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ARAŞTIRMA / RESEARCH

Complement C2 polymorphisms in children with Henoch Schönlein Purpura

Henoch Schönlein Purpurası tanılı çocuklarda kompleman C2 gen polimorfizmleri

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Abstract

Purpose: The aim of this study was to investigate whether C2 polymorphisms influence the symptoms and disease outcomes in children with Henoch Schönlein purpura (HSP).

Materials and Methods: This cross-sectional study included 49 children with HSP, diagnosed and followed for at least 6 months in our department between July 2016 and March 2018. Sanger sequencing was performed for detecting C2 gene polymorphisms. Statistical analysis was performed for comparison of clinical and laboratory parameters between patients according to having C2 polymorphisms.

Results: Only 6 patients (12.2%) had following C2 gene polymorphisms: rs9332739 (n=3), rs36221133 (n=2), rs146054348 (n=1). Age at disease onset, gastrointestinal and joint involvement, serum complement levels, renal involvement, requirement of systemic steroids and disease relapse were found similar between the patients with and without C2 gene polymorphism. We found higher serum IgM level and lower leukocyte counts in HSP patients with confirmed C2 polymorphisms than the patients with normal C2 gene.

Conclusion: Although C2 gene polymorphisms were not related to clinical manifestations and disease outcome in children with HSP, we speculate that C2 gene polymorphisms may be associated with elevated serum IgM levels in patients with HSP.

Keywords:. C2 gene, Henoch Schönlein Purpura, IgA vasculitis, polymorphism

Öz

Amaç: Bu çalışmada Henoch Shönlein purpurası (HSP) tanılı çocuklarda C2 gen polimorfizmlerinin semptomlar ve hastalık şiddeti üzerine etkilerinin incelenmesi amaçlanmıştır.

Gereç ve Yöntem: Bu kesitsel çalışmaya kliniğimizde Temmuz 2016 ve Mart 2018 tarihleri arasında HSP tanısı almış ve en az 6 ay takip edilmiş olan 49 çocuk hasta dahil edilmiştir. Kompleman C2 gen polimorfizmlerini değerlendirmek amacı ile Sanger sekanslama tekniği kullanılmıştır. Hastalar C2 gen polimorfizm varlığına gruplara ayrılarak klinik ve laboratuvar verileri karşılaştırılmıştır.

Bulgular: Çalışmamızda sadece 6 (%12,2) hastada C2 gen polimorfizmleri saptanmıştır. Bunlar; rs9332739 (n=3), rs36221133 (n=2), rs146054348 (n=1) olarak sıralanabilir. C2 gen polimorfizmleri varlığına göre hasta grupları arasında yaş, gastrointestinal ve eklem tutulumları, serum kompleman düzeyleri, böbrek tutulumu, sistemik steroid gereksinimi ve hastalık relaps oranları benzer bulunmuştur. C2 gen polimorfizmi olan hastalarda, olmayanlara göre istatistiksel anlamlı olarak daha yüksek serum IgM düzeyi ve daha düşük lökosit sayısı saptanmıştır.

Sonuç: Konusunda ilk olan çalışmamızda her ne kadar C2 gen polimorfizmlerinin HSP'li hastalarda klinik bulgular ve hastalık seyri üzerine etkisi gösterilememiş olsa da, HSP tanılı hastalarda yüksek serum IgM düzeyi C2 gen polimorfizmi ile ilişkili olabilir.

Anahtar kelimeler: C2 geni, Henoch Schönlein Purpurası, IgA vasküliti, polimorfizm

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INTRODUCTION

Immunoglobulin A (IgA) vasculitis, previously referred as Henoch Schönlein purpura (HSP) is the most common primary systemic vasculitis in childhood. The annual incidence is approximately 20.4 per 100000 children and twice as frequent during fall and winter that suggests an environmental trigger[1,2]. The disease has often a self-remitting course and predominantly involves skin, gastrointestinal tract, joints and kidneys. Patients usually represent with palpable purpura, abdominal pain, arthralgia and subcutaneous edema[3]. Relapses may occur, most frequently with skin involvement and nephritis is responsible for the late term prognosis in HSP patients[4]. Although the pathogenesis of the disease still remains unclear, mesangial deposition of galactose deficient IgA1, immune complex-mediated complement activation and leukocytoclastic vasculitis are thought as causative mechanisms[2]. It had been previously believed that complement activation occurs within the lectin and alternative pathways in HSP, however several studies also suggested activation of classical complement pathway[5-7].

Complement Component (C)2 forms a bridge between lectin and classical pathway by attaching activated C1 and formation of C3 convertase to activate subsequent components of complement pathway. C2 deficiency is relatively frequent and milder compared to other early complement deficiencies (C1Q, C1R, C1S, C4) resulting broad manifestations of autoimmunity[8,9]. C2 gene polymorphisms have been widely investigated in systemic lupus erythematosus (SLE) and suggested to associate with clinical heterogeneity[10]. Hereditary type II C2 deficiency, resultant from biallelic missense mutations in C2 gene had been reported with a prevalence of 1:10000-20000, whereas type I C2 deficiency due to heterozygote deletions in C2 gene, accounts for 7/1000 in general population [11,12].

Although there is no information on whether a possible relationship is present between C2 deficiency and HSP, we think that alterations in C2 gene still may be involved in pathogenesis of HSP similar to other immune-mediated disorders. Besides, being the first study on this topic, thus, we aimed to study whether C2 polymorphisms influence symptoms and severity of HSP in children.

MATERIALS AND METHODS

This cross-sectional study included 49 children with HSP, diagnosed and followed for at least 6 months at our department between July 2016 and March 2018. All patients fulfilled both the American College of Rheumatology (ACR) classification criteria for HSP and European League Against Rheumatism/Paediatric Rheumatology International Trials Organisation/Paediatric Rheumatology European Society (EULAR/PRINTO/PRES) 2010 criteria for HSP. ACR criteria was met if at least two of the following items were present: palpable purpura, onset before 20 years old, abdominal pain and histopathological evidence of granulocytes in small vessel wall. EULAR/PRINTO/PRES 2010 criteria for HSP necessitates purpura without thrombocytopenia with lower limb predominance as a mandatory criteria and plus two of the following items, including abdominal pain, arthritis or arthralgia, histopathological evidence of leukocytoclastic vasculitis and renal involvement [13,14]. Age at disease onset, presence of gastrointestinal, renal, articular and skin involvement, subcutaneous edema, serum complement C3, C4, erythrocyte Sedimentation Rate, C-reactive protein, immunoglobulin G, immunoglobulin M (IgM), immunoglobulin E and IgA levels at diagnosis were recorded retrospectively from the medical files of the patients. Hematuria was defined as presence of at least five red blood cells per high power field in urine sediment and proteinuria was defined as a spot urinary protein/creatinine ratio higher than 0.2.

Renal biopsy was performed if a rapid deterioration in renal function or persistent massive proteinuria were present. Kidney biopsy results were graded according to the International Study of Kidney Disease in Children classification for HSP nephritis. In this classification, grade I is defined as minimal alterations; grade II, as pure mesangial proliferation; and grade III to V, as focal segmental (a) or diffuse mesangial proliferation (b) with less than 50%, 50% to 75%, or greater than 75% crescents formation, respectively. Grade VI is associated with glomeruli with a membranoproliferative pattern of injury[15]. Disease relapses and relapse symptoms were also recorded. Disease relapse was considered when a new flare of cutaneous lesions or other manifestations occurs in a patient at least four asymptomatic weeks after the initial HSP episode.

The study was approved by local ethics committee of Cukurova University medical faculty (no: 68/5, date: 08.09.2017) and conducted in relevance with the ethical standards released in the 1964 Declaration of Helsinki and its later amendments. Parents of the patients accepted to participate to the study and signed written informed consents prior to the study.

Table 1. Demographic and clinical characteristics of 49 patients with Henoch Schönlein Purpura

Parameters	mean±SD
Hemoglobin (g/dl)	12.6±0.9
Platelets (/mm3)	390000±98000
Leukocytes (/mm3)	12200±3960
Erytrocyte sedimentation rate (mm/h)	8.9±8.8
C-reactive protein (mg/dl)	1.3±0.1
Complement C3 (mg/dl)	150.9±39.2
Complement C4 (mg/dl)	29.4±9.6
SD; Standard deviation	
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Molecular testings

The genetic analysis including the related C2 gene was employed as a molecular diagnostics tool by Sanger sequencing (3130XL, Applied Biosystems). The test comprised all exons, at least 50 nucleotides upstream and downstream of each exon and 1 kb of both the 5' promoter regions and the 3' UTRs. Sequencing was utilized on the leukocyte DNA collected from the patients' peripheral blood samples.

ClinCalc (http://clincalc.com) was used to determine the post-hoc power of the study and to apply the Bonferroni correction. Statistical analyses were performed using the GraphPad Prism software (GraphPad Software, Inc. USA), while the statistical significance was defined at $p \leq 0.05$. Hardy-Weinberg equilibrium analysis was performed for each polymorphism identified. A modified version of the human genome (www.varsome.com) was used as the major allele population-specific reference. Confidence interval (CI) as 95% was used to estimate the precision of the odds ratio. The chi-square test was also used to test the frequencies of the alleles and genotypes.

Statistical analysis

The remaining statistical analysis were performed by SPSS 20.0 statistical software package (IBM SPSS Statistics). Categorical variables were stated as numbers and percentages, besides continuous variables were notified as mean and standard deviation or median and minimum-maximum where appropriate. The distribution for continuous variables was tested by the Kolmogorov-Smirnov test. Comparisons of continuous variables between two groups were made with Mann-Whitney U test. Besides, Fisher's exact test was used to compare categorical variables between two groups. Significance of statistical level for all tests was considered to be 0.05.

Table 2. Laboratory parameters of the patients with Henoch Schönlein Purpura at disease onset.

Parameters	mean±SD
Hemoglobin (g/dl)	12.6±0.9
Platelets (/mm3)	390000±98000
Leukocytes (/mm3)	12200±3960
Erytrocyte sedimentation rate (mm/h)	8.9±8.8
C-reactive protein (mg/dl)	1.3±0.1
Complement C3 (mg/dl)	150.9±39.2
Complement C4 (mg/dl)	29.4±9.6

SD; Standard deviation

RESULTS

Present study included 49 HSP patients (25 females and 24 males). The mean age at disease onset was 8.1±3.1 years. The main demographic and clinical properties of 49 HSP patients were shown in Table 1. Disease reoccurred in seven patients (14.3%), two of them presented with abdominal pain and the remaining 5 relapses occurred with only purpura. Of 9 patients with renal involvement, only one of them underwent renal biopsy and resulted as grade 2 HSP nephritis. Table 2 shows laboratory evaluation results at the time of diagnosis of HSP patients. None of our patients had low serum complement C3 levels, whereas 5 (10.2%) patients had low C4 levels at

diagnosis. Renal involvement was present in 40% (2/5) of the patients with C4 hypocomplementemia and 15.9 % (7/44) with normal serum complement levels (p=0.23).

Among 49 HSP patients, only 6 patients (12.2%) had following C2 gene polymorphisms: rs9332739 (n=3), rs36221133 (n=2), rs146054348 (n=1). Comparison of clinical characteristics between HSP patients with or without C2 gene polymorphisms were demonstrated in Table 3. Leukocyte count was statistically lower and serum IgM levels were statistically higher in patients with C2 gene polymorphisms than the patients with normal C2 gene. Remaining clinical and laboratory parameters were similar between these groups.

Table 3. Comparison of disease characteristics between Henoch Schönlein Purpura patients according to the presence of C2 gene polymorphisms.

	Patients with confirmed C2 gene polymorphism (n=6)	Patients with normal C2 gene (n=43)	p
Age at disease onset (years), median (range)	8.5 (6.2-12.4)	7.5 (2.7-14.8)	0.211
Arthralgia, % (n)	50 (3)	48.8 (21)	0.646
Abdominal pain, % (n)	50 (3)	72.1 (31)	0.257
Positive occult blood in the stool, % (n)	33.3 (2)	41.9 (18)	0.527
Invagination, % (n)	0	2.3 (1)	0.878
Subcutaneous edema, % (n)	0	9.3 (4)	0.575
Renal involvement, % (n)	16.7 (1)	18.6 (8)	0.698
Treatment with systemic steroids, % (n)	50 (3)	53.5 (23)	0.605
Relapse, % (n)	16.7 (1)	14 (6)	0.625
Complement C3 (mg/dl), median (range)	159.5 (132-188)	142 (83-350)	0.318
Complement C4 (mg/dl), median (range)	24.7(14.3-44)	29.8 (11-51)	0.681
IgG (mg/dl), median (range)	1143 (932-1689)	965 (536-1575)	0.151
IgA (mg/dl), median (range)	230 (126-350)	179 (67-277)	0.452
IgM (mg/dl), median (range)	133 (103-242)	91 (35-178)	**0.021**
IgE (mg/dl), median (range)	98 (22-351)	83.8 (5-1650)	0.985
CRP (mg/dl), median (range)	1.28 (0.2-2.3)	0.8 (0.1-11.8)	0.867
ESR (/mm3), median (range)	8 (4-36)	6 (2-33)	0.374
Leukocytes (/mm3), median (range)	9465 (5060-11510)	12100 (5100-22550)	**0.035**
Hemoglobin (mg/dl), median (range)	12.9 (11.4-13.6)	12.7 (9.8-14.3)	0.927
Platelets (/mm3), median (range)	319000 (226000-594000)	394000 (227000-614000)	0.120

CRP; C-reactive protein, ESR; Erythrocyte Sedimentation Rate, Ig: Immunoglobulin
Significant p values are given in bold.

DISCUSSION

Although exact mechanism still remains poorly understood, several pathways had been investigated in HSP pathogenesis so far. It is widely suggested that HSP is an immune-mediated disease, according to the information on pathologic and laboratory findings, which include perivascular IgA deposition and polymorphonuclear leukocytes infiltration on small vessels, increased serum IgA and proinflammatory cytokines[16].

Besbas et al. suggested that T helper 1 (Th1)/T helper 2 (Th2) imbalance and excessive Th2 activation lead to cytokine secretion and B cell differentiation in HSP[17]. In a recent study, complement activation secondary to immune complexes was suggested to play an important role in renal injury[18]. In our cohort, renal involvement was frequent in HSP patients with C4 hypocomplementemia although the difference was statistically insignificant. Although C4 hypocomplementemia was present in 10.2% in our study, small study size lead to the lack of significant results about clinical outcomes. Similarly, owing the reason that only a few HSP patients had been reported to have hypocomplementemia in the literature, the role of complement system may not be understood[7,19,20]. In a recent meta-analysis, several possible genetic predispositions to HSP were elaborately reviewed. Human leukocyte antigen (HLA), Mediterranean fever (MEFV), cytokine and cytokine receptor genes were the most frequently

investigated ones[21]. Besides, we have recently showed that HSP patients carrying MEFV variants in exon 10 have more abdominal pain and intussusception than the ones without[22]. Thus, we proposed that there may be other genetic factors other than MEFV, affecting clinical variability and outcomes of HSP patients. In this view, two recent independent studies reported alterations in complement C4 gene to be associated with HSP and HSP nephritis[23,24]. In our study; we did not investigate if C2 polymorphisms predispose to HSP, however we found that the age at disease onset, gastrointestinal and joint involvement, serum complement levels, renal involvement, requirement of systemic steroids and disease relapse did not differ between patients regarding the presence of C2 gene polymorphism.

C2 deficiency is the most common complement deficiency worldwide and characterized by recurrent infections in early childhood[10]. Autoimmune disorders, particularly SLE were reported with C2 deficiency, but less frequently than the deficiencies of other early complement components[25,27]. Additionally, C2 gene polymorphisms were previously investigated in SLE and did reveal convincing results[9,27].

Despite the absence of evidence about the effects of C2 gene polymorphisms on clinical course and outcomes of HSP; we found higher IgM levels in HSP patients with confirmed C2 polymorphisms than patients with normal C2 gene in present study. Similarly, a recent report showed serum IgM elevation explained by increased immunoglobulin D (IgD)+CD27+ mantle zone (MZ) B cells in a 6 year-old-patient with recurrent palpable purpura and abdominal pain resembling HSP, eventually diagnosed and treated as SLE and C2 deficiency[28]. IgD+CD27+ MZ B cells are distinct splenic B cells responding to T-independent antigens and leading to IgM production[29]. We did not study the relationship between serum IgM elevation and B cell subsets including IgD+CD27+ MZ B cells in our patient. Nevertheless, we speculate that both C2 mutations and polymorphisms which may subsequently present with autoimmunity such as SLE may be associated with elevated serum IgM levels.

Another recent study revealed that direct immunofluorescence was positive in 60% of the skin biopsies of the HSP patients. IgA was the most commonly deposited component in the vessel walls. Other frequent deposits were C3 in 30.3%, IgM in 24.7%, and IgG 18.6% in HSP patients[30]. Therefore, it is still noteworthy to declare that HSP is an immune mediated vasculitis and somehow involves immunoglobulin and complement activation and accumulation in tissues. Although it is an ancient disease, there is an ongoing need for clarifying the exact mechanisms of HSP in the future.

To the best of our knowledge, this is the first study, designed to identify C2 gene polymorphisms in HSP. However, we could not demonstrate any relevance between clinical findings and C2 gene polymorphisms. Major limitations of the study are the small study size, lack of functional studies and information on whether C2 gene polymorphisms cause low serum C2 levels. This may be alleviated in future studies investigating a large number of HSP patients, with a multicenter participation design to generalize the results to general population.

In conclusion, C2 gene polymorphisms may be associated with elevated serum IgM levels in HSP patients. Further studies are needed to clarify whether high serum IgM levels in HSP patients might be a cue for C2 deficiency, which may subsequently present with autoimmunity.

Yazar Katkıları: Çalışma konsepti/Tasarımı: RMKE, AB, SB; Veri toplama: RMKE, SB, BA; Veri analizi ve yorumlama: BA, SB, AKB; Yazı taslağı: RMKE, SB, AB, DD, DUA; İçeriğin eleştirel incelenmesi: AKB, SB, BA, AB; Son onay ve sorumluluk:: RMKE, SB, BA, AKB, DD, DUA, AB; Teknik ve malzeme desteği: SB, AB; Süpervizyon: RMKE, AB; Fon sağlama (mevcut ise): yok.
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