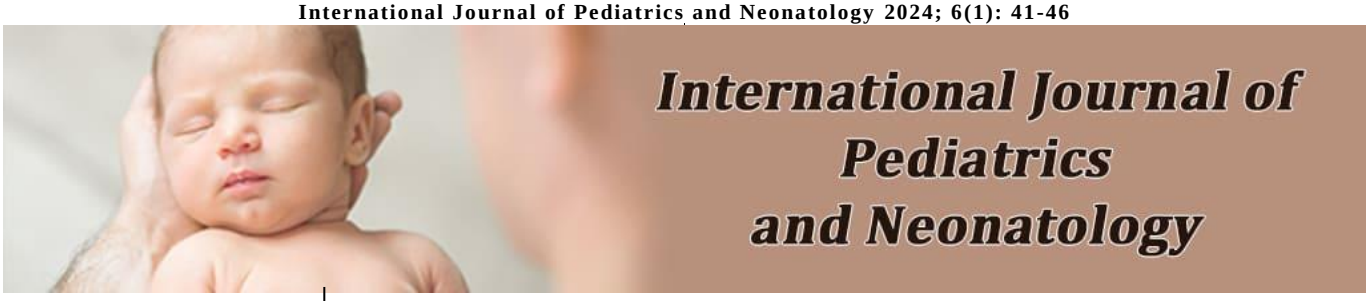

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Clinical significance of ischemia-modified albumin levels in children with steroid-sensitive nephrotic syndrome

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Abstract
Background: Elevated IMA levels are a valuable biochemical indicator for diagnosing myocardial ischemia. Additionally, they are associated with several clinical diseases involving type 2 diabetes, metabolic syndrome, cancer, as well as chronic kidney disease.
The aim of this work was to investigate clinical significance of IMA levels as an oxidative stress' marker among SSNS children throughout remission as well as relapse.
Methods: Our cross sectional descriptive and analytical study involved 90 cases whose ages are older than 2 years, both genders, developing SSNS who were subdivided into two subgroups: [Group AI: Relapse and Group AII: remission and 90 children apparently healthy with normal kidney functions being matched with cases regarding age, sex and socioeconomic status.
Results: IMA exhibited a significant positive association with SBP, DBP, serum cholesterol as well as urinary protein creatinine ratio while negative correlation with serum albumin in the cases group. Serum IMA can significantly predict oxidative stress in children with SSNS at cut-off >95 addressing 100% sensitivity, specificity, PPV and NPV. Cases with relapse exhibited greater IMA level as opposed to others with remission. IMA exhibited significantly greater values within cases group as opposed to controls (p<0.001).
Conclusions: Ischemia modified albumin might be utilized as non-invasive biomarker while diagnosing NS among children, being higher in level in those cases. Ischemia modified albumin could also be used as biomarker for identifying the course of the disease, being higher in cases with disease relapse.

Keywords: Ischemia-modified albumin levels, children, steroid-sensitive nephrotic syndrome, remission, relapse

Introduction
Nephrotic syndrome (NS) represents a renal condition that affects around 1.15-16.9 out of every 100,000 children. Key symptoms of such a condition involve significant amounts of proteins in the urine, low levels of albumin in the blood, swelling, along with changes in lipid levels. The NS etiology remains not completely understood, however research indicates it could be linked to immunological processes, podocyte-related factors, as well as genetic variations [1].
Several research provide evidence that oxidative stress exhibits a significant factor in several kidney disorders' progression, involving NS [2, 3].
Oxidative stress occurs when t an imbalance exists between the formation of reactive oxygen species (ROS) and their removal through antioxidants, leading to harmful cellular structures' circumstances [4].
Higher oxidative stress could induce glomerular hemodynamics' alterations as well as an improved glomerular permeability to proteins**.** Research has shown a strong connection between several oxidative stress indicators as well as proteinuria among those having NS [5, 6].
Albumin, the predominant protein in blood, exhibits a crucial role in transporting various substances, involving medicines, hormones, as well as transition metal ions throughout organisms.

The N-terminal f albumin segment, particularly the N–Asp–Ala–His–Lys sequence, acts as a binding site with strong attraction for transition metal ions involving cobalt, copper, as well as nickel. During ischemia, hypoxia, acidosis, along with enhanced ROS production, the N-terminal segment of this protein undergoes modifications that decrease its capacity to bind transition metal ions. Ischemia-modified albumin (IMA) represents a variant albumin form, reflecting cobalt binding ability of albumin [1].

Elevated IMA levels are a valuable biochemical indicator for diagnosing myocardial ischemia. Additionally, they are associated with several clinical diseases involving type 2 diabetes, metabolic syndrome, cancer, as well as chronic kidney disease [7, 8].

We anticipated that cases having NS could exhibit raised IMA levels due to the correlation of NS with increased oxidative stress. This work was aimed at assessing IMA levels as a marker of oxidative stress among steroid-sensitive nephrotic syndrome (SSNS) children throughout remission as well as relapse.

The aim of this work was to investigate clinical significance of IMA levels as an oxidative stress' marker among SSNS children throughout remission as well as relapse.

Patients and Methods

Our cross sectional descriptive and analytical study involved 90 cases whose ages are older than 2 years, both genders, with SSNS in addition to 90 children apparently healthy with normal kidney functions being matched with cases regarding age, sex and socioeconomic status. The research commenced following the Ethical Committee's approval at Menoufia University Hospital, Menoufia, Egypt. All cases' relatives were allowed to sign an informed consent.

Exclusion criteria involved cases having steroid-resistant NS, tumors, chronic conditions, acute/chronic infection, hepatic disorders, cardiac insufficiency, or secondary or congenital NS along with others consuming immunosuppressive medications as well as NS who were administered albumin, blood, or antioxidant supplements at a two-week timeframe before commencing the research.

Patients underwent further subdivided into two equal groups: Groups A (Cases group): children with SSNS who were subdivided into two subgroups: [Group AI: Relapse was characterized by the presence of 3+ or 4+ protein on dipstick or proteinuria over 40 mg/m2 per hour for a total of 3 days. [9] as well as Group AII: defined SSNS remission as either no detectable protein on dipstick or proteinuria below 4 mg/m² per hour for a total of 3 days] and Group B (Control group): Included 90 children apparently healthy with normal kidney functions being matched with cases regarding age, sex and socioeconomic status.

All subjects underwent a comprehensive medical history taking, clinical assessment as well as lab testing [CBC, serum creatinine, albumin, total protein, cholesterol, urea, alkaline phosphatase (ALP), total calcium, phosphorus, urine sample for 3 consecutive days and urinary protein/ creatinine ratio or 24 hr urine protein test].

Assessment of ischemia modified albumin levels

Utilize a serum separator tube and let the samples coagulate for two h at room temperature or overnight at 4 °C before spinning them in a centrifuge for twenty min at about 1,000 × g. Retrieve the supernatant for analysis. Hemolyzed samples deemed to be unsuitable for this test. Keep undiluted samples at -20 °C or below. Minimize several freeze-thaw cycles.

Ensure all reagents as well as samples are brought to room temperature with no added heating then blended completely via a gentle swirling prior to pipetting for preventing foaming. Follow the instructions provided in the preceding paragraphs while preparing all reagents as well as samples. Dispense 50 microliters of phosphate-buffered saline (0.02 M, pH 7.0-7.2) into an empty well. Dispense five µL of Balance Solution into each fifty µL sample of cell culture supernatant or tissue homogenate, if necessary, and thoroughly blend. Exclude plasma or serum samples, standards, along with the Blank. Dispense fifty microliters of Standard or Sample into each well. Dispense 100 µL of HRP-conjugate into each well, except the Blank well. Seal the plate and incubate at 37 °C for an hour. Remove the liquid from each well then rinse five times. Add about 350 µL of 1x Wash Buffer utilizing a squirt bottle, multi-channel pipette, manifold dispenser, or automated washer for washing. Let each wash stand for ten seconds prior to a full aspiration. Aspirate to remove any leftover Wash Buffer following the final wash, then flip the plate and tap it on clean absorbent paper. Dispense fifty µL of Substrate A along with a fifty µL of Substrate B into every well, involving the Blank. A gentle mixing is accomplished to achieve good blending, then incubate in darkness at 37 °C for fifteen to twenty min. Dispense fifty microliters of Stop Solution into each well. The blue hue will promptly transition to yellow. Tap the plate lightly to ensure complete mixing if the color change was not uniform. Incorporate the Stop Solution to the wells in the same sequence and at the same time as the substrate solution was added. Measure the optical density (OD value) of each well promptly utilizing a microplate reader set at 450 nm.

Statistical analysis

Data were transferred to computer then underwent analysis utilizing IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) Qualitative data were exhibited utilizing number as well as percent. The Kolmogorov-Smirnov test was employed for verifying the normality of distribution Quantitative data were exhibited utilizing range (minimum and maximum), mean, SD, median as well as interquartile range (IQR). The obtained results' significance was set at 5% level.

Results

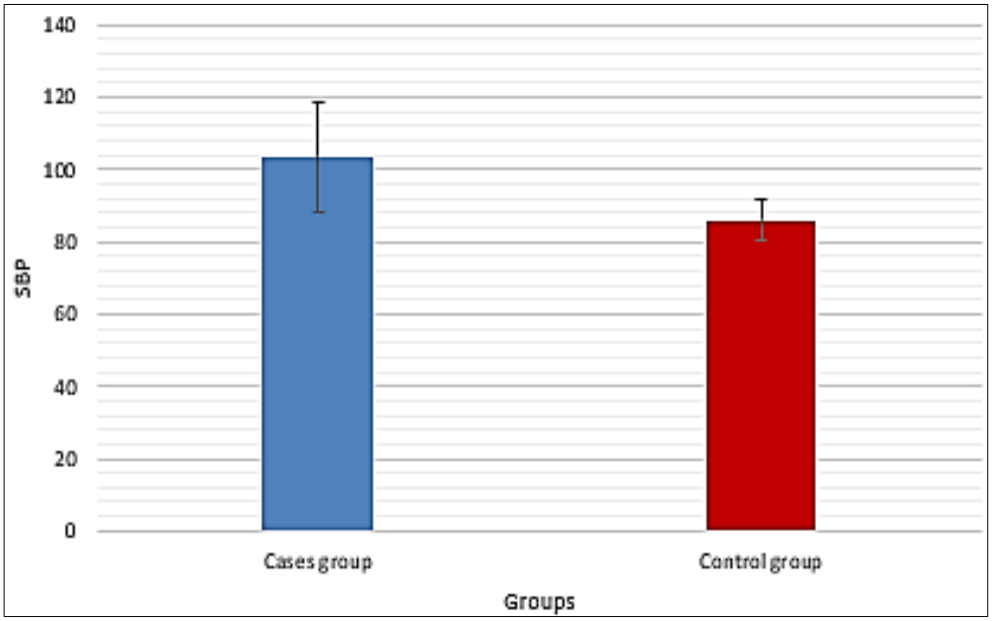
No statistically significant variance was documented among both groups as regards the age, parents' consanguinity, and body weight. There were no cases with positive similar condition in family members of both study groups. The mean height of the body exhibited significantly lower values within the cases group in comparison with controls (p= 0.004). The sex as well as BMI showed significantly greater within cases as opposed to controls (p= 0.002). In the cases group, there were 52 cases in relapse (57.8%) and 38 cases in remission (42.2%). The cases group's median age exhibited 5.0 (2.0-7.0) years. Table 1

Table 1: Comparison among both groups based on demographic data as well as anthropometric measurement

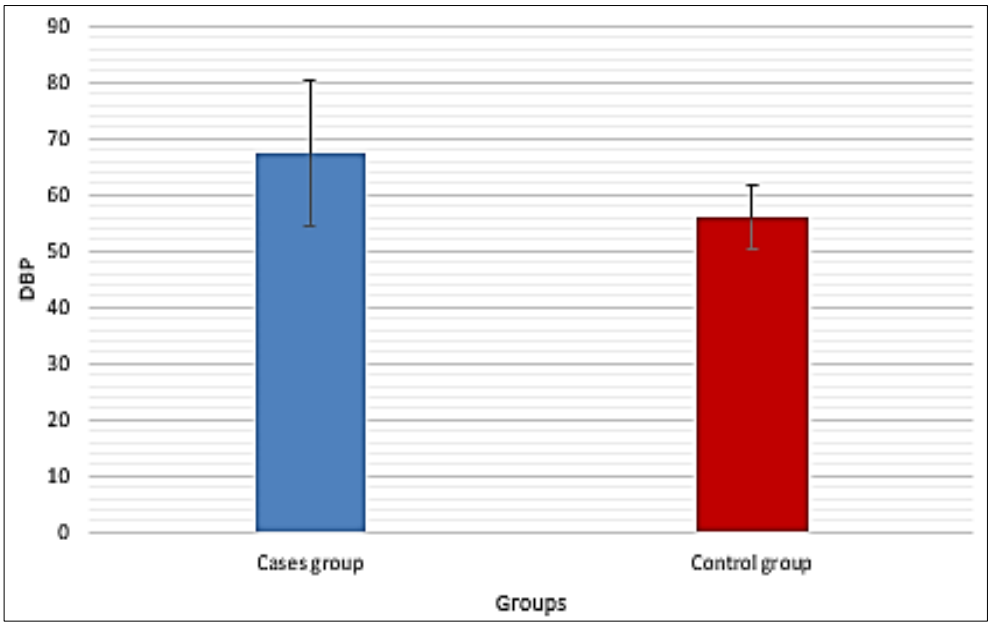
		Cases (n = 90)	Controls (n = 90)	p
Age (years)		8.56 ± 3.57	9.53 ± 3.33	0.054
Sex	Male	66 (73.3%)	48 (53.3%)	0.005*
	Female	24 (26.7%)	42 (46.7%)	
Parents consanguinity		18 (20.0%)	17 (18.9%)	0.851
Similar condition in family members		0 (0.0%)	0 (0.0%)	--
Weight (Kg)		33.82 ± 13.53	36.11 ± 12.44	0.307
Height (cm)		121.6 ± 23.64	131.0 ± 19.66	0.004*
BMI (kg/m^2)		22.06 ± 4.57	20.26 ± 2.69	0.002*
		Cases group (n = 90)		
Course	Relapse	52 (57.8%)		
	Remission	38 (42.2%)		
Onset (years)		5.0 (2.0-7.0)		

Data are exhibited as mean ± SD or frequency (%) or median (IQR). * Significant p value <0.05, BMI: Body mass index.

The mean SBP and DBP exhibited significantly higher values among the cases' group as opposed to the controls (p<0.001). Figure 1.



(A)



(B)

Fig 1: (A) systolic blood pressure and (B) diastolic blood pressure in the two study groups

An insignificant variance was documented among both groups as regards the HB level, WBCs count as well as creatinine level. The mean platelets count, serum cholesterol level, urinary protein creatinine ratio and IMA exhibited significantly greater values among cases as opposed to controls (p= 0.004, <0.001, <0.001 and < 0.001 respectively). The serum albumin level exhibited a significant reduction among cases as opposed to control (p<0.001). Table 2.

Table 2: Comparison among both groups based on laboratory parameters

	Cases (n = 90)	Controls (n = 90)	p
HB (g/dl)	11.83 ± 1.12	11.80 ± 1.04	0.874
WBCS (×10³/µl)	8.15 ± 2.44	7.43 ± 1.75	0.177
PLTS (×10³/µl)	391.5 ± 89.13	331.3 ± 78.37	<0.001*
Serum Albumin (gm/dl)	2.72 ± 1.15	3.95 ± 0.26	<0.001*
Serum Cholesterol (mg/dl)	408.2 ± 96.32	97.94 ± 20.20	<0.001*
Serum Creatinine (mg/dl)	0.50 (0.40 – 0.60)	0.50 (0.40 – 0.60)	0.350
Urinary protein creatinine ratio	7728.7 ± 7312.6	185.1 ± 41.04	<0.001*
IMA (ku/l)	446.2 ± 77.74	65.22 ± 17.24	<0.001*

Data are exhibited as mean ± SD or frequency (%) or median (IQR). * Significant p value <0.05, HB: haemoglobin, WBCs: white blood cell, PLTS: platelets, IMA: ischemia-modified albumin.

As regards cases group, a significant positive association was documented between IMA and SBP, DBP, serum cholesterol as well as urinary protein creatinine ratio whereas a significant negative association was documented among IMA and serum albumin. other correlations weren't statistically significant. Table 3.

Table 3: Correlation among serum IMA as well as different parameters within cases group

	Serum IMA	
	r	p
Age (years)	0.053	0.622
Onset (years)	0.040	0.711
Weight (kg)	-0.022	0.835
Height (cm)	-0.067	0.531
BMI (kg/m^2)	0.035	0.744
Systolic (mmHg)	0.265	0.012*
Diastolic (mmHg)	0.250	0.018*
HB (g/dl)	-0.129	0.226
WBCS (×10³/µl)	0.192	0.070
PLTS (×10³/µl)	-0.060	0.575
Serum albumin (gm/dl)	-0.550	<0.001*
Serum cholesterol (mg/dl)	0.389	<0.001*
Serum creatinine	0.059	0.578
Urinary protein Creatinine ratio	0.481	<0.001*

r: Pearson coefficient, *: Statistically significant at $p \leq 0.05$, BMI: Body mass index, HB: Haemoglobin, WBCs: White blood cell, PLTS: platelets, IMA: ischemia-modified albumin, SBP: systolic blood pressure, DBP: diastolic blood pressure.

As regards cases group, no statistically significant variance was documented as regards serum IMA according to sex and parents' consanguinity. However, the cases with relapse showed higher IMA level as compared to the cases with remission. Table 4.

Table 4: Association between Serum IMA as well as different parameters in cases group

		N	Serum IMA	P
Sex	Male	66	443.48 ± 72.54	0.582
	Female	24	453.75 ± 91.82	
Course	Relapse	52	481.54 ± 46.42	<0.001*
	Remission	38	397.89 ± 86.11	
Parents consanguinity	Negative	72	450.14 ± 78.42	0.342
	Positive	18	430.56 ± 75.01	
Albumin in urine	Remission (Nil + Trace)	38	397.89 ± 86.11	<0.001*
	Relapse	52	481.54 ± 46.42	

Data are exhibited as mean ± SD. *: Statistically significant at $p \leq 0.05$, IMA: ischemia-modified albumin.

Serum IMA can significantly predict oxidative stress among steroid-sensitive NS (SSNS) children throughout remission as well as relapse (P value <0.001) and AUC = 1) at cut-off >95 addressing 100% sensitivity, 100% specificity, 100% PPV and 100% NPV. Figure 2.

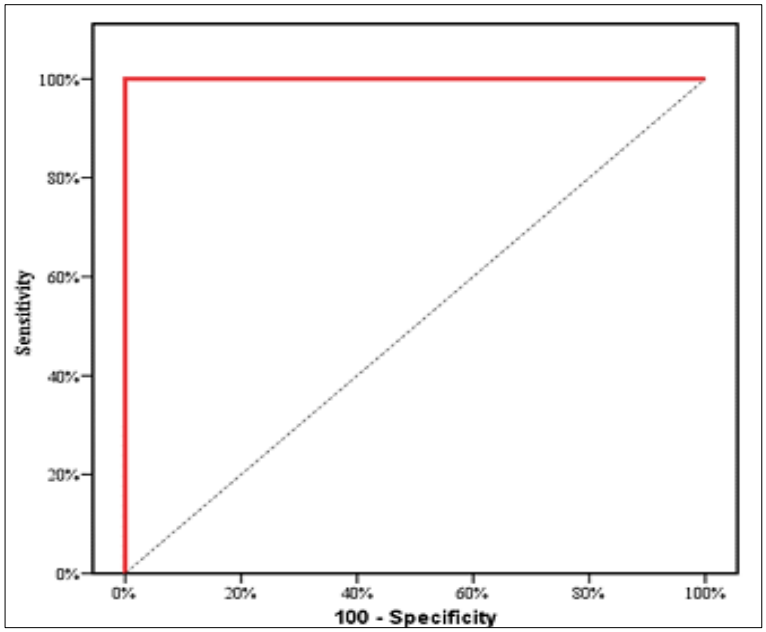


Fig 2: ROC curve for Serum ischemia-modified albumin (IMA) to discriminate patients (n = 90) from control (n = 90)

Discussion

IMA represents a novel oxidative stress marker, assessed using the albumin cobalt binding test, rising as a result of cardiac as well as skeletal muscle ischemia [10]. IMA levels have been studied in inflammatory conditions, involving obesity, asthma, appendicitis, as well as irritable bowel syndrome. IMA was also assessed in infectious disorders such bacteremia, neonatal sepsis, as well as pneumonia [11-13].

Our research addressed insignificant variation among both groups as regards the hemoglobin levels and WBCs count. The mean platelets count exhibited statistically significantly higher within the cases group in comparison with controls. This agreed with Elmogy [14] who showed significant increases as regards hemoglobin levels as well as platelets within cases as opposed to controls, whereas no significant variance was documented as regards as regards serum creatinine levels as well as WBCs between both groups. High platelet count supported the research by Andre *et al.* [15] explaining the thrombotic risk that was documented within some NS cases. Our research also addressed, the serum albumin exhibited significantly lower levels within patients as opposed to controls, which supported Tahir *et al.* [16] addressing a highly significant reduction ($p< 0.001$) of serum albumin as well as serum total protein among newly-diagnosed NS children in comparison with controls. Within the same line, Elmogy [14] reported a highly significant reduction could be observed within serum albumin levels among cases having primary NS in comparison with healthy controls.

Regarding our research, the mean urinary protein creatinine ratio exhibited statistically significantly higher values among cases in comparison with controls. This agreed with El Ghannam *et al.* [17] who addressed a statistically significant rise within nephrotic group as opposed to controls regarding Albumin/Creatinine Ratio. As regards our research, the serum cholesterol exhibited statistically significantly greater levels among the cases in comparison with controls.

This supported Tahir *et al.* [16] addressing a highly significant increase of Serum Triglycerides and Serum Low Density Lipoprotein Cholesterol among newly-diagnosed NS children as opposed to controls. Moreover, they reported a significant decrease as regards S.HDL-C among newly-diagnosed NS children in comparison with controls. The current results also agreed with Elmogy [14] reporting a highly significant rise as regards triglycerides as well as cholesterol serum levels among cases primary NS in comparison with healthy controls. Regarding our research, the mean serum IMA exhibited statistically significantly greater values among cases as opposed to controls, supporting Cakirca *et al.* [18] who addressed, IMA as well as adjusted-IMA exhibited significantly greater values. Additionally, albumin also showed significantly reduced values within SSNS-relapse group as opposed to the SRNS-remission group as well as controls. Moreover, IMA as well as adjusted-IMA exhibited significantly greater values and albumin exhibited significantly reduced values within the SSNS-remission group as opposed to controls. Ischemia causes a lack of oxygen in cells, leading to changes within albumin molecule structure. This alteration enhances albumin's ability to create more chemical bonds with metal ions, often nickel, in laboratory experiments, enabling the IMA test to identify the existence of ischemia [19]. IMA is believed to be created by an interaction with reactive oxygen species and/or hydroxyl radicals [20].

In the current study, within cases group, a statistically significant positive association was documented between IMA and SBP, DBP, serum cholesterol as well as urinary protein creatinine ratio whereas a statistically significant negative association was documented among IMA and serum albumin. Other correlations weren't statistically significant, which supported Cakira *et al.* [18] addressing, IMA levels exhibit an inverse correlation with albumin levels among SSNS cases.

The current findings align with other studies addressing elevated protein oxidation levels among NS cases as opposed to controls. For example, Fan *et al.* [21] observed greater levels of advanced oxidation protein products. Also, Yazilitas *et al.* [22] addressed an elevated thiol oxidation among NS cases as opposed to controls.

Regarding our research, the cases with relapse showed higher IMA level as compared to the cases with remission. This agreed with Cakira *et al*. [18] showed that IMA along with adjusted-IMA exhibited significantly greater levels while albumin levels exhibited a significant reduction within SSNS-relapse group as opposed to the SRNS-remission group as well as controls.

Limitations: A single-center study with a relatively modest sample. Therefore, we recommend further studies including larger number of cases from many centres over large geographic area. Further prospective studies should be carried out for better assessing the IMA role in prediction of the disease for treatment. Further studies should be conducted to test other non-invasive biomarkers while diagnosis of NS and monitoring the disease course.

Conclusions

Ischemia modified albumin might be utilized as non-invasive biomarker while diagnosing NS among children, being higher in level in those cases. Ischemia modified albumin could also be used as biomarker for identifying the course of the disease, being higher in cases with disease relapse.

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Conflict of Interest: Nil.

References

1. Noone DG, Iijima K, Parekh R. Idiopathic nephrotic syndrome in children. The Lancet. 2018;392:61-74.
2. Mishra OP, Gupta AK, Prasad R, Ali Z, Upadhyay RS, Mishra SP, *et al*. Antioxidant status of children with idiopathic nephrotic syndrome. Pediatric Nephrology. 2011;26:251-256.
3. Bakr A, Abul Hassan S, Shoker M, Zaki M, Hassan R. Oxidant stress in primary nephrotic syndrome: Does it modulate the response to corticosteroids? Pediatric Nephrology. 2009;24:2375-2380.
4. Özcan O, Erdal H, Çakırca G, Yönden Z. Oxidative stress and its impacts on intracellular lipids, proteins and DNA. Journal of Clinical and Experimental Investigations. 2015;6:331-336.
5. Kamireddy R, Kavuri S, Devi S, Vemula H, Chandana D, Harinarayanan S, *et al*. Oxidative stress in pediatric nephrotic syndrome. Clinica Chimica Acta. 2002;325:147-150.
6. Begenik H, Soyoral YU, Erkoc R, Emre H, Taskın A, Tasdemir M, *et al*. Serum malondialdehyde levels, myeloperoxidase and catalase activities in patients with nephrotic syndrome. Redox Report. 2013;18:107-112.
7. Zurawska-Płaksej E, Grzebyk E, Marciniak D, Szymańska-Chabowska A, Piwowar A. Oxidatively modified forms of albumin in patients with risk factors of metabolic syndrome. Journal of Endocrinological Investigation. 2014;37:819-827.
8. Cichota LC, Moresco RN, Duarte MM, da Silva JE. Evaluation of ischemia-modified albumin in anemia associated with chronic kidney disease. Journal of Clinical Laboratory Analysis. 2008;22:01-05.
9. Noone DG, Iijima K, Parekh R. Idiopathic nephrotic syndrome in children. The Lancet. 2018;392:61-74.
10. Öncel EDA, Tüz A, Keleş Y, Şahin A, Üstündağ G, Taşar S, *et al*. Evaluation of ischemia-modified albumin in the diagnosis and clinical severity of COVID-19 in children. Turkish Journal of Pediatrics. 2023;65:15-19.
11. Aykac K, Ozsurekci Y, Demir OO, Avcioglu G, Erel O, Ceyhan M. Oxidant and antioxidant balance in children with bacteremia. Minerva Pediatrica. 2020;17:15-20.
12. Bolatkale M, Duger M, Ülfer G, Can Ç, Acara AC, Yiğitbaşı T, *et al*. A novel biochemical marker for community-acquired pneumonia: Ischemia-modified albumin. The American Journal of Emergency Medicine. 2017;35:1121-1125.
13. Wang IK, Chang Y-C, Liang C-C, Chuang F-R, Chang C-T, Lin H-H, *et al*. Bacteremia in hemodialysis and peritoneal dialysis patients. Internal Medicine. 2012;51:1015-1021.
14. Elmougy R. Genetic studies of nephrotic syndrome in Egyptian children. Journal of Applied Biomedicine. 2021;19:228-233.
15. Andre E, Voisin P, Andre JL, Briquel ME, Stoltz JF, Martinet N, *et al*. Hemorheological and hemostatic parameters in children with nephrotic syndrome undergoing steroid therapy. Nephron. 1994;68:184-191.
16. Tahir N, Hashim RK, Bohan A, Mahmood L. Assessment of Cystatin-C level in Newly Diagnosed Iraqi Children with Nephritic Syndrome. Open Access Macedonian Journal of Medical Sciences. 2022;10:639-643.
17. El Ghannam MZ, Ali AAEM, El Samanoudy MI, Kamel AR. Assessment of The Level of Protein C in Children with Nephrotic Syndrome. Egyptian Journal of Hospital Medicine. 2019;76:3828-834.
18. Cakirca G, Guzelcicek A, Yilmaz K, Nas C. Increased ischemia-modified albumin levels in children with steroid-sensitive nephrotic syndrome. Pakistan Journal of Medical Sciences. 2020;36:1490-1499.
19. Pan G, Chen J, Lv C, Lin X, Huang J, Lin B, *et al*. The clinical significance of ischaemia-modified albumin in acute coronary syndrome and hypertension. Cardiology in the Young. 2023;175:01-06.
20. Holte C, Szafranska K, Kruse L, Simon-Santamaria J, Li R, Svistounov D, *et al*. Highly oxidized albumin is cleared by liver sinusoidal endothelial cells via the receptors stabilin-1 and-2. Scientific Reports. 2023;13:40-49.
21. Fan A, Jiang X, Mo Y, Tan H, Jiang M, Li J. Plasma levels of oxidative stress in children with steroid-sensitive nephrotic syndrome and their predictive value for relapse frequency. Pediatric Nephrology. 2016;31:83-88.
22. Yazılıtaş F, Oztek-Celebi FZ, Erel Ö, Çakıcı EK, Alışık M, Bülbül M. Dynamic thiol/disulphide homeostasis in children with nephrotic syndrome. Nephron. 2019;142:17-25.

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