ANALYSIS OF HIGH DOSE AND LONG-TERM PREDNISONE THERAPY ON TRAP 5B LEVEL CHANGE IN CHILDREN WITH STEROID SENSITIVE NEPHROTIC SYNDROME

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ABSTRAK

Sindrom nefrotik adalah suatu kondisi yang ditandai dengan kebocoran protein dari darah ke urin melalui glomeruli. Ini menyebabkan hipoproteinemia dan edema menyeluruh. Pasien dengan sindrom nefrotik membutuhkan dosis tinggi dan glukokortikoid jangka panjang, seperti prednison. Dosis tinggi dan glukokortikoid jangka panjang dapat meningkatkan resorpsi tulang. Penanda biologis adalah alat yang berharga untuk mengevaluasi kemanjuran terapi. TRAP 5B adalah penanda biologis yang sensitif untuk resorpsi tulang karena mencerminkan jumlah osteoklas. TRAP 5B tidak terpengaruh oleh disfungsi ginjal dan makanan. Ini juga memiliki variasi diurnal yang rendah dibandingkan penanda resorpsi tulang lainnya. Tujuan dari penelitian ini adalah untuk menganalisis perubahan tingkat TRAP 5B pada fase induksi dan fase alternatif pada anak-anak dengan sindrom nefrotik sensitif steroid. Penelitian prospektif observasional ini dilakukan dari Mei hingga Oktober 2016. Sampel darah vena diperoleh pada pukul 08.00-10.00 pagi. Level TRAP5B diukur sebelum dan sesudah fase induksi dan setelah fase alternatif menggunakan ELISA. Lima belas pasien dilibatkan dalam penelitian ini (60% anak laki-laki). Mayoritas usia mereka adalah 6 - <12 tahun dan 40% tergantung NS steroid. Level serum TRAP 5B dalam fase induksi meningkat sebesar 37,41%±56,22%. Pada fase alternatif, kadar serum TRAP 5B meningkat sebesar 28,75%±66,55% dibandingkan dengan fase induksi. Namun, perubahan tingkat kedua fase itu tidak signifikan. Sebagai kesimpulan, tingkat TRAP 5B meningkat pada fase induksi dan fase alternatif setelah dosis tinggi dan pengobatan prednison jangka panjang pada sindrom nefrotik. ***(FMI 2018;54:116-122)***

Kata kunci*: Sindrom nefrotik; pediatri; prednisone; resorpsi; TRAP 5B*

ABSTRACT

Nephrotic syndrome is a condition which is characterized by protein leakage from the blood to the urine through glomeruli. It leads to hypoproteinemia and generalised oedema. Patients with nephrotic syndrome need high dose and long term glucocorticoid such as prednisone. High dose and long term glucocorticoid can increase bone resorption. Biological marker is a valuable tool to evaluate efficacy of therapy. TRAP 5B is a sensitive biological marker for bone resorption because it reflects the number of osteoclasts. TRAP 5B is not affected by renal dysfunction and food. It also has a low diurnal variation than other bone resorption marker. The aim of this study was to analyze the changes of TRAP 5B levels at induction and alternate phase in children with steroid sensitive nephrotic syndrome. This observational prospective study was conducted from May to October 2016. Venous blood samples obtained at 08.00-10.00 am. TRAP5B levels were measured before and after induction phase and after alternate phase using ELISA. Fifteen patients were included in this study (60% boys). Majority of their age was 6 - <12 years and 40% were dependent steroid NS. TRAP 5B serum levels in induction phase increased by 37.41%±56.22%. In alternate phase, TRAP 5B serum levels increased by 28.75%±66.55% compared to the induction phase. However, the level change of both phases were not significant. As a conclusion, TRAP 5B levels increased in induction and alternate phase after high dose and long-term prednisone treatment in nephrotic syndrome. ***(FMI 2018;54:116-122)***

Keywords*: Nephrotic syndrome; pediatric; prednisone; resorption; TRAP 5B*

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INTRODUCTION

Nephrotic syndrome (NS) is a condition which is characterized by protein leakage from the blood to the urine through glomeruli (Hahn et al 2015). It leads to hypoproteinemia and generalised edema. NS is characterized by heavy proteinuria, hypoalbuminemia (serum albumin <2.5 g/dl), hyperlipidemia, and edema (Sinha et al 2015). The incident of NS was reported 6 per 100.000 per year in Indonesian's children at age less than 14 years. Boys are susceptible than girls to suffer from NS with ratio 2:1 (Trihono et al 2012).

Proteinuria in NS is caused by podocytes damage or genetic mutation of gene which produced podocytes protein. Podocytes are epithelial cell which is differen-

tiated and located outside glomerular capillary (Pais & Avner 2015). Podocytes are target of glucocorticoid. Co-incubation podocytes with glucocorticoid increase nefrin transport, reduce actin alteration and increase its polimerisation, prevent upregulation of miRNA-30, and increase podocytes recovery (Hahn et al 2015). Prednisone is often used as therapy in NS. The dosage of prednisone in NS is 60 mg/m2/day or 2 mg/kg/day or maximum 60 mg per day for 4-6 weeks, then following by alternate day prednisone (40 mg/m2 or 1.5 mg/kg) for 2 until 5 months with tapering dose (Eknoyan & Lameire 2012).

High dose and long-term prednisone therapy has potency to cause side effect like osteoporosis. Glucocorticoid induced-osteoporosis will increase fracture risk in children (Hansen et al 2014). It disturbs number, life span and function of osteoblasts and osteoclasts. It has been controversial whether glucocorticoid affects bone resorption. Glucocorticoid may reduce apoptosis of mature osteoclast, then increase osteoclasts formation and increase bone resorption (Canalis et al 2007). Biologic marker of bone turnover can be used for monitoring efficacy of therapy before evaluating the bone mineral density (BMD) (Wheather et al 2013).

Tartrate-resistant acid phosphatase 5B (TRAP 5B) is an enzyme which is secreted by osteoclasts. It is a sensitive marker which reflects the number and resorption activity of osteoclast (Henriksen et al 2007). TRAP 5B has a low diurnal variation, and it is not affected by food and renal dysfunction. The aim of this study was to analyze the changes of TRAP 5B serum level in children with sensitive steroid nephrotic syndrome (SNSS) after high dose and long-term prednisone therapy.

MATERIALS AND METHODS

All subjects provided written informed consent, and the study was approved by Ethics Committee of Dr. Soetomo Hospital, Surabaya, Indonesia. The study sample comprised a consecutive series of children who were admitted to the Nephrology Division of Pediatric Department. This observational prospective study was conducted from June to October 2016. The inclusion criteria of this study were children with SSNS including initial attack, infrequent relapse, frequent relapse and dependent steroid at age $\leq$ 18 years who received high dose and long term prednisone therapy (more than 30 days) in Nephrology Division, Departement of Pediatric, Dr. Soetomo Hospital, Surabaya, and the patient's guardians were pleased to sign the information for consent.

There were 15 patients who met the inclusion criteria, and 1 patient dropped out because of nephritic lupus. The exclusion criterion of this study was patients with Steroid-Resistant Nephrotic Syndrome (SRNS). The independent variables in this study were high dose and long-term prednisone therapy, while dependent variable was TRAP 5B serum level.

In this study, the patients received therapy in accordance with guideline therapy of SN in Indonesia. Patients received high dose and long-term prednisone therapy. Patients received prednisone therapy of 2 mg/kg/day or 60 mg/m2/day for±4 weeks in induction phase then followed by prednisone 1.5 mg/kg/day or 40 mg/m2 alternate day for±4 weeks. All patients' past medical and treatment histories, patients' clinical and laboratory data were collected from medical record and assessment with the patients' guardian. We also recorded the history of calcium lactate consumption, because it can affect bone mineral metabolism. Venous blood (1 ml) samples were collected from SSNS patients prior to the treatment and after induction phase and after alternate phase at 08.00 –10.00 a.m. All samples were centrifuged at 2,000 rpm for 20 min to remove the cellular components, and the supernatants were frozen in aliquots at 80°C until analysis.

The serum levels of TRAP 5B (Bioassay Technology Laboratory, Shanghai Korain Biotech co, Ltd, Shanghai, China) were measured with enzyme-linked immunosorbent assays in accordance with the manufacturer's protocol. The lowest limit detected in this assay was 0,05 U/L. The highest limit detected in this assay 20 U/L. The sensitivity limit detected in this assay was 0,03 U/L. The intra-assay precision was <10%, and the inter-assay precision was <12%.

SPSS 20.0 (IBM, Corp., Armonk, NY, USA) was used for all statistical analyses. Continous data were expressed as the mean±standard deviation. Student's t–test was used to compare the normally distributed continous variables of the two groups. In all tests, p< 0,05 and CI (confidence interval) 95% indicated a statistically significant difference.

RESULTS

Table 1 shows the baseline characteristics of the subjects. Fifteen patients were included in this study. Sixty percent of them were boys. Fifty three percent of the patients were 7.9±1.6 years old, while 53% of the patients had mean body weight as much as 16.4±13.3 kg. Forty percents of the patients were diagnosed with dependent steroid NS and 40% of the patients also had mean TRAP 5B serum levels as much as 1.726±0.120 U/L prior to the treatment.

Table 1. Subjects' characteristics

Characteristics		Total patients (N = 15)		Mean±SD
		Number (n)	Percentage (%)	
Gender	Male	9	60	–
	Female	6	40	–
Age range	< 2 years	0	0	–
	2 – < 6 years	4	27	3.8±1.4
	6 – < 12 years	8	53	7.9±1.6
	12 – 18 years	3	20	14.6±1.0
Body weight	≤ 20.9 kg	8	53	16.4±3.13
	21 – 40.9 kg	5	33	26.9±8.05
	41 – 60 kg	2	14	48.5±2.12
Diagnosis	Initial attack nephrotic syndrome	4	27	–
	Infrequent relapse nephrotic syndrome	3	20	–
	Frequent relapse nephrotic syndrome	2	13	–
	Dependent steroid nephrotic syndrome	6	40	–
TRAP 5B serum level	1.000 – 1.999 U/L	6	40	1.726±0.120
	2.000 – 2.999 U/L	5	33	2.187±0.138
	> 3.000 U/L	4	27	3.285±0.154
Duration of prednisone treatment 6 months prior to induction	Not taking prednisone	5	33	–
	1 – 75 days	3	20	49.3±5.4
	76 – 105 days	6	40	92.8±5.6
	>105 days	1	7	–
Cumulative dosage of prednisone 6 months prior to induction	Not taking prednisone	5	33	–
	< 1.800 g	5	33	0.581±0.430
	1.801 – 2.55 g	4	27	2.048±0.133
	2.551 – 3.3 g	1	7	–
	3.3 – 3.9 g	–	–	–
	3.90 – 4.35 g	–	–	–
	4.351– 4.651 g	–	–	–
Cumulative dosage of calcium lactate 6 months prior to induction	Not taking calcium lactate	7	47	–
	0.5 – 45 g	0	0	–
	45.5 – 90 g	1	7	–
	90.5 –135 g	2	13	102±12
	135.5 –180 g	0	0	–
	180 – 225 g	5	33	183±2.04
	255 – 270 g	0	0	–
Total calcium serum prior to induction	No measurement	2	13	–
	Normal (0.5 – 0.6 mmol/L)	13	87	9.3±0.6
	Below or above normal (<0.5 or >0.6 mmol/L)	0	0	–
Phosphate serum prior to induction	No measurement	5	33	–
	Normal (2.7 – 6.5 mg/dL)	8	54	5.1±0.6
	Below or above normal (<2.7 or >6.5 mg/dL)	0	0	–
Edema	No edema	1	7	–
	Palpebra	4	26	–
	Upper extremity	0	0	–
	Lowe extermity	1	7	–
	Anasarca	9	60	–
Complaining bone pain	Complaining bone pain	5	33	–
	Not complaining bone pain	10	67	–

We also recorded the history of prednisone treatment from the patients six months prior to the treatment. Most of the patients (33%) never received prednisone therapy, while 40% of the patients had received prednisone for 92.8±5.6 days. Thirty three percent of them had received average prednisone cumulative dosage as much as 0.581±0.430 g. Forty seven percents of the patients took calcium lactate before induction phase. Thirty three percent of them had received average calcium lactate cumulative dosage as much as 183±2.04 g. Based on the clinical condition, 60% of the patients had showed proteinuria 3+ until 4+ which was characterized by

anasarca oedema, while 67% of the patients never complained bone pain.

Table 2 shows mean of TRAP 5B serum level profile of 15 patients with NS. Mean of TRAP 5B serum levels before induction was 2.368±0.688 U/L, while mean of TRAP 5B serum levels after induction phase was 3.271±1.795 U/L. Mean of TRAP 5B serum level after alternate phase was 3.756±2.316 U/L. Mean changes of TRAP 5B serum level in induction phase was 0.945±1.495, while mean changes of TRAP 5B serum level in alternate phase was 0.617±2.147. Mean percentage changes of TRAP 5B serum level in induction phase was 37.41%±56.22%, while percentage mean changes of TRAP 5B serum level in alternate phase was 28.75%±66.56%. All percentage mean changes of TRAP 5B serum levels are shown in Fig. 1. There are no significant differences of mean changes and percentage mean changes of TRAP 5B between induction and alternate phase which are shown in Fig. 2 and Fig. 3.

Table 2. TRAP 5B serum level profile and mean changes of TRAP 5B serum level in 15 patients

Parameters	Time/Phase	Mean±SD
TRAP 5B serum level	t=0	2.368±0.688
	t=1	3.271±1.795
	t=2	3.756±2.316
Mean changes of TRAP 5B serum level	Δ t=1 – t=0	0.945±1.495
	Δ t=2 – t=1	0.617±2.147
Mean percentage changes of TRAP 5B serum level	Δ t=1 – t=0	37.41%±56.22%
	Δ t=2 – t=1	28.75%±66.56%

Note: Δ t=1 – t=0, induction phase; Δ t=2 – t=1, alternate phase

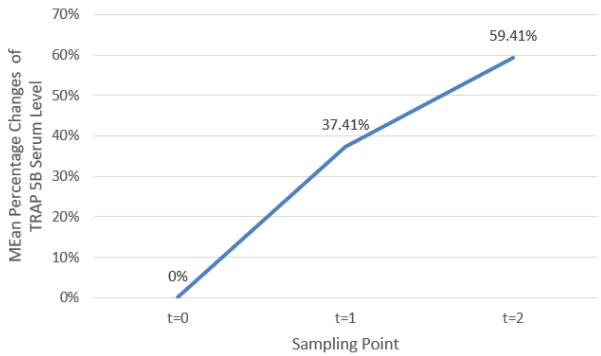

Fig. 1. Mean percentage changes of TRAP 5B serum level in all phases.

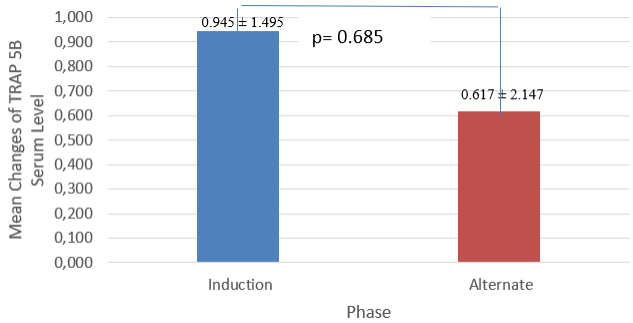

Fig. 2. Paired T-test of mean changes of TRAP 5B serum level after induction and alternate.

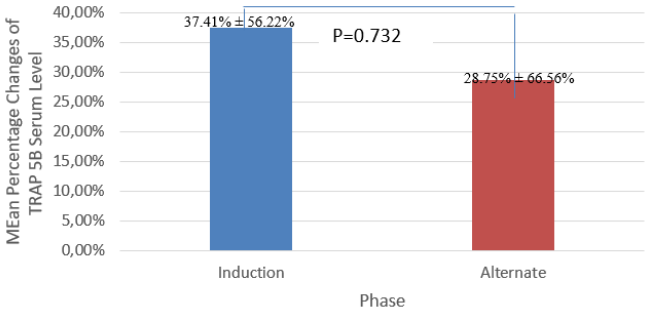

Fig. 3. Paired T-test of mean percentage changes of TRAP 5B serum level after induction and alternate.

Table 3. Paired T-test of mean changes and mean percentage changes of TRAP 5B serum level in group unexposed and exposed to prednisone

Parameters	Unexposed to Prednisone	Exposed to Prednisone	p
	Mean±SD (U/L)	Mean±SD (U/L)	
TRAP 5B serum level before induction (baseline)	3.061±0.552	2.022±0.444	0.003*
TRAP 5B serum level after induction phase	4.310±1.810	2.752±1.546	0.130
TRAP 5B serum level after alternate phase	3.852±0.916	3.709±2.760	0.918
Changes of TRAP 5B serum level in induction phase	1.374±1.540	0.730±1.425	0.468
Changes of TRAP 5B serum level in alternate phase	-0.423±1.193	1.137±2.322	0.211
Percentage changes of TRAP 5B serum level in induction phase from baseline	44.17%±44.43%	34.03%±60.99%	0.763
Percentage changes of TRAP 5B serum level in alternate phase from baseline	19.66 %±26.21%	79.28 %±129.94%	0.425

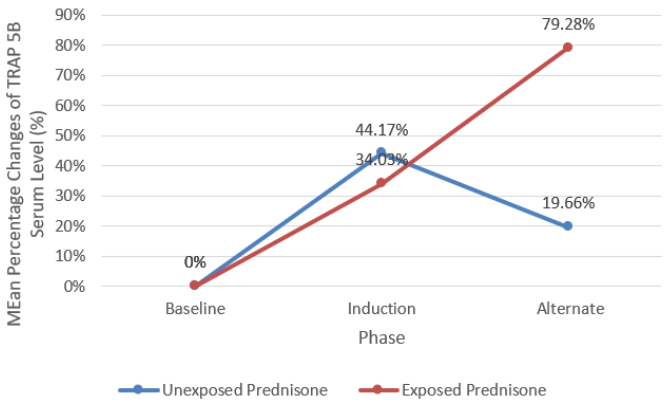

Fig. 4. Mean percentage changes of TRAP 5B serum levels in group of exposed and not exposed to prednisone.

We tried to analyze further whether there was effect of history prednisone treatment to TRAP 5B serum level or not. We classified patients into two groups, those who were exposed and not exposed to prednisone 6 months prior to the treatment. After we analyzed those grops statistically using Independent T-test, there were significant differences of TRAP 5B serum level before induction between those groups (p=0.003). However, there were no significant differences of mean changes and mean percentage changes of TRAP 5B serum level which is shown in Table 3. The mean percentage changes of TRAP 5B serum level profile in those groups is shown in Fig. 4.

DISCUSSION

The aim of this study was to analyze the changes of TRAP 5B levels at induction and alternate phase in children with steroid sensitive nephotic syndrome after high dose prednisone treatment after induction and alternate phase. In this study, there were 15 patients who met the inclusion criteria, and 1 patient dropped out

because of nephritic lupus. Sixty percent of them were boys. It was matched with a previous study which stated that boys are susceptible to suffer from NS than girls with ratio 2:1 (Trihono et al 2012). Fifty three percent of the patients were 7.9±1.6 years old. It was matched with the study which reported that children with age less than 14 years had the highest incident of NS (Trihono et al 2012). Fifty three percent of the patients had mean body weight as much as 16.4±13.3 kg. We measured 'dry' body weight because body weight was used to determine dosage of prednisone since glucocorticoid immunosuppressive action depends on dosage. Prednisone doses to stabilize podocytes cytoskeleton maybe lower than those to immunosuppressive action. Glucocorticoid stabilizes podocytes cytoskeleton by synthesizing glycosilated nephrin (Raman et al 2015).

Forty percents of the patients also had mean TRAP 5B serum levels as much as 1.726±0.120 U/L prior to the treatment. All TRAP 5B serum levels in this study was lower than those in all the studies. In a study, TRAP 5B normal value was based on Chinese healthy children, because there were no study in profiling TRAP 5B normal value in Indonesia. Mean TRAP 5B serum levels in Indonesian children with NS was lower than those in China. We also classified the patients based on the duration of prednisone treatment and cumulative dosage of prednisone 6 months prior to the treatment. Based on the study by Zhang et al (2016) TRAP 5B serum in healthy children is not significantly different from that in patients with SN initial attack 3 and 6 months after induction. It is indicated that TRAP 5B serum level can turn back to normal level at the time of remission due to the reduction dosage of glucocorticoid and reduction of total protein leakage (Zhang et al 2016).

Mean percentage changes of TRAP 5B serum level in induction phase in 15 patients was 37.41%±56.22%, while mean percentage changes of TRAP 5B serum level in alternate phase was 28.75%±66.56%. Elevation of TRAP 5B serum levels after induction and alternate phase in NS patients showed that high dose and long term prednisone can increase bone resorption. Alternate phase glucocorticoid causes reduction of bone mineral density in children with SN (Jeon et al 1998). Resorption affects bone mineral density because one of resorption steps is demineralisation. Demineralisation is a process of proton secretion into bone surfaces in order to reduce bone mineral density (Kim et al 2006). Thus, TRAP 5B serum levels still increase in alternate phase, but the values was lower than those in induction phase. However, the changes of TRAP 5B serum level between induction and alternate phase was not significantly different. It may be caused by big variance between samples, difference history of prednisone treatment, and difference quality of bone mineral density.

The difference history of prednisone treatment may cause the differences of TRAP 5B serum levels between group of exposed and not exposed to prednisone. Mean changes of TRAP 5B serum levels before induction in group of unexposed prednisone was higher than those in group of exposed prednisone. It happened because of the elevation of resorption depth (elevation of bone resorption number per bone multicellular unit) in the first high dose glucocorticoid therapy (Kim et al 2006). Otherwise, patients in group of unexposed to prednisone never consumed calcium lactate which can affect osteoclasts. Calcium, through cation sensitive receptor, can directly inhibit osteoclasts-mediated bone resorption (Marie 2010). However, the changes of TRAP 5B serum levels between induction and alternate phase in group of unexposed prednisone is not significantly different.

However, in group of exposed prednisone mean TRAP 5B serum levels showed low rate of resorption. Bone resorption reflects the sum of osteoclast recruitment and death rate in which the average cells degrade the matrix. Antiapoptotic effect only needs short-term exposure of steroid in late differentiation of osteoclasts. Although that effect was so long but osteoclasts which is exposed by glucocorticoid can suppress resorptive activity both in vitro and in vivo. Glucocorticoid induced RANKL and M-CSF expression and disturb osteoclast-inhibited osteoprotegerin (Kim et al 2006).

CONCLUSION

TRAP 5B levels increase in induction and alternate phase after high dose and long-term prednisone treatment in nephrotic syndrome.

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