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Original article

The Study of Hemopexin Concentration as a Predictor for Relapsing Nephrotic Syndrome in Children

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Abstract

Background: Nephrotic syndrome (NS) is characterized by progressive loss of proteins, albumin, & other plasma components of comparable mass in patients who present with a syndrome complex, which includes elevated blood lipid concentrations, urinary lipids, & edema. The study aimed to assess the predictive value of urine Hemopexin concentration in kids with Idiopathic Nephrotic Syndrome in relation to disease recurrence and steroid response. **Methods:** This was a comparative study that was performed on 40 children attending Pediatric Nephrology Unit & outpatient Clinic of Benha University Hospital from August 2022 to February 2023 and 20 apparently healthy control kids who were selected from outpatient clinic. Hemopexin concentrations in urine of all subjects (uHpx) were determined by ELISA. **Results:** An extremely significant difference in urinary hemopexin concentrations among the groups ($F=701.562$, $p<0.001$). Both children with idiopathic NS and those with relapses have significantly higher hemopexin concentrations in urine in comparison to the control group ($P1<0.001$, $P2<0.001$), with no significant distinction among the two NS groups ($P3=0.000$). There wasn't statistically significant distinction among types of relapses regarding Hemopexin concentrations in urine. There was statistically significant positive association among Hemopexin concentrations in urine & age. There was statistically significant negative association among Hemopexin concentrations in urine and protein concentrations. **Conclusion:** Our study demonstrated that Hemopexin concentrations in urine were significantly greater in both kids with NS & those with relapses compared to the control group, indicating its potential as a biomarker for disease activity.

Key words: Hemopexin - relapsing nephrotic syndrome- predictor.

Introduction:

NS is characterized by the presence of excessive protein in the urine, widespread swelling, low levels of albumin in the blood, and high levels of lipids, all while maintaining normal kidney function (1)

Neurological symptoms can generally be categorized into main & secondary causes. Primary nephrotic syndrome (PNS), often referred to as idiopathic nephrotic syndrome (INS), is characterized by glomerular disorders that originate within the kidney and are not caused by systemic factors ***(2)***

Idiopathic nephrotic syndrome is the predominant kind of podocytopathy observed in children. It represents over ninety percent of cases in the age group between one and ten years, and roughly fifty percent in the age group of children older than ten years. The estimated incidence rate is approximately sixteen cases per 100,000 individuals in the pediatric population, with a range of two to seven new cases per hundred thousand kids under the age of fifteen. The diagnostic criteria consist of significant proteinuria over 50 mg/kg/day, hypoalbuminemia with levels below 2.5 gram per liter & the presence of edema ***(3)*** **.**

A majority of children diagnosed with nephrotic syndrome have one or many relapses, with the majority of these relapses occurring within the initial six months ***(4)***

Hemopexin (Hpx) is a glycoprotein found in the plasma that circulates in the blood. It has a molecular weight of 60 kDa and is produced by a gene located on the eleventh chromosome (pp. 15.4–15.5). The synthesis of this compound primarily occurs in hepatocytes as a solitary polypeptide chain. The spatial configuration of the molecule is dictated by two disulfide bonds that relate structurally similar domains, one at the C-terminus and the other at the N-terminus, while also attaching the heme group ***(5)*** px has serine protease activity. It demonstrates both anti-inflammatory and pro-inflammatory properties, and it prevents the death of multinuclear granulocytes and the sticking together of cells. Recently, there has been a suggestion that Hpx, when present in the bloodstream, may have an impact on the permeability of the glomerular filtration barrier & the development of proteinuria ***(6).***

Children diagnosed with minimal change nephrotic syndrome (MCNS) exhibited heightened activation of circulating hemopexin (Hx), suggesting that this protein plays a role in the development of the disease **(7).**

This work aimed to assess the urine Hemopexin concentration in kids with Idiopathic Nephrotic Syndrome as a predictor of disease recurrence and response to steroid.

Patients and Methods

This study utilized a cross-sectional design and included a sample of forty kids who were receiving care at the Pediatric Nephrology Unit and Clinic at Benha University Hospitals between August 2022 & February 2024. Additionally, twenty healthy control children of the same age & sex were

included for comparison. A comprehensive history was obtained from all subjects. Information required includes the following: age, sex, age of onset, duration, residency, family history of nephrotic syndrome, kind of treatment, dates, number of relapses, and if the individual has steroid-dependent or steroid-resistant nephrotic syndrome. A full clinical examination was conducted, encompassing general assessment of vital
signs, anthropometric measurements, p resence of edema & ascites, as well as evaluation for bone pain. A full local examination was conducted, which included assessments of the chest, heart, abdomen, and neurological system. The laboratory work was performed in the Clinical Pathology Department at Benha University Hospitals. The study participants were separated into separate groups as follows: The study included three groups: Group I consisted of twenty cases suffering their first episode of nephrotic syndrome, Group II consisted of twenty cases suffering their second episode of nephrotic syndrome, and Group III consisted of twenty healthy kids serving as the control group. The inclusion criteria for the study were laboratory criteria for idiopathic nephrotic syndrome, which included significant proteinuria (> 40 mg/m2/h), hypoalbuminemia (< 2.5 g/L), hypercholesterolemia (> 250 mg/dL), & edema. Additionally, participants were required to have normal renal function, as determined by normal glomerular filtration rate calculated using the Schwartz method (8). Both genders were included, and their age was below eighteen years. The exclusion criteria encompassed secondary nephrotic syndrome.

Ethical Consideration:

Prior to enrolling the children in the study, ethical approval was acquired from the parents, who were fully informed about all research protocols and provided their agreement. The ethical committee of the Faculty of Medicine, Benha University Hospitals granted approval for this investigation. Parents provided informed written consent. The ethical approval code number is (MS 42-7-2022).

Method and sampling:

Urine was collected using a sterile container, centrifugation twenty-minute at the speed of 2000-3000 revolutions per minute was done, then the supernatant was removed, and the samples were stored at <-20°C. Hemopexin concentration levels in urine were measured using an ELISA kit. Five ml of fresh venous blood was collected into the test tube and left for 20 minutes for coagulation, and then it was subjected to centrifuging at a speed of 5000 rpm for 5 minutes to generate serum. Measurements were taken for albumin, creatinine, CRP (C-reactive protein), total cholesterol, urine creatinine, & protein concentrations. GFR was estimated using the Schwartz formula (9). Proteinuria was evaluated using the urinary protein creatinine ratio (uPCR) on a urine sample collected in the morning. A uPCR more than 2 mg/mg was considered the threshold for identifying nephrotic proteinuria. (10). Hemopexin concentrations in urine

(uHpx) were determined by an enzyme-linked immunosorbent assay kit for human (Human Hemopexin (HPX) ELISA Kit); Sun Red Biotechnology, Company Catalogue No. 201-12-3937. Sensitivity: 13.72ng/ml. Assay range:15ng/ml→4000ng/ml.

Statistical Analysis:

The data that was gathered was systematically organized and assessed utilizing SPSS version 24 (SPSS Inc., Chicago, ILL Company) software. For categorical data, percentages and figures were utilized. Analyzing categorical variables required the chi-square test (X2). The measures utilized to express quantitative data were range, mean ± standard deviation, and median. In order to analyze normally distributed variables between two independent groups, the student "t" test was applied. Rho, which stands for Spearman's correlation coefficient, was employed to measure the correlation among non-parametric variables. With regression analysis, the risk of association was examined. To identify cutoff values with optimal sensitivity and specificity, a ROC curve was implemented. In this study, a significance level of 0.05 was established (P <0.05 was deemed to be significant).

Results:

Group 1: Children with idiopathic NS first attack

Group 2: Children with Relapses group

Group 3: Controls group

Table (1) shows that there was no statistically significant distinction among cases & control regarding age & sex. The mean of Hemopexin concentrations in urine was statistically significantly greater amongst cases than control (**table 1)**.

This table showed an extremely significant difference in hemopexin concentrations among the groups (F=701.562, p<0.00). Both children with idiopathic NS and those with relapses have significantly higher hemopexin concentrations in urine compared to the control group (P1<0.001, P2<0.001), with significant variance among the two NS groups (P3=0.000) (**table 2) & Fig 1**.

This table showed that there was no statistically significant variances among types of relapses as regard Hemopexin concentrations in urine (**table 2)**.

This table demonstrations that there statistically significant beneficial association among Hemopexin concentrations in urine and (age, SBP, DBP, total cholesterol, GFR, and protein creatinine ratio) and there statistically significant negative correlation between Hemopexin concentrations in urine and (HR, temperature, RR, albumin, protein concentrations), while there was no statistically significant correlation between Hemopexin concentrations in urine and other parameters (**table 3)**.

Regarding the Diagnostic accuracy of Hemopexin concentrations in urine, Sensitivity was 80%, Specificity was 64.5%, PPV was 100%, NPV was 45.5% and accuracy was 72.5% (**table 4) & Fig 2**.

Table (1): Comparison among Cases & Controls as regards demographic data and Hemopexin concentrations in urine and age of duration & onset among cases group

			Cases No=40	Controls No=20	P. value
Age (years)		**Mean ± SD**	9.57± 4.64	7.70± 2.56	**.099**
Sex	**Female**	**No.**	12	10	**.130**
		%	30.0%	50.0%	
	Male	**No.**	28	10	
		%	70.0%	50.0%	
Hemopexin concentrations in urine (ng/ml)		**Mean ± SD**	47.59± 23.19	15.49± .735	**.000**
Age of onset (years)			**Mean ± SD**		**5.13± 3.37**
Duration (years)			**Mean ± SD**		**4.50± 3.11**

Table (2): Comparison between Children with idiopathic NS first attack group, Children with Relapses group and Controls group regarding Hemopexin concentrations in urine and between types of relapses regarding Hemopexin concentrations in urine.

		Children with idiopathic NS first attack group	Children with Relapses group	Controls group	P. value	LSD
Hemopexin concentrations in urine (ng/ml)	**Mean ± SD**	25.44±2.21	69.74± 8.11	15.49± .735	.000	**P1=.000 P2=.000 P3=.000**
		SDNS No=5	**SRNS No=4**	**FRNS No=7**	**INFRNS No=4**	**P. value**
Hemopexin concentrations in urine (ng/ml)	**Mean ± SD**	**68.3±0.9**	**72.1± 0.7**	**69.6± 0.5**	**0.65**	**0.49**

(SDNS= steroid-dependent nephrotic syndrome, SRNS=steroid-resistant nephrotic syndrome, FRNS =frequent relapsing nephrotic syndrome, INFRNS = infrequent relapsing nephrotic syndrome)

Table (3): Correlation between Hemopexin concentrations in urine & other variable.

Correlation	Pearson's correlation	
	r	p
Age (years) * Hemopexin concentrations in urine	.405	**.001**
Age of onset (years) * Hemopexin concentrations in urine	.267	**.096**
Duration (years) * Hemopexin concentrations in urine	.076	**.641**
SBP * Hemopexin concentrations in urine	.492	**.000**
DBP * Hemopexin concentrations in urine	.463	**.000**
HR * Hemopexin concentrations in urine	-.282-	**.029**
Temerature * Hemopexin concentrations in urine	-.420-	**.001**
RR * Hemopexin concentrations in urine	-.443-	**.000**
Serum creatinine * Hemopexin concentrations in urine	-.110-	**.404**
Albumin * Hemopexin concentrations in urine	-.600-	**.000**
Total cholesterol * Hemopexin concentrations in urine	.646	**.000**
Urine creatinine * Hemopexin concentrations in urine	-.045-	**.735**
Protein concentrations (mg/dl) * Hemopexin concentrations in urine	-.623-	**.000**
GFR * Hemopexin concentrations in urine	.366	**.004**
Protein creatinine ratio * Hemopexin concentrations in urine	.481	**.000**
Hb* Hemopexin concentrations in urine	.125	**.343**
TLC* Hemopexin concentrations in urine	-.002-	**.987**
Platlets* Hemopexin concentrations in urine	**.178**	**.173**

Table (4): Diagnostic accuracy of Hemopexin concentrations in diagnosis of Relapse of NS.

	Cut off value	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy
Hemopexin concentrations in urine	25	.91	80%	64.5%	100%	45.5%	72.5%

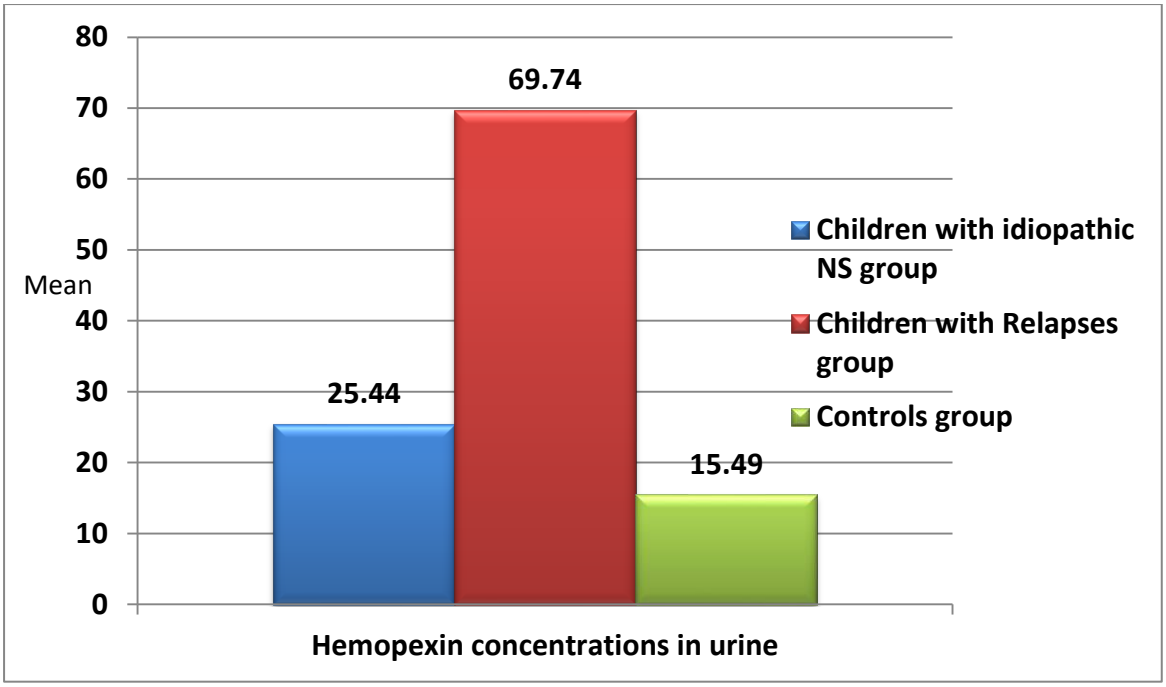

Fig 1: Comparison between Children with idiopathic NS first attack group, Children with Relapses group and Controls group regarding Hemopexin concentrations in urine.

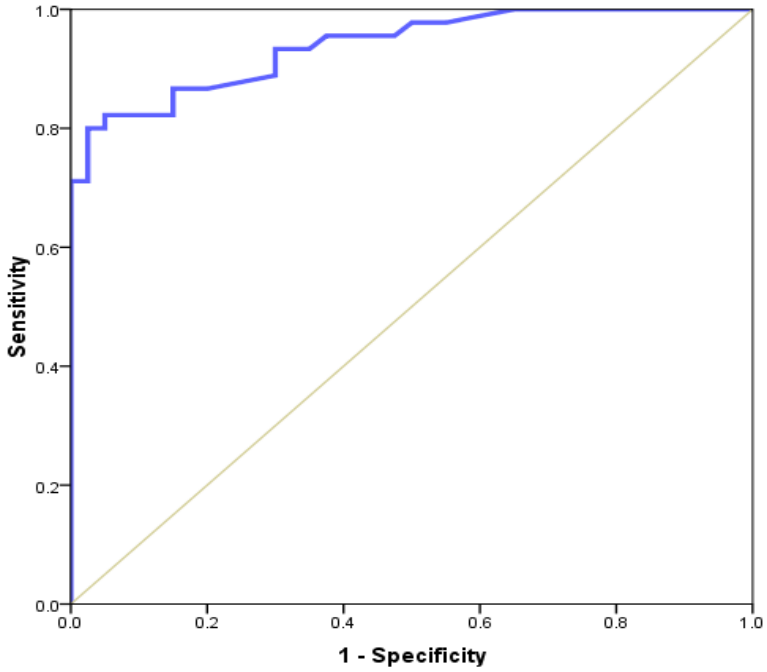

Fig 2: ROC curve of accuracy of Hemopexin concentrations in urine in diagnosis of Relapse of NS.

Discussion

The study found no statistically significant distinction in age among the cases & control groups. The mean age in the cases group was 9.57± 4.64, while in the control group it was 7.70± 2.56.

These findings align with the study conducted by other researchers which

revealed that the age range of cases with NS was between two and fifteen years, with a median age of 4.5. There wasn't statistically significant distinction in age among the patients & control groups (p>0.05) **(11)*.***

Most cases in this investigation were male, comprising seventy percent of the total. There wasn't statistically significant disparity in terms of gender between the cases & control groups***.***

The findings of **some** studies **(12)** & **(13)** corroborated the notion that INS impacts men compared to women.

This also aligns with other researchers who stated that there was a slight male preponderance **(14).**

In this study, an extremely significant difference in urinary hemopexin concentrations among the groups (F=701.562, p<0.001. Both children with idiopathic NS and those with relapses have significantly higher hemopexin concentrations in urine compared to the control group (P1<0.001, P2<0.001), with no significant distinction among the two NS groups (P3=0.000).

In this research, there was no statistically significant distinction among types of relapses regarding Hemopexin concentrations in urine.

These findings support the results of the study done in 2021 **(3)**, where it was observed that there was a significant rise in uHpx levels in the relapse group of the INS study comparison to the control group. During the remission phase, the uHpx values reduced, although they remained considerably elevated compared to those of healthy kids. This observation implies that Hpx may have a role in the development of proteinuria. Additionally, they tried to quantify Hpx levels in infants with nephrotic proteinuria in the context of several glomerulopathies. The urinary Hpx levels are notably elevated in kids who experience relapses after remission, in comparison to kids who have their first episode of INS***.***

A study on the same groups of kids with INS was conducted **(15)** and found similar results. They observed that IL-13, a circulating factor known to increase glomerular permeability in minnimal change disease, had significant effects. The excretion of urinary Hpx was notably greater during relapse compared to remission in children who were solely treated with glucocorticosteroids. The rise in urinary Hpx during remission could be explained to the continued presence of elevated levels of these cytokines in the affected area in children who received combination treatment. This notion is supported by the elevated urine IL-13 levels observed in this case group throughout remission. However, it is important to highlight that these levels are much lower compared to those observed throughout relapse ***.***

It was found that urine Hpx levels were greater in cases suffering a relapse compared to those in remission, but lower compared to the control group **(16)***.*

Hpx causes a restructuring of the cytoskeleton of podocytes by rearranging the actin inside it and

decreasing the glycocalyx, in a manner that depends on nephrin. The researchers also discovered that when podocytes were exposed to plasma from healthy individuals before being treated with Hpx, there was a significant decrease in the extent of cytoskeletal reorganization. This indicates that in cases with minimal change disease lesions, protective plasma factors may be depleted, making podocytes more susceptible to the effects of active Hpx (**7)** .

Hemopexin (Hpx) can be partially released into the bloodstream by glomerular mesangial cells, as demonstrated by the findings of the study done in 2003 **(17).** They confirmed the presence of Hpx in the supernatant of glomerular mesangial cells obtained from healthy persons after being exposed to TNF-alpha. These cells are expected to produce Hpx locally, which in turn directly affects the glomerular filtration barrier, including podocytes**.**

The research found a significant beneficial association among the levels of Hemopexin in urine & age**.**

The level of urinary Hpx varies with age. In kids, it is roughly eighty percent of the adult value, which ranges from 0.4 to 1.5 grams per liter (18).The study found a significant negative association among the levels of Hemopexin in urine and the quantities of protein**.**

A correlation was established among uHpx & proteinuria in their investigation of 557 renal transplant recipients who were at risk of graft loss. Individuals with greater uHpx levels exhibited markedly increased proteinuria & had more rapid malfunction of the transplanted kidney, in contrast to cases with lower Hpx values (19)**.**

It was observed that Hpx influences the permeability of the glomerular filtration barrier, resulting in the occurrence of proteinuria. Hence, it is reasonable to expect a correlation among the examined glycoprotein & proteinuria. Nevertheless, no such correlation was proven. This could be attributed to the limited sample size of the research group **(3)**. The association among Hpx & the cytokine network is substantiated by the findings of other research investigating inflammatory diseases, such as sepsis **(20).**

In this research, there is a statistically significant positive association between Hemopexin concentrations in urine and (SBP, DBP, total cholesterol, GFR, protein creatinine ratio) & there statistically significant negative association among Hemopexin concentrations in urine and (HR, temperature, RR, albumin), while there was no statistically significant correlation between Hemopexin concentrations in urine and other parameters.

In the study done in 2021 **(3),** no statistically significant correlations between uHpx & biochemical markers of nephrotic syndrome across the entire patient population was found.

5. Conclusion:

This study demonstrated that Hemopexin concentrations in urine

were significantly greater in both kids with NS & those with relapses compared to the control group, indicating its potential as a biomarker for disease activity. Additionally, there were significant correlations between Hemopexin concentrations in urine and various clinical parameters, such as age, total cholesterol, blood pressure, glomerular filtration rate, and protein creatinine ratio. However, no significant difference in Hemopexin concentrations was found between the two NS groups (first attack and relapses). These findings suggest that urine Hemopexin concentration could serve as a useful indicator for monitoring disease activity in kids with Idiopathic Nephrotic Syndrome.

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