

PROINFLAMMATORY CYTOKINES IL-8 AND TNF α IN HENOCH-SCHONLEIN PURPURA IN CHILDREN

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Abstract

Background. Henoch-Schonlein purpura (HSP) belongs to a group of systemic vasculitis with predominant damage to small caliber vessels (EULAR/Pres, 2006). Difficulties in diagnosis in the early stages and the likelihood of developing complications leaves HSP in the top of the current issues of pediatrics today. Kidney damage is observed in 20–50% of patients with HSP and leads to complications. In recent years, the question of prognostic markers of progression of HSP remains open. **Subjects&methods.** The study involved examination of total of 83 HSP patients aged between 2 and 17, divided into two groups: patients with HSP without nephrotic syndrome (HSPWN, n = 58.35 of which were boys and 23 – girls) and a group of patients with HSP with renal syndrome (HSPN, n = 25.14 of them boys and 11 girls) in acute and remission periods. **Results.** The Kraskal-Wallis analysis recorded a highly statistically significant H criterion for IL-8 in the acute period (H = 17.421, p = 0.0002) and the remission period (H = 13.035, p = 0.0015). IL-8 levels in both groups of patients with HSP WN and HSPN were significantly higher in the acute period than in the control group, and the difference was statistically significant (p = 0.0004 and p = 0.0002, respectively). No significant difference was found between the medians in both periods regarding TNF- α level (H = 4.136, p = 0.1264; H = 0.133, p = 0.9356). **Conclusions.** In the group HSPN in the acute phase, a high IL-8 level in serum has been recorded compared to the group HSPWN. There was no significant difference in TNF- α level in both groups.

Keywords: children, Henoch–Schonlein purpura, Ig-A vasculitis, nephritis.

Introduction

Henoch–Schonlein purpura (HSP) belongs to a group of systemic vasculitis with predominant damage to small caliber vessels (EULAR/Pres, 2006). Clinical presentation is characterized by the presence of hemorrhagic rash on the skin, which is combined with the joint damage and nephrotic syndromes. HSP develops more often in children and occurs between the ages of 4 and 8 years. Difficulties in diagnosis in the early stages and the likelihood of developing complications leaves HSP in the top of the current issues of pediatrics today. Kidney damage is observed in 20–50% of patients with HSP and leads to complications [1]. The progress to chronic renal

failure observed in 25–30% of patients who had nephritis due to HSP [2]. Long-term prognosis of HSP is determined by the severity of kidney damage, which varies significantly from patient to patient [3].

In recent years, the question of prognostic markers of progression of HSP remains open. In particular, the IL-10 levels were determined, IgA-IgG complexes [4], IL-6, serum amyloid A [5], antistreptolysin I, C-reactive protein, antibodies to anticardiolipin [6], (IL)-17, IL-18, IL-23 [7, 8]. Many studies have identified some changes to different indicators, but no specific marker has been found.

Increase in the level of tumor necrosis factor α (TNF α) in the acute phase of HSP induces a series of functional and morphological changes in the nephrons and can be used as a marker of activity in renal dysfunction [9].

TNF- α is a cytokine with multiple immune response functions. Many studies have found that TNF- α plays a major role in many systemic

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inflammatory diseases, including systemic vasculitis [10].

Recent studies have suggested that TNF α hyperproduction causes severe pathological reactions in the body. Further study of the biological activity of TNF α will allow the use of this predictor of the acute phase of inflammation in the complex diagnosis of early manifestations of complications in diseases [11].

Many cells can produce IL-8 under the influence of many factors, which include proinflammatory cytokines, such as TNF- α or IL-1 [12].

2. Purposes, subjects and methods:

2.1. Purpose was to study the levels of proinflammatory cytokines IL-8 and TNF α as specific markers of nephrotic syndrome in children with HSP.

2.2. Subjects & Methods

The research was conducted in Kharkiv City Clinical Children's Hospital No. 16 from 1 January 2015 to 1 November 2018. A total of 83 HSP patients aged between 2 and 17 were examined. Patients with HSP were divided into two groups: patients with HSP without nephrotic syndrome (HSPWN, $n = 58.35$ of which were boys and 23 – girls) and a group of patients with HSP with renal syndrome (HSPN, $n = 25.14$ of them boys and 11 girls). The control group consisted of 20 healthy children, underwent a planned medical examination or received vaccination in the Kharkiv City Clinical Children's Hospital No. 16.

The diagnosis of HSP has been established according to the criteria defined by the European League against Rheumatism and Pediatric Rheumatology European Society [13]. The criteria for inclusion in the study were the established diagnosis of HSP, the presence of informative consent on the part of patients and / or parents. Criteria for exclusion from the study were the refusal of patients and / or parents to take a part in the study; the presence in patients of hereditary diseases of the blood system and other acute or chronic inflammatory diseases.

The main methods of examination were assessment of presentation, past history and present history, examination findings, clinical, laboratory and instrumental data. Major syndromes were identified, such as skin and joint pain. Detection of hematuria (erythrocyte amount $> 5 / \text{mm}^3$) and / or proteinuria, nephritis during the first month of PSH was diagnosed as nephrotic syndrome. Standard tests included routine clinical blood and urine tests, acute inflammation proteins. Clinical examination was performed twice: upon

admission to the hospital (without nephrotic syndrome) and during remission (after disappearance of the skin syndrome). TNF α , IL-8 levels were studied upon the admission to hospital and remission period in patients with HSP and once in the control group. The proinflammatory markers of TNF α , IL-8 were determined by enzyme-linked immunosorbent assay using standard VECTOR BEST kits, Russia (A-8756, A-8762). All samples were frozen at $- 40^\circ$ until the time of the study.

Ethical aspects. All participants and / or their parents were informed about the goals, objectives and scope of the study and gave written informed consent. The study was approved by the Ethics Committee of Kharkiv National Medical University (Ethics Committee Protocol No. 8 as of 5.10.2016) and conducted in accordance with the recommendations of the Declaration of Helsinki (1975).

Statistical analysis. Statistical analysis was performed using StatSoft STATISTICA Version 8 (Tulsa, OK). Gauss's law on distribution was performed using the Shapiro-Wilk test. Nonparametric variables were represented as median (Me), interquartile range (Lq – lower quartile; Uq – upper quartile). A Kruskal-Wallis analysis of variance (ANOVA) was used to determine the statistically significant difference between the medians across all groups. A nonparametric Mann-Whitney test was used to compare the two independent variables; non-parametric Wilcoxon test was used to compare two dependent samples (T). All p-values were two-sided and values < 0.05 were considered statistically significant. The relationship between the series of indicators was assessed using Spearman's rank correlation methods (r).

Conflict of interests

The authors of the article declare no conflict of interest.

3. Results

The study involved examination of 83 children between the ages of 2 and 17. The distribution of children by gender among the total number of patients did not show a statistically significant difference: boys – 49 and girls – 34 (59.04%, 40.96% respectively, $\delta = 0.1105$). HSP in children was significantly diagnosed at the age of 12 years $86.6\% \pm 4.3\%$ ($p = 0.003$). An analysis of HSP clinical presentations showed that skin syndrome was recorded in 100% of cases and represented by symmetric palpatory purpura. In 83.13% (69/83) patients, skin syndrome was combined with articular syndrome with signs of arthritis and

arthralgia. Representation of the abdominal syndrome were distinguished by abdominal pain, nausea and vomiting and were recorded in 43.37% (36/83), and nephrotic syndrome appeared in 30.12% (25/83).

There was no significant difference between sex and age, in terms of leukocyte and platelet levels, fibrinogen (*Table 1*). There was a significant decrease in ESR (erythrocyte sedimentation rate) in group HSPN compared to group HSPWN ($p = 0.009$).

was significantly higher in patients with HSPN compared to the HSP WN and control group ($p = 0.0327$ and $p = 0.0005$, respectively), and higher IL-8 level in the HSPN group compared to the group of patients with HSPWN ($p = 0.0375$). The Wilcoxon test showed that IL-8 levels were significantly higher in groups during both the acute and remission periods ($T = 0.00$; $p = 0.00006$ and $T = 16.0$, $p = 0.00247$).

On the contrary, no significant difference was found between the medians in both periods

Table 1

Clinical and laboratory data of children with HSP in the acute period

| Indicator | HSPWN, n=58 | HSPN, n= 25 | p |
|-----------------------------|-------------------|---------------------|-------|
| Age, years | 6.9 (3.2;15.5) | 7.1 (3.8;9.8) | 0.43 |
| Boys | 35 (60.34 %) | 14 (56 %) | 0.714 |
| Girls | 23 (39.66%) | 11(44%) | 0.713 |
| Leukocytes, $\times 10^9/L$ | 6.5 (5.3;9.6) | 6.3 (5.0;10.3) | 0.813 |
| ESR, mm / h | 15.0 (9.0;20.0) | 9.0 (5.0;15.0) | 0.009 |
| Fibrinogen, mg/dL | 3.5 (2.6; 4.0) | 3.9 (2.4;4.4) | 0.127 |
| Platelets, $\times 10^9/L$ | 211 (187.0;253.5) | 194.5 (161.0;280.0) | 0.547 |

The Kraskal–Wallis analysis determined a highly statistically significant H criterion for IL-8 (*Table 2*) in the acute period ($H = 17.421$, $p = 0.0002$) and the remission period ($H = 13.035$, $p = 0.0015$), which indicated the difference between the medians in all groups.

IL-8 levels in both groups of patients with HSP WN and HSPN were significantly higher in the acute period than in the control group, and the difference was statistically significant ($p = 0.0004$ and $p = 0.0002$, respectively), but no significant difference was observed between groups of patients with HSPWN and HSPN ($p = 0.1087$). During remission period, IL-8 level

regarding TNF- α level ($H = 4.136$, $p = 0.1264$; $H = 0.133$, $p = 0.9356$). TNF- α level tended to increase in the acute period in patients with HSPN compared to the patients with HSP WN and control group, but was not statistically significant ($p > 0.05$). The level of TNF α in both HSP periods in all groups did not change significantly. During the remission period, TNF- α level was close to correspondent level of the control group and was statistically unreliable ($p > 0.05$). The Wilcoxon test showed that TNF- α levels were significantly higher in groups both in acute and remission periods ($T = 0.00$, $p = 0.007$ and $T = 0.00$ $p = 0.0002$).

Table 2

*TNF α , IL-8 * levels in the acute and remission period in children with HSP WN and HSPN and the control group*

| Indicators' levels | | HSPWN n=22 | HSPN n= 18 | Control group n= 20 | P |
|------------------------|----|------------|------------|---------------------|--|
| Acute period | | | | | |
| IL-8, (pg/ml) | Me | 19.5 | 27.8 | 8.8 | $p_{HSP\ WN-HSPN} = 0.1087$ $p_{HSP\ WN-c} = 0.0004$ $p_{HSPN-c} = 0.0002$ |
| | Lq | 9.6 | 14.5 | 6.2 | |
| | Uq | 36.4 | 77.5 | 9.1 | |
| TNF α , (pg/ml) | Me | 6.9 | 7.5 | 4.9 | $p_{HSP\ WN-HSPN} = 0.5497$ $p_{HSP\ WN-c} = 0.0574$ $p_{HSPN-c} = 0.0849$ |
| | Lq | 5.4 | 4.6 | 3.6 | |
| | Uq | 9.1 | 13.3 | 6.3 | |
| Remission period | | | | | |
| IL-8, (pg/ml) | Me | 11.4 | 24.1 | 8.8 | $p_{HSP\ WN-HSPN} = 0.0375$ $p_{HSP\ WN-c} = 0.0327$ $p_{HSPN-c} = 0.0006$ |
| | Lq | 6.8 | 10.8 | 6.2 | |
| | Uq | 23.9 | 56.4 | 9.1 | |
| TNF α , (pg/ml) | Me | 4.8 | 4.4 | 4.9 | $p_{HSP\ WN-HSPN} = 0.7034$ $p_{HSP\ WN-c} = 0.8879$ $p_{HSPN-c} = 0.8676$ |
| | Lq | 3.4 | 3.7 | 3.6 | |
| | Uq | 6.7 | 10.3 | 6.3 | |

* TNF α , IL-8 was studied in HSPWN group (22 patients), HSPN group (18 patients).

There was a direct correlation between IL-8 and TNF- α in patients with HSPN during acute period ($r = 0.527$, $p \leq 0.05$) and remission period ($r = 0.658$, $p = 0.05$), $p \leq 0.05$, respectively. A direct correlation between IL-8 and TNF- α was observed in the acute phase of HSP ($r = 0.396$, $p \leq 0.05$).

4. Discussion

The study showed that non-invasive biomarkers could be used to safely diagnose nephrotic syndrome in children with HSP. Many studies have found that HSP is not a self-curable disease and can transform in chronic kidney disease (CKD) in childhood. [14]. The pathogenic mechanism underlying HSP is still not fully understood, so it is important to find specific markers for the development of kidney complications in HSP patients, because many researchers consider HSP as one of the serious and common causes of kidney damage in children [15].

With regard to pro-inflammatory cytokines, it has recently been found that serum IL-8 levels are significantly increased in cases of kidney damage [6] and our study confirmed these results. Our patients were found to have an increased level of IL-8 in serum, not only in the HSP group with nephritis, but also without kidney damage in comparison with the control group, the same data was obtained by French researchers. IL-8 concentrations were higher in patients with HSP WN and HSPN than in the control group [4]. Based on our results, IL-8 level may be useful as a marker for monitoring the progression of nephrotic syndrome in children with HSP.

We have not determined a significant difference between the median levels of TNF- α

in both groups of patients in the acute and remission periods compared to the control group, which has been also confirmed in studies by our colleagues. A slight increase in TNF- α level during the acute period in the nephrotic syndrome group compared to the group without nephrotic syndrome indicates that increased TNF- α level in serum causes a number of functional and morphological changes in the glomerular cells in the acute phase and cannot be used as a marker to monitor the activity of HSP disease with severe kidney damage, which is in contrast to the studies of our colleagues. [16]. Thus, it can be stated that in addition to TNF- α , in serum of active HSP there may be other major factors that can activate endothelial cells to produce IL-8 [17].

The activation of inflammation leads to the activation of the coagulation system, which also markedly affects inflammatory activity. This is considered important in the pathogenesis of vascular diseases [18, 19].

Conclusions

1. In the group with nephrotic syndrome in the acute phase, a high IL-8 level in serum was detected as compared to the group without nephrotic syndrome and the control group.

2. There was no significant difference in TNF- α level in both groups in the acute phase as compared to the control group.

3. In the follow-up, the IL-8 level was decreasing in both groups of the study, but still remained high in the group with nephrotic syndrome in remission period, which can indicate accurate prognostic effectiveness in the detection of patients with HSP with kidney damage at the time of diagnosis.

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