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SERUM MALONDIALDEHYDE LEVELS IN CHILDREN WITH NEPHROTIC SYNDROME IN FULL DOSE CORTICOSTEROID AND FINISHED THERAPY

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ABSTRACT

A cross-sectional study of 50 nephrotic syndrome (NS) patients that were admitted to Pediatric Nephrology Department of Adam Malik General Hospital, Medan, Indonesia was conducted to determine the difference of Malondialdehyde (MDA) levels in NS patients who were receiving full-dose corticosteroid therapy and finished therapy. Diagnosis criteria for NS are massive proteinuria (>40mg/ m2/ hour or urine albumine creatinine ratio (UACR) >2 mg/g or dipstick ≥2+), hypoalbuminemia ≤ 2.5 g/dL, generalized edema, and may accompanied by hypercholesterolemia.

MDA levels were significantly higher in NS patients who were receiving full dose corticosteroid therapy than those who finished therapy. Furthermore, there was a significant negative correlation between albumin and MDA levels, and significant positive correlations between UACR and total cholesterol levels with MDA levels.

Introduction

Nephrotic syndrome (NS) is the most common kidney disease in pediatric patients. The incidence were about 2-7 cases per 100,000 children under 16 years. Approximately 90% of patients with NS are idiopathic and steroid sensitive with a good prognosis.[1] There are several symptoms of NS such as massive proteinuria (> 40 mg /m^2/ hour or urine albumine creatinine ratio (UACR) >2mg/g or dipstick ≥2+), hypoalbuminemia ≤ 2.5 g/dL, generalized edema, and may be accompanied with hypercholesterolemia.[2] The pathogenesis of NS remains controversial. The clinical response of NS to systemic immunosuppressant supports that NS is an inflammatory disease. The presence of recruitment and accumulation of proinflammatory cytokines causes podocyte damage as well as the cause of massive proteinuria in NS. Inhibition of proinflammatory cytokine synthesis and receptors becomes the basis of immunosuppressive therapy in NS.[3]

Significant inflammatory reactions can induce reactive oxidative species (ROS) that can cause further tissue damage, cell metabolism disruption, and DNA damage. Previous study suggests that accumulation of free radicals were responsible for nephrosis injury. Hence the paradigm emerges that inflammation and increased free radicals involved in the pathogenesis of NS.[4]

Free radicals have very short half-lives that are difficult to measure with laboratory studies. Damage done to lipid tissues due to free radicals can be measured with malondialdehyde (MDA) which is a lipid peroxidation product. The production of free radicals is indirectly assessed by lipid peroxidation.[5] Research on MDA levels in NS patients especially on pediatric patients was not widely known, and studies for comparison of MDA levels between pediatric patients with NS receiving full dose corticosteroid therapy and finished therapy was limited. In this study, MDA levels were measured in patients with NS who were receiving full-dose corticosteroid therapy and finished therapy, and assessed for correlation of MDA levels with albumin, total cholesterol, and UACR levels, as early predictors of suspicion of high free radicals to account for whether or not to provide antioxidant supplementation to the NS patients.

Methods

Patient Selection

This cross-sectional study was conducted on 50 NS patients that were admitted to Pediatric Nephrology Department of Adam Malik General Hospital, Medan, Indonesia between April and December 2017. Inclusion criteria were NS patients who were receiving full dose and finished treatment of corticosteroid, aged 1-18 years, cooperative, and parents consent for interview. Diagnosis criteria for NS are massive proteinuria (>40 mg/ m^2 /hour or urine albumine creatinine ratio (UACR) >2 mg/g or dipstick ≥ 2+), hypoalbuminemia ≤ 2.5 g/dL, generalized edema, and may accompanied by hypercholesterolemia. NS patients and control subjects were not on vitamins or minerals supplementation. NS in full dose corticosteroid therapy is a NS without steroid contraindication receiving prednisone therapy 60 mg/ m^2/ day or 2 mg/ kg/ day in divided doses to induce remission. NS patients that finished therapy had undergone either initial phase therapy or relapse therapy and remission with negative proteinuria or trace for 3 consecutive days within 1 week. Exclusion criteria included patients with end-stage renal disease with glomerular filtration rate <60 ml/ minute/ 1.73 m^2 body surface area; systemic diseases such as malignancy, pulmonary tuberculosis, severe malnutrition, cardiac abnormalities, liver disorders. Written informed consent was obtained from all participants and the study protocol was approved by the research ethics committee of Universitas Sumatera Utara. Blood samples were taken from patients for albumin, total cholesterol, urea, creatinine, MDA, and urine examination for UACR evaluation.

Detection of Serum MDA Level

High performance liquid chromatographic (HPLC) analysis with isocratic method was performed using an Agilent 1200 HPLC system (San Jose, CA, USA) with commercial MDA kits. (Immundiagnostik AG, Bensheim, Germany). The initial step in determining MDA is a sample preparation with a derivatization reagent that transforms MDA into a fluorescent product. Afterwards, the pH was optimized and the reaction mixture (20 ml) was chromatographed on a reversed phase C18 column (18.5 mm, 125 × 4 mm) at 30°C. Flow rate was 0.8 ml/min. Fluorimetric detection was performed with excitation at 515 nm and emission at 553 nm. The detection limit was 0.15 µmol/L.[6]

Statistical Methods

Data analysis was performed using the SPSS 22 version (SPSS Inc., Chicago) with a 95% confidence interval. Analysis was performed using chi square test, mann whitney u test, spearman correlation with significance $p<0.05$.

Results

Both groups, who were receiving full dose corticosteroid therapy and who finished therapy were in a homogeneous condition. Gender, nutritional status, and age did not differ significantly in both groups ($p>0.05$). There were significant differences in albumin, UACR, total cholesterol, and MDA levels between NS

patients with full dose and finished therapy. Significantly lower albumin levels in patients with full dose corticosteroid therapy were compared with those who finished therapy (p <0.001). UACR, total cholesterol, and MDA levels were significantly higher in NS patients with full dose therapy than finished therapy (p<0.001). Unfortunately, no significant differences in urea and creatinine levels was found between NS patients with full dose corticosteroid therapy and finished therapy (p>0.05).

Table 1. Basic characteristics and laboratory parameters in NS patients with full dose corticosteroid and finished therapy

Variabel	Nephrotic Syndrome		p
	Full Dose Corticosteroid Therapy	Finished Therapy	
Gender [a]			0.981
Males	19 (55.9%)	15 (44.1%)	
Females	9 (56.3%)	7 (43.8%)	
Nutritional Status [a]			0.994
Underweight	3 (37.5%)	5 (62.5%)	
Normal Overweight	20 (60.6%) 5 (55.6%)	13 (39.4%) 4 (44.4%)	
Age (years) [b]	5 (2 – 8)	4 (2 – 6)	0.389
Urea (g/dL) [b]	21 (15 – 48)	19 (13 – 45)	0.944
Creatinine (g/dL) [b]	0.7 (0.45 – 1.1)	0.65 (0.45 – 0.86)	0.266
Albumin (g/dL) [b]	2 (1 – 2.6)	3 (2.3 – 3.6)	<0.001 *
UACR (mg/g) [b]	3 (2.3 – 5.2)	0.35 (0.2 – 1.3)	<0.001 *
Total cholesterol (mg/dL) [b]	288.5 (120 – 400)	164 (120 – 190)	<0.001 *
MDA (µmol/L) [b]	1.39 (1 – 2.91)	1.15 (0.96 – 1.38)	<0.001 *

[a] n (%)
[b] median (minimum-maximum)
*p<0.05

There were significant positive correlations between UACR and total cholesterol levels with MDA levels, where the higher UACR and total cholesterol levels, the higher MDA levels in NS patients (p <0.001). Significant negative correlation was also found between albumin levels with MDA levels, where the lower albumin levels, the higher MDA levels in NS patients (p = 0.004). However, no significant correlations was found between urea and creatinine levels with MDA levels in NS patients (p> 0.05). (Table 2)

Table 2. Correlation of laboratory parameter with MDA level in NS patients

Laboratorium	r	p
Urea, g/dL	-0.106	0.462
Creatinine, g/dL	0.147	0.307
Albumin, g/dL	-0.503	0.004*
UACR, mg/g Total cholesterol, mg/dL	0.497 0.592	<0.001* <0.001*

*p<0.05

Discussion

NS is characterized by massive proteinuria, hypoalbuminemia, generalized edema, and hypercholesterolemia. The molecular mechanisms involved in pathogenesis of NS are still being studied. A significant inflammatory reaction can induce the occurrence of reactive oxidative species causing podocyte damage.[4] Glomerular capillary wall permeability is influenced by free radical formation resulting in oxidative stresss, which affect the occurrence of proteinuria.[7,8] The role of oxidative stress in the pathogenesis of NS has been reported through in vitro studies previously.[9,10] This study aims to determine the level of MDA in NS patients, who were receiving full dose corticosteroid therapy and finished therapy, and correlation of MDA levels with albumin, total cholesterol, and UACR levels, as a predictor of high free radicals to take into account whether or not to provide antioxidant supplementation to the patient.

Remission NS was defined as a negative to trace urine dipstick for protein for 3 consecutive days or a spot urinary protein-to-creatinine ratio <0.2.[11] Consequently, higher UACR and lower albumin levels were found in NS patients who underwent full-dose corticosteroid therapy compared to finished therapy. Meanwhile, total cholesterol levels were significantly higher in NS patients who underwent full-dose corticosteroid therapy than those who finished therapy. The hypercholesterolemia response due to hepatic lipogenesis is triggered by a decrease in albumin levels and plasma oncotic pressure. The degree of hyperlipidemia is inversely related to decreased oncotic pressure because low oncotic pressure directly stimulates transcription of the apoprotein B gene in the liver. The hyperlipidemic conditions can be reversible as the resolution of NS, occurs either spontaneously or induced by drug.[12,13]

There was no difference in renal function (urea, creatinine) between NS patients who underwent full-dose corticosteroid therapy and finished therapy. Begenic et al and Dogra et al reported no differences in glomerular filtration rates between NS patients and control group.[7,14]

Previous studies reported significantly higher MDA levels in NS patients than controls.[14-16] As well as higher MDA levels in active NS than remission NS patients. MDA levels significantly decreased after treatment.[4,17-21] High levels of MDA in NS patients will have an impact on decreasing levels of antioxidants due to consumption of oxidative stress so that antioxidant administration may be considered in NS patients.

There was a significant positive correlation between UACR levels and MDA levels. This is in line with previous studies. ROS contributes to the incidence of proteinuria, even the severity of lipid peroxidation correlates with the degree of proteinuria.[22-24] ROS can induce proteinuria by lowering the electronegative charge of the glomerular filtration barrier or through other mechanisms that remain unknown.[25] Hypoalbuminemia in NS occurs due to leakage of protein in the urine due to increased glomerular permeability. Albumin is a potent antioxidant containing thiol, capable of binding transitional metals.[26] There was a significant negative correlation between albumin levels and MDA levels. These results are in line with previous studies showing patients with hypoalbuminemia will be accompanied by oxidative damage.[15,27] Nevertheless, total cholesterol levels and MDA levels have a significant positive correlation instead . Zachwieja et al reported that NS patients had decreased antioxidants due to consumption of oxidative stress correlated with lipid abnormalities.[28] Dyslipidemia that occurs in NS patients will aggravate the state of oxidative stress.[7,18] NS patients had a five-fold increased risk for coronary deaths primarily due to hypercholesterolaemia and hypercoagulability.[12,13] Further studies are needed to evaluate the role of various antioxidants to obtain the best antioxidants that can significantly reduce MDA levels in NS patients.

Conclusion

MDA levels were significantly higher in NS patients who were receiving full dose corticosteroid therapy than those who finished therapy. There was a significant negative correlation between albumin levels with MDA levels, and significant positive correlations between UACR and total cholesterol levels with MDA levels.

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