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# A POSSIBLE RELATIONSHIP BETWEEN SERUM HOMOCYSTEINE LEVEL AND IgA NEPHROPATHY IN CHILDREN

## ÇOCUKLARDA SERUM HOMOSİSTEİN DÜZEYİ İLE IgA NEFROPATİSİ ARASINDAKİ OLASI İLİŞKİ

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### ABSTRACT

**Objective:** The evidences from experimental and epidemiological studies suggests that elevated serum homocysteine levels may lead to renal injury and may be a significant risk factor for the development of chronic kidney disease. The aim of this study was to investigate a possible relationship between serum homocysteine level and crescent formation in children with IgA nephropathy and Henoch-Schonlein purpura nephritis.

**Material and Methods:** A total of 31 patients diagnosed as biopsy proven IgA nephropathy and Henoch-Schonlein purpura nephritis and idiopathic crescentic glomerulonephritis in three Pediatric Nephrology centers within the last five years and 25 healthy controls were enrolled in the study.

**Results:** Homocysteine levels of patients were higher than the upper limit of normal value and also higher than the controls (p=0.0001). There was no significant difference between the patients with or without crescent formation regarding homocysteine levels (p>0.05). Presence or severity of proteinuria was not related to homocysteine levels (p>0.05).

**Conclusion:** Serum homocysteine levels are elevated in patients with IgA nephropathy and Henoch-Schonlein purpura nephritis.

### ÖZET

**Amaç:** Deneysel ve epidemiyolojik çalışmalar, yüksek serum homosistein düzeylerinin renal hasara yol açabileceğini ve kronik böbrek hastalığı gelişimi için önemli bir risk faktörü olabileceğini göstermektedir. Çalışmamızın amacı IgA nefropatisi ve Henoch-Schönlein purpura nefriti olan hastalarda serum homosistein düzeyleri ile kresent oluşumu arasında bir ilişki olup olmadığını belirlemektir.

**Gereç ve Yöntemler:** Üç pediatrik nefroloji merkezinde son beş yılda biyopsi ile IgA nefropatisi, Henoch-Schönlein purpura nefriti ve idiopatik kresentik glomerulonefrit tanısı alan 31 hasta ve 25 sağlıklı kontrol çalışmaya alındı.

**Bulgular:** Hastaların homosistein düzeyleri normal değerin üst sınırından ve kontrollerden de daha yüksekti (p=0,0001). Biyopside kresent görülen ve görülmeyen hastalar arasında da homosistein düzeyleri açısından anlamlı fark yoktu (p>0,05). Proteinüri varlığı ve şiddeti ile homosistein düzeyleri arasında ilişki saptanmadı (p>0,05).

**Sonuç:** Serum homosistein düzeyleri IgA nefropatisi ve Henoch-Schonlein purpura nefriti olan hastalarda yüksek bulunmuştur. Sonuçlarımız, bu hasta grubunda yüksek serum homosistein

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Our results suggest that elevated serum homocysteine levels may be related to segmental glomerulosclerosis in these patient groups.

**Keywords:** IgA nephropathy, homocysteine, crescent

düzeylerinin segmental glomerüloskleroz ile ilişkili olabileceğini düşündürmektedir.

**Anahtar Kelimeler:** IgA nefropatisi, homosistein, kresent

## INTRODUCTION

IgA nephropathy (IgAN), one of the most common glomerulonephritis in adults and children, may progress to end-stage renal disease (ESRD) in 20-50% of patients within 20 years (1-3). Although IgAN usually has a slow progression course, acute deterioration in renal function due to crescent formation may occur during the course of the disease (3, 4). It has been reported that crescent formation in IgAN may influence renal outcomes and aggressive immunosuppressive treatment may be required if the number of crescents involves >50% of the glomeruli in a renal biopsy (5). The Kidney Disease Improving Global Outcomes (KDIGO) guidelines recommend that Henoch-Schonlein Purpura Nephritis (HSPN) should be treated the same as IgAN because it has the same histopathologic features as IgAN in kidney biopsies (5). Crescent formation in glomeruli begins with the emergence of ruptures of glomerular capillary walls (6). These ruptures permit coagulation factors and cells including monocytes and lymphocytes to enter the Bowman's space, which results in crescent formation (6). There is some evidence to suggest that elevated concentration of serum homocysteine (Hcy) leads to endothelial cell injury, which results in stimulation of the coagulation system and resistance of anticoagulation activity on the endothelial surface (7, 8). Moreover, it has been reported that hyperhomocysteinemia causes glomerular and tubulointerstitial damage in several ways, such as increasing oxidative stress, stimulating monocyte chemoattractant protein-1 (MCP-1) expression, and nuclear factor kappa B activation (NF-B) (9-14).

We observed hyperhomocysteinemia in two patients with IgAN and crescent formation in their renal biopsy. Using observations from these two patients, we hypothesized that crescent formation may be related to hyperhomocysteinemia in IgAN. The aim of the study was to investigate whether a relationship exists between serum Hcy level and crescent formation in children with IgAN and HSPN.

## MATERIALS AND METHODS

Thirty-one patients with renal biopsy-proven IgAN (n=17), HSPN (n=12), and idiopathic crescentic glomerulonephritis (ICGN; n=2) in three pediatric nephrology centers were enrolled in the study. The control group was consisted of 25 age matched healthy children without medical history of any chronic disease or renal disorder.

This study was approved by the Ethical Committee of Istanbul University Istanbul Faculty of Medicine (Date: 27.05.2013, No: 640). Informed consent was obtained from the parents of all participants. A physical examination was performed, and the patients' medical history and clinical findings were recorded at the time of the sampling.

Baseline investigations including serum urea, creatinine, electrolytes, lipids, total protein, albumin, Hcy, urinalysis and 24-hour urinary protein were performed in the patient group. Moreover, serum levels of vitamin B12 and folic acid, thyroid function tests, methylenetetrahydrofolate reductase *(MTHFR)* 677 and *MTHFR* 1298 genetic mutation analysis were also performed because hyperhomocysteinemia may be caused by deficiency of folic acid and vitamin B12, hypothyroidism or decreased enzyme activity of *MTHFR* due to genetic mutations. A physical examination was performed and serum urea, creatinine, Hcy, B12 and urinalysis were performed in the control group.

IgAN, HSPN, and ICGN were diagnosed based on clinical, laboratory and immunehistopathologic findings. Mesangial hypercellularity with predominant IgA deposition in the mesangium and/or capillary wall was described as IgAN in patients without systemic involvement. The MEST score was evaluated as mesangial hypercellularity (M0, M1), endocapillary hypercellularity (E0, E1), segmental glomerulosclerosis (S0, S1) and tubular atrophy/interstitial fibrosis (T0, T1), in accordance with the Oxford classification (4). Presence or absence of crescents was recorded. Henoch-Schonlein Purpura was diagnosed using the EULAR/PReS consensus criteria (15). Extracapillary proliferation of cells in the Bowman's space with more than two cell layers was described as cellular crescent (16). Idiopathic crescents in more than 50% of glomeruli on renal biopsies were described as ICGN.

Hypertension was defined as average clinic measured systolic and/or diastolic blood pressure ≥95$^{th}$ percentile on the basis of age, sex and height percentiles (17).

The mean estimated glomerular filtration rate (eGFR) was calculated according to the Schwartz formula (18). Hematuria was defined as five or more red blood cells per high power field in a urine specimen (19). Proteinuria was determined as a positive dipstick reading of ≥1+, urinary excretion ≥4 mg/m$^2$/per hour in urine over a period of 24 hours (19).

Venous blood samples were collected in tubes from the antecubital vein, followed by overnight fasting. The tubes were centrifuged at 2000 relative centrifugal force (RCF) (10 minutes) to remove the serum. The blood and serum samples were analyzed within an hour. Serum glucose, urea, creatinine, total cholesterol, HDL cholesterol, triglyceride, total protein, albumin, thyrotropin (TSH), free triiodothyronine (T3), free thyroxine (T4), and other biochemical parameters were determined using a Beckman Coulter AU5800 Clinical Chemistry, Dxl 800 Immunoassay Auto-Analyzer and commercial kits (Beckman Coulter, CA, USA). Urine protein levels were measured using Siemens BNII nephelometric system (Siemens Healthcare Diagnostics, USA) using reagents and protocols provided by the manufacturer. Serum Hcy, vitamin B12, and folic acid were determined using an Immulite 2000 chemiluminescence auto-analyzer and commercial kits (Siemens, USA). Hyperhomocysteinemia was defined as serum levels of Hcy >12µg/L (20). Deficiency of vitamin B12 was defined as <200 pg/mL and deficiency of folic acid was defined as <3 ng/mL (21, 22).

Genomic deoxyribonucleic acid (DNA), was isolated from peripheral blood leukocytes using the High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany) for C677T and A1298C of *MTHFR* gene mutations. Detection of gene mutations was achieved using rapid capillary PCR with melting curve analysis using fluorescence-labeled hybridization probes in a Light Cycler (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analysis in this study was performed with the package program Number Cruncher Statistical System (NCSS) 2007 Statistical Software (Utah, USA). Patients mentioned in the background section were excluded from statistical analyses because of their low eGFR to provide group homogeneity. In addition to standard descriptive statistical calculations (mean, standard deviation, median and IQR), the Kruskal-Wallis test was used in the comparison of groups, the Mann-Whitney U test was used in the comparison of two groups, and the Chi-square test was performed to evaluate qualitative data. Statistical significance level was established at $p<0.05$.

## RESULTS

The median age of the patients and controls were 11.6 years (range: 3.53-19.37 years) and 12.60 years (range: 9.53-14.84), respectively. There was no significant difference between patient and control groups regarding age and gender distribution (p=0.145 and p=0.186; respectively). The median follow-up duration of the patients was 61.5 months (range: 3-365 months). The patient's characteristics are given in Table 1.

Mean serum Hcy level was significantly higher in the patients than in the controls (p=0.0001) (Table 2, Figure 1). Hcy levels were not significantly different in the patients with IgAN and HSPN (Table 2). The presence or severity of proteinuria were not related to Hcy levels (p>0.05) (Table 2).

**Table 1:** Characteristics of the study group

| Patients characteristics | n (%) |
|---|---|
| **Total** | 31 (100) |
| **Histopathologic diagnosis** | |
| IgA nephropathy | 17 (54.8) |
| Henoch-Schonlein nephritis | 12 (38.7) |
| Idiopathic crescentic glomerulonephritis | 2 (6.5) |
| **Presence of crescent** | **11 (35.5)** |
| IgA nephropathy | 3 (27.3) |
| Henoch-Schonlein nephritis | 6 (54.5) |
| Idiopathic crescentic Glomerulonephritis | 2 (18.2) |
| **Presenting symptoms** | |
| Macroscopic hematuria | 12 (38.7) |
| Hypertension | 6 (19.3) |
| Rash | 12 (38.7) |
| Arthritis/arthralgia | 9 (29) |
| Proteinuria | 17 (54.8) |
| Nephrotic proteinuria | 3 (9.6) |
| Deterioration of kidney function | 4 (12.9) |
| **Actual situation of the patients** | |
| Microscopic hematuria | 9 (29) |
| Non-nephrotic proteinuria | 18 (58) |
| Hypoalbuminemia | 0 (0) |
| Hypertension | 2 (6.4) |
| Hyperlipidemia | 7 (22.5) |
| Deterioration of kidney functions | 1 (3.2) |

Eleven (35.5%) of the patients had crescent formation in renal biopsy. Crescents existed in ≥50% of glomeruli in two patients with ICGN, in one patient with IgAN, and in one patient with HSPN. Other patients with IgAN and HSPN had crescents in ≤25% of glomeruli. There was no significant difference between the patients with and without crescent formation regarding Hcy levels (p=0.17) (Table 2).

According to the MEST score, 40.7% of the patients were assessed as M1, 48.1% as E1, 22.2% as S1, and 7.4% as T1. Serum Hcy level was significantly higher in patients who were assessed as S1 (29.36±11.99 µg/L) than in those assessed as S0 (19.74±4.15µg/L) with regard to MEST score (p=0.009) although other parameters of the score were not related to Hcy level (p>0.05).

As an interesting observation, Hcy levels were higher than the upper limit of normal values in all our patients (Figure 1). Mean vitamin B12 level was significantly lower in the patient group than in the controls (238.77±127.24 vs 378.88±69.63; p=0.0001)(Table 2). Vitamin B12 level was lower than 200 ng/mL in 12 patients (38.7%) in our

**Table 2:** Serum homocysteine, folic acid, and vitamin B12 levels in controls and patients according to histopathology and proteinuria

| | Homocysteine mean±SD (µg/L) | Vitamin B12 mean±SD (pg/mL) | Folic acid mean±SD (ng/mL) |
|---|---|---|---|
| **Patients** (n=29) | 23.18±8.66 | 238.77±127.24 | 7.52±2.78 |
| **Controls** (n=25) | 8.88±1.79 | 378.88±69.63 | 8,01±1.46 |
| p | 0.0001 | 0.0001 | 0.427 |
| **Histopathologic diagnosis** | | | |
| IgAN[a] (n=17) | 22.67±8.49 | 222±69.88 | 8.87±3.74 |
| HSPN[b] (n=12) | 21.34±5.44 | 249.33±191.52 | 7.52±2.78 |
| ICGN[c] (n=2) | 38.65±15.76 | 318±41.01 | 9.05±0.40 |
| p | 0.790 | 0.413 | 0.376 |
| **Presence of crescent** | | | |
| Crescent (+) (n=11) | 26.71±12.16 | 261±168.08 | 7.69±2.39 |
| Crescent (-) (n=20) | 21.25±5.43 | 226.55±105.38 | 8.73±3.69 |
| p | 0.173 | 0.695 | 0.536 |
| **Presence of proteinuria** | | | |
| Proteinuria (+) (n=18) | 22.56±8.41 | 218.56±102.02 | 8.24±4.03 |
| Proteinuria (-) (n=13) | 24.06±9.28 | 266.77±160.07 | 8.52±1.98 |
| p | 0.575 | 0.378 | 0.328 |
| **Severity of proteinuria** | | | |
| <150 mg/24h (n=13) | 24.06±9.28 | 266.77±160.07 | 8.52±1.98 |
| 150-500 mg/24h (n=10) | 20.26±3.59 | 198.5±41.26 | 7.16±1.99 |
| 500-1000 mg/24h (n=8) | 25.44±11.75 | 243.63±147.64 | 9.59±5.53 |
| p | 0.480 | 0.625 | 0.424 |

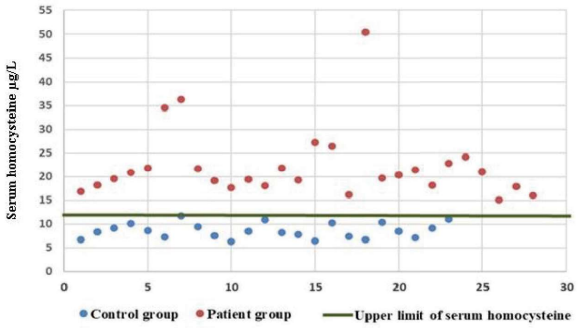



**Figure 1:** Serum homocysteine levels of the controls and patients

study groups. There was no significant difference between patients who had normal vitamin B12 levels and those who had low vitamin B12 in terms of crescent formation (p=0.641). Serum folic acid level and thyroid function tests were within the normal range in all our patients. The frequency of 677CC, 677CT, and 677TT genotypes was 32.2%, 61.2%, and 6.4%, respectively. The results for the 1298AA, 1298AC, and 1298CC genotypes were 19.3%, 77.4%, and 3.2%, respectively.

## DISCUSSION

Crescent formation may occur at any time during the course of IgAN and HSPN. We previously observed crescents in two patients with both IgAN and hyperhomocysteinemia in our outpatient clinic. It has been reported that elevation of serum Hcy level has many harmful effects on the endothelium (23). Some of these effects are activation of coagulation and inhibition of fibrinolysis, increase of vascular tonus, and stimulation of oxidative stress (23). The effects of Hcy have also been demonstrated in glomeruli via some experimental studies (11, 13, 14). Hence, we expected that serum Hcy levels might be higher in patients with crescents than in those without among children with IgAN and HSPN. On the contrary, Hcy levels were not different between patients with or without crescents in their glomeruli in our study group.

Interestingly, all of our patients had elevated serum Hcy levels. Therefore, we evaluated the well-known risk factors for hyperhomocysteinemia such as hypothyroidism, vitamin B12 and folic acid deficiency, and *MTHFR* gene polymorphisms (24). TT polymorphism of *MTHFR* C677T was found to be associated with decreased activity of *MTHFR*, which results in higher Hcy and lower folic acid levels. Also, it has been reported that the CC variant of *MTHFR* A1298C might be associated with lower enzyme activity, but results in the literature are less conclusive than for C677T (25-27). As possible causes of hyperhomocysteinemia, vitamin B12 deficiency was found in 12 patients (38.7%) in our study group. Moreover, TT polymorphisms were detected

in two patients and CC polymorphism in one patient. However, serum Hcy levels were also found elevated in the remaining 16 patients who did not have features that may predispose to hyperhomocysteinemia. We speculate that hyperhomocysteinemia may be related to IgAN itself because there was no other reason for hyperhomocysteinemia in these patients.

Endothelial cells have an important role in the maintenance of vascular structure integrity in glomeruli (28, 29). It has been considered that endothelial cells are related to the progression of IgAN. Kusano T et al. evaluated the number of glomerular endothelial cells in patients with IgAN by examining the number of nuclei of CD34+ glomerular endothelial cells in renal biopsies (6). The authors demonstrated that the loss of CD34+ endothelial cells was associated with glomerular necrosis, segmental and global sclerosis in IgAN (6). Also, there were fewer CD34+ endothelial cells found in glomeruli with chronic lesions than in normal glomeruli in the biopsy samples (6). Moreover, hyperhomocysteinemia may aggravate endothelial damage and sclerotic modification in glomeruli (11, 13, 30). According to Oxford Classifications, segmental glomerulosclerosis was related to the poor prognosis in IgAN (4). Hcy levels were significantly higher in patients with segmental sclerosis in their kidney biopsies in our study group, which suggests that hyperhomocysteinemia may be related to sclerosis and chronic changes in glomeruli and consequently poor prognosis in IgAN.

Proteinuria is another important factor that is known to impact the prognosis of patients with IgAN (31). Thus, the relationship between the serum Hcy levels and proteinuria was evaluated in our study. According to our results, there were no differences between the presence or absence of proteinuria and between the different levels of proteinuria in patients with IgAN in terms of serum Hcy levels. In our previous study, we evaluated whether there was Hcy elevation in idiopathic nephrotic syndrome in children. The serum Hcy levels were not higher in nephrotic children than in the control group (32). Kong et al. demonstrated that hyperhomocysteinemia was an independent risk factor of decreased eGFR in the elderly population, although there was no relationship between proteinuria and elevated serum Hcy levels in their study (33). On the contrary, Shuwei et al. reported that hyperhomocsyteinemia was associated with lower eGFR and also initial proteinuria in patients with IgAN (34). Although these studies had different results about the relationship between serum Hcy levels and proteinuria, they concluded that hyperhomocysteinemia was related to decreasing in eGFR and poor prognosis.

Our study had some limitations. One of these limitations was that our patients were not in the acute phase of the illness. The mean duration between kidney biopsy and blood sampling for the study was 35 months. We believe that further studies in larger groups of patients in the acute phase of the illness may reveal a relationship between these pathologic prognostic factors. The second limitation was our relatively small sample size.

In conclusion, we demonstrated that serum Hcy levels are elevated in patients with IgAN and HSPN. Our results suggest that elevated serum Hcy levels may be related to segmental glomerulosclerosis in these patient groups.

**Ethics Committee Approval:** This study was approved by the Clinical Research Ethical Committee of the Istanbul University, Istanbul Faculty of Medicine (Date: 27.05.2013, No: 640).

**Informed Consent:** Written consent was obtained from the participants.

**Peer Review:** Externally peer-reviewed.

**Author Contributions:** Conception/Design of Study- Z.Y.Y., A.Y., A.G., Y.Ö., S.E.; Data Acquisition- C.P., N.A., N.K.; Data Analysis/Interpretation- B.Y.A., Y.Ö., A.A.Ö., I.K.; Drafting Manuscript- C.P., N.A., N.K., A.K., B.Y.A., A.Ö., G.Ö.; Critical Revision of Manuscript- A.Y., Z.Y.Y., S.E., I.K., Y.Ö., A.G.; Final Approval and Accountability- C.P., Z.Y.Y., A.Y., A.G., N.K., M.K., A.Y., G.Ö., Y.Ö., I.K., A.A.Ö., B.Y.A., S.E.

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