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Original Article

Heat shock protein 70 levels in children with nephrotic syndrome

Bağdagül Aksu[1,2], Zeynep Nagehan Yürük Yıldırım[2], Asuman Gedikbaşı[1], Alev Yılmaz[2]

[1]Department of Pediatric Basic Sciences, Institute of Child Health, İstanbul University, İstanbul; [2]Division of Pediatric Nephrology, İstanbul Faculty of Medicine, İstanbul University, İstanbul, Türkiye.

ABSTRACT

Background. Idiopathic nephrotic syndrome (NS) is the most prevalent glomerular disease in children. Heat shock protein 70 (HSP70) is synthesized in response to diverse stress factors like infections and oxidative stress. We aimed to evaluate serum and urine levels of HSP70 in children with steroid-sensitive nephrotic syndrome (SSNS) and to assess changes in HSP70 levels with prednisolone treatment. Additionally, we seek to determine whether serum and urine levels of HSP70 can differentiate between frequently relapsing and infrequently relapsing cases in children with SSNS.

Methods. A total of 36 patients with SSNS and 35 healthy children were included in the study. Samples were taken from all patients at four time points; before corticosteroid treatment (day 0) and on days 15, 30, and 90 after the initiation of corticosteroid treatment. Serum and urine levels of HSP70 were measured by enzyme-linked immunosorbent assay (ELISA).

Results. In the NS group before steroid treatment (day 0), urine HSP70 (uHSP70) levels and urine HSP70/creatinine (uHSP70/Cre) ratios were significantly higher ($p<0.0001$), whereas serum HSP70 (sHSP70) levels were lower ($p=0.002$), compared to the healthy group. uHSP70 levels decreased gradually during prednisolone treatment in the patient group ($p<0.0001$). There was no difference in terms of sHSP70, uHSP70, and uHSP70/Cre ratios between patients with frequently relapsing and infrequently relapsing.

Conclusions. Our study demonstrates that uHSP70 levels are elevated in SSNS prior to treatment and decrease with prednisolone therapy, reflecting reduced renal stress and damage. uHSP70 may be a useful biomarker for monitoring renal damage and treatment response. Serum and urine levels of HSP70, as well as uHSP70/Cre ratios, did not differentiate between frequent and infrequent relapses.

Key words: pediatric, steroid-sensitive nephrotic syndrome, heat shock protein 70, HSP70, relapse, prednisolone.

Idiopathic nephrotic syndrome (NS) is the most prevalent glomerular disease in children, characterized by edema, nephrotic-range proteinuria, and hypoalbuminemia.[1] The molecular mechanisms underlying NS involve significant alterations in podocytes, including effacement of foot processes, cytoskeletal modifications, and reorganization of the slit diaphragm, leading to pronounced proteinuria.[2] Podocyte dysfunction may be idiopathic, genetic, or reactive etiologies stemming from mechanical stress, medications, toxins, viral infections, and unidentified circulating proteins.[3] Heat shock protein 70 (HSP70) is expressed in various renal cell types, including podocytes, mesangial cells, and different tubular epithelial cells.[4] As a member of the heat shock protein family, HSP70 is synthesized in response to stressors

✉ Bağdagül Aksu ▪ bagdagul@yahoo.com

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such as infections and oxidative stress, playing crucial roles in the structural shaping of new proteins, restoring partially denatured proteins, and degrading irreversibly damaged proteins.[5] Increased HSP70 expression is observed under stressful conditions and in diseases like chronic glomerulonephritis, tubulointerstitial nephritis, diabetic nephropathy, and chronic kidney disease, suggesting its influence on glomerular disease progression and response mechanisms.[4,6,7] Glucocorticosteroids are the primary treatment for NS, offering renoprotective effects by modulating podocyte gene expression and promoting prolonged podocyte survival, thereby reducing proteinuria.[8] Approximately 85–90% of NS patients achieve complete remission within 4–6 weeks of glucocorticoid treatment, classifying them as having steroid-sensitive nephrotic syndrome (SSNS).[9] However, around 76%-93% of these patients experience a relapse, with a substantial proportion (57%) facing frequent recurrences.[10] Relapses represent a significant clinical challenge in the long-term management of children with idiopathic NS. Firstly, children experiencing relapses are at an elevated risk of glucocorticoid-related adverse effects due to their exposure to higher cumulative steroid doses. Secondly, frequent relapses may develop, necessitating the initiation of additional immunosuppressive therapies. Identifying predictors of relapse risk could guide more personalized treatment strategies, allowing clinicians to optimize therapy choices before drug administration and mitigate the adverse effects associated with prolonged steroid use. Unfortunately, there is currently a lack of clinical or laboratory biomarkers that reliably predict the likelihood of frequent relapses. While various studies suggest potential predictors of relapse frequency[11-13], no biomarker has yet been validated to accurately forecast which patients are likely to experience recurrent relapses or require ongoing steroid and/or other immunosuppressive treatments.

This study aims to evaluate serum and urine levels of HSP70 in pediatric patients with SSNS and to assess changes in HSP70 levels in response to prednisolone treatment. Additionally, it seeks to determine whether HSP70 levels can distinguish between frequently relapsing and infrequently relapsing cases in children with SSNS.

Materials and Methods

Ethics committee approval was obtained in 2013 by the Clinical Research Ethics Committee of Istanbul University Istanbul Faculty of Medicine (2013/706), and sample collection began for our study. Serum and urine samples were collected from patients during their first episode of NS between 2014 and 2023 and then stored at -80 °C. In 2023, additional ethics committee approval was obtained to use the collected samples in the current study to investigate HSP70 as a biomarker by the Clinical Research Ethics Committee of Istanbul University Istanbul Faculty of Medicine (2023/2222), in accordance with the Declaration of Helsinki. Idiopathic NS was diagnosed in all patients in accordance with the criteria recommended by the International Study for Kidney Diseases in Children.[14] SSNS was defined as complete remission within 4 weeks of prednisolone treatment at a standard dose (60 mg/m²/day or 2 mg/kg/day, maximum 60 mg/day). Frequently relapsing was defined as having ≥2 relapses in the first 6 months following remission of the initial episode, or ≥3 relapses in any 12-month period.[15] Infrequently relapsing was defined as having <2 relapses in the 6 months following remission of the initial episode or fewer than 3 relapses in any subsequent 12-month period.[15]

Patients who experienced their initial episode of SSNS between January 2014 and December 2023 and fulfilled the inclusion criteria were enrolled in the study. Serum and urine samples were taken from all patients at four time points; prior to starting corticosteroid therapy (day 0) and on

days 15, 30, and 90 after starting corticosteroid therapy. All patients received standard oral steroids (2 mg/kg/day) for 4 weeks as induction therapy. Since proteinuria was negative in patients with NS, corticosteroid treatment was continued on alternate days for 4 weeks, then the dose was gradually tapered off, and the steroid was discontinued. Patients were divided into two groups: frequently relapsing and infrequently relapsing. Subjects in the control group were healthy children without any acute or chronic diseases.

The inclusion criteria were as follows:

- Patients with idiopathic NS
- Patients with SSNS
- Age 1-18 years
- Glomerular filtration rate (eGFR) ≥ 90 mL/min/1.73 m^2

The exclusion criteria were as follows:

- Secondary causes of NS (e.g., Henoch-Schönlein purpura, lupus nephritis)
- Patients with steroid-resistant nephrotic syndrome (SRNS)
- Presence of any acute or chronic infection or systemic disease

Written informed consent was obtained from the parents of the patients and the controls.

Measurement of serum and urine HSP70

Blood and urine samples were collected from the patient and promptly delivered to the laboratory. Blood samples were centrifuged at 2,500 g for 10 minutes to separate the serum. Following centrifugation, aliquots were stored at -80°C until analysis. On the day of analysis, samples were thawed at room temperature, and measurements were performed using the Human Heat Shock Protein 70 antibody (HSP70-Ab) ELISA Kit (Cat no: KTE62748) purchased from Abbkine (Abbkine, Inc, China) in accordance with the manufacturer's instructions. The manufacturer's guidelines for sample preparation and storage recommend storing samples at -80 °C if they are to be preserved for three years or longer. Accordingly, the samples were stored at -80 °C for long-term preservation, and repeated freeze-thaw cycles were strictly avoided. All samples were handled and stored under identical preanalytical conditions, ensuring uniform exposure and minimizing potential preanalytical effects on the results. HSP70 levels were expressed as ng/mL. The detection and quantification limits for HSP70 were set at <0.05 ng/mL. The intra-assay coefficient of variations (CV) for HSP70 were 7.9% and 8.6%; respectively. Urine creatinine levels were measured using an Architect c16000 analyzer (Abbott Laboratories, Abbott Park, IL, USA), with results reported in mg/dL. The urine HSP70/creatinine (uHSP70/Cre) ratio was expressed in ng/mg.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 22.0 for Windows (IBM Corp., Armonk, N.Y., USA). The normality of the parameters was tested using the Shapiro-Wilk test. Descriptive analyses were presented as medians and interquartile range (IQR) for non-normally distributed and ordinal variables. Mann–Whitney U test was used for between-group comparisons. Chi-square test was performed to evaluate the qualitative data. The relationships among variables were analyzed using Spearman's correlation coefficients. Friedman test was performed to evaluate the course of serum, urine HSP70 (uHSP70) and uHSP70/Cre ratio on days 0, 15, 30, and 90 following the initiation of corticosteroid treatment. For significant pairwise comparisons, Wilcoxon test was applied, with Bonferroni correction used to adjust for multiple comparisons. Post-power analysis was performed and the power was found to be 72%. p values < 0.05 were considered statistically significant.

Results

A total of 36 patients with SSNS and 35 healthy children were included in the study. Age and sex distribution were comparable between the NS and control groups (p>0.05, Table I). In the NS group prior to starting corticosteroid therapy (day 0), uHSP70 levels and uHSP70/Cre ratios were significantly higher compared to the healthy group (p=0.001 and p=0.034, respectively, Table I). Conversely, serum HSP70 (sHSP70) levels were lower in patients with NS than in the controls (p=0.002). In the patient group, there was a negative correlation between age and uHSP70/Cre ratio (r=-0.520, p=0.002), yet no correlation was detected between sHSP70 and uHSP70 (p>0.05). There was also a negative correlation between serum albumin and uHSP70 (r=-0.352, p=0.045), and a positive correlation between the spot urine protein creatinine ratio (UPCR) and uHSP70 (r=0.347 p=0.048) in NS patients prior to starting corticosteroid therapy (Table II). sHSP70, uHSP70 levels, and uHSP70/Cre ratio were independent of sex (p=0.624, p=0.381, p=0.870; respectively).

When we evaluated uHSP70 levels on days 0, 15, 30, and 90 after starting corticosteroid therapy, we observed a gradual decrease in uHSP70 levels in the patient group (p<0.0001) (Fig. 1). sHSP70 levels and uHSP70/Cre ratios showed a fluctuating course, and there was no statistically significant difference between day 0 and day 90 of the steroid treatment (p>0.05, Fig. 1).

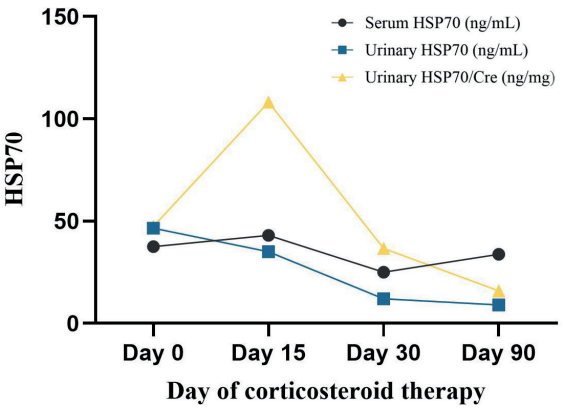

Fig. 1. Course of serum and urine levels of HSP70 with corticosteroid therapy.

GraphPad Prism version 10.4.1 (free trial) was used to produce this figure. HSP70: heat shock protein 70.

Table I. Comparison of age, sex, and HSP70 levels between the nephrotic syndrome patients prior to starting corticosteroid therapy (day 0) and controls.

Parameters	Nephrotic syndrome (n=36)	Controls (n=35)	p
Age (years)	5.3 (3.2-9.3)	7.5 (4.9-9.2)	0.067
Female/Male (%)	36/64	51/49	0.193
Serum albumin (g/dL)	1.8±0.5 (1.0-2.9)	-	
Urine protein creatinine ratio (mg/mg)	8.1±4.5 (2.2-19.5)	-	
Serum HSP70 (ng/mL)	37.5 (31.2-44.1)	49.2 (39.9-68.7)	0.002
Urine HSP70 (ng/mL)	46.5 (37.5-55.6)	33.9 (31.3-40.0)	0.001
Urine HSP70 creatinine ratio (ng/mg)	47.4 (31.3-96.2)	37.2 (21.6-60.5)	0.034

Data are given as percent, mean ± SD (min-max) or median (interquartile range) as appropriate. NS: nephrotic syndrome, HSP70: heat shock protein 70.

Table II. Correlations between HSP70 levels and various parameters in the nephrotic syndrome patients prior to starting corticosteroid therapy (day 0).

Parameters		Serum HSP70	Urine HSP70	Urine HSP70 creatinine ratio
Age	r	-0.103	-0.103	-0.520
	p	0.564	0.563	0.002
Serum albumin	r	0.112	-0.352	-0.192
	p	0.535	0.045	0.292
Urine protein creatinine ratio	r	0.149	0.347	-0.041
	p	0.407	0.048	0.824

HSP70: heat shock protein 70; r: Spearman's correlation coefficient.

The total follow-up period of the patients was 7.4±1.5 years (range: 4.3-9.4 years), and the total number of attacks was 3±2 (range: 1-11). The total number of attacks in frequently relapsing patients was 5±3 (range: 3-11), while in infrequently relapsing patients, it was 2±2 (range: 1-6). Patients with frequent and infrequent relapsing were compared in terms of HSP70 levels, and no significant differences were found between the two groups in terms of sHSP70, uHSP70, and uHSP70/Cre (p>0.05) on days 0, 15, 30, and 90 (Table III).

Discussion

This study aimed to evaluate serum and uHSP70 levels, as well as the uHSP70/Cre ratio in children with SSNS and to assess changes in these levels with prednisolone treatment. Our results revealed significantly higher uHSP70 levels and uHSP70/Cre ratios in the NS group before steroid treatment compared to healthy controls. Conversely, sHSP70 levels were lower in NS patients.

The elevated uHSP70 levels prior to treatment suggest that HSP70 might serve as a marker of renal damage and stress, reflecting glomerular dysfunction and podocyte injury. This finding aligns with previous research showing HSP70 overexpression in various renal pathologies, including chronic glomerulonephritis and diabetic nephropathy.[6,16] The increased uHSP70 levels may reflect mechanical stress and injury in the kidneys. In NS, decreased anionic charge on the glomerular filtration barrier contributes to increased permeability and proteinuria.[17] Normally, albumin, a small protein with a molecular weight of 66.5 kDa, does not pass through the glomerular barrier due to its negative charge. In conditions like minimal change disease, where the negative charge on podocytes is lost, albumin and other small proteins such as IgG and transferrin are excreted in the urine. HSP70, with a molecular

Table III. Comparison of HSP70 levels prior to starting corticosteroid therapy (day 0) between nephrotic syndrome patients with frequently relapsing disease and those with infrequently relapsing disease.

Parameters	Frequently relapsing (n=11)	Infrequently relapsing (n=25)	p
Age, years	5.9 (2.4-13.7)	4.9 (3.7-7.8)	0.396
Female/Male (n)	4/7	9/16	0.983
Day 0 of corticosteroid treatment			
Serum HSP70, ng/mL	37.4 (31.2-52.5)	37.6 (31.2-43.0)	0.818
Urine HSP70, ng/mL	51.4 (33.9-56.8)	44.1 (38.0-52.2)	0.696
Urine HSP70 creatinine ratio, ng/mg	60.6 (35.7-158.6)	40.6 (29.2-92.0)	0.414
Day 15 of corticosteroid treatment			
Serum HSP70, ng/mL	42.9 (37.2-58.6)	43.0 (37.6-49.2)	0.689
Urine HSP70, ng/mL	37.2 (33.9-48.9)	32.9 (29.9-44.7)	0.178
Urine HSP70 creatinine ratio, ng/mg	107.3 (50.9-364.5)	109.2 (49.8-174.7)	0.756
Day 30 of corticosteroid treatment			
Serum HSP70, ng/mL	35.2 (19.2-49.3)	24.3 (9.1-38.6)	0.235
Urine HSP70, ng/mL	16.3 (10.7-30.9)	10.8 (6.4-25.0)	0.305
Urine HSP70 creatinine ratio, ng/mg	33.7 (18.0-59.9)	48.9 (22.1-139.4)	0.345
Day 90 of corticosteroid treatment			
Serum HSP70, ng/mL	33.8 (24.0-82.5)	34.5 (19.2-50.2)	0.188
Urine HSP70, ng/mL	11.5 (4.8-15.3)	8.9 (7.3-18.3)	0.593
Urine HSP70 creatinine ratio, ng/mg	15.6 (4.9-24.4)	24.0 (7.7-75.0)	0.219

Data are given as median (interquartile range). HSP70: heat shock protein 70.

weight of approximately 70 kDa, is similar in size to albumin and carries a neutral or slightly positive charge. The glomerular filtration barrier selectively filters based on both molecular size and charge, which affects the passage of proteins like HSP70, similar to albumin.[18]

In our previous study on sHSP70 levels in IgA nephropathy (IgAN) and idiopathic NS, higher sHSP70 levels were observed in IgAN and IgA vasculitis nephritis (IgAVN) compared to idiopathic NS.[19] However, when comparing the NS and control groups, sHSP70 levels in patients with idiopathic NS were slightly higher than in controls.[19] These findings are contrary to the findings of the current study. This discrepancy could be attributed to differences in patient demographics, disease profiles, and treatment statuses. Notably, our NS group comprised younger patients with more cases of minimal change disease and steroid-sensitive cases, which may account for these differences. It was interpreted that HSP70 was detected at low serum levels because it was excreted from the glomerulus like albumin in NS.[18]

Our study observed a gradual decline in uHSP70 levels on the 15th, 30th, and 90th days following the initiation of corticosteroid treatment. The observation that uHSP70 levels decrease with prednisolone treatment suggests that uHSP70 might be a more sensitive marker for renal damage and treatment response compared to sHSP70. While uHSP70 levels decreased with treatment, indicating reduced renal stress, sHSP70 levels remained stable, highlighting the potential of uHSP70 as a useful biomarker for monitoring kidney damage and therapeutic efficacy.

Frequent relapsing patients require long-term immunosuppressive treatment and are at risk for complications such as osteoporosis, hypertension, cataracts, and sperm abnormalities.[20] Biomarkers that predict relapse patterns at initial presentation are crucial. Our study found no significant differences in HSP70 levels between frequently and infrequently relapsing SSNS patients, suggesting that HSP70 may not effectively differentiate relapse frequency. This highlights the need for additional biomarkers or clinical parameters to predict relapse patterns more accurately.

The most significant aspect of our study is its longitudinal design, which permitted us to monitor changes in HSP70 levels over time in children with idiopathic NS who received steroid treatment during their initial episode. One of the limitations of our study was the post-power analysis of 72%, which is suboptimal as values below 80%. Another potential limitation pertained to the long-term storage of serum and blood samples at -80 °C. As the ELISA kit manufacturer advised storage at -80 °C for periods exceeding three years, the samples were maintained at this temperature for extended durations. Repeated freeze-thaw cycles were avoided, and all samples were analyzed concurrently. Thus, it was assumed that all samples experienced uniform conditions, minimizing any pre-analytical variation in results. However, data regarding the stability of proteins like HSP70 during long-term storage at -80 °C remain limited.

In conclusion, our study shows that uHSP70 levels are elevated in SSNS prior to treatment and decrease with prednisolone therapy, reflecting reduced renal stress and damage. While HSP70 may be a useful biomarker for monitoring renal damage and treatment response, it does not differentiate between frequent and infrequent relapses. Further research is needed to confirm these findings and identify additional biomarkers for better prediction and management of SSNS.

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Ethical approval

The study was approved by the Ethical Committee of Istanbul University Istanbul Faculty of Medicine (2013/706 and 2023/2222). Written informed consent was obtained from the parents of the patients and the controls.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: BA, ZNYY, AG, AY; data collection: BA, ZNYY, AY; analysis and interpretation of results: BA, ZNYY, AG, AY; draft manuscript preparation: BA, ZNYY, AG, AY. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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