Correlation between serum inflammatory factors TNF-α, IL-8, IL-10 and Henoch-Schonlein purpura with renal function impairment

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Abstract. The changes of tumor necrosis factor-α (TNF-α), interleukin-8 (IL-8), interleukin-10 (IL-10) in the serum of Henoch-Schonlein purpura nephritis (HSPN) patients were analyzed to explore the correlation between the above inflammatory factors and progression of the disease. The present study used the double antibody sandwich enzyme-linked immunosorbent assay (ELISA) method to detect the serum levels of TNF-α, IL-8, IL-10 and urine protein in 112 cases of patients with Henoch-Schonlein purpura (HSP), including 54 cases of HSP combined with renal function impairment (group HSPN), and 58 cases not combined with renal function impairment (NHSPN), as well as 50 healthy patients who were selected as the control group. The concentration of TNF-α, IL-8, and IL-10 in the serum of HSP patients were higher than that of the control group, and the difference was statistically significant (P<0.05). There was no significant difference in the levels of IL-10, and IL-8 between the HSPN group and the NHSPN group (P>0.05), but the level of TNF-α in the serum of HSPN group was significantly higher than that of NHSPN group (P<0.05). TNF-α, IL-8 and IL-10 levels of the acute nephritis, chronic nephritis and nephrotic syndrome groups were all higher than the simple proteinuria group. In addition, the levels of the three factors of the acute nephritis group were all higher than those of the chronic nephritis and nephrotic syndrome groups (P<0.05). IL-8, IL-10, and TNF-α were positively correlated with the urinary protein levels. The results indicated that the levels of serum TNF-α, IL-8 and IL-10 are correlated with HSPN, and serum TNF-α concentration can be used as an indicator of the severity of HSPN.

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Introduction

Henoch-Schonlein purpura (HSP) is common in children, the main pathological feature is the small blood vessel inflammation caused by the accumulation of a variety of cytokines, and it recurs easily, mostly occurring in children aged 3-10 years, with less incidence among adults (1).

The main clinical features of the disease are non-thrombocytopenic purpura, arthritis, and internal organs involved, including gastrointestinal tract and kidney (2). The disease is usually acute and self-limiting, however, kidney involvement often leads to serious clinical consequences, and its prognosis depends on the severity of kidney involvement (3). HSP patients may occur with secondary renal function impairment, hematuria or proteinuria, the renal disease at this time is also known as Henoch-Schonlein purpura nephritis (HSPN) (4). IL-8 is a proinflammatory factor, IL-10 is an anti-inflammatory factor, and both of these are altered with the change in the degree of inflammation. TNF-α has a very important regulatory role for the immune function, and the level of urinary protein is closely related to the degree of renal function impairment.

In the present study, by detecting the levels of TNF-α, IL-8 and IL in serum of HSP combined with renal function impairment patient (HSPN group) and HSP combined without renal function impairment (NHSPN group) and healthy people, we explored the relationship between inflammatory factors such as IL-8, IL-10, TNF-α and HSPN.

Materials and methods

General information. Patients with Henoch-Schonlein purpura (HSP) admitted into The Affiliated Hospital of Jining Medical University from June, 2016 to June, 2017 were included, divided into the NHSPN group (n=58) and HSPN group (n=54) according to whether or not combined with HSPN. The NHSPN group comprised 31 males and 27 females, aged 7.2±2.5 years, and the HSPN group had 28 males and 26 females, aged 7.1±2.3 years. In addition, 50 healthy individuals were randomly selected as the control group, including 27 males and 25 females, with an average age of 7.0±2.1 years. There was no significant difference in sex and age in the three groups (P>0.05).

This study was approved by the ethics committee of The Affiliated Hospital of Jining Medical University (Shandong, China). Signed written informed consents were obtained from all the participants before the study.

According to the classification of HSPN at the Zhuhai meeting in 2000, 15 cases of simple proteinuria, 11 cases of acute nephritis, 13 cases of chronic nephritis, and 15 cases of nephrotic syndrome were diagnosed in HSPN group. Of these, 15 cases had light nephritis, and 39 cases had severe nephritis. There was no significant difference in age, sex and weight between the subgroups of HSPN group (P>0.05). Specific distribution is provided in Table I. All the included cases were consistent with the Henoch-Schonlein purpura classification criteria of the American Rheumatology Association (5). Patients were excluded from the HSPN group if systemic vasculitis, thrombocytopenic purpura, other systemic immune diseases (such as systemic lupus erythematosus), dermato-myositis, diabetes was detected, and if renal biopsy results did not render them eligible for this group.

Methods. Venous blood (2 ml) was taken from the subjects in the morning following fasting, and centrifuged at 2,300 x g for 10 min. The serum at the lower part of the test tube was stored at -20˚C. The collected blood samples were assayed for cytokine level using the ELISA kit (Sigma, St. Louis, MO, USA). Determination of the urinary protein level was performed by MA-4210 urine analyzer (Guilin Huatong Medical Instrument Co., Ltd., Guangxi, China), the urine reagent strip was immersed in urine for 1 min, and then removed and placed into the urine analyzer for quantitative detection.

Statistical analysis. SPSS 11.0 statistical software (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Measurement data are presented as mean ± SD. Comparisons between groups was tested with ANOVA and SNK test was used as post hoc test. Enumeration data were analyzed using Chi-square analysis. The correlation between urinary protein content and serum factor in patients with HSPN combined with renal impairment was analyzed by Pearson's linear correlation analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

TNF-α levels in the three groups. The levels of TNF-α in the HSPN and NHSPN groups were significantly higher than those in the control group (P<0.05). The level of TNF-α in HSPN group was higher than that in NHSPN group (P<0.05) (Fig. 1).

IL-8 levels in three groups. The levels of IL-8 in the HSPN and NHSPN groups were significantly higher than those in the control group, and the difference was statistically significant (P<0.05). There was no significant difference in IL-8 level between HSPN group and NHSPN group (P>0.05) (Fig. 2).

IL-10 level in the three groups. The levels of IL-10 in the HSPN and NHSPN groups were significantly higher than those in the control group, and the difference was statistically significant (P<0.05). There was no significant difference in IL-10 level between the HSPN and NHSPN groups (P>0.05) (Fig. 3).

Table I. General information of patients with HSPN (mean ± SD).

Type of nephritis	No. of cases	Sex (M/F)	Age (year)	Weight (kg)
Light nephritis				
Simple proteinuria	15	9/6	6.9±3.1	23.2±3.5
Severe nephritis				
Acute nephritis	11	7/4	7.0±2.9	24.1±3.2
Chronic nephritis	13	8/5	7.1±3.2	22.8±3.9
Nephrotic syndrome	15	8/7	7.1±3.1	23.5±3.1
t/χ^2 test	-	0.354	2.321	3.011
P-value	-	P>0.05	P>0.05	P>0.05

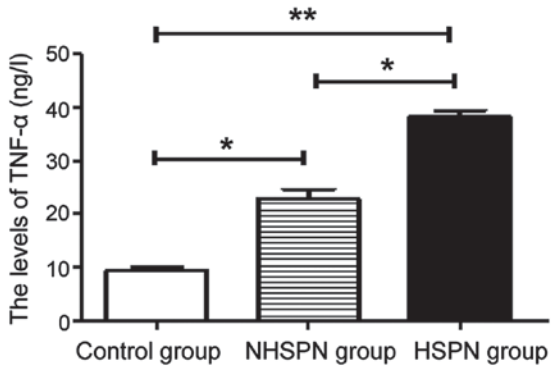

Figure 1. TNF-α levels in the three groups. The levels of TNF-α in the HSPN and NHSPN groups were significantly higher than those in the control group, and the difference was statistically significant (*P<0.05, **P<0.01). The content of TNF-α in the HSPN group was higher than that in the NHSPN group, and the difference was statistically significant (*P<0.05).

Levels of TNF-α, IL-8 and IL-10 in HSPN group. The levels of TNF-α, IL-8 and IL-10 in the acute nephritis, chronic nephritis and nephrotic syndrome groups were higher than those in the simple proteinuria group (P<0.05, P<0.01). Levels of TNF-α, IL-8 and IL-10 in the acute nephritis group were significantly higher than those in the chronic nephritis and nephrotic syndrome groups (P<0.05). There was no significant difference between the chronic nephritis and nephrotic syndrome groups (P>0.05) (Fig. 4).

Correlation between urinary protein content and serum TNF-α, IL-8 and IL-10 in HSPN patients. The r values of IL-8, IL-10, TNF-α and urinary protein were 0.61, 0.413 and 0.428, respectively, and the test standard was α=0.05 (Table II and Fig. 5).

Discussion

Henoch-Schonlein purpura (HSP) is the most common small vasculitis in childhood, which mainly causes skin, bowel and kidney changes (6,7). Although the pathogenesis is not clear

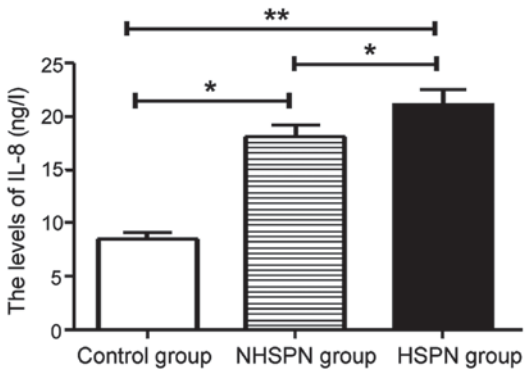

Figure 2. IL-8 levels in three groups. The levels of IL-8 in the HSPN and NHSPN groups were significantly higher than those in the control group (*P<0.05, **P<0.01). There was no significant difference in IL-8 level between the HSPN and NHSPN groups (P>0.05).

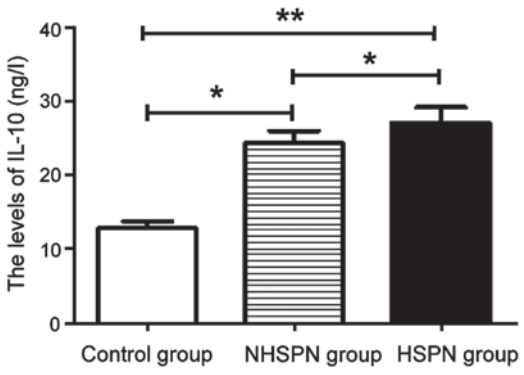

Figure 3. IL-10 levels in three groups. The levels of IL-10 in the HSPN and NHSPN groups were significantly higher than those in the control group (*P<0.05, **P<0.01). There was no significant difference in IL-10 level between the HSPN and NHSPN groups (P>0.05).

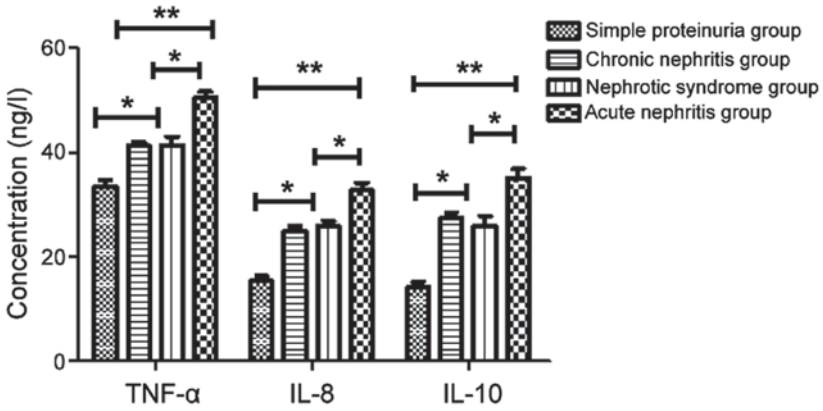

Figure 4. The levels of TNF-α, IL-8 and IL-10 in the different types of HSPN groups. The levels of TNF-α, IL-8 and IL-10 in the acute nephritis, chronic nephritis and nephrotic syndrome groups were higher than those in the simple proteinuria group (*P<0.05, **P<0.01), and TNF-α, IL-8 and IL-10 in the acute nephritis group were significantly higher than those in the chronic nephritis and nephrotic syndrome groups (*P<0.05).

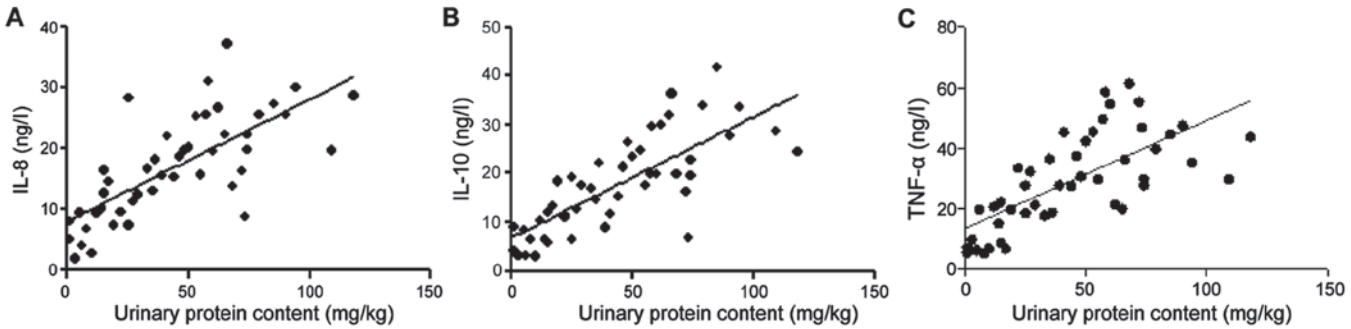

Figure 5. Analysis of the correlation between urinary protein content and serum TNF-α, IL-8 and IL-10 levels. (A) Results show that IL-8 was positively correlated with urinary protein. (B) IL-10 was positively correlated with urinary protein. (C) TNF-α was positively correlated with urinary protein.

currently, in various related research on the pathogenesis, cytokines have proven to have a certain role on the development of the disease. Clinical experiments have confirmed that cytokines such as TNF-α, IL-8 and IL-10 are involved in the pathogenesis of HSP (8,9). They are likely to be released by vascular endothelial cells, which trigger and promote the inflammatory response. These proinflammatory factors are stimulated and release chemokines, adsorbing inflammatory cells, inducing the expression of endothelial cell adhesion

Table II. Pearson's linear correlation analysis of HSPN and serum TNF-α, IL-8 and IL-10.

Group	r	Pearson	Sig
IL-8	0.61	0.017	0.001
IL-10	0.413	0.045	0.012
TNF-α	0.428	0.520	0.038

molecules, promoting the adhesion of endothelial cells to vascular walls (10,11).

IL-8 is a cytokine that appears in the acute phase of HSP, mainly infiltrated by peripheral leukocytes and polymorphonuclear cells, released to activate endothelial cells (12), which is also a protein peptide. Elevated levels of IL-8 in serum cause neutrophil chemotaxis and release associated proteases, causing systemic small vessel damage (13). The results of the present study show that serum IL-8 levels in patients with HSPN are significantly higher than those of NHSPN and normal healthy individuals, and IL-8 is correlated with HSPN urine protein content. Since the content of urine protein and the degree of renal function impairment were positively correlated, IL-8 is closely associated with the pathogenesis of HSP with nephritis. IL-10, as an immune regulator secreted by TH2 cells, mainly plays anti-inflammatory and immunosuppressive roles (14). Kimura *et al* have confirmed that serum levels of IL-10 in patients with HSP mainly exert an inhibitory effect on the antigen-presenting function of macrophages, and can effectively inhibit the production of other proinflammatory factors (15).

This study confirmed that serum IL-10 levels in HSPN patients were significantly higher than those in NHSPN and healthy individuals, and its content was positively correlated with the level of urine protein, which indicates the degree of renal function impairment, suggesting that IL-10 is involved in the pathogenesis of HSP, especially playing a greater role in the combination of renal dysfunction. TNF-α is an effective proinflammatory cytokine produced by a variety of cell types, including monocytes/macrophages, mesangial cells and renal epithelial cells. TNF-α can also cause changes in renal endothelial and mesangial cells (16). Previous findings have found that serum TNF-α levels are elevated in HSP patients, suggesting that cytokine TNF-α may be involved in vascular injury (17). Yang *et al* have shown that the activation of inflammatory cells in glomerular and renal interstitial cells can lead to local TNF-α production, leading to glomerular barrier damage and increased permeability (18).

The present findings have shown that serum TNF-α levels in patients with HSPN were significantly higher than the NHSPN and control group. TNF-α levels in the HSPN group serum was significantly higher than that in the NHSPN group. Urinary protein content also increased correspondingly, indicating that TNF-α and HSP renal function impairment is closely related (19,20). Urine protein is an indicator of renal dysfunction, and with the increase in urinary protein level, the degree of renal function damage also increases. Thus, the above three inflammatory immune factors are involved in kidney function impairment. Our results showed that the levels of TNF-α, IL-8 and IL-10 in the severe nephritis group (acute nephritis, chronic nephritis and nephrotic syndrome groups) were higher than those in the light nephritis group (i.e., simple proteinuria group) ($P<0.05$, $P<0.01$). The levels of TNF-α, IL-8 and IL-10 in the acute nephritis group were higher than those in the chronic nephritis and nephrotic syndrome groups.

In summary, the pathogenesis of HSP is complex, serum TNF-α, IL-8, and IL-10 in acute phase HSP patients was significantly higher than that in the healthy group. The TNF-α level in the HSPN group was significantly higher than that in the NHSPN group, indicating that TNF-α may cause a series of functions and morphological changes in glomerular cells. Therefore, serum TNF-α concentration can be used as an indicator of disease activity.

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Availability data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

LY and QW analyzed and interpreted the patients data. SZ wrote the manuscript. LZ revised the manuscript for important intellectual content. SZ and LZ contributed to the conception and design of the study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of The Affiliated Hospital of Jining Medical University (Shandong, China). Signed written informed consents were obtained from all the participants before the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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