

The predictive value of β 2-MG and TGF- β for elderly hypertensive nephropathy

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Abstract. Predictive value of β 2-microglobulin (β 2-MG) and transforming growth factor- β (TGF- β) for elderly hypertensive nephropathy was investigated. The clinical data of 56 patients with hypertensive nephropathy and admitted to Affiliated Hospital of Chengde Medical College from December 2015 to December 2017, were retrospectively analyzed and the clinical data were used as the study group, the clinical data of 50 patients with hypertension, but not nephropathy, were selected as the control group. The expression levels of β 2-MG and TGF- β in the serum were detected by ELISA. The correlation between β 2-MG and TGF- β was analyzed by Pearson's correlation. The sensitivity and specificity of β 2-MG, TGF- β and combined application in the diagnosis of hypertensive nephropathy were analyzed by ROC curve. The expression levels of β 2-MG and TGF- β in the serum of the patients in the study group were significantly higher than those in the control group ($P < 0.001$). There was a positive correlation between the expression levels of β 2-MG and TGF- β in the serum of the patients in the study group ($r = 0.619$, $P < 0.001$). The AUC of β 2-MG in the diagnosis of hypertensive nephropathy was 0.786. The AUC of TGF- β in the diagnosis of hypertensive nephropathy was 0.793. The AUC of the combined application of β 2-MG and TGF- β in the diagnosis of hypertensive nephropathy was 0.860. β 2-MG and TGF- β were highly expressed in the patients with hypertensive nephropathy, and the expression levels of β 2-MG and TGF- β were positively correlated ($r = 0.619$, $P < 0.001$). The combined application of β 2-MG and TGF- β in the diagnosis of hypertensive nephropathy could reduce or even avoid the missed diagnosis caused by single detection. The two indicators complemented and confirmed each other, which had a

great significance for improving the positive diagnosis rate of hypertensive nephropathy.

Introduction

Hypertension is a common chronic disease (1), and its morbidity increases year by year with the development of society aging, the change of dietary structure and the gradual improvement of living standards, which threaten the life and health of the elderly (2). The morbidity of hypertensive nephropathy has also increased, and it is the most common and most serious complication of hypertension (3). If the glomeruluses are in a permanent state of high pressure, high infusion, and hyperfiltration caused by hypertension, the renal tubules and glomerular filtration membranes will be damaged and then result in hypertensive nephropathy (4,5). The early clinical manifestation of hypertensive renal injury generally is nocturia, but it is very difficult to attract patients' attention, and it usually shows normal during the routine examinations. However, generally speaking, the kidneys are already severely damaged when renal function shows abnormalities (6,7). Thus, the normality in the renal function indicators and urine protein of the patients with hypertension do not indicate that the kidneys are not pathologically damaged (8). So it is particularly important to seek the sensitive indicators for the diagnosis of renal injury of the patients with hypertension.

In recent years, research which uses β 2-microglobulin (β 2-MG) and transforming growth factor- β (TGF- β) on the diagnosis of hypertensive nephropathy has gradually increased (9-11). It is reported in the literature that β 2-MG is a good indicator in the diagnosis of early hypertensive renal injury (12). It is a small molecular globulin that is reabsorbed by renal proximal convoluted tubules after being freely filtered through the glomerulus, and finally is degraded into amino acids by renal tubular endothelial cells. The increase of the level of β 2-MG in the serum can reflect the impaired filtration function or increased filtration load of the glomerulus (13). Studies have shown that hypertensive nephropathy is closely related to TGF- β , which is highly expressed in hypertensive nephropathy and affects the development course of hypertensive nephropathy and the prognosis of patients (6). It is also reported in the literature that the main pathological changes of hypertensive nephropathy are renal interstitial fibrosis

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and glomerulosclerosis, and the main factor that regulates extracellular matrix deposition and stimulates the production of collagen is TGF- β (14,15). At the same time, TGF- β is a hematological indicator that can be detected conveniently and operated repeatedly.

Relative study of the combined application of β 2-MG and TGF- β in the diagnosis of elderly hypertensive nephropathy has not been established, therefore, the aim of this study was to investigate the diagnostic value of β 2-MG and TGF- β in elderly hypertensive nephropathy, and to provide some theoretical bases and references for the early clinical diagnosis, treatment and prognosis.

Materials and methods

General data. The clinical data of 56 patients, who were more than 60 years old, with hypertensive nephropathy, and admitted to Affiliated Hospital of Chengde Medical College (Chengde, China) from December 2015 to December 2017, were retrospectively analyzed and the clinical data were used as the study group, including 34 males and 22 females. The systolic pressure of all the patients was 140 mmHg or more and the diastolic pressure of them was 90 mmHg or more on average, and the 24 h urine protein of the patients in the study group was more than 30 mg/l or any urine protein was more than 37 mg/l, moreover, the clinical data of 50 patients, who were more than 60 years old, with hypertension, but did not have nephropathy, were selected as the control group, including 29 males and 21 females. There were no significant difference in each indicator of the general data of the patients in the two groups ($P > 0.05$). Some physiological conditions of the patients were excluded, including the insufficiency of the cardiac and hepatic functions, the incompleteness of clinical data, the existence of cancer, coagulopathy and severe metabolic diseases as well as other renal organic lesions.

This study was approved by the Ethics Committee of Affiliated Hospital of Chengde Medical College. Patients who participated in this research had complete clinical data. The signed informed consents were obtained from the patients or the guardians. The general data of the selected persons are shown in Table I.

Detection methods. The fasting venous blood of the patients was taken in the morning (33 ml in a vacuum blood collection tube). The blood was naturally agglutinated for 10 to 20 min at indoor temperature and centrifuged at 3,000 \times g for 5 min at 4°C and the supernatant was taken. The processes of sample loading were as follows: Firstly, 100 μ l of the sample diluent was added into the blank well, and then 100 μ l of the sample to be tested was respectively added into the standard well and the sample well, and 100 μ l of the test solution was added into each well after the sample to be tested was drained and dried, next, the test solution was incubated for 1 h at 37°C, then it was drained and dried again, and rinsed 3 times using PBS, after being dried, 100 μ l of another test solution was added, then the mixed solution was incubated for 1 h at 37°C and was drained and dried, and rinsed 3 times using PBS, then 90 μ l of the substrate solution was added, and the color was developed in the dark at 37°C, finally 50 μ l of the stop solution was added to terminate the translation. ELISA and Multiskan

Spectrum Microplate Spectrophotometer (SPECTROstar® Omega; Boqi Biotechnology Co., Ltd.) were used to detect β 2-MG (QY-MB12035; Shanghai Qiaoyu Biotechnology Co., Ltd., Shanghai, China) and TGF- β (YM-E3369W; Shanghai Yunmai Biotechnology Co., Ltd., Shanghai, China) and the serum of the patients was measured, as OD value at 450 nm wavelength. All the operations were strictly carried out according to the specifications.

Statistical analysis. The analysis was performed by using SPSS 19.0 (SPSS, Inc., Chicago, IL, USA) statistical software. Chi-square test was used for enumeration data and t-test was used for measurement data. Pearson's analysis was used for correlation analysis. The diagnostic value of β 2-MG, TGF- β and their combined application were analyzed, using ROC curve. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

The expression levels of β 2-MG and TGF- β in the serum of the patients in the two groups. The expression levels of β 2-MG and TGF- β in the serum of the patients in the study group (2.86 ± 1.18 mg/l and 73.46 ± 15.63 μ g/l) were significantly higher than those in the control group (1.87 ± 0.65 mg/l and 52.89 ± 13.58 μ g/l), the difference was statistically significant ($P < 0.001$; Fig. 1 and Table II).

There was a positive correlation between the expression levels of β 2-MG and TGF- β in the serum of the patients who had hypertensive nephropathy ($r = 0.619$, $P < 0.001$; Fig. 2).

The sensitivity and specificity of β 2-MG and TGF- β in the diagnosis of hypertensive nephropathy. The analysis result of the ROC curve of β 2-MG in the diagnosis of hypertensive nephropathy showed that the AUC of β 2-MG in the diagnosis of hypertensive nephropathy was 0.786; the 95% confidence interval was from 0.699 to 0.871, and the cut-off value was 2.480; there were 33 positive cases in the study group and 4 in the control group; the sensitivity was 58.93%, and the specificity was 92.00%.

The analysis of the ROC curve of TGF- β in the diagnosis of hypertensive nephropathy showed that the AUC of TGF- β in the diagnosis of hypertensive nephropathy was 0.793; the 95% confidence interval was from 0.709 to 0.877, and the cut-off value was 59.330; there were 39 positive cases in the study group and 10 in the control group; the sensitivity was 69.64%, and the specificity was 80.00%.

The analysis result of the ROC curve of the combined application of β 2-MG and TGF- β in the diagnosis of hypertensive nephropathy showed that the AUC of the combined application of β 2-MG and TGF- β in the diagnosis of hypertensive nephropathy was 0.860; the 95% confidence interval was from 0.791 to 0.928, and the cut-off value was 0.303; there were 53 positive cases in the study group and 17 in the control group; the sensitivity was 94.64%, and the specificity was 66.00% (Fig. 3 and Table III).

Discussion

Hypertension is a disease that is affected by environmental and genetic factors (16). According to the statistics, there are

Table I. Comparison of general data between two groups of patients [n(%)].

Factors	Observation group (n=56)	Control group (n=50)	t/ χ^2	P-value
Sex			0.081	0.844
Male	34 (60.71)	29 (58.00)		
Female	22 (39.29)	21 (42.00)		
Average age, year	65.68±4.27	65.13±4.68	0.633	0.528
The course of hypertension, year	10.68±2.23	11.05±1.95	0.904	0.368
BMI, kg/m ²	23.86±1.64	23.75±1.52	0.357	0.722
Smoking			0.968	0.339
Yes	26 (46.43)	28 (56.00)		
No	30 (53.57)	22 (44.00)		
Drinking			1.772	0.242
Yes	23 (41.07)	27 (54.00)		
No	33 (58.93)	23 (46.00)		
The place of residence			0.089	0.844
City	32 (57.14)	30 (60.00)		
Country	24 (42.86)	20 (40.00)		

Table II. The expression levels of β 2-MG and TGF- β in the serum of the patients in the two groups.

Groups	Case	β 2-MG (mg/l)	TGF- β (μ g/l)
Study	56	2.86±1.18	73.46±15.63
Control	50	1.87±0.65	52.89±13.58
t		5.261	7.192
P-value		<0.001	<0.001

β 2-MG, β 2-microglobulin; TGF- β , transforming growth factor- β .

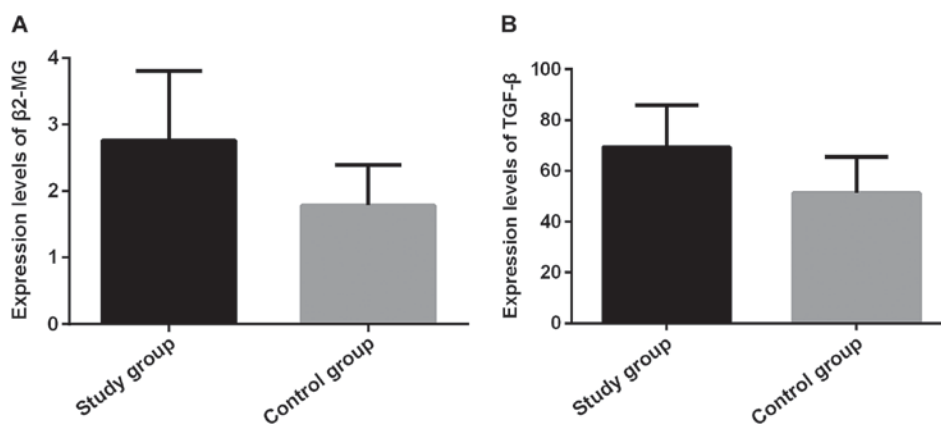


Figure 1. The expression levels of β 2-MG and TGF- β in the serum of the patients in the two groups. (A) The results of ELISA showed that the expression level of β 2-MG in the serum of the patients in the study group was significantly higher than that in the control group ($P<0.001$). (B) The expression level of TGF- β in the serum of the patients in the study group was significantly higher than that in the control group ($P<0.001$). $P<0.001$. β 2-MG, β 2-microglobulin; TGF- β , transforming growth factor- β .

approximately 1.1 billion adult patients with hypertension in the world, among which approximately 700 million adult patients with hypertension are in the developing countries and approximately 400 million adult patients with hypertension

are in the developed countries (17). In the state of persistent hypertension, the organism will suffer from arteriolosclerosis, however, the impairment of the kidney is particularly obvious when compared with all the visceral organs. The regulatory

Table III. The sensitivity and specificity of β 2-MG, TGF- β in the diagnosis of hypertensive nephropathy.

Indicator	n	The positive case	Sensitivity	Specificity
β 2-MG	56	33	58.93	92.00
TGF- β	56	39	69.64	80.00
β 2-MG+TGF- β	56	53	94.64	66.00

β 2-MG, β 2-microglobulin; TGF- β , transforming growth factor- β .

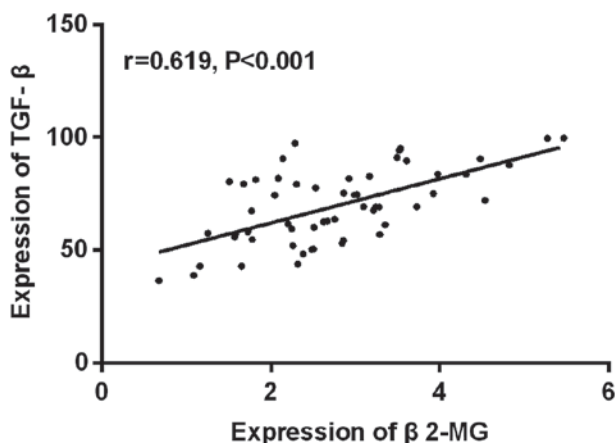


Figure 2. The correlation analysis of β 2-MG and TGF- β in the serum of the patients with hypertensive nephropathy. The result of Pearson correlation analysis showed that the expression levels of β 2-MG and TGF- β in the serum of the patients with hypertensive nephropathy were positively correlated ($r=0.619$, $P<0.001$). β 2-MG, β 2-microglobulin; TGF- β , transforming growth factor- β .

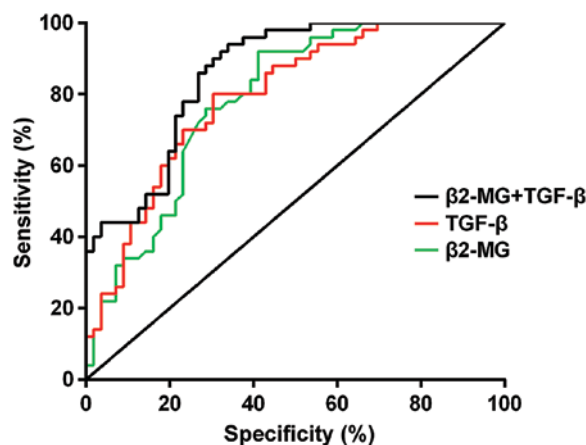


Figure 3. The ROC curve of β 2-MG and TGF- β in the diagnosis of hypertensive nephropathy. The AUC of β 2-MG in the diagnosis of hypertensive nephropathy was 0.786; The AUC of TGF- β in the diagnosis of hypertensive nephropathy was 0.793; The AUC of the combined application of β 2-MG and TGF- β in the diagnosis of hypertensive nephropathy was 0.860. β 2-MG, β 2-microglobulin; TGF- β , transforming growth factor- β .

functions of the kidney will be weakened by a state of high pressure, high filtration, and high perfusion, which will lead to the disequilibrium of hemodynamics and the abnormality of functions in the kidney (18,19). According to the reports in the literature, generally β 2-MG is rarely found in the blood and urine of healthy people, and the resultant rate is stable, and there is no significant difference in its changes between the morning and evening. Therefore, the evaluation of the functions of renal tubular and glomerulus will be more stable and accurate when using β 2-MG as indicator (20). It is also found in other studies that TGF- β is overexpressed in diabetic hypertensive nephropathy and is involved in the pathogenesis of diabetic hypertensive nephropathy (21).

This study showed that the expression levels of β 2-MG and TGF- β in the serum of the patients in the study group were significantly higher than those in the control group, and the difference was statistically significant ($P<0.001$). Study has shown that the rise of β 2-MG in the serum indicates that the filtration function of glomerulus is damaged or is overloaded (22). TGF- β plays a major role in many renal diseases and the formation of hypertensive renal scars, and is an early indicator of hypertensive benign renal disease (23). The study of Rouse *et al* showed that the expression level of β 2-MG in the serum of the patients who had hypertensive nephropathy was significantly higher than that of patients with only hypertension (6). The study of Kurts *et al* showed that the expression

level of TGF- β in the serum of the patients with hypertensive nephropathy was significantly higher than that of patients who had hypertension, but did not have renal disease (24). The results of these studies were consistent with our findings. There was a positive correlation between the expression levels of β 2-MG and TGF- β in the serum of the patients with hypertensive nephropathy ($r=0.619$, $P<0.001$). At present, no similar literature has been found to support the findings, but the results of our study demonstrated that if the expression levels of β 2-MG and TGF- β in the serum of the patients increased, the impairment of renal functions is most likely to occur. The combined application of the two in the diagnosis could help to detect the occurrence of hypertensive nephropathy.

The analysis of the ROC curve of β 2-MG in the diagnosis of hypertensive nephropathy showed that the AUC of β 2-MG in the diagnosis of hypertensive nephropathy was 0.786; the 95% confidence interval was from 0.699 to 0.871, and the cut-off value was 2.480; there were 33 positive cases in the study group, the sensitivity was 58.93%, and the specificity was 92.00%. The result of the ROC curve of TGF- β in the diagnosis of hypertensive nephropathy showed that the AUC of TGF- β in the diagnosis of hypertensive nephropathy was 0.793; the 95% confidence interval was from 0.709 to 0.877, and the cut-off value was 59.330; there were 39 positive cases in the study group, the sensitivity was 69.64%, and the specificity was 80.00%. The result of the ROC curve of the

combined application of β 2-MG and TGF- β in the diagnosis of hypertensive nephropathy showed that the AUC of the combined application of β 2-MG and TGF- β in the diagnosis of hypertensive nephropathy was 0.860; the 95% confidence interval was from 0.791 to 0.928, and the cut-off value was 0.303; there were 53 positive cases in the study group, the sensitivity was 94.64%, and the specificity was 66.00%. This indicated that TGF- β was not a specific indicator in the diagnosis of hypertensive nephropathy, but it still had screening value. The sensitivity of β 2-MG in the diagnosis of hypertensive nephropathy was low, but the specificity was good. The sensitivity of the combined application of β 2-MG and TGF- β in the diagnosis of hypertensive nephropathy was high, but the specificity was poor, which provided a more effective and comprehensive diagnostic basis for hypertensive nephropathy.

In summary, β 2-MG and TGF- β were highly expressed in the patients with hypertensive nephropathy, and the expression levels of β 2-MG and TGF- β were positively correlated ($r=0.619$, $P<0.001$). The sensitivity of the combined application of β 2-MG and TGF- β was highest in the diagnosis of hypertensive nephropathy, but the specificity was worst. Therefore, the combined application of β 2-MG and TGF- β in the diagnosis of hypertensive nephropathy could reduce or even avoid the missed diagnosis caused by single detection. The two indicators complemented and confirmed each other, which had a great significance for improving the positive diagnosis rate of hypertensive nephropathy.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JC wrote the manuscript. JC, RH and CZ performed ELISA. JL and KZ collected and analyzed the general data of patients. HJ, YF and YW were responsible for statistical analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Affiliated Hospital of Chengde Medical College (Chengde, China). Patients who participated in this research had complete clinical data. The signed informed consents were obtained from the patients or the guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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