ORIGINAL ARTICLE

Genetic Determinants of Homocysteine and Proinflammatory Cytokines in Henoch–Schönlein purpura: A Study on the Role of MTHFR and MTRR Polymorphisms

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Abstract

Introduction: Henoch–Schönlein purpura (HSP) remains a disease with an unclear etiology in many patients, which makes prompt diagnosis, treatment, and prevention challenging. During the acute phase of HSP, patients exhibit increased levels of vascular endothelial growth factor, tumor necrosis factor-alpha (TNF-α), and interleukin (IL)-6. This study aimed to assess the levels of proinflammatory cytokines in patients with HSP who had genetically determined hyperhomocysteinemia (HHcy) and received standard basic treatment with folic acid. **Materials and Methods:** This study included 145 patients with HSP treated at the Department of Hematology of the National Center of Oncology and Hematology, Kyrgyz Republic. Diagnosis was based on clinical and laboratory tests, including complete blood count, urine test, biochemical blood test, hemostasiogram, cytokine and homocysteine level studies, and methylenetetrahydrofolate reductase and methionine synthase reductase gene studies using real-time polymerase chain reaction. Treatment included bed rest, a hypoallergenic diet, anticoagulants, antiplatelet agents, fibrinolysis activators, and steroid hormone drugs at medium doses. **Results:** The blood serum of patients with cutaneous, articular, abdominal, and renal syndromes of HSP showed a significant increase in IL-6 and TNF-α levels compared with the control group and patients with cutaneous and articular syndromes of HSP ($P < 0.001$ and $P < 0.05$, respectively). However, the IL-1β levels were within the normal range. In patients with a generalized form of HSP, treatment resulted in 10% and 18.3% decreases in the concentrations of IL-6 and TNF-α, respectively ($P < 0.001$). However, the IL-6 level remained above normal values. **Conclusion:** Proinflammatory cytokines, such as IL-6, TNF-α, and IL-1β, are important in HSP pathogenesis and genetically determined HHcy may serve as a predisposing factor for this condition.

Key words: Henoch–Schönlein purpura, hyperhomocysteinemia, methionine synthase reductase, methylenetetrahydrofolate reductase, proinflammatory cytokines

INTRODUCTION

Henoch–Schönlein purpura (HSP) is a form of hypersensitivity systemic vasculitis that primarily affects small-caliber arteries, such as arterioles, capillaries, and venules.[1-5] HSP can affect anyone, including elderly people,[6] but it is most common in children between the ages of 2 and 6 years.[7,8] It occurs about twice as often in

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boys as girls.[9] The incidence of HSP in children is about 20/100,000 children per year, making it the most common form of vasculitis in children.[8]

HSP is an immunocomplex characterized by aseptic inflammation in the microvessels. This inflammation leads to vessel wall destruction, thrombosis, and the appearance of purpura in various body parts. These effects are caused by circulating immune complexes and activated components of the complement system.[10,11] Although the precise mechanisms responsible for HSP are not entirely understood, alterations in the immune complex, delayed-type hypersensitivity, autoimmunity, and para-allergic processes play significant roles in disease development. Vascular damage caused by immune complexes in HSP can result from various factors such as bacterial and viral infections, drug usage, and exposure to cold factors. However, the etiology of HSP remains ambiguous in several patients, impeding prompt diagnosis, treatment, and prevention of the disease.

Patients with HSP have elevated levels of vascular endothelial growth factor (VEGF), tumor necrosis factor-alpha (TNF-α), and interleukin (IL)-6 during the acute phase of the disease.[12] Various triggers including IL-1 and IL-6 can induce elevated VEGF levels. Dysregulation of vascular tone in HSP is linked to increased endothelial peptide and vasoconstrictor synthesis in response to increased TNF-α concentrations.

Approximately 20% of thrombophilia cases are associated with elevated plasma homocysteine (Hcy) levels primarily caused by hyperhomocysteinemia (HHcy), which leads to endothelial dysfunction. This dysfunction causes increased collagen production in the blood vessel walls, decreased flexibility, and reduced dilation capacity.[13] HHcy concentrations indicate a disturbance in Hcy metabolism and are linked to an increased risk of cardiovascular, cerebrovascular, and thromboembolic disorders.[14,15]

Elevated levels of proinflammatory cytokines in the brain, such as TNF-α, IL-1β, IL-6, and chemokine monocyte chemotactic protein-1, have been observed in Wistar rats with mild HHcy.[16] There is a link between Hcy levels, fibrinogen, von Willebrand factor, and D-dimer, suggesting activation of intravascular coagulation.[17]

The most important enzyme, methylenetetrahydrofolate reductase (MTHFR) regulates the conversion of Hcy to methionine, and the enzyme methionine synthase (MTR) catalyzes the remethylation of Hcy to methionine and tetrahydrofolate.[18] Methionine synthase reductase (MTRR) catalyzes the regeneration of methylcobalamin, a cofactor of MTR. Thus, MTR activity is maintained by MTRR.

The two most common genetic variations in MTHFR and MTRR are the C677T and A66G polymorphisms, respectively. These variations have been shown to reduce the activity of enzymes produced by these genes.[19] HHcy can be caused by various factors such as deficiencies in folic acid, Vitamins B6 and B12, excess methionine in food, diseases such as diabetes mellitus, thyroid diseases, psoriasis, kidney pathology, smoking, excessive consumption of caffeine and alcohol, and a sedentary lifestyle.

This study aimed to measure the levels of proinflammatory cytokines in patients with HSP who had genetically determined HHcy and received routine basic treatment with folic acid.

MATERIALS AND METHODS

This cross-sectional study included 145 individuals diagnosed with HSP who received treatment at the hematology department of the National Center of Oncology and Hematology under the Ministry of Health in the Kyrgyz Republic. The largest proportion of patients (25.5%) was between the ages of 20 and 29 years. Only 10.3% of the patients, mostly those aged 70 years or above, received inpatient care.

Skin capillary damage was evident in all the individuals. The articular form, characterized by microthrombosis in significant load-bearing joints, was observed in 84.8% of the patients. In addition, 15.8% of patients experienced damage to the microvessels of the gastrointestinal tract and hemorrhage, while 8.2% of patients exhibited parallel intravascular thrombus formation in the glomeruli of the kidneys.

All the patients underwent thorough clinical and laboratory examinations. The diagnosis was based on clinical and laboratory findings. Standard laboratory methods were used to assess the patients' general blood count, platelet count, general urine test, biochemical blood tests (including bilirubin and its fractions, alanine aminotransferase, aspartate transaminase, total protein, sugar, urea, creatinine, and lactate dehydrogenase), viral Hepatitis B and C markers, HIV, and general hemostasiogram (platelet aggregation, prothrombin time, prothrombin index, international normalized ratio, thrombin time, APTT, fibrinogen, and RFMC). In addition, special studies were conducted using the following methods:

1. Cytokine analysis using enzyme-linked immunosorbent assay kits (Quantikine® ELISA Kits, R&D Systems, United States),
2. Hcy assessment using enzyme-linked immunosorbent assay kit (Hcy Enzyme Immunoassay Kit, Bio-Rad Lab, United States),
3. MTHFR and MTRR gene analyses were performed using real-time polymerase chain reaction.

The treatment plan for patients with HSP at the department of our hospital involved a combination of bed rest and semi-bed rest, along with a hypoallergenic diet, if necessary, to avoid damaging the capillaries in the gastrointestinal tract. The patients also underwent complete starvation and intestinal

decontamination using antibacterial drugs that were not absorbed by the body. Basic therapy included the use of anticoagulants, antiplatelet agents, fibrinolysis activators, prostacyclin, and steroid hormonal drugs at moderate doses with the support of anticoagulants, antiplatelet agents, and plasmapheresis sessions. The treatment plan was designed for patients with II and III degrees of autoimmune and immune complex process activity.

Statistical analysis of the study data was performed using Statistica v8.0 (StatSoft Inc., Tulsa, USA). Data are presented as *n* (%) or the mean ± standard deviation. The Student's *t*-test was used to assess any intergroup differences in characteristics that followed a continuous distribution. Statistical significance was calculated as follows: Values of different groups of patients or $P < 0.05$ (*), patients compared to controls or $P < 0.01$ (**), group of patients at different periods of treatment, or $P < 0.001$ (***). The study was conducted with the full consent of the patients' parents and was approved by the Bioethics Committee of the I.K. Akhunbaev Kyrgyz State Medical Academy (Protocol No. 32, dated April 12, 2022).

RESULTS

Patients were divided into two groups based on the localization of microvascular lesions: Cutaneous and joint forms of HSP ($n = 46$) and generalized form of HSP ($n = 35$). The concentration of cytokines in the blood serum of patients with cutaneous, articular, abdominal, and renal syndromes of HSP showed a significant increase in IL-6 and TNF-α, compared with the control group and patients with cutaneous and articular syndromes of HSP ($P < 0.001$ and $P < 0.05$, respectively).

The levels of IL-1β in the blood serum of patients in both groups were within the normal range. In patients with a generalized form of HSP, treatment resulted in a decrease in the concentrations of IL-6 and TNF-α by 10% and 18.3%, respectively ($P < 0.001$); however, the level of IL-6 remained above normal values [Table 1].

A total of 64 patients with HSP were evaluated for Hcy metabolism, focusing on MTHFR (C677T polymorphism) and MTRR (A66G polymorphism) genes. Among these patients, 71.9% had HHcy. In addition to the standard therapy, patients with HHcy received folic acid supplementation at a dose of 5 mg/day.

The MTHFR C677T anomaly was detected in 27 (42.1%) patients with HSP, which involves the replacement of the cytosine (C) base with thymine (T) at position 677. This substitution results in changes to the biochemical properties of an enzyme, leading to the replacement of the amino acid alanine with valine at the folate-binding site.

A study of the MTRR gene, which encodes the MTRR responsible for the reverse conversion of Hcy to methionine, revealed the presence of the genetic abnormality MTRR A66G in 35 patients (64.9% of whom were heterozygotes and 44.9% were homozygotes) with HSP [Table 2]. The A66G genetic marker, which involves the replacement of adenine (A) with guanine (G) at position 66, leads to changes in the biochemical properties of the enzyme, with the amino acid isoleucine replaced by methionine. As a result, 65.6% of patients with HSP ($n = 42$) had HHcy in their blood.

DISCUSSION

In patients with HSP, proinflammatory cytokines, such as IL-1β, IL-4, IL-6, IL-8, IL-12p70, IL-17A, TNF-α, and IFN-γ, are elevated. The role of TNF-α is complex and not straightforward; conflicting results have been reported, particularly when TNF-α inhibitors are used to treat HSP. TNF plays a role in cytokine production triggered by HSP.[20]

Patients with IgA nephropathy show higher levels of TNF-α, IL-1β, and IL-6 in their serum compared to healthy individuals, which suggests that cytokines play a role in the vascular damage observed under these conditions.[21] Activation of the NF-κB, ERK1/2, and MEK/REK pathways by IgA-containing complexes leads to neutrophil infiltration and downstream signaling in HSP.[22,23] Chemokines produced by endothelial cells in response to cytokines trigger endothelial cells glycocalyx shedding and increase the expression of cell adhesion molecules, which enhances cell attachment to the vascular wall.

Table 1: Concentration of proinflammatory cytokines in patients with HSP during therapy

	Groups	IL-1ß, pg/mL	IL-6, pg/mL	TNF-α, pg/mL
1.	Control (*n*=15)	5.8±0.032	7.2±0.103	4.6±0.091
2.	Skin-articular form (*n*=46, before treatment)	7.2±0.004*	8.7±0.006***	5.3±0.094*
3.	Skin-articular form (*n*=46, after treatment)	6.7±0.058	8.1±0.331	4.7±0.005
4.	Generalized form (*n*=35, before treatment)	9.8±0.002**	11.9±0.008**	7.1±0.074**
5.	Generalized form (*n*=35, after treatment)	8.1±0.094	10.7±0.001***	5.8±0.034***

Values are presented as the mean±standard deviation. *P<0.05, **P<0.01, ***P<0.001. HSP: Henoch–Schönlein purpura, IL-1ß: Interleukin-1ß, IL-6: Interleukin-6, TNF-α: Tumor necrosis factor alpha

Table 2: Indicators of genetic abnormalities in patients with HSP

	Genetic polymorphism	Group of patients with normal homocysteine in the blood (*n*=18) (%)	Group of patients with HHcy in the blood (*n*=46) (%)
1.	MTHFR C677T homozygous	1 (5.5)	7 (15.2)*
2.	MTHFR C677T heterozygote	5 (27.7)*	14 (30.4)*
3.	MTRR A66G homozygous	3 (16.6)*	13 (28.3)*
4.	MTRR A66G heterozygote	7 (38.8)*	12 (26.1)*

Values are presented as the *n* (%). *P<0.05. HSP: Henoch–Schönlein purpura, MTHFR: Methylenetetrahydrofolate reductase, MTRR: Methionine synthase reductase

TNF-α specifically induces ROS generation in vascular endothelial cells, stimulates transient ICAM-1 expression, and activates the ERK1/2 and p38 MAPK signaling pathways, resulting in endothelial cell death.[24] Conversely, TNF-α increases the attachment of the IgA AECA to ECs, stimulates IL-8 production, and finally to vascular injury. The balance between anticoagulation and procoagulation of ECs is influenced by the presence of an IF factor, which is released in response to TNF-α to activate ECs, leading to thrombin generation and fibrin deposition.[25]

Specifically, the C677T polymorphism is located in exon 4 of MTHFR and has been associated with decreased folate availability for methionine synthesis.[26,27] Similarly, the A66G polymorphism in MTRR leads to a reduced rate of methionine synthesis and increased Hcy levels in the plasma.[28,29]

Specific genetic variations in MTHFR have been linked to an increased risk of cancer. The C677T variant is associated with a higher risk of breast cancer in Caucasians; however, there is no substantial link between the C677T variant and ovarian cancer in Caucasian populations.[30] Molecular investigation of the MTRR gene revealed the presence of the A66G polymorphism, which was found to be significantly associated with lung cancer in a population of Turkish individuals and a significant risk factor for colorectal cancer in a cohort of Japanese individuals.[31,32]

CONCLUSION

Patients with HSP have elevated levels of IL-6 and TNF-α, confirming the complex immunological etiology of the disease, 71.9% of these patients had HHcy. Among patients with HSP, 42.1% had the MTHFR C677T mutation and 61.9% had the MTRR A66G abnormality, causing HHcy. Therapy led to 10% and 18.3% decreases in IL-6 and α-TNF levels, respectively. Folic acid supplements were administered to the patients with HHcy in the HSP group.

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AUTHOR CONTRIBUTIONS

Conception, design of the work, manuscript preparation, and data acquisition: Ormonbek Dzhakypbaev, Oskon Salibaev, Klara Kuttubaeva, Rysbek Sadyjev, Otkurbek Tursunaliev, Kalbubu Arzymatova, Yethindra Vityala. Clinical management: Ormonbek Dzhakypbaev, Oskon Salibaev, Klara Kuttubaeva, Rysbek Sadyjev, Otkurbek Tursunaliev, Kalbubu Arzymatova.

REFERENCES

1. Blanco R, Martínez-Taboada VM, Rodríguez-Valverde V, García-Fuentes M, González-Gay MA. Henoch-Schönlein purpura in adulthood and childhood: Two different expressions of the same syndrome. Arthritis Rheum 1997;40:859-64.
2. Kang Y, Park JS, Ha YJ, Kang MI, Park HJ, Lee SW, *et al*. Differences in clinical manifestations and outcomes between adult and child patients with Henoch-Schönlein purpura. J Korean Med Sci 2014;29:198-203.
3. Liu Z, Wei YD, Hou Y, Xu Y, Li XJ, Du YJ. Differences in pathological characteristics and laboratory indicators in adult and pediatric patients with Henoch-Schönlein purpura nephritis. J Huazhong Univ Sci Technolog Med Sci 2016;36:659-66.
4. Saulsbury FT. Henoch-Schönlein purpura in children. Report of 100 patients and review of the literature. Medicine (Baltimore) 1999;78:395-409.
5. Pina T, Blanco R, González-Gay MA. Cutaneous vasculitis: A rheumatologist perspective. Curr Allergy Asthma Rep 2013;13:545-54.
6. Min Z, Garcia RR, Murillo M, Uchin JM, Bhanot N. Vancomycin-associated Henoch-Schönlein purpura. J Infect Chemother 2017;23:180-4.
7. Stone HK, Mitsnefes M, Dickinson K, Burrows EK, Razzaghi H, Luna IY, *et al*. Clinical course and management of children with IgA vasculitis with nephritis. Pediatr Nephrol 2023;38:3721-33.
8. Gardner-Medwin JM, Dolezalova P, Cummins C, Southwood TR. Incidence of Henoch-Schönlein purpura, Kawasaki disease, and rare vasculitides in children of different ethnic origins. Lancet 2002;360:1197-202.
9. Piram M, Mahr A. Epidemiology of immunoglobulin

A vasculitis (Henoch-Schönlein): current state of knowledge. Curr Opin Rheumatol 2013;25:171-8.
10. Gedalia A. Henoch-Schönlein purpura. Curr Rheumatol Rep 2004;6:195-202.
11. Sabry A, Sheashaa H, El-Husseini A, Mahmoud K, Eldahshan KF, George SK, *et al*. Proinflammatory cytokines (TNF-alpha and IL-6) in Egyptian patients with SLE: Its correlation with disease activity. Cytokine 2006;35:148-53.
12. Topaloglu R, Sungur A, Baskin E, Besbas N, Saatci U, Bakkaloglu A. Vascular endothelial growth factor in Henoch-Schonlein purpura. J Rheumatol 2001;28:2269-73.
13. Veeranki S, Gandhapudi SK, Tyagi SC. Interactions of hyperhomocysteinemia and T cell immunity in causation of hypertension. Can J Physiol Pharmacol 2017;95:239-46.
14. Bostom AG, Carpenter MA, Kusek JW, Levey AS, Hunsicker L, Pfeffer MA, *et al.* Homocysteine-lowering and cardiovascular disease outcomes in kidney transplant recipients: Primary results from the folic acid for vascular outcome reduction in transplantation trial. Circulation 2011;123:1763-70.
15. Park WC, Chang JH. Clinical implications of methylenetetrahydrofolate reductase mutations and plasma homocysteine levels in patients with thromboembolic occlusion. Vasc Specialist Int 2014;30:113-9.
16. Scherer EB, Loureiro SO, Vuaden FC, da Cunha AA, Schmitz F, Kolling J, *et al.* Mild hyperhomocysteinemia increases brain acetylcholinesterase and proinflammatory cytokine levels in different tissues. Mol Neurobiol 2014;50:589-96.
17. McCully KS. Homocysteine, vitamins, and prevention of vascular disease. Mil Med 2004;169:325-9.
18. Coppedè F, Grossi E, Migheli F, Migliore L. Polymorphisms in folate-metabolizing genes, chromosome damage, and risk of Down syndrome in Italian women: Identification of key factors using artificial neural networks. BMC Med Genom 2010;3:42.
19. Zidan HE, Rezk NA, Mohammed D. MTHFR C677T and A1298C gene polymorphisms and their relation to homocysteine level in Egyptian children with congenital heart diseases. Gene 2013;529:119-24.
20. Sugino H, Sawada Y, Nakamura M. IgA vasculitis: Etiology, treatment, biomarkers and epigenetic changes. Int J Mol Sci 2021;22:7538.
21. Pillebout E, Jamin A, Ayari H, Housset P, Pierre M, Sauvaget V, *et al*. Biomarkers of IgA vasculitis nephritis in children. PLoS One 2017;12:e0188718.
22. Chen T, Guo ZP, Jiao XY, Jia RZ, Zhang YH, Li JY, *et al.* CCL5, CXCL16, and CX3CL1 are associated with Henoch-Schonlein purpura. Arch Dermatol Res 2011;303:715-25.
23. Yang YH, Huang YH, Lin YL, Wang LC, Chuang YH, Yu HH, *et al.* Circulating IgA from acute stage of childhood Henoch-Schönlein purpura can enhance endothelial interleukin (IL)-8 production through MEK/ERK signalling pathway. Clin Exp Immunol 2006;144:247-53.
24. Izawa-Ishizawa Y, Ishizawa K, Sakurada T, Imanishi M, Miyamoto L, Fujii S, *et al.* Angiotensin II receptor blocker improves tumor necrosis factor-α-induced cytotoxicity via antioxidative effect in human glomerular endothelial cells. Pharmacology 2012;90:324-31.
25. Ge W, Wang HL, Sun RP. Pentraxin 3 as a novel early biomarker for the prediction of Henoch-Schönlein purpura nephritis in children. Eur J Pediatr 2014;173:213-8.
26. Jaiswal SK, Sukla KK, Mishra SK, Lakhotia AR, Kumar A, Rai AK. Association of genetic polymorphisms in genes involved at the branch point of nucleotide biosynthesis and remythylation with Down syndrome birth risk - a case-control study. J Mol Genet Med 2016;10:207.
27. Tayeb MT. The methylenetetrahydrofolate reductase gene variant (677 C-T) in risk mothers with down syndrome among Saudi population. Egypt J Med Hum Genet 2012;13:263-8.
28. James SJ, Pogribna M, Pogribny IP, Melnyk S, Hine RJ, Gibson JB, *et al.* Abnormal folate metabolism and mutation in the methylenetetrahydrofolate reductase gene may be maternal risk factors for down syndrome. Am J Clin Nutr 1999;70:495-501.
29. Kaur A, Kaur A. Prevalence of methylenetetrahydrofolate reductase 677 C-T polymorphism among mothers of down syndrome children. Indian J Hum Genet 2013;19:412-4.
30. He L, Shen Y. MTHFR C677T polymorphism and breast, ovarian cancer risk: A meta-analysis of 19, 260 patients and 26,364 controls. Onco Targets Ther 2017;10:227-38.
31. Aksoy-Sagirli P, Erdenay A, Kaytan-Saglam E, Kizir A. Association of three single nucleotide polymorphisms in MTR and MTRR genes with lung cancer in a Turkish population. Genet Test Mol Biomarkers 2017;21:428-32.
32. Matsuo K, Hamajima N, Hirai T, Kato T, Inoue M, Takezaki T, *et al.* Methionine synthase reductase gene A66G polymorphism is associated with risk of colorectal cancer. Asian Pac J Cancer Prev 2002;3:353-9.

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