EXPRESSION OF THE SERUM TNFR1 AND TNFR2 IN PATIENTS WITH IDIOPATHIC MEMBRANOUS NEPHROPATHY AND THEIR RELATIONSHIP WITH CLINICOPATHOLOGICAL PARAMETERS AND PROGNOSIS

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ABSTRACT

Objective: *To analyse the expression of tumour necrosis factor receptor-l (TNFRl) and tumour necrosis factor receptor-2 (TNFR2) in the serum of patients with idiopathic membranous nephropathy and their relationship with the clinicopathological parameters and prognosis.*

Methods: *Patients with idiopathic membranous nephropathy that were treated in our hospital during the timeframe of January 2015 to February 2016 were selected as part of the IMN group (n=88), patients with non-idiopathic membranous nephropathy who had renal biopsy at the same time were selected as part of the non-IMN group (n=50), and healthy adults who had a physical examination acted as a control group (n=45). The levels of serum creatinine, glomerular filtration rate, serum albumin, 24h urinary protein (24hpro), and TNFR1 and TNFR2 were compared among the three groups. After treatment, the IMN patients were divided into 24hpro <3.5g group (n=30), 24hpro ≥3.5g group (n=58), and the quantitative relationship between TNFR1, TNFR2, and 24h urinary protein was analysed. After treatment, the IMN patients were divided into complete remission group (n=42), partial remission group (n=28) and ineffective group (n=18), according to the 24hpro level. The relative expression levels of TNFR1 and TNFR2 were also analysed.*

Results: *Compared with the control group, the serum albumin level of the IMN group and non-IMN group was significantly lower, and the 24hpro was significantly higher (P<0.05). When compared with the non-IMN group, the serum albumin level of the IMN group was significantly lower, and the 24hpro was significantly higher (P<0.05). There was no significant difference in the serum creatinine and glomerular filtration rate among the three groups (P>0.05). Compared with the control group, the relative expression level of the TNFR1 and TNFR2 in both the non-IMN group and IMN group increased significantly (P<0.05); compared with the non-IMN group, the relative expression level of TNFR1 and TNFR2 in the IMN group was seen to increased significantly (P<0.05). There was no significant difference in the relative expression of TNFR1 and TNFR2 between the control group and the IMN group (P>0.05). In contrast, a comparison with the control group showed that the relative expression level of TNFR1 and TNFR2 in stage III and IV patients were significantly higher (P<0.05). Compared with the <3.5g group, the expression levels of TNFR1 and TNFR2 in ≥ 3.5g group were also significantly higher (P<0.05). There was a positive correlation between the expression of the TNFR1 and TNFR2 and 24-hour urine protein. Compared with the complete remission group, the relative expression levels of TNFR1 and TNFR2 in the partial remission group and the ineffective group were also significantly higher (P<0.05).Finally, when compared with the partial remission group, the relative expression levels of TNFR1 and TNFR2 in the ineffective group were significantly higher (P<0.05).*

Conclusion: *The relative expression level of TNFR1 and TNFR2 in the serum of patients with idiopathic membranous nephropathy is significantly increased, and the expression of TNFR1 and TNFR2 is correlated with the pathological stage, 24hpro, and prognosis of patients. This may be an important biomarker for evaluating the occurrence, development, and prognosis of idiopathic membranous nephropathy.*

Keywords: *Idiopathic membranous nephropathy, TNFR1, TNFR2, prognosis.*

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Introduction

Idiopathic membranous nephropathy can be seen at any age, with the characteristics being that of slow onset, a common cause of primary nephrotic syndrome. According to some investigations, the total incidence rate of idiopathic membranous nephropathy accounts for about 10% of primary glomerulonephritis, while the proportion in foreign countries ranges as high as 30%[(1)]. The incubation period of the disease can range from weeks to months, and the clinical symptoms vary in severity. Most of the patients have a large number of proteinuria, hyperlipidemia, and other nephrotic syndrome

manifestations, and some of the patients have deep vein thrombosis, decreased resistance, increased risk of infection, and other phenomena. Indeed, idiopathic membranous kidney disease has two prognosis results: self-remission and the gradual deterioration of renal function[2]. However, the pathogenesis of idiopathic membranous nephropathy has not been fully elucidated. At present, our understanding mainly begins from the cellular and molecular level.

The drug treatment of specific membranous nephropathy has different sensitivities, many side effects, and other shortcomings, and as such, the treatment of this disease is controversial[3]. In addition, immunosuppressive therapy should be given to patients with specific membranous nephropathy with nephrotic syndrome and renal insufficiency[4]. At present, there are many immunosuppressants available for the treatment of specific membranous nephropathy, but the effect of each inhibitor is different.

Therefore, early diagnosis and treatment are crucial for an analysis of the biomarkers that are related to idiopathic membranous nephropathy, which would also allow for a better understanding of its pathogenesis. The tumour necrosis factor receptor-1 (TNFRl) has a death structure range, while TNFR2 and TNFR1 have a totally different intracellular structure.

The interaction of the two receptors can thus have an effect on the process of tumour proliferation, neovascularization, and migration[5-6]. However, there is concern that these two receptors play an important role in an idiopathic membranous kidney. The role of TNFR1 and TNFR2 in the pathogenesis of idiopathic membranous nephropathy is thus rarely reported in this study.

Materials and methods

General information

From January 2015 to February 2016, 88 patients that had idiopathic membranous nephropathy from our hospital were collected to form the IMN group, including 48 males and 40 females, with an average age of (49.22±12.77) years.

Inclusion criteria:

• Idiopathic membranous nephropathy was confirmed by renal biopsy and pathological examination;

• Age >18; (3) Patients agreed to participate in the study and signed their informed consent.

Exclusion criteria:

• Exclusion of patients treated with hormone and immunosuppression before puncture;

• Exclusion of patients with metabolic diseases and tumours;

• Exclusion of patients with secondary membranous nephropathy.

In addition, 50 patients with non-idiopathic membranous nephropathy who underwent renal biopsy during the same time were selected as part of the non-IMN group and 45 healthy adults who underwent physical examination acted as the control group. The total included 28 males and 22 females in the non-IMN group with an average age of (48.96±13.86) years; and 25 males and 20 females in the control group with an average age of (50.13±12.56) years. There were no significant differences in general data among the three groups (P>0.05).

Method

Western blotting: the fasting venous blood of the three groups of experimental subjects was extracted and the serum was separated, with the protein being subsequently extracted from these. The protein was centrifuged in a centrifuge, then electrophoretic separation and membrane transfer steps were carried out. After that, TNFR1 and TNFR2 antibodies were added for immunohybridization experiment, and development was finally carried out.

Observation index

• The serum creatinine level of the three groups was measured by the creatine oxidase method, and the serum albumin and 24 h urine protein quantitative (24hpro) level of the three groups were measured by the automatic biochemical analyser.

• The relative expression of TNFR1 and TNFR2 was detected by protein immunoblotting.

• After treatment, 24hpro=3.5 was divided into 24hpro <3.5g group (n=30) and 24hpro ≥3.5g group (n=58). This was to analyse the quantitative relationship between TNFR1, TNFR2, and 24h urinary protein in patients with idiopathic membranous nephropathy.

• The patients with 24hpro ≤0.5g/24h were treated as the complete remission group (n=42), 24hpro ≤3.0g/24h as the partial remission group (n=28), 24hpro >3.0g/24h as the ineffective group (n = 18). The relative expression levels of TNFR1 and TNFR2 in three groups were also analysed.

Statistical methods

($\bar{x}\pm s$) was used to express the relative expression level of TNFR1 and TNFR2 in the three groups, and F was used to test the data comparison among the

three groups; Pearson was used to analyse the quantitative relationship between the TNFR1, TNFR2, and 24h urinary protein in patients with idiopathic membranous nephropathy. P<0.05, the difference was statistically significant.

Results

Comparison of three biochemical indexes

Compared with the control group, the serum albumin level of IMN group, and the non-IMN group was significantly lower, 24hpro was significantly higher (P<0.05); indeed, compared with the non-IMN group, the serum albumin level of IMN group was significantly lower, and the 24hpro was significantly higher (P<0.05).

There were no significant differences in the serum creatinine and glomerular filtration rates among the three groups (P>0.05). See Table 1.

Group	n	Serum creatinine (mg/dL)	Glomerular filtration rate (mL/min/1.73m²)	Serum albumin (g/dL)	24hpro (g/gCr)
Control	45	0.90±0.26	93.22±22.92	4.63±0.39	0.02±0.03
Non-IMN	50	0.87±0.29	90.75±17.45	4.04±0.54	1.77±0.52
IMN	88	0.96±0.37	85.30±13.62	2.59±0.60	4.70±0.67
F		0.120	2.450	257.94	401.258
P		0.329	0.090	<0.001	<0.001

Table 1: Comparison of biochemical indexes of three groups ($\bar{x}\pm s$).

Comparison of the relative expression levels of TNFR1 and TNFR2 in three groups

Compared with the control group, the relative expression level of TNFR1 and TNFR2 in the non-IMN group and IMN group increased significantly (P<0.05); and compared with the non-IMN group, the relative expression levels of TNFR1 and TNFR2 in the IMN group had increased significantly (P<0.05). See Table 2.

Group	n	TNFR1	TNFR2
Control	45	0.56±0.08	0.55±0.09
Non-IMN	50	0.94±0.14	0.80±0.30
IMN	88	1.72±0.37	1.65±0.45
F		147.372	146.656
P		<0.001	<0.001

Table 2: Comparison of relative expression levels of serum TNFR1 and TNFR2 in three groups ($\bar{x}\pm s$).

Comparison of relative expression of TNFR1 and TNFR2 in the IMN group at different pathological stages

There were no significant differences in the relative expression of TNFR1 and TNFR2 between the control group and the IMN group (P>0.05).

Compared with the control group, the relative expression level of TNFR1 and TNFR2 in stage III and IV patients was significantly higher (P<0.05). See Figure 1.

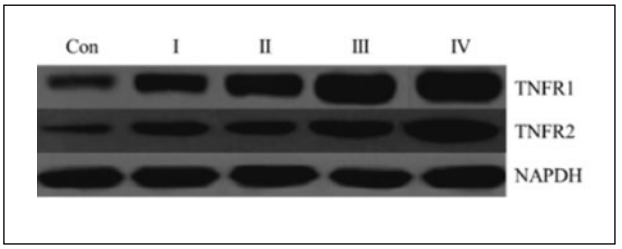

Figure 1: Comparison of relative expression levels of TNFR1 and TNFR2 in three groups at different pathological stages.

The relationship between TNFR1, TNFR2 and 24hpro in the IMN group

Compared with <3.5g group, the expression levels of TNFR1 and TNFR2 in ≥3.5g group were significantly higher (P<0.05), as seen in Table 3. The expression level of TNFR1 and TNFR2 was positively correlated with 24hPro. See picture 2AB.

24hpro	n	TNFR1	TNFR2
<3.5g	30	1.32±0.33	1.28±0.31
≥3.5g	58	1.92±0.50	1.79±0.44
t		3.005	2.843
P		0.008	0.012

Table 3: The relationship between TNFR1, TNFR2, and 24hpro in the IMN group.

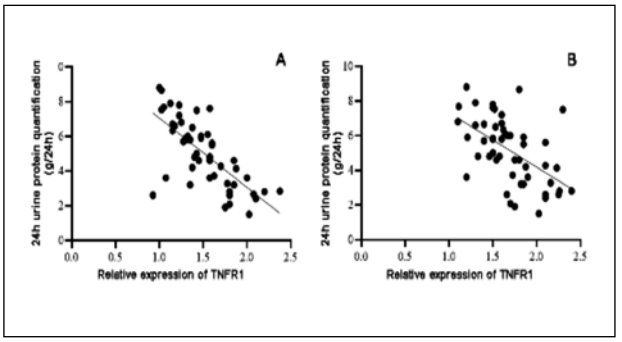

Figure 2: The quantitative relationship between TNFR1, TNFR2, and 24h urinary protein in the IMN group.
A: the quantitative relationship between TNFR1 and 24h urine protein; B: the quantitative relationship between TNFR2 and 24h urine protein.

Comparison of the relative expression levels of TNFR1 and TNFR2 in three prognosis groups

Compared with the complete remission group, the relative expression levels of TNFR1 and TNFR2 in the partial remission group and the ineffective group were significantly higher (P<0.05); compared with the partial remission group, the relative expression levels of TNFR1 and TNFR2 in the ineffective group were also significantly higher (P<0.05). See Table 4.

Group	n	TNFR1	TNFR2
Complete remission	42	0.99±0.34	0.85±0.32
Partial remission	28	1.35±0.37	1.09±0.38
Invalid	18	1.74±0.45	1.34±0.43
F		60.15	27.42
P		<0.001	<0.001

Table 4: Comparison of relative expression levels of serum TNFR1 and TNFR2 in three groups ($\bar{x}\pm s$).

Discussion

Idiopathic membranous nephropathy is characterized by a complicated condition, prolonged course, alternation of remission and recurrence, and great differences in its prognosis. Although it is a benign process, some patients run the risk of developing end-stage nephropathy[7]. Therefore, we need to explore the pathogenesis of the disease and look for related biomarkers to provide a reference for clinical treatment. TNFR1 and TNFR2 are two receptors of tumour necrosis factor α. TNFR1 was expressed in the glomeruli of healthy volunteers, but TNFR2 was not expressed[8-9]. When the kidney is damaged, TNFR1 and TNFR2 are expressed in glomerulus and renal tubules[10]. In the study of IgA nephropathy, up-regulating the expression of TNFR1 and TNFR2 can induce renal tubulointerstitial injury, which can subsequently lead to renal injury[11]. Many studies have shown that TNFR1 and TNFR2 are closely related to inflammatory kidney disease, but the relationship between the clinicopathological characteristics of patients with idiopathic membranous nephropathy and the expression of TNFR1 and TNFR2 is lesser[12].

The combination of TNF-α with TNFR1 and TNFR2 can promote the release of inflammatory mediators and chemokines, leading to direct renal injury[13]. In addition, the tumour necrosis factor pathway can cause cell damage and promote cell apoptosis, and an aggregation of the inflammatory cells eventually leads to changes in the renal tubulointerstitial[14]. There are immune complexes in the development of idiopathic membranous nephropathy, and these can lead to the activation of multiple signal pathways, the change of glomerular filtration rate, and a large number of proteinuria.

Clinical research has also shown that if 24hpro ≥ 3.5g continuously, it can be regarded as an independent risk factor for the development of idiopathic membranous nephropathy[15].

In this study, 88 patients with idiopathic membranous nephropathy, 50 patients with non-idiopathic membranous nephropathy, and 45 healthy volunteers were included. Through the detection of various indicators, it was found that when compared with the control group, the serum albumin level of the IMN group and non-IMN group decreased significantly, and the 24hpro increased significantly (P<0.05). Furthermore, when compared with the non-IMN group, the serum albumin level of the IMN group decreased significantly, and 24hpro increased significantly (P<0.05). It also increased (P<0.05). There were no significant differences in the serum creatinine and glomerular filtration rate among the three groups (P>0.05). There was also a positive correlation between the expression of TNFR1 and TNFR2 and the 24-hour urine protein. It is thus suggested that the increased expression of TNFR1 and TNFR2 may be related to the development of proteinuria and ultimately accelerates the progression of idiopathic membranous nephropathy.

Compared with the control group, the relative expression level of TNFR1 and TNFR2 in the non-IMN group and IMN group was seen to have increased significantly (P<0.05); and when compared with the non-IMN group, the relative expression level of TNFR1 and TNFR2 in the IMN group increased significantly (P<0.05). It is thus suggested that TNFR1 and TNFR2 are involved in the progression of idiopathic membranous nephropathy. There were no significant differences in the relative expression of TNFR1 and TNFR2 between the control group and the IMN group (P>0.05).

Compared with the control group, the relative expression level of TNFR1 and TNFR2 in stage III and IV patients were also found to be significantly higher (P<0.05). It is further suggested that the increased expression of TNFR1 and TNFR2 is closely related to the late development of idiopathic membranous nephropathy, which may be an important marker to evaluate the occurrence and development

of idiopathic membranous nephropathy. In addition, there was a positive correlation between the expression of TNFR1 and TNFR2 and the 24-hour urine protein. Compared with the complete remission group, the relative expression levels of TNFR1 and TNFR2 in the partial remission group and the ineffective group were significantly higher ($P<0.05$); compared with the partial remission group, the relative expression levels of TNFR1 and TNFR2 in the ineffective group were also significantly higher ($P<0.05$). It is thus suggested that the expression level of TNFR1 and TNFR2 is related to the prognosis of patients with idiopathic membranous nephropathy, which may be an important marker for the evaluation of the prognosis of patients with idiopathic membranous nephropathy.

To sum up, the relative expression of TNFR1 and TNFR2 in the serum of patients with idiopathic membranous nephropathy is significantly increased, and the expression of TNFR1 and TNFR2 is correlated with pathological stage, 24hpro, and prognosis of patients, and this may become an important biomarker for evaluating the occurrence, development, and prognosis of idiopathic membranous nephropathy.

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