallow acupuncture, which originated in Guangxi region, is an important part of Zhuang medicine and is relatively common among Zhuang people. The shallow needling method of Zhuang medicine is mainly based on medical theories and requires the use of needles. Therefore, the shallow needling method is closely related to new and old stone tools. The earliest place where primitive needles were discovered was Guangxi Zhuang Autonomous Region. However, due to limited medical equipment and local policies in the primitive society, the medical needles were crude. Although diagnoses were not affected, the treatment effect was significantly affected. During the New Paleolithic Age, needles were used instead of manual acupoint pressure therapy, and its curative effect was remarkable compared to manual acupoint pressure therapy. The rate at which it was used was also rising, thus creating certain research value for the development of related medical instruments.

3.2. Development of acupuncture instruments

3.2.1. Bone needles

From the Paleolithic Age to the Qin and Han Dynasties, bone needles were common tools. In daily life, bone needles were used not only to sew clothes, but also in medicine. The main materials are animal and fish bones, which were mainly polished according to their shapes and then shaped into needles. It was difficult to make them due to the shape of the bones. Since the needles were relatively simple, they were unable to play an auxiliary role but owing to the conditions during the Paleolithic Age, bone needles were still used as the main needles in shallow needling.

3.2.2. Pottery needles

Before the Warring States Period, pottery needles were widely used. Pottery needles refer to the treatment of patients using pottery and porcelain pieces instead of needles. Before the Warring States Period, the materials for needles were relatively scarce; hence, medical skills were unutilized. Most patients were not cured, and serious adverse outcomes occurred. As the pottery needles cannot be polished due to their material, the feedback from patients is extremely poor; thus, they were not used as the treatment of choice. When necessary, bone needles were still used for shallow needling.

3.2.3. Bronze needles

During the Xia, Shang, and Zhou Dynasties, bronze was widely used. Hence, the medical use of bronze needles improved. These needles were mainly used as medical instruments for treating patients. The main material is bronze. Bronze was not only the main material in needles, but also one of the main materials in daily utensils. Therefore, the craftsmanship of bronze needles was more refined. There were further improvements to bronze needles, making it easier to grasp the patient's acupoints during treatment and further contributing to medicine. Before using any bronze needle for acupuncture, the bronze needle would be sterilized. Due to the superiority of bronze material and manufacturing methods as well as certain improvements in the treatment of Chinese medicine, more attention was paid to medical hygiene. Hence, the feedback of patients on bronze needles was better. In July 1976, Guangxi archaeologists found three silver needles in the funerary objects of No. 1 Han Tomb in Luobowan, Guigang City, with similar external shapes, noose-shaped needle handles, and sharp, conical needle bodies of 0.2-cm diameter. The lengths of the three needles were measured to be 9.3 cm, 9.0 cm, and 8.6 cm, respectively. From their appearance, there is a circular hole at the top of the handle of all three needles. The shape of the silver needle is similar to modern acupuncture needles, but it is relatively thick, thus confirming that it is a medical needle. This is the earliest metal needle with a rope-shaped needle handle discovered in China thus far. This kind of needle handle has had a profound influence on the shape of needle handles in later generations, and it is used to this day. It is of great significance to the history of needling instruments in China [4].

3.2.4. Electroacupuncture

Electroacupuncture is highly sought after by patients in this age. Since it is a relatively new technology, electroacupuncture is in the stage of perfection in terms of its medical theory and technology. With electroacupuncture, the acupoints can be controlled precisely, and the pain caused by shallow needling can be reduced. Different treatment options are offered for different conditions. Shallow needling has a millennial history in medicine, and its theoretical basis is centered on natural therapy. Based on the patient's disease, relevant acupoints are stimulated to achieve a healing effect. According to relevant data, shallow needling has certain significance in clinical satisfaction and curative effect and is well received by patients [5].

4. Discussion

With the continuous development of medical technology, Zhuang medicine has improved its instruments for acupuncture from the most primitive bone needles to electroacupuncture. From the Paleolithic Age to Qin and Han Dynasties, bone needles were made from animal bones. Bone needles were relatively crude, and the curative effect was only average. Before the Warring States Period, pottery needles drew more interest as their curative effect was better than bone needles in acupuncture treatment. From the Western Zhou Dynasty to the Spring and Autumn Period, bronze needles were introduced. Since the Western Zhou Dynasty was the heyday of bronze, bronze needles were the most popular needles. The curative effect also improved with the use of bronze needles. Compared with the aforementioned acupuncture instruments, electroacupuncture is an improvement in the appliance itself. It can effectively reduce pain during treatment and improve patient satisfaction. In this article, we mainly expound the origin and development of the shallow needling method of Zhuang medicine and describe the evolvement of medical needles from bone needles, pottery needles, and bronze needles to electroacupuncture. Through the different eras of acupuncture from using crude to fine needles, the curative rate has improved. The shallow needling method of Zhuang medicine is mainly based on the relevant medical theory of shallow needling of Zhuang medicine since its needles are yet to be perfected and the needling effect only accounts for a part of the healing process. However, with the continuous improvement of medical technology, shallow needling will be more accurate. Its role in medical treatment is constantly changing, and it will eventually become an irreplaceable part of acupuncture.

The shallow needling method of Zhuang medicine originated in Guangxi and developed on the basis of accumulated acupuncture experience from Xiou, Luoyue, and other ethnic groups in Guangxi in ancient times. Exquisitely, the ground stone needles and bone needles that existed in the Neolithic Age in Guangxi are the best explanation for the origin of acupuncture in the Zhuang area of Guangxi. Ground stone and bone needles were developed from the accumulation of acupuncture experience. Without the practice of acupuncture in the Paleolithic Age, there would be no ground stone and bone needles in the Neolithic Age. The law of development from ground stone, bone, pottery, and bronze to silver needles is consistent with the development of human civilization. The historical process of the development of acupuncture in Zhuang medicine is similar to that of acupuncture and moxibustion in Chinese medicine. However, in the process of development, due to differences in humanity, geographical, and social development, they developed in different directions.

The most important connotation of the acupuncture theory in Zhuang medicine is the theory of synchronization of the three Qis: heaven, earth, and human. In Zhuang medicine, the whole human body can be divided into upper, middle, and lower parts: the upper part represents heaven, the lower part represents earth, and the middle part represents human. Physiologically, the Qi produced by heaven is at the top, and it descends; the Qi produced by earth is at the bottom, and it ascends; the Qi produced by human is at the middle, and it dominates harmony, taking the Qis from both heaven and earth and harmonizing them. The heaven, earth, and human parts of the body operate synchronously with the natural world (heaven and earth), restricting the growth of metaplasia and sustaining life. If the descend and ascend of Qi are congruous, and they meet in the middle, Qi and blood will be harmonious, yin and yang will be balanced, and the viscera will be at ease, adapting to the changes in nature; this is the normal state of human health. On the contrary, if the Qi from heaven does not descend, and the Qi from earth does not ascend, the Qi that meets in the human part is not harmonious, and the three Qis cannot run synchronously; this is a pathological state, which can cause various diseases. Man must be consistent with the law of change of heaven, earth, and nature, which is emphasized by the synchronization of the three Qis. In Zhuang medicine, the ring acupoints and other acupoints for acupuncture as well as the techniques of acupuncture are inseparable from the theoretical guidance of the synchronization of heaven, earth, and human Qi.

In conclusion, the long history of medical treatment in China has brought about certain research value and reference significance to China's medical undertakings. With the continuous advancement of science and technology, acupuncture in Zhuang medicine will also continue to develop, along with the theoretical aspect, methods, and instruments used in acupuncture, thus enriching the acupuncture and moxibustion treatments used in Zhuang medicine and promoting the development of Zhuang medicine itself.

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References

[1] Qiao M, Zeng R, Chen C, et al., 2019, Discussion on Shallow Pricking to Relieve Itching from "The Itching Is Positive, Shallow Pricking". World Latest Medical Information Abstracts, 19(86): 199–200.

[2] Yi X, Zhu H, Lu L, et al., 2019, Analysis of Shallow Needling in Huangdi Neijing. Chinese Medical Journal, 34(09): 1812–1816.

[3] Lin S, Wan W, 2019, Discussion on the Relationship Between Acupuncture Depth and Acupoint Properties. Chinese Medicine Bulletin, 18(04): 33–35 + 43.

[4] Sun J, Li Y, Zhu M, 2019, Current Situation of Application of Placebo Acupuncture in Acupuncture Clinical Trials. World Traditional Chinese Medicine, 14(08): 1959–1962 + 1968.

[5] Yang X, Fu Y, 2019, A Case of Superficial Needling on the Skin in the Treatment of Peripheral Neuropathy After Chemotherapy. Hunan Journal of Traditional Chinese Medicine, 35(04): 95–96.

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Relationship Between Postprandial Blood Glucose, Fasting Insulin, and Glycated Hemoglobin Levels and Early Diabetic Nephropathy in Patients with Type 2 Diabetes Mellitus

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Abstract: *Objective:* To investigate the relationship between postprandial blood glucose (PBG), fasting insulin (FINS), and glycated hemoglobin (HbA1c) levels and early diabetic nephropathy in patients with type 2 diabetes. *Methods:* 96 cases of type 2 diabetes mellitus treated in our hospital from May 2021 to May 2022 were selected as the research subjects. The patients were divided into two groups according to the urinary albumin excretion rate (UAER), with 53 cases in the type 2 diabetes group (UAER < 30 μg/min) and 43 cases in the early diabetic nephropathy group (30 μg/min ≤ UAER < 300 μg/min). PBG, FINS, and HbA1c levels were detected in 87 healthy patients. *Results:* The levels of PBG, FINS, and HbA1c in the early diabetic nephropathy group were higher than those in the control group ($P < 0.01$) and the type 2 diabetes group ($P < 0.01$). *Conclusion:* PBG, FINS, and HbA1c are factors affecting the occurrence of diabetic nephropathy in patients with type 2 diabetes; thus, controlling the levels of PBG, FINS, and HbA1c can effectively prevent the occurrence of diabetic nephropathy in type 2 diabetes mellitus.

Keywords: Type 2 diabetes mellitus; Diabetic nephropathy; Postprandial blood glucose

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1. Introduction

Diabetes is a common chronic disease in China, which seriously affects people's health and threatens lives. Type 2 diabetes, which is mainly caused by pancreatic islet cells, accounts for more than 90% of the incidence of diabetes in China. Type 2 diabetes develops as a result of the compensatory mechanism of islet cells, where more insulin is secreted to reduce the effect of insulin resistance. Hence, there will be increased insulin levels in the blood. When fasting, the blood sugar level is normal, but it will be higher than normal two hours after a meal. At this time, if it is not controlled, the islet cells will eventually lose their compensatory function. Diabetic nephropathy (DN), which is most common microvascular complication of diabetes, accounts for 35% of diabetic complications. DN has become one of the main diseases affecting the health of people in China. It has a complex pathogenesis and is the main cause of end-stage renal disease (ESRD). At present, more than one-third of diabetic patients in China die of renal failure and uremia, and it is believed that DN is caused by the combination of genetic factors, environmental

factors, and immune dysfunction. Postprandial blood glucose (PBG), fasting insulin (FINS), and glycated hemoglobin (HbA1c) are the main components of the extracellular matrix (ECM). They participate in various biological processes in the human body [1-5]. Previous studies have shown that PBG and FINS are associated with the occurrence of early DN, while HbA1c is involved in the pathogenesis of early DN and is related to kidney damage. However, the mechanism of how PBG, FINS, and HbA1c leads to renal injury in early DN is still unclear.

2. Materials and methods

2.1. General information

A total of 96 cases of type 2 diabetes mellitus treated in our hospital from May 2021 to May 2022 were selected as the research subjects. According to the urinary albumin excretion rate (UAER), the patients were divided into two groups, with 53 cases in the type 2 diabetes group (UAER < 30 μg/min) and 43 cases in the early diabetic nephropathy group (30 μg/min ≤ UAER < 300 μg/min). The levels of PBG, FINS, and HbA1c were detected in 87 healthy subjects.

2.2. Methods

5 mL of fasting venous blood was drawn from all subjects, and HbA1c level was detected by high performance liquid chromatography (HPLC) using Lifotronic H9 HBA1c Analyzer and its kit. Roche e602, an automatic electrochemiluminescence immunoassay analyzer, and its accessories were used. FINS levels were measured using reagents, while PBG levels were measured by Roche cobas 8000 modular analyzer, an automatic biochemical analyzer, and its supporting reagents. All operations were carried out according to the instructions of the reagents or instruments.

2.3. Observation indicators

2.3.1. Postprandial blood glucose

In medicine, PBG is used as one of the criteria used to diagnose diabetes. Generally, the 2-hour postprandial blood glucose in a normal person is less than 7.8 mmol/L. Elevated blood glucose observed 2 hours after a meal is common in patients with type 2 diabetes.

2.3.2. Fasting insulin

FINS refers to the amount of insulin produced by the pancreas in the fasting state of the human body. Insulin is the only substance that can lower blood sugar in the human body. In a fasting state, insulin is secreted at a constant rate without the stimulation of food and glucose in the blood. Food, after consumption, is converted into glucose and absorbed into the blood. As a result, the pancreas is stimulated, and a large amount of insulin is secreted within a short period of time to control postprandial blood sugar. Glycogen is continuously synthesized by the liver at night; only when basal insulin counteracts the output of liver glycogen can there be normal fasting blood sugar.

2.3.3. Glycated hemoglobin

HbA1c is produced by hemoglobin in the blood and sugar (mainly glucose) in the blood. Since the non-enzymatic reaction of HbA1c is continuous, slow, and irreversible, its level depends on whether a person is fasting, whether insulin is injected, whether hypoglycemic drugs are taken, *etc*. It is generally believed that in the last 8–12 weeks, HbA1c can be used as a good indicator and monitoring method for diabetes. HbA1c is expressed as the percentage of hemoglobin in adults.

2.4. Statistical analysis

All the data in this study were processed by SPSS 22.0. The measurement data were expressed as mean ± standard deviation and tested by t-test, while the enumeration data were tested by chi-squared test.

3. Results

3.1. Comparison of postprandial blood glucose, fasting insulin, and glycated hemoglobin levels between the study group and the control group

The levels of PBG, FINS, and HbA1c in the early diabetic nephropathy group were higher than those in the control group ($P < 0.01$), as shown in **Table 1**.

Table 1. Comparison of PBG, FINS, and HbA1c levels between the early diabetic nephropathy group and healthy subjects

Group	PBG (mmol/L)	FINS (mU/L)	HbA1c (%)
Early diabetic nephropathy group (n = 43)	11.80 ± 3.21	11.73 ± 2.46	8.04 ± 1.37
Control group (n = 87)	6.72 ± 0.65	7.73 ± 1.25	4.86 ± 0.62
t	3.698	4.021	3.025
P	< 0.01	< 0.01	< 0.01

Abbreviations: FINS, fasting insulin; HbA1c, glycated hemoglobin; PBG, postprandial blood glucose.

3.2. Comparison of postprandial blood glucose, fasting insulin, and glycated hemoglobin levels between the type 2 diabetes group and the early diabetic nephropathy group

The levels of PBG, FINS, and HbA1c in the early diabetic nephropathy group were higher than those in the type 2 diabetes group ($P < 0.01$), as shown in **Table 2**.

Table 2. Comparison of PBG, FINS, and HbA1c levels between the type 2 diabetes group and the early diabetic nephropathy group

Group	PBG (mmol/L)	FINS (mU/L)	HbA1c (%)
Early diabetic nephropathy group (n = 43)	13.42 ± 3.15	13.55 ± 2.17	9.13 ± 1.52
Type 2 diabetes group (n = 53)	10.49 ± 2.58	10.26 ± 1.96	7.15 ± 1.26
t	4.698	3.021	4.025
P	< 0.01	< 0.01	< 0.01

Abbreviations: FINS, fasting insulin; HbA1c, glycated hemoglobin; PBG, postprandial blood glucose.

4. Discussion

4.1. Relationship between glycated hemoglobin and diabetic nephropathy

HbA1c is a multifunctional protein that can regulate glucose metabolism, energy production, and cell growth. When renal function is abnormal, it can increase the glomerular filtration rate and reduce the clearance efficiency of serum proteins. HbA1c can combine with different types of lipids and proteins, such as plasma proteins and tissues, to form complexes. HbA1c can increase the permeability of the glomerular cell membrane, resulting in decreased glomerular filtration rate; at the same time, it can reduce the interactions between glomerular cells, resulting in the inhibition of glomerular cell proliferation or apoptosis. In addition, HbA1c levels are influenced by diet. Studies have shown that HbA1c levels are associated with chronic kidney disease (CKD) and ESRD in diabetic patients. Hence, HbA1c is one of the risk factors leading to CKD and ESRD [6-11].

4.2. Relationship between postprandial blood glucose and fasting insulin levels and diabetic nephropathy

Studies have shown that the levels of PBG and FINS are correlated with glomerular filtration rate and PGA expression was significantly increased in the FINS group compared to the PBG group [10,11]. In human patient samples, the expression of PGA in the type 2 diabetes mellitus group was significantly higher than that in the healthy control group. At the same time, serum and urine PGA levels were also significantly higher in the type 2 diabetes mellitus group compared to the healthy control group. However, no studies have confirmed the specificity and sensitivity of PBG and FINS levels in DN patients. A renal histopathological analysis of type 2 diabetic rats has revealed that PBG and FINS have a significant positive correlation with DN progression. Further studies have found that insulin can reduce urinary albumin excretion by upregulating the expression of PGA in urine cells, thereby reducing the risk of DN progression in type 2 diabetes mellitus. In a histological analysis of the kidneys of type 2 diabetic rats, the level of PGA was found to be significantly elevated in the T2DM group compared with the normal control group, with no significant change in PBG [12,13].

4.3. Relationship between postprandial blood glucose and fasting insulin levels and renal injury

The kidney is a complex system comprising many important cells, blood vessels, and nerves. The kidney plays an important role in maintaining fluid balance through glomerular filtration, renal tubular secretion, and glomerular reabsorption. This complex organ that is composed of abundant ECM plays an important role in maintaining the stability of the internal environment. PBG is mainly secreted by epithelial cells and is the main component of the renal barrier. As people's understanding of PBG continues to grow, its influence on human bodily functions has garnered widespread attention.

FINS is mainly synthesized by fibroblasts. Fibroblasts can secrete various growth factors and collagen, and they are one of the main components of the ECM in the kidney. In renal injury, with the changes in ECM composition, renal tubular reabsorption capacity weakens or is impaired, which in turn results in decreased urine concentrating function. The kidney is highly sensitive to glucose toxicity and hypoxia-induced inflammatory response, which is also one of the main pathological mechanisms of DN. Injury to renal tubular epithelial cells can cause a series of clinical changes, such as tubular structural changes and epithelial-mesenchymal transition. DN and diabetic kidney injury are very similar in pathology, but there are certain differences in their pathological mechanisms. The early stage of DN manifests as decreased glomerular filtration rate, glomerulosclerosis, and progression of interstitial fibrosis, but the manifestations of kidney injury vary in different stages, and its occurrence is associated with many different factors, such as diabetes, inflammation, oxidative stress, and metabolic disorders. Patients with CKD are more likely to develop clinical manifestations in the early stage (1–3 years), while patients with type 2 diabetes have a higher probability of developing early kidney disease (3–4 years); in addition, the rate of progression is faster. However, studies have shown no significant difference in patients with advanced renal insufficiency. The pathogenesis of DN is very complex and has not been fully elucidated. The pathological changes of diabetic kidney include glomerular injury, renal tubular cell injury, and microvascular disease, and these changes eventually lead to renal tubular epithelial cell apoptosis and glomerulosclerosis, which are the main causes of early DN. FINS, a pro-inflammatory cytokine, participates in various pathological processes, such as glomerular injury, tubular epithelial cell injury, tubular apoptosis, and tubular fibrosis; it plays an important role in early DN. PBG, on the other hand, is not only involved in renal interstitial injury, but also related to renal interstitial fibrosis. Studies have found that PBG may also contribute to the development of early DN [12-16].

4.4. Significance of postprandial blood glucose, fasting insulin, and glycated hemoglobin in the prevention of early diabetic nephropathy

HbA1c can bind to a variety of enzymes that regulate ECM metabolism and promote its degradation, thus participating in kidney injury. The serum levels of PBG, FINS, and HbA1c in patients with type 2 diabetes were significantly elevated. In a mouse model of type 2 diabetes, PBG was positively correlated with HbA1c concentration, whereas FINS was not significantly correlated with HbA1c [15,16]. Serum PBG, FINS, and HbA1c levels were elevated in patients with type 2 diabetes, and serum PBG levels were positively correlated with acute kidney injury ($P < 0.05$); FINS was found to be associated with the progression of DN. Serum creatinine, blood urea nitrogen, creatinine clearance rate, and glomerular filtration rate in patients with type 2 diabetes were significantly higher than those in the normal healthy group ($P < 0.05$); meanwhile, a positive correlation was observed between urinary protein excretion rate and PBG ($P < 0.05$). Hyperglycemia can activate the nuclear factor kappa light chain enhancer of activated B cells (NF-κB) pathway, cause renal oxidative stress, generate a large amount of reactive oxygen species, and lead to apoptosis as well as the destruction of renal tubular epithelial cells, while PBG may contribute to renal injury by inhibiting apoptosis. The exposure to inflammatory factors may cause infiltration of a large number of inflammatory cells and tissue damage and may result in increased permeability of the glomerular membrane and thickened glomerular basement membrane.

In conclusion, PBG, FINS, and HbA1c are factors that affect the occurrence of DN in patients with type 2 diabetes, and the occurrence of DN in type 2 diabetes can be effectively prevented by controlling the levels of PBG, FINS, and HbA1c.

Disclosure statement

The authors declare no conflict of interest.

References

[1] Zhang Y, Xu M, 2022, The Predictive Value of ApoB/ApoA1 Ratio for Early Diabetic Nephropathy in Patients with Type 2 Diabetes. Medical Theory and Practice, 35(24): 4258–4260.

[2] Zhang R, 2022, Analysis of Risk Factors of Early Diabetic Nephropathy in Patients with Type 2 Diabetes Mellitus. Chinese Medical Engineering, 30(02): 25–27.

[3] Guo J, Wei H, Liang Z, et al., 2022, Influencing Factors of Early Diabetic Nephropathy in Patients with Type 2 Diabetes. Chinese Health Engineering, 21(01): 99–100 + 103.

[4] Yang L, Shi W, Zhao L, et al., 2022, Clinical Observation on the Effect of Visceral Fat Area on Early Diabetic Nephropathy in Patients with Type 2 Diabetes. Yunnan Medicine, 43(01): 40–43.

[5] Ding J, Gao W, Huang Z, et al., 2021, Analysis of Related Influencing Factors of Patients with Type 2 Diabetes Developing Early Diabetic Nephropathy. Electronic Journal of Modern Medicine and Health Research, 5(18): 111–113.

[6] Wang B, 2021, Clinical Effect Analysis of Saxagliptin Tablets Combined with Telmisartan Tablets in the Treatment of Early Diabetic Nephropathy in Type 2 Diabetes. Chinese Modern Drug Application, 15(15): 164–166.

[7] Liu S, Wang S, Zuo H, et al., 2021, Diagnostic Value of Cystatin C in Early Nephropathy in Patients with Type 2 Diabetes. Clinical Medicine Research and Practice, 6(03): 36–39.

[8] Cai L, 2020, Application Observation of Neutrophil/Lymphocyte Ratio in Early Diagnosis of Type 2 Diabetic Nephropathy. Chinese Medical Innovation, 17(02): 60–63.

[9] Liu Y, 2019, Correlation Analysis Between Early Diabetic Nephropathy and TCM Constitution in Patients with Type 2 Diabetes Combined with SCH, thesis, Liaoning University of Traditional Chinese Medicine.

[10] Yang S, Wang F, 2019, Study on Related Risk Factors of Type 2 Diabetes Complicated with Early Diabetic Nephropathy. Electronic Journal of Clinical Medicine Literature, 6(08): 26.

[11] Luo Z, 2018, Clinical Exploration of Saxagliptin Combined with Telmisartan on Patients with Type 2 Diabetes and Early Diabetic Nephropathy. Chinese Modern Drug Application, 12(16): 84–86.

[12] Wang L, 2018, Analysis of Related Risk Factors of Type 2 Diabetes Combined with Early Diabetic Nephropathy. Imaging Research and Medical Application, 2(05): 209–211.

[13] Su D, 2018, The Value of Resting Heart Rate in Patients with Type 2 Diabetes in Predicting Early Diabetic Nephropathy. Chinese Medicine Herald, 15(05): 58–61.

[14] Wang H, Wan L, Gao W, et al., 2017, Therapeutic Effect of Saxagliptin Combined with Telmisartan on Patients with Type 2 Diabetes and Early Diabetic Nephropathy. Jiangsu Medicine, 43(06): 446–448.

[15] Zhang N, Gao Z, 2016, Analysis of the Incidence and Risk Factors of Early Diabetic Nephropathy in Patients with Type 2 Diabetes. Journal of Aerospace Medicine, 27(12): 1473–1476.

[16] Niu Y, Zhang J, Wen X, et al., 2021, Clinical Effect of Telmisartan Combined with Calcitriol in the Treatment of Patients with Early Diabetic Nephropathy and Its Effect on Serum Lp(a) and TGF-β1 Levels. Clinical Misdiagnosis and Mistreatment, 34(3): 31–35.

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Risk Factors of Infection in Nephrotic Syndrome

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Abstract: *Objective:* To determine the risk factors of infection in patients with nephrotic syndrome. *Methods:* A retrospective study was conducted on 155 patients with nephrotic syndrome under our department from January 2019 to December 2019. Among them, 43 cases had infection, and the rate of infection was 27.74%. The risk factors of infection were analyzed. *Results:* Among the 155 patients with nephrotic syndrome, 43 cases developed infection, including 3 cases of upper respiratory tract infection (6.98%), 33 cases of lower respiratory tract infection (76.74%), 3 cases of skin infection (6.97%), 3 cases of urinary tract infection (6.97%), and 1 case of facial nerve infection (2.32%). Compared with the group without infection, the group with infection had lower serum albumin and immunoglobulin G (IgG) levels as well as higher serum creatinine and 24-hour urinary protein levels ($P < 0.05$). Multivariate logistic regression analysis showed that decreased serum albumin (odds ratio [OR] = 1.14; $P < 0.01$) and IgG (OR = 1.1; $P < 0.144$) were independent risk factors for infection. *Conclusion:* Respiratory infection is the most common infection in nephrotic syndrome, and the decrease in serum albumin and IgG are independent risk factors for infection.

Keywords: Nephrotic syndrome; Infection; Risk factors

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1. Introduction

Nephrotic syndrome is characterized by proteinuria, hypoproteinemia, edema, and hyperlipidemia. Infection is one of the most common complications of nephrotic syndrome, which affects the prognosis of such patients, easily leads to recurrence, and increases the hospitalization and mortality rates of such patients [1]. In this study, 155 patients with nephrotic syndrome from January 2019 to December 2019 were retrospectively analyzed, among which 43 patients developed infection as a complication. Risk factors related to these patients were analyzed to develop a better understanding of the susceptibility of patients with nephrotic syndrome to infection, improve the prevention of infection, reduce the occurrence of infection, improve the remission rate of kidney disease, and reduce the financial burden on patients.

2. Patients and methods

A retrospective study was conducted on 155 patients with primary nephrotic syndrome under our department from January 2019 to December 2019. The diagnostic criteria of nephrotic syndrome were as follows: massive proteinuria > 3.5 g and hypoproteinemia < 30 g/L, which may be accompanied by edema and hypertension.

The general data of the patients (sex, age, course of immunosuppressant treatment, and hormone dose) and laboratory test results (white blood cell count, platelet count, neutrophil count, lymphocyte count, urine protein quantification, serum albumin, serum creatinine, C-reactive protein [CRP], immunoglobulin G [IgG], *etc.*) were collected.

The patients were classified into different groups: without infection, with infection, existing infection on admission, infection after treatment (hormone or immunosuppressant), and intensive care unit (ICU) admission. The patients were further categorized according to the site of infection: upper respiratory tract infection, lower respiratory tract infection, urinary tract infection, skin and soft tissue infection, *etc*.

SPSS 22.0 was used for statistical analysis. If the measurement data were in accordance with normal distribution and homogeneity of variance, they were expressed as mean ± standard deviation, and the comparison between groups was analyzed by Student-Newman-Keuls (SNK) test. If the measurement data did not obey the normal distribution and/or meet the homogeneity of variance, they were expressed as median (25%–75% quantile), and Kruskal-Wallis H test was used for comparison between groups. The indicators with statistically significant differences in univariate analysis were included in logistic regression for multivariate analysis to explore the risk factors for infection in patients with nephrotic syndrome. $P < 0.05$ was considered statistically significant.

3. Results

A total of 155 patients with nephrotic syndrome were retrospectively analyzed, among which 43 developed infection, and the rate of infection was 27.74%, as shown in **Table 1**.

Table 1. Incidence of infection in patients with nephrotic syndrome

Total number of cases	Infected cases	Proportion (%)
155	43	27.74

Among the 43 patients with infection, 3 cases had upper respiratory tract infection, 33 cases had lower respiratory tract infection, 3 cases had skin infection, 3 cases had urinary tract infection, and 1 patient had facial nerve infection. See **Table 2** for more details.

Table 2. Distribution of infection sites

Site of infection	Number of cases (n, %)
Upper respiratory tract	3 (6.98)
Lower respiratory tract	33 (76.74)
Skin infection	3 (6.97)
Urinary tract infection	3 (6.97)
Facial nerve infection	1 (2.32)

Among the 43 cases, 18 had infection on admission, accounting for 41.86%, 21 cases developed infection after receiving hormone or immunosuppressant, accounting for 48.83%, 3 cases were admitted to the ICU, accounting for 6.97%, and 1 case died, accounting for 2.32%. See **Table 3** for more details.

Table 3. Distribution of infection timing

Timing of infection	Number of cases (n, %)
Existing infection on admission	18 (41.86)
Infection after treatment	21 (48.83)
Admission to the ICU	3 (6.97)
Number of deaths	1 (2.32)

We also found significant differences in serum albumin, 24-hour urinary protein, IgG, and serum creatinine among the groups ($P < 0.05$); the aforementioned indicators were all lower in the group with infection than in the group without infection ($P < 0.05$). However, there was no significant difference among the group with existing infection on admission, the group with infection after treatment, and the group admitted to the ICU. **Table 4** shows the comparison of data.

Table 4. Comparison of the clinical data of each group

Groups / Indicators	Without infection	Existing infection on admission	Infection after treatment	Admitted to the ICU	With infection	P	P1	P2	P3
Age	52.09 ± 13.15	52.72 ± 18.92	53.70 ± 12.81	63.00 ± 4.74	53.23 ± 16.53	0.678	0.665	0.837	0.247
White cells ($\times10^9$/L)	6.92 (5.31–8.27)	7.49 (4.63–8.00)	7.59 (6.66–9.84)	12.57 (10.11–13.2)	7.64 (5.67–8.53)	0.007	0.024	0.242	0.063
Neutrophils ($\times10^9$/L)	3.82 (3.07–5.96)	4.17 (2.65–6.01)	5.03 (3.91–6.52)	10.45 (8.40–11.05)	5.01 (3.81–6.44)	< 0.001	0.002	0.365	0.036
Lymphocytes ($\times10^9$/L)	1.89 (1.30–2.75)	1.53 (1.23–2.77)	1.66 (1.24–2.09)	1.02 (0.85–1.52)	1.57 (1.13–2.05)	0.069	0.029	0.279	0.67
Platelets ($\times10^{12}$/L)	251.84 ± 74.09	253.66 ± 95.58	262.85 ± 69.82	318.75 ± 140.26	265.39 ± 91.11	0.524	0.364	0.733	0.219
Serum albumin (g/L)	20.00 ± 0.00	20.00 ± 5.69	27.00 ± 0.00	19.75 ± 4.32	20.55 ± 4.98	0.003	0.002	0.591	0.641
Urine protein (g/dL)	6.39 (4.40–8.00)	7.34 (6.11–9.85)	7.51 (5.54–9.00)	7.45 (6.25–9.51)	7.45 (5.69–9.65)	0.048	0.016	0.629	0.938
Serum creatinine (μmol/L)	68 (58.25–77.55)	74 (61.5–78)	82.5 (64–112)	113.5 (94.25–141.75)	77.0 (63–100)	0.002	0.01	0.095	0.163
IgG (g/L)	6.16 (4.67–7.95)	4.75 (2.66–6.88)	4.41 (2.50–5.97)	5.91 (4.79–7.14)	5.03 (2.64–6.85)	0.034	0.019	0.579	0.296

Abbreviations: ICU, intensive care unit; IgG, immunoglobulin G; P1, comparing between the group without group and the group with infection; P2, comparing between the group with existing infection on admission and the group with infection after treatment; P3, comparing between the group with infection after treatment and the group admitted to the ICU; P, comparing among groups.

The multivariate logistic regression analysis of infection-related factors showed that serum albumin (odds ratio [OR] = 1.14; $P < 0.01$) and IgG (OR = 1.1; $P < 0.144$) were independent risk factors for infection (**Table 5**).

Table 5. Multivariate logistic regression analysis of infection-related factors in the group with infection

Variable	B (K)	SE (B)	χ^2	*P*-value	OR	95% CI
Serum albumin	0.134	0.041	1	0.001	1.14	1.238
Urine protein (g/dL)	-0.11	0.047	1	< 0.001	0.9	0.817–0.981
Serum creatinine (μmol/L)	0.026	0.008	1	0.001	0.98	0.96–0.99

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Variable	B (K)	SE (B)	χ^2	*P*-value	OR	95% CI
IgG (g/L)	0.099	0.068	1	0.144	1.1	0.967–1.261

Abbreviations: CI, confidence index; B, unstandardized beta; IgG, immunoglobulin G; K, number of predictor terms; OR, odds ratio; SE, standard error.

4. Discussion

Infection is one of the most common complications in nephrotic syndrome [2-6]. Infection is the leading cause of all-cause mortality in primary nephrotic syndrome. Through multicenter cohort studies, Yamamoto [7] revealed that infection is the leading cause of death in patients with nephrotic syndrome and is more common in patients with minimal change disease than in those with membranous nephropathy, suggesting that patients with minimal change disease are susceptible to infection. Xu [8,9] showed the incidence of complications in nephrotic syndrome, attributing 27.14% to infection, 3.68% to thromboembolism, 9.38% to acute renal insufficiency, and 67.17% to lipid metabolism disorder. Infection is a common complication in nephrotic syndrome, and the common infection sites are the respiratory tract, urinary tract, and skin. Gulati [11] showed that children with complications of infection in nephrotic syndrome have significantly lower serum albumin levels compared with those without infection and suggested that hypoproteinemia is an independent risk factor for infection in patients with nephrotic syndrome. Singh *et al*. [12] found that low IgG level is a risk factor for nephrotic syndrome complicated with severe infection. Our analysis showed that the group with infection, compared with the group without infection, had lower serum albumin and IgG levels and higher serum creatinine and urine protein levels; the differences between the groups were statistically significant. Multivariate logistic regression analysis showed that serum albumin and IgG are independent risk factors for infection. This finding is consistent with previous research results. We suggest that hypoalbuminemia and serum IgG levels are important risk factors of infection in patients with nephrotic syndrome.

The impairment of immune function by glucocorticoids increases the incidence of opportunistic infections [19-21], which are also considered a significant cause of mortality in patients. With long-term use of glucocorticoids, the body's humoral and cell-mediated immune response are affected to varying degrees, and the decrease of IgG levels in humoral immunity is an independent risk factor of infection in patients with nephrotic syndrome [22-24]. In terms of cell-mediated immunity, glucocorticoids may cause $CD4^+$ T cells apoptosis, which increases the susceptibility to infection [25,26].

Both Chen *et al*. and Grijalva *et al*. established that the risk of infection in various autoimmune diseases is positively correlated with the dose of glucocorticoids used [27,28]. The study showed that among all the patients with infection, the proportion of patients using glucocorticoids was about 72.09%, and the serum IgG level in the infected patients was significantly lower. The logistic regression analysis showed that low IgG level is an independent risk factor for infection.

In conclusion, infection is one of the common complications in nephrotic syndrome, with the respiratory system being the most common targeted site. Our analysis showed that the serum albumin and IgG levels of patients with infection were lower than those of patients without infection, while the serum creatinine and urine protein were higher in the former group compared to the latter. This suggests that serum albumin and IgG are independent risk factors for infection. In the diagnosis and treatment of patients with nephrotic syndrome, the factors predisposing to infection should be considered before the use of glucocorticoids or immunosuppressants to prevent the occurrence of infection, which may affect the prognosis of patients and even lead to the death of patients.

Disclosure statement

The authors declare no conflict of interest.

References

[1] Cutolo M, Seriolo B, 2008, Use of Glucocorticoids and Risk of Infections. Autoimmun Rev, 8(2): 153–155.

[2] Waldman M, Crew RJ, Valeri A, et al., 2007, Adult Minimal-Change Disease: Clinical Characteristics, Treatment, and Outcomes. Clin J Am Soc Nephrol, 2: 445–453.

[3] Shinzawa M, Yamamoto R, Nagasawa Y, et al., 2013, Age and Prediction of Remission and Relapse of Proteinuria and Corticosteroid-Related Adverse Events in Adult-Onset Minimal-Change Disease: A Retrospective Cohort Study. Clin Exp Nephrol, 17: 839–847.

[4] Maas RJ, Deegens JK, Beukhof JR, et al., 2017, The Clinical Course of Minimal Change Nephrotic Syndrome with Onset in Adulthood or Late Adolescence: A Case Series. Am J Kidney Dis, 69: 637–646.

[5] Fenton A, Smith SW, Hewins P, 2018, Adult Minimal-Change Disease: Observational Data from a UK Centre on Patient Characteristics, Therapies, and Outcomes. BMC Nephrol, 19: 1–8.

[6] Ozeki T, Ando M, Yamaguchi M, et al., 2018, Treatment Patterns and Steroid Dose for Adult Minimal Change Disease Relapses: A Retrospective Cohort Study. PLoS ONE, 2018: 13.

[7] Yamamoto R, Imai E, Maruyama S, et al., 2020, Incidence of Remission and Relapse of Proteinuria, End-Stage Kidney Disease, Mortality, and Major Outcomes in Primary Nephrotic Syndrome: The Japan Nephrotic Syndrome Cohort Study (JNSCS). Clinical and Experimental Nephrology, 2020: 6.

[8] Lee H, Kim DK, Oh K-H, et al., 2013, Mortality and Renal Outcome of Primary Glomerulonephritis in Korea: Observation in 1943 Biopsied Cases. Am J Nephrol, 37: 74–83.

[9] Xu Q, 2011, To Analyze the Incidence and Influencing Factors of Nephrotic Syndrome Complications, thesis, Fujian Medical University.

[10] Ogi M, Yokoyama H, Tomosugi N, et al., 1994, Risk Factors for Infection and Immunoglobulin Replacement Therapy in Adult Nephrotic Syndrome. Am J Kidney Dis, 24: 427–436.

[11] Gulati S, Kher V, Gupta A, et al., 1995, Spectrum of Infections in Indian Children with Nephrotic Syndrome. Pediatr Nephrol, 9: 431–434.

[12] Singh JA, Hossain A, Kotb A, et al., 2016, Risk of Serious Infections with Immunosuppressive Drugs and Glucocorticoids for Lupus Nephritis: A Systematic Review and Network Meta-Analysis. BMC Med, 14(1): 137.

[13] Ye WL, Tang N, Wen YB, et al., 2016, Underlying Renal Insufficiency: The Pivotal Risk Factor for Pneumocystis Jirovecii Pneumonia in Immunosuppressed Patients with Non-Transplant Glomerular Disease. Int Urol Nephrol, 48(11): 1863–1871.

[14] Vaziri ND, Pahl MV, Crum A, et al., 2012, Effect of Uremia on Structure and Function of Immune System. J Ren Nutr, 22(1): 149–156.

[15] Yoon JW, Gollapudi S, Pahl MV, 2006, Naive and Central Memory T-Cell Lymphopenia in End-Stage Renal Disease. Kidney Int, 70(2): 371–376.

[16] Girndt M, Sester M, Sester U, et al., 2001, Molecular Aspects of T- and B-cell Function in Uremia. Kidney Int Suppl, 78: S206–211.

[17] Massry S, Smogorzewski M, 2001, Dysfunction of Polymorphonuclear Leukocytes in Uremia: Role of Parathyroid Hormone. Kidney Int Suppl, 78: S195–196.

[18] Smogorzewski M, Massry SG, 2001, Defects in B-Cell Function and Metabolism in Uremia: Role of Parathyroid Hormone. Kidney Int Suppl, 78: S186–189.

[19] Li J, Zhang Q, Su B, 2017, Clinical Characteristics and Risk Factors of Severe Infections in Hospitalized Adult Patients with Primary Nephrotic Syndrome. J Int Med Res, 2017: 30.

[20] Lim CC, Liu PY, Tan HZ, et al., 2017, Severe Infections in Patients with Lupus Nephritis Treated with Immunosuppressants: A Retrospective Cohort Study. Nephrology (Carlton), 22(6): 478–484.

[21] Lionakis MS, Kontoyiannis DP, 2003, Glucocorticoids and Invasive Fungal Infections. The Lancet, 362(9398): 1828–1838.

[22] Wang Y, Wang J, 2015, Clinical Observation of Glucocorticoid Combined with Compound A-Keto Acid in the Treatment of Primary Nephrotic Syndrome. Journal of Practical Medicine, 31(19): 3185–3188.

[23] Li X, Hao L, Wang D, 2015, Bone Metabolism in Patients with Nephrotic Syndrome and the Early Effects of Corticosteroids. Journal of Anhui Medical University, 50(4): 495–499.

[24] Chen L, Dong H, Xu S, 2018, Clinical Characteristics and Risk Factors of Nephrotic Syndrome Complicated with Severe Infection. Journal of Kidney Disease and Dialysis Kidney Transplantation, 27(1): 18–23.

[25] Yang Q, Zhang L, Zhang Z, 2015, Application of Immunosuppressive Agents in the Treatment of Pulmonary Infection After Kidney Transplantation. Tissue Engineering Research in China, 19(2): 262–266.

[26] Wang X, Xue W, Ding X, 2015, The Significance of ATP Level in CD4+ T Lymphocytes in Individualized Immunosuppressive Therapy of Renal Transplant Recipients. Chinese Journal of Organ Transplantation, 36(8): 453–457.

[27] Chen L, Delzell E, Baddley JW, et al., 2011, Initiation of Tumor Necrosis Factor Alpha Antagonists and the Risk of Hospitalization for Infection in Patients with Autoimmune Diseases. JAMA, 306(21): 2331–2339.

[28] Grijalva CG, Kaltenbach L, Arbogast PG, et al., 2010, Initiation of Rheumatoid Arthritis Treatments and the Risk of Serious Infections. Rheumatology (Oxford), 49(1): 82–90.

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[2] Gamelin F.X., Baquet G., Berthoin S., et al. Effect of high intensity intermittent training on heart rate variability in prepubescent children. European Journal of Applied Physiology, 2009, 105: 731–738.

Journal article (online) with one to three authors

[3] Jackson D., Firtko A., Edenborough M. Personal resilience as a strategy for surviving and thriving in the face of workplace adversity: a literature review. Journal of Advanced Nursing, 2009, 60(1): 1–9,

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[4] Hargreave M., Jensen A., Nielsen T.S.S., et al. Maternal use of fertility drugs and risk of cancer in children—A nationwide population-based cohort study in Denmark. International Journal of Cancer, 2015, 136(8): 1931–1939.

Book

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[5] Schneider Z., Whitehead D., Elliott D. Nursing and midwifery research: methods and appraisal for evidence-based practice. 3rd edn. 2009, Elsevier Australia, Marrickville, NSW.

Book with more than three authors

[6] Davis M., Charles L., Curry M.J., et al. Challenging spatial norms. 2013, Routledge, London.

Chapter or Article in Book

[7] Knowles M.S. Independent study. In Using learning contracts. 1986, Jossey-Bass, San Francisco, 89–96.

Others

Proceedings of meetings and symposiums, conference papers

[8] Chang S.S., Liaw L. and Ruppenhofer J. (eds). Proceedings of the twenty-fifth annual meeting of the Berkeley Linguistics Society, February 12–15, 1999: general session and parasession on loan word phenomena. 2000, Berkeley Linguistics Society, Berkeley.

Conference proceedings (from electronic database)

[9] Bukowski R.M. Prognostic factors for survival in metastatic renal cell carcinoma: update 2008. Innovations and challenges in renal cancer: proceedings of the third Cambridge conference. Cancer, 2009, 115 (10): 2273, viewed 19 May 2009, Academic OneFile database.

Online Document with author names

[10] Este J., Warren C., Connor L., et al. Life in the clickstream: the future of journalism, Media Entertainment and Arts Alliance, 2008. viewed 27 May 2009, http://www.alliance.org.au/documents/foj_report_final.pdf

Online Document without author name

[11] Developing an argument n.d., viewed March 30 2009, http://web.princeton.edu/sites/writing

/Writing_Center/WCWritingResources.htm

Thesis/Dissertation

[12] Gale L. The relationship between leadership and employee empowerment for successful total quality management. 2000, University of Western Sydney.

Standard

[13] Standards Australia Online. Glass in buildings: selection and installation. AS 1288–2006. 2006, SAI Global database.

Government Report

[14] National Commission of Audit. Report to the Commonwealth Government, Australian Government Publishing Service, 1996, Canberra.

Government report (online)

[15] Department of Health and Ageing. Ageing and aged care in Australia, 2008, viewed 10 November 2008, http://www.health.gov.au/internet/main/publishing.nsf/Content/ageing

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[16] Guide to agricultural meteorological practices. 2nd edn, Secretariat of the World Meteorological Organization, 2010, Geneva.

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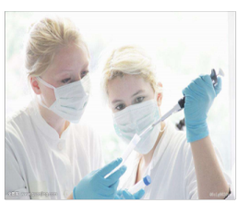

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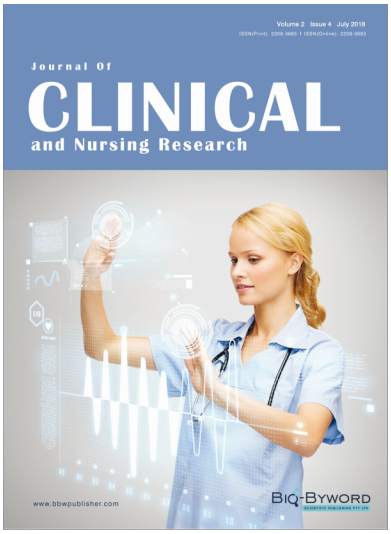

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