Pediatric Dermatology Vol. 30 No. 6 695–699, 2013

Levels of Interleukin-18 and Endothelin-1 in Children with Henoch-Schönlein Purpura: A Study from Northern India

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Abstract: **Henoch-Schönlein purpura (HSP) is an acute systemic vasculitis with unknown etiology, although several studies have found HSP to be related to cytokines such as tumor necrosis factor α, interleukin (IL)-1, and adhesion molecules. In the present study we determined the levels of cytokines such as IL-18 and endothelin-1 (ET-1) in children with HSP. Subjects were divided into three groups (group 1, 20 subjects with HSP; group 2, 10 subjects belonging to group 1 during their follow-up 4 to 6 months later; and group 3, 16 controls who were healthy siblings of the subjects). IL-18 and ET-1 levels were determined using enzyme immunoassay and expressed as mean ± standard deviation. We observed higher IL-18 levels in children with HSP (767.6 ± 145.1 pg/mL) than in controls (614.6 ± 66.54 pg/mL, $p > 0.05$), but IL-18 levels were found to be significantly lower in subjects with HSP in remission (502.7 ± 60.81 pg/mL) than in those who were in an active phase (1,050 ± 244.5 pg/mL, $p < 0.05$, $n = 10$). ET-1 levels were found to be significantly higher in subjects with HSP (1.93 ± 0.19 pg/mL) than in controls (1.10 ± 0.13 pg/mL, $p < 0.05$), although no significant difference was observed in ET-1 levels between subjects in group 1 (1.88 ± 0.30 pg/mL) and group 2 (1.91 ± 0.120, $p > 0.05$, $n = 10$). A positive correlation was observed between IL-18 and ET-1 levels in subjects with HSP (correlation coefficient [r] = 0.5254, $p < 0.01$). These results suggest that levels of IL-18 and ET-1 are worth monitoring during the clinical course of the disease, but caution must be exercised in extrapolating data based on small study samples.**

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DOI: 10.1111/pde.12222

Henoch-Schönlein purpura (HSP) is an acute, self-limited, systemic, small-vessel vasculitis of childhood with a reported incidence of 10 to 20 cases per 100,000 children per year. The etiology of HSP remains unknown. Several environmental and genetic influences may have a role in the pathogenesis of this condition and these influences may vary between geographical regions (1–4). Only a few studies from India have reported cases of HSP. Earlier we reported 45 cases of HSP over 4 years, which indicated that the condition is perhaps as common in India as it is in the West (5). HSP is generally benign and self-limited in children, whereas it is more severe in adults, and the frequency of severe renal manifestations or renal insufficiency during the course of the disease has been found to be significantly higher in adults than in children (6).

Interleukin (IL)-18, a member of the IL-1 superfamily, has several biological activities that initiate and promote host defense and inflammation after infection or injury (7). Cells known to express IL-18 include macrophages, Kupffer cells, keratinocytes, and glucocorticoid-secreting adrenal cortex cells. IL-18 is biologically and structurally related to IL-1β (7). There have been several reports relating the upregulation of IL-18 in human inflammatory and autoimmune diseases, including rheumatoid arthritis, type I diabetes, atherosclerosis, chronic heart failure, and idiopathic thrombocytopenic purpura (8–10). An association between the IL-1β gene and IL-1 receptor antagonist gene polymorphisms and severe renal involvement in patients with HSP has been reported (11,12). A potential role of the IL-18 gene polymorphism has also been reported. An association between the *IL18* gene promoter −607A/C polymorphism and HSP has also been reported, although the results were not conclusive (13).

Endothelin (ET)-1 is a potent vasoconstrictor, proinflammatory, and proliferative endothelial cell-derived peptide, which is important in the regulation of vascular function. Its main site of vascular production is the endothelial cell, but other cell types, including vascular smooth muscle cells and epicardial cells, also produce it (14,15).

Therefore, keeping in mind the lack of any conclusive evidence for or against various proposed hypotheses for the pathogenesis of this disease and scanty data from India on subjects with HSP, we decided to investigate the role of proinflammatory cytokine IL-18 and a potent vasoconstrictor and proinflammatory molecule such as ET-1 in children with HSP in a small study cohort in the context of a clinical setting in northern India.

MATERIALS AND METHODS

Subjects diagnosed with HSP in the Pediatric Rheumatology and Immunology Clinic of the Postgraduate Institute of Medical Research and Education (Chandigarh, India) were enrolled as described previously (2). The present study included three groups (group 1, 20 subjects with HSP; group 2, 10 subjects belonging to group 1 in follow-up 4 to 6 months later; and group 3, 16 controls who were healthy siblings of the subjects who were nearest in age). The institute's Ethics Committee approved the study protocol. After obtaining informed consent from the parents of the children, venous blood was collected after an overnight fast at the time of enrollment during the acute phase of the illness and subsequently during remission at the time of follow-up visits after 4 to 6 months. Enzyme-linked immunosorbent assay kits were used to determine serum levels of IL-18 (MBL, Japan, catalog no. 7620) and ET-1 (R&D System, Minneapolis, MN, catalog no. DET100). The results were given as mean ± standard deviation (SD). Unpaired and paired *t* tests were used to compare the difference between the two groups as and when required. The correlation was evaluated using Pearson's correlation. Prism statistical software (GraphPad Software, La Jolla, CA) was used to analyze the results.

RESULTS

The median age of the children was 5 years (range 3–12 years) and the male:female ratio was 1.5:1. Three of 20 children with HSP (15%) had antistreptolysin O titers greater than 200, and 2 (20%) were positive for parvovirus B19 immunoglobulin M (IgM) serology. Skin biopsies were performed in 17 of 20 patients in the acute stage from the site of the lesion. Histopathology showed leukocytoclastic vasculitis in 11 of 17 patients (65%). Immunofluorescence showed IgA 2+ to 3+ in 15 patients (88%), IgM 1+ to 2+ in 11 patients (65%), and IgG and C3 were found in 3 patients (18%). A typical HSP rash was reported in 19 (95%) patients. Two subjects with HSP (10%) developed arthritis before appearance of the rash. Arthritis preceded the onset of purpura in 2 patients (10%) and arthritis was present in 15 patients (75%). Twelve (60%) patients had abdominal pain, but none had any gastrointestinal complication. The majority of patients recovered within 6 to 8 weeks, although one had continuous symptoms for 3 months. Scrotal swelling was observed in one case of HSP and subsided gradually without treatment.

We did not observe any significant difference in IL-18 levels between subjects with HSP and controls, although IL-18 levels were higher in subjects with HSP (767.6 ± 145.1 vs 614.6 ± 66.54 pg/mL, $p > 0.05$; Fig. 1A). IL-18 levels were found to be significantly lower in subjects with HSP in remission than in those in the active phase (502.7 ± 60.81 vs 1,050 ± 244.5 pg/mL, $p < 0.05$, $n = 10$; Fig. 1B). We observed significantly higher levels of ET-1 in subjects with HSP (1.93 ± 0.19 pg/mL) than in controls (1.10 ± 0.13 pg/mL; Fig. 1C). In contrast to IL-18, no statistically significant difference was observed in ET-1 levels between subjects with HSP in an active phase (1.88 ± 0.30 pg/mL) and those in a remission phase (1.91 ± 0.20 pg/mL, $p > 0.05$, $n = 10$). Individual ET-1 levels in study subjects are depicted in Fig. 1D.

Correlation analysis was performed to determine whether IL-18 and ET-1 are associated with each other. We observed a positive and significant association between IL-18 and ET-1 levels in subjects with HSP ($r = 0.525$, $p < 0.01$; Fig. 1E).

DISCUSSION

HSP is defined as an immune-mediated vasculitis that is the result of an immune complex–mediated reaction to various antigenic stimuli in a susceptible person,

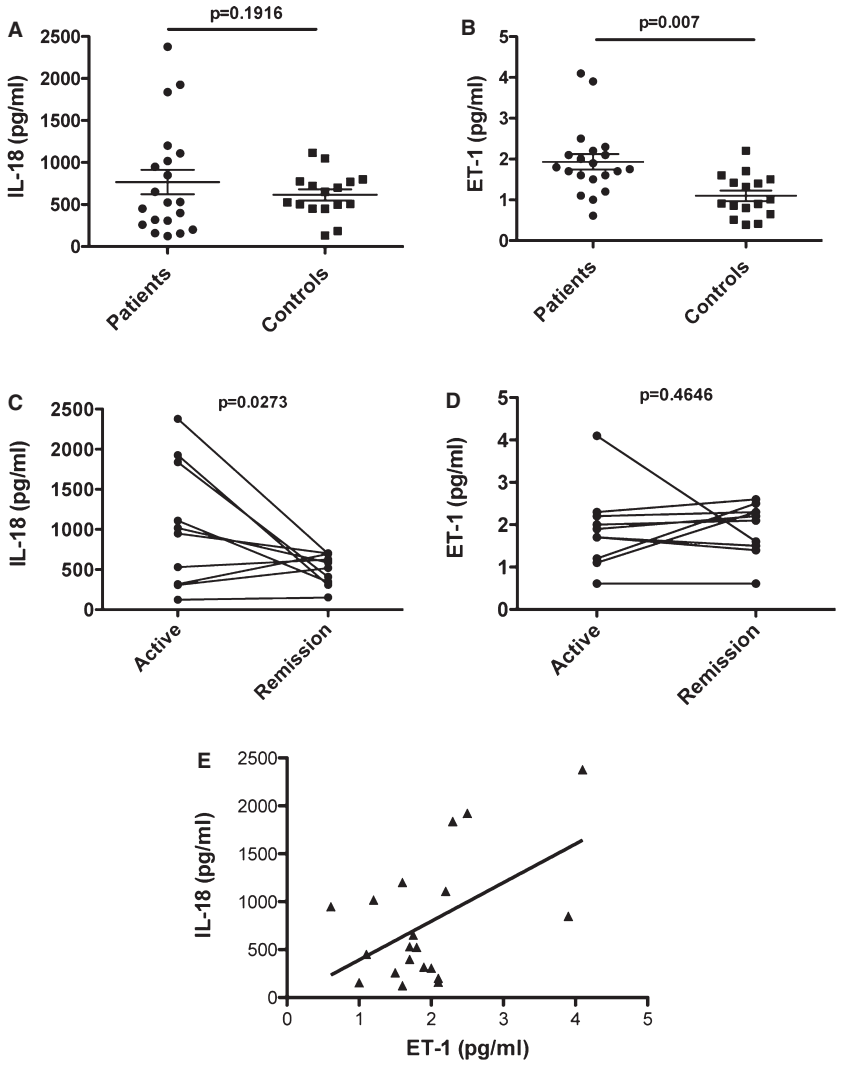

Figure 1. (**A, B**) Scatter plots for circulating IL-18 and ET-1 levels in subjects with HSP ($n = 20$) in the acute phase and controls ($n = 16$). (**C, D**) IL-18 and ET-1 levels in patients ($n = 10$) with HSP studied during the active and convalescent phases. (**E**) Correlation between IL-18 and ET-1 levels in subjects with HSP who were in the active phase.

but recent evidence suggests the involvement of certain cytokines, such as tumor necrosis factor α, IL-1, and adhesion molecules, which may cause vascular inflammation, and specific antigens and causes remain to be determined (1,3,4).

IL-18, a member of IL-1β family, is primarily a proinflammatory cytokine because of its ability to stimulate the expression of genes associated with inflammation and autoimmune diseases (9,10). Our results demonstrate the absence of a significant difference in circulating levels of IL-18 between subjects with HSP and healthy controls. Wang et al (16) reported higher IL-18 and IL-18 binding protein (IL-18BP) levels in subjects with HSP than in controls (16) and did not observe any significant difference in IL-18 levels between patients with active disease and those in remission. In our study we observed a significant decline in IL-18 levels with remission. This anomaly can be explained to some extent by the fact that Wang et al (16) did not compare individual values for each subject but reported their results as mean levels in a group of 19 subjects in remission versus 30 subjects who were in an active phase.

In a Turkish population, Muslu et al (17) observed higher ET-1 levels in subjects with HSP during the acute phase than when they were in the remission phase or in controls. Moreover, ET-1 levels did not correlate with clinical and laboratory findings except in a minority of patients with severe disease. Fessatou et al (18) reported the lack of a significant difference in plasma and urine ET-1 levels in HSP subjects and controls in a Greek population. Our results are in agreement with Muslu et al (17), as we also observed significantly higher ET-1 levels in subjects with HSP in an active phase than in controls. The expression of ET-1 and its receptor has been shown to be markedly altered during disease progression, highlighting the importance of the ET-1 system in HSP (15).

Several reports support a role of ET-1 in the formation of various reactive species that cause endothelial dysfunction (19). Considering this together with its proinflammatory effects, it could be an important mechanism in the development of HSP. Our group previously reported significantly higher serum and urine reactive nitrogen intermediate and citrulline levels in the active phase of HSP than in controls (20).

Studies determining IL-18 and ET-1 levels in subjects with HSP have been previously documented, but measuring these two molecules in the same study subjects makes our study unique. Tumor necrosis factor α, another inflammatory cytokine, has been shown to increase the production of ET-1 in cultured bovine endothelial cells (21). We speculate that inflammatory molecules such as IL-18 could be one of the cytokines that may augment ET-1 in the acute phase. We observed that IL-18 levels were higher in children with HSP than in controls, although this difference did not reach statistical significance. We also observed a positive correlation between IL-18 and ET-1 in subjects with HSP who were in the active phase (Fig. 1E). A positive correlation between these two inflammatory molecules points toward the possibility of their involvement in the pathogenesis of this disease, but a small study cohort is the main limitation of the study, and the data need to be validated in a larger number of subjects.

Also, we need to emphasize that our finding of an association may be viewed as a hypothesis-generating observation rather than taken as a definitive conclusion in itself. Caution must be exercised in extrapolating data based on small study samples, although vasculitic disorders in children are uncommon, and in a single-center-based study like ours, this limitation must be borne in mind. We would like to emphasize that HSP has been reported all over the world, with wide variation in its prevalence in different geographical regions and an absence of conclusive etiology. Comparing and thoroughly analyzing the results from various studies reported worldwide is needed to provide a direction for future research on HSP.

ACKNOWLEDGMENTS

We thank the Post Graduate Institute of Medical Education and Research for providing financial support.

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