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Soluble urokinase plasminogen activator receptor (suPAR) is prospective biomarker to evaluate treatment progress in children with pulmonary tuberculosis during maintenance phase

Mardining Raras Tri Yudani, Marrecar Aunilla Hamid, Muhammad Slamet Chandra Kusuma, Ery Olivianto

ABSTRACT

Aims: Childhood pulmonary tuberculosis (TB) is still a major problem in developing countries. One of the difficulty in TB management is lack of biomarkers to evaluate treatment response.This study aimed to investigate the role of plasma soluble urokinase-type plasminogen activator receptor (suPAR) as a biomarker to evaluate response to anti tuberculosis treatment in children with pulmonary TB. Methods: Twenty five children aged 2–10 years with pulmonary TB in Dr Saiful Anwar Hospital, Malang from February 2012 to January 2013 were enrolled into the study. The plasma suPAR level was measured using ELISA prior to anti-tuberculosis treatment and two, four, six months after treatment initiation. Clinical parameters were observed including coughs, lymphadenitis, nutritional status and X-ray. Ten healthy children served as a control group. Results: All TB patients showed high plasma suPAR levels prior to anti TB treatment (4.77 +1.51 ng/mL) and did not change (p = 0.0001>0.05) after two months (4.89+1.61 ng/mL) of treatment. However, it decreased significantly after four months (2.51+1.81 ng/mL) and six months of treatment (0.27+0.64 ng/mL). By the end of therapy the level of suPAR reached a value that was lower than control group (1.58+0.09 ng/mL). The reduce of suPAR level was not parallel to clinical improvement after two months treatment. Conclusion: the suPAR level is elevated in childhood pulmonary TB and significantly decreased after four months of anti tuberculosis treatment. This imply that suPAR is not appropriate biomarker to evaluate TB treatment response in childhood TB after intensive phase of therapy. However, it is a prospective biomarker to monitor the efficacy of therapy in the maintenance phase after two months therapy.

Keywords: Biomarker, Children, Pulmonary tuberculosis, Soluble urokinase plasminogen activator receptor (suPAR)

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INTRODUCTION

Pulmonary tuberculosis is still counted as significant source of morbidity and mortality among children in Indonesia. Successful control of childhood TB depends on accurate early diagnosis, effective therapy and robust method to evaluate the response to therapy. Because the long duration of therapy may adversely affect patient adherence to treatment and cease the treatment earlier, hence urges a suitable biomarkers as predictor the response to anti-TB treatment that may allow the duration of treatment to be shortened accordingly. In the last decade, several biological markers have been intensively studied [1]. Such markers could contribute the improvement of the quality of clinical trials; and therefore enable development and validation of new therapeutic strategies.

Urokinase plasminogen activator receptor (uPAR) is one of biomarker that has been intensively investigated in the last decade. It is a cellular receptor for serine protease urokinase plasminogen activator (uPA). Several types of immune cell express uPAR such as neutrophils, eosinophils, monocytes, macrophase, activated T lymphocytes and NK cell [2, 3], endothelial cells [4] and megakaryocytes [5]. uPAR is cleaved from cell surface by a number of protease, yielding a soluble form of the receptor (suPAR) that can be found in body fluids [6]. Although suPAR is not specific for TB, but elevated level of this marker in several infectious diseases such as TB and HIV associated with the severity of disease and may hence be used as a prognostic tool. The level of suPAR reflect the activation level of the immune and inflammatory systems [7]. It has been demonstrated that plasma suPAR is elevated and has prognostic value in predicting disease severity and outcome in various conditions, such as autoimmune diseases, cancer and bacterial, parasitic and viral infections [7, 8]. Study by Eugen-Olsen showed elevated levels of suPAR in pulmonary TB patient with higher suPAR levels in smear microscopy-positive patients compared to smear-negative patients [9].

Until now, studies concerning the use suPAR to monitor the progress of therapy in children with TB are very limited. Assuming that serum levels of suPAR reflect clinical improvement during treatment in children with pulmonary TB, it was worthwhile to know whether this biomarker may be used to evaluate the response to treatment in children with TB by measuring its serum level prosprectively.

In the present study, the plasma suPAR of TB patients were measured during six month therapy and compared to other routine clinical evaluation including lymphadenitis, nutritional status and coughing.

MATERIALS AND METHODS

Subjects

We enrolled all children aged 2–10 years who were admitted to Dr Saiful Anwar Hospital, Malang for pulmonary TB between February 2012 to January 2013. These patients was diagnosed as clinically confirmed pulmonary TB by the presence of clinical symptoms, such as chronic cough, poor weight gain, malnutrition, unexplained fever, and cervical lymphnode enlargement; history of contact source case and suggestive chest X-ray. The inclusive criteria were pulmonary TB, age between 2-10 years, agreed to be the subject of research and the parent signed the informed consent. Healthy children with negative tuberculin skin test served as negative controls.

Patients who also suffered from other diseases such as severe bacterial pneumonia, HIV-AIDS, heart disease, diabetes mellitus, and liver or kidney problems were excluded.

Tuberculosis treatment

The anti tuberculosis regimen consisted of a fixed drugs combination (FDC) of INH, rifampicin and pyrazinamide for two months intensive phase followed by rifampicin and INH for four month maintenance phase. The given dose of FDC were in accordance to WHO new recommendation of anti tuberculosis doses.

Adherence to anti tuberculosis treatment was accessed by observing remains of FDCs package each time the patients came to the outpatient clinic (i.e, once a month). The patients considered as non-adherent if they were not taking FDCs at least for consecutive two weeks in intensive phase or in maintenance phase. Such patients would be ruled out of the study observation.

Clinical examination

The clinical symptoms including coughs, decreased body weight, fever, lymphnode enlargement; history of contact source case and chest X-ray examination were documented at the time of diagnosis [10]. Symptoms of coughs, fever, and lymphnode enlargement were evaluated and body weight was measured at second, fourth, and sixth months visit.

Sampling handling

As much as 3 ml blood specimens were collected from new patients during hospitalization and at the outpatient clinic visit through aspiration using injective needle prior anti-tuberculosis therapy and at two, four and six month after the initiation of anti-TB drugs treatment based on WHO guideline [10, 11]. Separation of serum was conducted by centrifugation (6000x g) at 4°C for 7 minutes and aliquots of 500 uL stored at -80°C until required.

Plasma suPAR levels were determined using a commercial enzyme-linked immunosorbent assay (suPARnostic® Standard kit; ViroGates A/S, Birkerød, Denmark) according to the manufacturer's instructions.

Enzyme linked immunoassaysorbant assays

Serum suPAR measurement was done in duplicate using commercially available ELISA kits according to the manufacture's protocol (suPARnosticÒ, ViroGates A/S, Copenhagen, Denmark). Plate reading was conducted using a Biotekmicroplate reader set to 450 nm, with the wavelength correction set to 650 nm. Concentrations of the respective analytes were determined using SPSS version 3.4 software.

Statistical analysis

All statistical analysis were carried out using SPSS 16 software version and the statistical programming language (SPSS Inc, Chicago, IL) program. The difference between suPAR level between patients and control was analyzed using one-way ANOVA. A p-value of ≤0.05 was considered significant.

Ethics

Written consent was obtained from the parents of all participants. The study was approved by the Ethics Committees, Faculty of Medicine, Brawijaya University.

RESULTS

Twenty-five patients with pulmonary TB were observed during the six months treatment. Characteristics of the patients are presented in Table 1. The number of female pulmonary TB patients who fulfilled the inclusion and exclusion criteria were (72%) higher than the number of male patients (28%). Distribution based on age in this study showed that TB patients were mainly dominated by those aged between 7–10 years. Tuberculin test showed that majority of children (92%) have been infected by M tuberculosis.

Table 1: Characteristics of patients

Characteristics	Frequency (n)	Percentage (%)
Subject counts (Age ≈ 7.2 years)	35	100
Healthy children (age≈ 6.5 years)	10	28.6
Female	5	50
Male	5	50
Subject (Age ≈ 7.5 years)	25	71.4
Female	18	72
Male	7	28
Tuberculin skin test		
positive	24	92
negative	1	4

Apparently coughing is the most common complaint (92%), however lymphadenitis (60%) and fever (64%) were also found. All patients were malnourished before anti tuberculosis treatment. The number of patients with cough reduced significantly at second month and after six month treatment the cough has totally disappeared. There was no patient complain of fever after six monts of treatment. The plasma suPAR level was significantly decreased after four months of treatment and this correlated significantly with the reduction of lymphnode enlargement and improvement of nutritional status (Table 2).

It was also found that the number of patients with poor nutrition status went down after two month (72%) and drastically dropped (12%) after four months. By the end of therapy, all patients showed good nutrition status (100%). It was found that the decrease in suPAR level was in accordance with the increasing number of children who recovered from malnutrition.

Measurement of suPAR concentration indicated that there was an elevated level of suPAR in infected patients before treatment (mean 4.77±1.51 ng/mL) and did not change after two month (mean 4.89±1.61 ng/mL). The suPAR level dropped significantly after four (mean 2.51±1.81 ng/mL) and sixt month of therapy (mean 0.27±0.09 ng/mL) (Figure 1). The healthy group showed suPAR level that remained the same throughout the course of therapy (mean 1.58±0.64 ng/mL).

DISCUSSION

To our knowledge, this is a first study concerning the use of suPAR as biomarker to evaluate response to anti tuberculosis treatment in TB therapy in children in Indonesia. In general, plasma suPAR level in TB patients childhood was high (4.75 ng/mL) with range between 2.8–5.8 ng/mL and that was much higher compared to healthy children (1.7 ng/mL). Increased suPAR levels prior therapy may be a result of migration of macrophage into bronchi and activation of immune response as well as inflammation caused by the active infection [9]. Adherence and migration of monocytes involves a functional interaction between uPAR and integrins [12]. The level of plasma suPAR in children with pulmonary TB in this study was lower than that in TB adult (ranged 8.5–28 ng/mL) [13]. The paucibaciliary nature of TB in children probably in part explain this condition [14]. Moreover, the immature cell mediated immunity in children may also play a role. It is demonstrated that children have reduced function in antigen-presenting cells (APC), neutrophils and TLRs and decreased blood complement levels. On the other hand adaptive immunity is thought to be altered to a helper T cell 2 type response, this will also influence the level of plasma suPAR in the TB patient [15].

It is surprising that suPAR level did not change after two months therapy, we suspected that the absent of

Table 2: Clinical symptoms and measurement of suPAR level during six months antituberculosis treatment in children with tuberculosis

Medical record and suPAR level		Duration of therapy (month)			
		0	2	4	6
Symptoms	- coughing	23(92%)	5(20%)	2(8%)	0(0%)
	- fever	16(64%)	0(0%)	0(0%)	0(0%)
Lymphnode enlargement	- yes	15(60%)	12(48%)	5(20%)	2(8%)
Nutritional status	- poor	25(100%)	18(72%)	3(12%)	0(%)
	good	0(0%)	7(28%)	22(88%)	25(100)
suPAR conc.(ng/mL)	patient	4.77+1.51	4.89+1.61	2.51+1.81	0.27+0.09
	healthy	1.5			

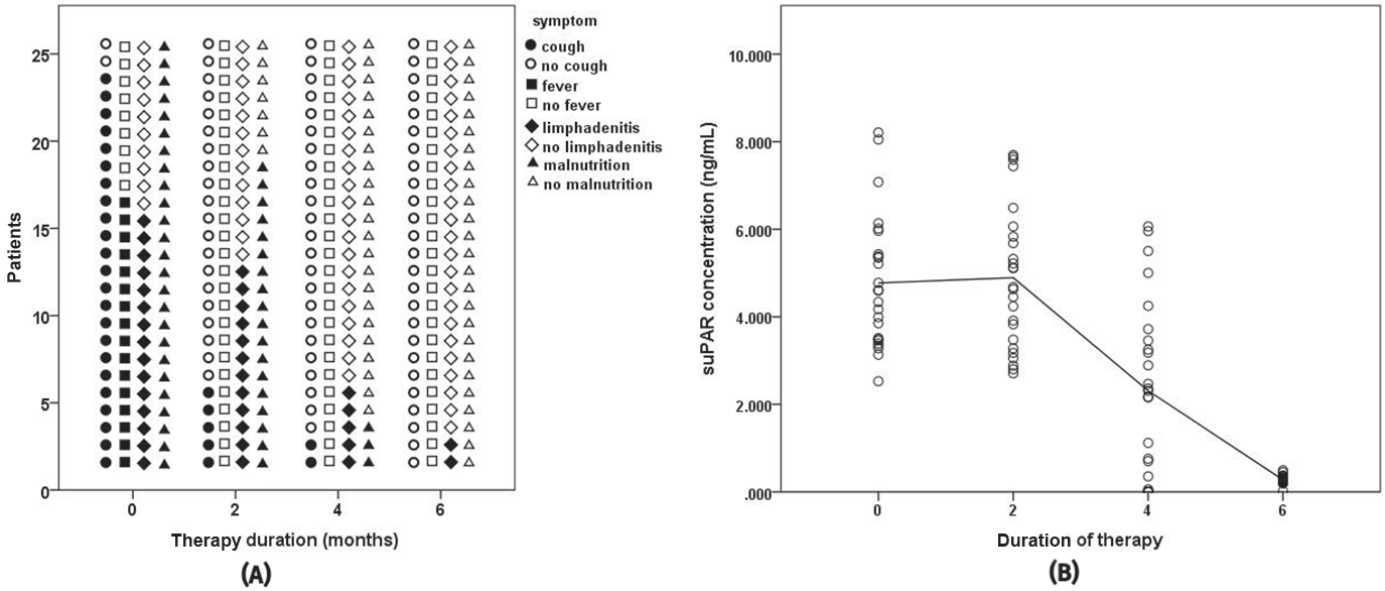

Figure 1: Comparison between (A) Clinical symptoms and (B) Level of plasma suPAR of children with tuberculosis during six month treatment.

ethambutol in the TB regimen is the possible answer. In hospital where subject was registered, ethambutol was not recommended to be included in the regimen for children due to ocular toxicity [16]. Ethambutol works by disturbing the synthesis of Mycobacterium cell wall that will result in the lysis of bacteria [17]. Lacking of ethambutol in regimen may lead to the retardation of bacterial clearance in parenchim that was reflected by the level of suPAR that continuing unchanged after 2 months.

The suPAR level then declined drastically at four and six month after therapy. A decrease in suPAR after 8-month of TB-treatment among responders was also found in the study of Eugen-Olsen et al [9].

The infection of Mycobacterium tuberculosis among the malnourished status of TB pediatric patient affect inflammatory as well as affects synthetic pathways that consequently promotes malnutrition. The present study indicated that the decrease in suPAR level was in accordance with the increasing number of children who recovered from malnutrition. Study on guinea pigs that were supplemented with low protein diet and then exposed to Mycobacterium tuberculosis showed lack of ability to mount Th1-type cell-mediated response including low lymphocyte proliferation, higher immunoglobulin G levels, and reduced cytokines such as IL-2, TNF-α, and IFN-γ [18]. Particularly in relation with T-cell proliferation in that study demonstrated the elevated level of Fc-γ T cells and TGF-β, both cytokine are considered as blocker function and T-cell proliferation.

Lymphnode enlargement in childhood pulmonary TB is of important sign that help physician to diagnose tuberculosis. It develops over weeks to months without apparent tenderness and signs of inflammation [19]. Pathologically it consist of Langhans giant cell, epitheloid cells and necrotic caseous tissue which indicate chronic inflammatory process in response to mycobacterium [20]. Our study demonstrated that lymphnode enlargement seems to resolve slower than other clinical symptoms. This may be due to poor drug penetration into the lymph node. Secondly, there might be enhanced cell mediated immunity in response to mycobacterial antigens release during anti tuberculosis treatment [20]. Hence, the delayed improvement of lymphnode enlargement in pulmonary TB may explain the slower decrease of plasma suPAR than previously expected.

CONCLUSION

The plasma suPAR level in children with pulmonary TB did not express treatment efficacy in early TB treatment. However suPAR showed a potential complement marker during maintenance phase in after two months treatment.

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Author Contributions
Mardining Raras Tri Yudani – Substantial contributions to conception and design, Analysis and interpretation of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published
Marrecar Aunilla Hamid – Acquisition of data, Analysis and interpretation of data, Drafting the article, Final approval of the version to be published
Muhammad Slamet Chandra Kusuma – Substantial contributions to conception and design, Revising it critically for important intellectual content, Final approval of the version to be published
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Guarantor
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Conflict of Interest
Authors declare no conflict of interest.

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