ORIGINAL ARTICLE

Oxidative stress markers in initial therapy and remission of nephrotic syndrome and serum malondialdehyde level predictor from routine laboratory test

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ABSTRACT

Aim To compare oxidative stress state of children with nephrotic syndrome at the first week of treatment and in remission, and to predict malondialdehyde (MDA) level from routine laboratory tests.

Methods This cross-sectional study involved 80 1-18 years old children with nephrotic syndrome, who were divided into two groups: initial group (40 children in the first week of therapy) and remission group (40 children in remission). Demographic characteristics of the patients were taken by a questionnaire. Laboratory tests were measured in the initial group; in the remission group negative or trace proteinuria was measured for three consecutive days. Serum urea, creatinine, albumin, total cholesterol, MDA, superoxide dismutase, glutathione peroxidase, and urine albumin-to-creatinine ratio (UACR) were measured and compared between the groups. Albumin, total cholesterol, and UACR were subjected to predict high serum MDA using a mean of all patients' MDA level as a cutoff.

Results There were higher albumin levels and lower UACR, total cholesterol, and MDA in the remission group compared to the initial group. Albumin and UACR showed good accuracy, and total cholesterol showed very good accuracy to predict serum MDA level more than 1.35 μmol/L.

Conclusion Children with nephrotic syndrome in the first week of therapy showed a higher oxidative stress state than the children in remission. Serum albumin, serum total cholesterol, and UACR can predict serum MDA level with good accuracy.

Key words: antioxidant, glutathione peroxidase, nephrosis, reactive oxygen species, superoxide dismutase

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INTRODUCTION

Nephrotic syndrome is a common paediatric kidney disease characterized by hypoalbuminemia, oedema, and hyperlipidemia arising from large urinary losses of protein (1,2). Mostly of idiopathic cause, it is classified based on patients' response to corticosteroid medication (3). Histological classification of idiopathic nephrotic syndrome in childhood includes minimal change disease (MCD), focal segmental glomerulosclerosis (FSGS), and other nephropathies (1).

Previous research has shown the association between oxidative stress and nephrotic syndrome (4). Persistent oxidative stress can result in DNA damage (5,6). Excessive oxidative stress in glomeruli can cause oedema, foot process fusion, and epithelial vacuolization thus contributing to the development of the nephrotic syndrome (7). Patients with high oxidative stress were correlated to a higher frequency of relapse (4,8) and are more likely to be steroid-resistant or steroid-dependent (9). Some studies reported the roles of malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPX) as oxidative stress markers (5,10,11). Lipid peroxidation produces MDA directly, therefore MDA is believed to be the optimal biomarker of oxidative stress (12). Antioxidant enzymes such as SOD and GPX degrade oxidative stress and its concentration altered during the development of oxidative stress in nephrotic syndrome children (13). However, oxidative stress biomarkers were expensive and not readily available, especially in developing countries. Therefore, other laboratory parameters were needed to predict the oxidative stress biomarkers in patients with nephrotic syndrome.

The aim of this study was to investigate oxidative stress state of children with nephrotic syndrome in the first week of treatment and to compare it with the children in remission, and to predict MDA level from routine laboratory tests.

PATIENTS AND METHODS

Patients and study design

This study was conducted as a cross-sectional study at the Paediatric Outpatient and Paediatric Ward Departments of the H. Adam Malik General Hospital, Medan, Indonesia during the period January - December 2018. All patients were 1 to 18 years old with nephrotic syndrome in the first week of therapy, confirmed by massive proteinuria (>40 mg/m^2 per hour or urinary protein/creatinine ratio (PrU/CrU) in urine >2 mg/mg or in urine dipstick ≥ 2+), hypoalbuminemia ≤ 2.5 g/dL, and oedema; remission (complete) confirmed by negative or trace proteinuria for three consecutive days. Chronic kidney disease patients with glomerular filtration rate ≤ 60 mL/minute per 1.73 m^2 and patients with systemic disease such as malignancy, pulmonary tuberculosis, severe malnutrition, obesity, cardiac diseases, liver diseases, systemic lupus erythematosus, and Henoch-Schonlein purpura were excluded.

A written informed consent was obtained from all patients' legal guardians prior to the study enrollment. The study was approved by the Health Research Ethical Committee, Faculty of Medicine, Universitas Sumatera Utara.

This study involved 40 nephrotic syndrome children in the first week of therapy (initial group) and 40 nephrotic syndrome children in complete remission (remission group). All patients or patients' legal guardians were interviewed using a structured questionnaire to note gender, age, nutritional status, and known systemic diseases. Weight-for-height was used to determine nutritional status based on the WHO paediatric growth indicators (14).

Methods

Six millilitres of blood samples were collected from all patients after 8-10 hours of fasting, and they were subjected to measuring serum urea, creatinine, albumin, total cholesterol, MDA, SOD, and GPX level. Serum urea, creatinine, albumin, and total cholesterol were measured using the SMAC autoanalyzer (Technicon, Tarrytown, NY, USA). Serum MDA level was measured by high performance liquid chromatographic (HPLC) using Agilent 1200 HPLC system (San Jose, CA, USA) with commercial MDA kits (Immundiagnostik AG, Bensheim, Germany). Serum SOD and GPX were measured using Advia 1800 instrument (Siemens Healthcare GmbH, Germany) with Ransel Glutathione Peroxidase kit (Randox Laboratories, London, UK) and Ransod kit (Randox Laboratories, London, UK). All urine samples were collected and subjected to measure albumin and creatinine by urine albumin-to-creatinine ra-

tio (UACR) assay kit (MyBioSource, US). Serum albumin, UACR, and total cholesterol were categorized with albumin <2.43 mg/dL, UACR >1.8 mg/g, and total cholesterol >220 mg/dL as the cutoff. Serum MDA was categorized using the mean value of all patients' serum MDA as the cutoff. Serum albumin, UACR, and total cholesterol were subjected to predict high serum MDA.

Renal biopsy was not performed as most children with steroid-sensitive idiopathic nephrotic syndrome fitted the standard clinical presentation of minimal change nephrotic syndrome that did not require a routine renal biopsy (15).

Statistical analysis

Demographic characteristics were analysed for differences between the initial group and the remission group by using the □2 test for categorical data, independent t-test for normally distributed numerical data, and Mann-Whitney U test if the distribution was not normal. Fisher's exact test was used if □2 test assumptions were not met. Serum urea, creatinine, albumin, UACR, total cholesterol, MDA, SOD, and GPX were compared between the initial group and the remission group by using independent t-test if data were normally distributed, otherwise, Mann-Whitney U test was used. Pearson's correlation test was used to determine the correlation between albumin, UACR, total cholesterol with MDA. Overall accuracy was evaluated using the area under curve (AUC) of receiver operating curve (ROC), and specific diagnostic accuracy was evaluated using sensitivity (Sn), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), and negative likelihood ratio (NLR), then diagnostic effectiveness (accuracy) was measured.

Differences were considered statistically significant at $p<0.05$.

RESULTS

Gender, age, and nutritional status (weight-for-height) were similar among the initial group and the remission group (Table 1). There were no significant differences in gender (p=0.317), age (p=0.390), and nutritional status (p=0.731) between the initial and the remission group.

Table 1. Demographic characteristics of 80 patients with nephrotic syndrome

Characteristic	Initial group	Remission group	p
Gender (No %)			
Male	31 (77.5)	27 (67.5)	0.317
Female	9 (22.5)	13 (32.5)	
Nutritional status (No, %)			
Underweight	5 (12.5)	4 (10)	0.731
Normal weight	32 (80)	31 (77.5)	
Overweight	3 (7.5)	5 (12.5)	
Mean age (SD) (years)	4.54 (1.58)	4.18 (1.22)	0.390

BMI, body mass index;

There were significantly higher albumin levels ($p<0.001$) and lower UACR ($p<0.001$), total cholesterol ($p<0.001$), and MDA levels ($p<0.001$) in the remission group compared to the initial group (Table 2).

Table 2. Serum biomarker in patients with nephrotic syndrome in the initial group and the remission group

Mean biomarker value (SD)	Initial group	Remission group	Mean difference (CI 95%)	p
Urea (g/dL)	24.96 (5.18)	24.91 (5.3)	0.05 (-4.61- 6.39)	0.986
Creatinine (g/dL)	0.7 (0.09)	0.64 (0.07)	0.06 (-0.001 - 0.16)	0.232
Albumin (g/dL)	1.8 (0.34)	3.04 (0.26)	-1.2 (-1.45 - -1.01)	<0.001
UACR (mg/g)	3.36 (0.41)	0.48 (0.16)	2.88 (2.65 - 3.24)	<0.001
Total cholesterol (mg/dL)	279.66 (41.32)	163.81 (10.07)	115.9 (96.13 - 152.23)	<0.001
Oxidative stress				
MDA (µmol/L)	1.52 (0.22)	1.14 (0.49)	0.38 (0.28 - 0.56)	<0.001
SOD (U/gHb)	1155.38 (184.9)	1279.89 (270.37)	124.5 (-28.1 - 198.9)	0.095
GPX (U/gHb)	30.71 (6.35)	33.56 (5.29)	2.85 (-1.02 - 4.8)	0.132

UACR, urine albumin/creatinine ratio; MDA, malondialdehyde; SOD, superoxide dismutase; GPX, glutathione peroxidase; CI, confidence interval;

There were no significant differences in urea (p=0.986), creatinine (p=0.232), SOD (p=0.095), and GPX level (p=0.132) between the groups.

The mean serum MDA level of all patients 1.35 µmol/L would be used as a cutoff point. Predictive analyses showed good accuracy of albumin and UACR (AUC 0.75 and 0.76, respectively), and very good accuracy of total cholesterol (AUC 0.84) (Figure 1).

There was moderate negative correlation between albumin and serum MDA level ($r=-0.596$; $p<0.001$). Moderate positive correlation was found between UACR and serum MDA level ($r=0.485$; $p=0.002$). A strong positive corre-

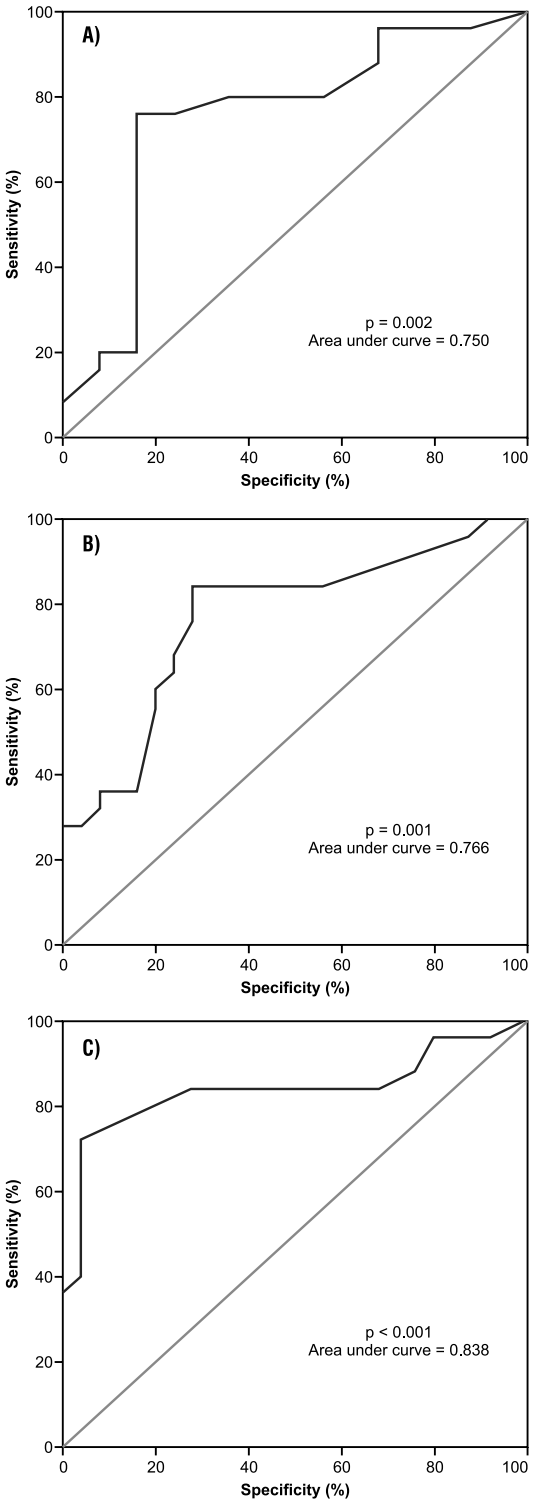

Figure 1. Receiver operating curves of A) albumin, B) urine albumin/creatinine ratio (UACR), C) total cholesterol level to predict serum MDA level higher than 1.35 μmol/L in nephrotic syndrome children

lation was found between total cholesterol and serum MDA level (r=0.674; p<0.001). Good performance diagnostic accuracy of albumin lower than cutoff score and UACR and total cholesterol higher than cutoff score to predict high MDA higher than 1.35 μmol/L were found (Table 3).

Table 3. Specific diagnostic accuracy of variables to predict high serum MDA level in nephrotic syndrome children*

Variable (cutoff point)	Sn (%)	Sp (%)	PPV (%)	NPV (%)	PLR	NLR	Accuracy (%)
Albumin (at 2,43 g/dL)	76	84	83	78	4.75	0.29	80
UACR (at 1,8 mg/g)	84	72	75	82	3	0.22	78
Total cholesterol (at 220 mg/dL)	72	96	95	77	18	0.29	84

*Serum MDA level of 1.35 μmol/L was used as cutoff ; Sn, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio

DISCUSSION

The association between nephrotic syndrome and oxidative stress state has been reported in previous studies (10–13). Overproduction of reactive oxygen species (ROS) and impairment of antioxidant enzymes can cause oxidative stress state (5,8,13). Excessive oxidative stress resulting from an inflammation reaction can cause the injury of glomerular filtration membrane through the destruction of the electrostatic barrier, and injury of endothelial cells and podocytes (16).

Lipid peroxidation, a particular reaction of ROS with lipids, produces MDA as its direct byproduct (12,17). This study showed the oxidative stress state in paediatric nephrotic syndrome patients, especially in the initial group with higher serum MDA level compared to the remission group. This is in concordance with a previous study by Reddy et al. (5) that showed higher MDA level in the active group (children with nephrotic syndrome during first episode/relapse) compared to the remission group, and even in the remission group compared to the control group (children without nephrotic syndrome). This result indicated that the changes in oxidative stress persist even after remission.

The first line of defence against ROS *in vivo* is antioxidant enzyme SOD (18). This enzyme protects the cell from harmful superoxide by redox reaction to decrease the level of superoxide and mitigate oxidative stress (19). Another antioxidant enzyme, GPX, is a selenium-dependent enzyme that reduces intracellular hydrogen peroxide and lipid peroxides by redox reaction (20). These antioxidant enzyme (SOD and GPX) activities were altered during the development

of oxidative stress in nephrotic syndrome (4). However, this study did not show significant differences in antioxidant enzymes (SOD and GPX) between the initial group and the remission group. These findings are discordant with previous studies by Reddy et al. (5) and Fydryk et al. (11,21). This might be due to the fast reaction rate and short half-life of SOD (19) and due to selenium dependency of GPX activities (21).

The correlation between high oxidative stress state with hypoalbuminemia, proteinuria, and hypercholesterolemia was found in our study. This is concordant to previous studies which revealed the association between oxidative stress with proteinuria (UACR), hypoalbuminemia, and hypercholesterolemia (22,23). A previous study by Zhou et al. (22) showed oxidative stress-induced podocyte dysfunction via Wnt/β-catenin activation, whereas, hypoalbuminemia decreases sphingosine 1 phosphate (S1P) availability in the endothelium thus inducing oxidative stress and increasing vascular permeability (24,25). Hypercholesterolemia, however, induces oxidative stress in endothelial cells and initiates peroxidation of cell membranes and unsaturated fatty acids by itself thus producing oxidized low-density lipoproteins intensifying the oxidative stress (23). Therefore, it is concordant to this study that showed good accuracy of UACR, albumin, and total cholesterol to predict the oxidative stress state of patients with nephrotic syndrome.

Some limitations of this study should be noted. This study did not compare MDA level in the same patient before and after therapy with controls, and also steroid-sensitive with steroid-resistant nephrotic syndrome patients.

In conclusion, this study is the first study to predict the oxidative stress by MDA level from routine laboratory tests. Children with nephrotic syndrome in the first week of therapy have higher oxidative stress state than those in remission. Oxidative stress state of children with nephrotic syndrome can be predicted by measuring routine laboratory tests such as albumin, total cholesterol, and UACR. Serum albumin level less than 2.43 mg/dL, serum total cholesterol more than 220 mg/dL, and UACR more than 1.8 mg/g can predict MDA level more than 1.35 μmol/L with good accuracy.

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CONFLICTS OF INTEREST

Competing interests: None to declare

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