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Increased Serum Macrophage Migration Inhibitory Factor (MIF) Concentrations as Potential Risk Factors in Steroid-Resistant Nephrotic Syndrome

Oke Rina Ramayani[1]*, Nanan Sekarwana[2], Partini Pudjiastuti Trihono[3], Ahmad Hamim Sadewa[4], Aznan Lelo[5] and Putri Chairani Eyanoer[6]

[1]*Department of Pediatrics, Sumatera Utara University, Indonesia*
[2]*Department of Pediatrics, Padjajaran University, Indonesia*
[3]*Department of Pediatrics, Indonesia University, Indonesia*
[4]*Department of Biochemistry, Gadjah Mada University, Indonesia*
[5]*Department of Pharmacology and Therapeutics, Sumatera Utara University, Indonesia*
[6]*Department of Community Medicine, Sumatera Utara University, Indonesia*

Abstract

Background: Patients with steroid-resistant nephrotic syndrome (SRNS) tend to progress to end-stage renal disease (ESRD). Although the risk of steroid resistance depends mainly on histopathology, other factors, such as cytokines, may contribute to this condition. Cytokine macrophage Migration Inhibitory Factor (MIF) acts to counter-regulate glucocorticoids, which have become the main drug therapy for NS. The aim of this study was to evaluate whether raised serum MIF levels represent a potential risk factor for SRNS patients.

Methods: A prospective study was conducted in a multi-centre hospital and school in Medan, Sumatera, Indonesia. A total of 99 subjects were included in the study consisting well child (n=31) and NS patients (n=68). Serum macrophage migration inhibitory factor (MIF) was collected and measured. Patient's data about demographics, blood pressure, threshold steroid dosage at inclusion, urinary albumin creatinine ratio, plasma angiotensin II and serum MIF were compared between groups.

Results: Majority of subjects showed MIF levels between 10.4 and 31.8 ng/ml. Group SRNS had significantly higher serum MIF (median 31.9 (14.3-117.2) ng/mL) compared to the levels in group SSNS (median 21.8 (10.4-31.8) ng/mL) and well child (median 24.1 (11.4-31.1) ng/mL. Half of SRNS subjects (n=20) showed higher levels of MIF. In logistic regression analysis, diastolic blood pressure and plasma angiotensin II levels were found to be independently associated with higher serum MIF. There was a weak positive liniear correlation between concentration of MIF serum and angiotensin II plasma.

Conclusions: The serum MIF levels in group SRNS is higher than SSNS and well child. Diastolic blood pressure and plasma angiotensin II levels were found to be independently factors associated with higher MIF serum.

Keywords: Macrophage; Migration inhibitory factor; Hypertension; Steroid resistant; Nephrotic syndrome

Introduction

Idiopathic Nephrotic Syndrome (NS) represents a heterogeneous group of glomerular disorders occurring mainly in children. Generally, NS is divided into steroid sensitive (SSNS) and Steroid Resistant Nephrotic Syndrome (SRNS), depending on the response to steroid therapy. SRNS accounts for more than 10% of children who progress to chronic kidney disease [1]. This group of NS has in common permanent loss of selectivity of the glomerular barrier to protein filtration. It has been recognized that patients with persistent high grade proteinuria are more likely to develop chronic kidney disease than patients with low grade or no proteinuria [2].

The risk of steroid resistance among NS children is affected by many factors. While the risk of steroid resistance depends mainly on histopathology, other factors, such as cytokines, may contribute to this condition. The production of MIF cytokine represents a physiologic counter regulator to the anti-inflammatory and immunosuppressive effects of glucocorticoids [3]. This cytokine is induced by glucocorticoids, and then acts to counter-regulate the inflammatory action of glucocorticoids [4]. Higher MIF levels can inhibit the action glucocorticoids [5]. While MIF is central to determining chronicity in steroid resistance [6], data regarding serum MIF levels in SRNS are scarce.

Renal macrophage infiltration may persist if there are persistent MIF cytokine, originally described as a T cell-derived cytokine. Liao et al. demonstrated an increase renal macrophage infiltration in angiotensin II-induced hypertension [7]. It was shown that T lymphocyte is required for angiotensin II to induce vascular remodeling. Furthermore, angiotensin II increased the expression of macrophage infiltration in the vascular neointima [8].

The role of higher serum MIF levels and their correlation with level of plasma angiotensin II have not yet been elucidated especially as risk factor for steroid resistance in NS. One study reported elevated serum MIF concentrations in chronic kidney disease patients [9]. However, that study focused on adults with a wide range of glomerular filtration rates. Furthermore, since most mechanical aspects of serum MIF action have not yet been fully elucidated, the current study sought to determine whether higher serum MIF levels are a potential risk factor for SRNS patients.

***Corresponding author:** Oke Rina Ramayani, Department of Pediatrics, Sumatera Utara University, Indonesia, Tel: 061-8365663; E-mail: oke_rina@yahoo.com

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Methods

Study participants

A total of 99 children were recruited, consisting of well-child subjects (n=31, 11.6 ± 3.8 years) and NS patients (n=68, 8.3 ± 4 years). The NS patients comprised both SRNS (n=40) and SSNS (n=28) patients. The well-child subjects were recruited at a school in Medan, North Sumatera when they underwent annual medical checkups. The NS patients were undergoing steroid and other immunosuppressant treatment, if needed. Details of the subjects recruited are shown in Figure 1.

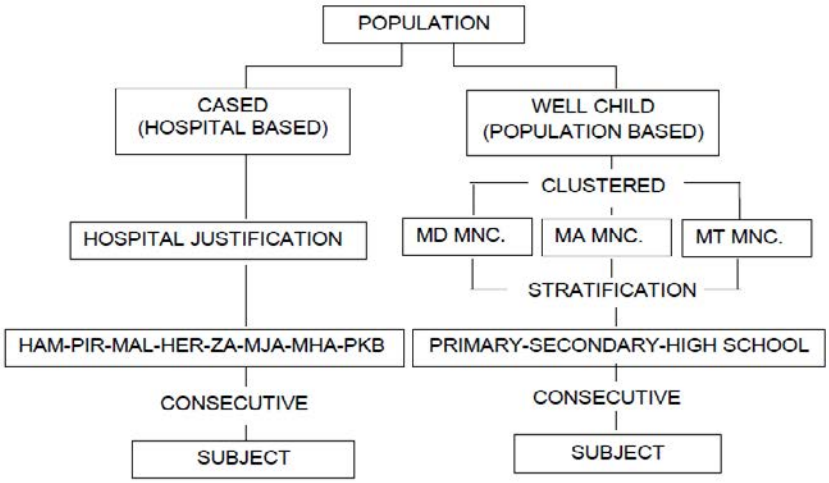
Each hospital was selected based on having NS patients in a similar age range, similar vision and mission for the management of NS, and an appropriate system for transporting samples to the laboratory. After the study subjects were identified, patient data were coded and labeled to anonymize study participants from the outset.

SRNS was diagnosed for children who had not responded to standard steroid treatment within 4 weeks of initiation. SSNS patients were defined as patients who went into remission after standard steroid treatment and remission was defined by proteinuria levels in the non-nephrotic range. "Well child" was defined as a person aged below than 18 years; with confirmed normal renal function (normal estimated Glomerular Filtration Rate (GFR), and spot Urinary Albumin Creatinine Ratio (UACR) value below than 150 µg/mg creatinine.

A well-trained doctor interviewed and enrolled the study subjects using a questionnaire/health examination to gather demographic and clinical data. The clinical records of the SSNS and SRNS cases were reviewed. The study parameters included age, sex, body weight and height, blood pressure, threshold steroid dosage at inclusion, spot UACR, plasma angiotensin II, and serum MIF.

Blood pressure was measured with a mercury sphygmomanometer, using an appropriately sized cuff for a child's upper-arm circumference. Blood pressure was measured after the children had rested for 10 minutes. An average of three blood-pressure readings was used for data analysis. For categorical analysis, blood pressure was divided into two categories: systolic/diastolic hypertension, and normotension. The children were categorized as having systolic/diastolic hypertension by computing systolic and diastolic blood pressure percentiles, adjusted for height, sex, and age. Hypertension was defined as systolic and/or diastolic blood pressure upper or equal 95th percentile [10]. Plasma angiotensin II concentrations were measured by commercial Enzyme-Linked Immunosorbent Assay (ELISA), with an analytical sensitivity of 1 pg/mL. The intra/inter-assay Coefficient Variation (CV) was 7%. Since a cut-off for plasma angiotensin II was not found, for the categorical analysis we analyzed fragmentation specificity and sensitivity values. Plasma angiotensin II levels were considered high if the value was upper than 18.2 pg/mL. Serum MIF concentrations were measured by commercial ELISA, at an analytical sensitivity of 1 ng/mL. The intra-assay CV was 5% and the inter-assay CV 7%. Proteinuria was expressed as the spot urinary ratio of protein to creatinine (UACR). The study subjects and their parents visited the laboratory in the morning (between 08.00 and 10.00). For SRNS and SSNS patients, blood and urine were collected at most 2 weeks after completing a full course of steroid treatment. The threshold dose of prednisone is amount of prednisone which had taken by patients at inclusion of study. This is measured by computed blisters of prednisone which patients taken and expressed in mg/m^2 body surface area every other day. All tests were performed according to standard protocols and established guidelines. The laboratory technicians were blinded to the test results.

Subjects were included into the study if they were aged between 1-17 years, with normal renal function confirmed by Schwartz formula [11] (GFR variated according to age, sex, and body proportion, based on National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) guidelines [12]), who did not suffer from any systemic illness, and with the appropriate parental informed consent. Patients and controls were excluded if they had proteinuria (transient, orthostatic, or non-renal), were in an unstable clinical condition, or were undergoing Angiotensin-Converting Enzyme Inhibitor (ACEI) or Angiotensin Receptor Blocker (ARB) drug treatment. The study protocol and the informed consent procedure were both approved by the Ethics Board of the Medical Faculty, Sumatera Utara University, Indonesia.



Abbreviations: HAM: H.Adam Malik Hospital; PIR: Pirngadi Hospital; MAL: Malahayati Hospital; HER: Herna Hospital; ZA: Zainul Abidin Hospital; MJA: M. Djamil Hospital; MHA: M. Hoesin Hospital; PKB: Pekanbaru Hospital; MD MNC: Medan Denai Municipality; MA MNC: Medan Area Municipality; MT MNC: Medan Tuntungan Municipality

Figure 1: Subject recruitment.

Statistical analysis

Descriptive analysis was performed as appropriate, and normally distributed variables were expressed as mean +/- SD. Nonparametric variables were expressed as median and range. Correlations between serum MIF level and plasma angiotensin II level were evaluated by Spearman's test. Logistic regression was used for multivariate analysis. Since the response variable was categorical (higher vs normal serum MIF), the logistic regression model was adjusted such that all variables with P value ≤ 0.25 were included. By stepwise elimination, variables that retained a significantly independent association with the presence of higher MIF were included in the final model. All P values were two-sided and P value <0.05 was considered statistically significant.

Results

Serum MIF levels in well child and NS patients group

General characteristics of the patients versus controls are summarized in Table 1. Measurement of the serum MIF levels in the pediatric serum samples revealed that most of the subjects had MIF levels of between 10.4 and 31.8 ng/ml. Half of the SNRS subjects (n=20) showed higher MIF levels, ranging from 33.8 to 117.2 ng/ml.

Group SRNS had higher threshold steroid dosage at inclusion of study (Table 1). The SRNS group had significantly higher serum MIF levels than SSNS and well-child groups (Figure 2). As seen in Table 2, systolic, diastolic hypertension and higher plasma angiotensin II levels were significantly related with higher serum MIF in bivariate analysis. Logistic regression testing showed that diastolic blood pressure and plasma angiotensin II levels were independently factors related to SRNS with high serum MIF (Table 3).

Correlation between MIF serum and plasma angiotensin II levels

Serum MIF and plasma angiotensin II were positive correlated, although correlation was weak (Figure 3).

Discussion

We conducted this study to document the potential risk factor of higher MIF among children with SRNS. In this study we found half of the SRNS subjects had higher serum MIF concentrations. Despite the huge threshold dosage of steroid at inclusion, it was noted that higher MIF concentrations obscure the effects of steroids in SRNS. These findings might support a role for this cytokine blunting steroid therapy [3]. Hence, although higher dosage levels of steroids are noted in the circulation, the effectiveness of the steroid in reducing proteinuria is limited.

Studies by Savin et al. [13] evaluated the possibility that circulating factors may cause glomerular injury. Savin's study showed that, although serum NS subjects derived from various histopathological findings but could still increase glomerular permeability to albumin. Carlotti et al. [14] showed that cytokine concentrations remained higher, even with high doses of glucocorticoids, and this affected tissue sensitivity to glucocorticoids [15]. The risk of steroid resistance becomes greater when podocyte sensitivity to glucocorticoids is impaired.

For these reasons, cytokines can result in glomerular injury. In the circulation, higher MIF concentrations influence the influx of macrophages into the vascular wall [16]. These findings were confirmed by the correlation between the influx of macrophages, and renal function. Although there is no evidence of subjects with decreased renal function in our study, the inflammatory process could occur with GFR upper than 90 ml/min/1.73 m^2.

Angiotensin II-induced hypertension also led to infiltration of macrophages in the glomerular and tubular cells accompanied by the induction of various cytokines [7,17]. Furthermore, the infusion of chronic doses of angiotensin II into rat models caused increased blood pressure and impaired vascular relaxation [18]. In children with proteinuria (such as SRNS patients), persistent elevation of systemic blood pressure, especially diastolic blood pressure, is a predictor of further kidney damage.

In this study, we found that diastolic blood pressure and plasma angiotensin II may affect MIF serum concentration. A correlation (albeit weak) between serum MIF concentrations and plasma angiotensin II has also been shown. This reinforces the explanation that macrophage adhesion to blood vessel walls is accompanied by cytokine MIF. Therefore, the role of systemic plasma angiotensin II on circulating MIF can explain how the equilibrium concentration of serum MIF

Characteristic	SRNS n=40	SSNS n=28	Well child n=31
Age at study, mean (SD) yrs	8.3(4.5)	8.5 (3.9)	12 (3.6)
Age at diagnosis, mean(SD) yrs	5.9 (3.3)	6.2 (3.2)	-
Sex, n (%)			
Boys	31(77.5)	17 (60.7)	16 (51.6)
Girls	9 (22.5)	11 (39.3)	15 (48.4)
Systolic blood pressure, mmHg (SD)	113.6(14.6)	103.2 (10.2)	106.8 (10.8)
Diastolic blood pressure, mmHg (SD)	75.3(12.8)	65.4 (7.9)	68.4 (9.3)
Serum MIF, median (min-max) ng/mL	31.9(14.3-117.2)[a]	21.8(10.4-31.8)	24.1 (11.4-31.1)
Plasma angiotensin II, median (min-max) pg/mL	22.8 (3.1-153.4)[b]	16.3 (2.9-139.5)	12.9 (5.5-58.8)
Patients' management, n (%) *Pred + CPA iv *Pred + CPA oral *Pred + CsA *Pulse MP + Pred + CPA oral *Pred AD only	 22 (55) 14 (35) 2 (5) 2 (5) -	 - - - - 28 (100)	 -
Threshold dose of prednisone at inclusion of study, median (min-max) mg/m^2 BSA e.o.d	33 (16-41)[c]	12 (5-34)	-
UACR, mean (SD) µg/mg creatinine	5898.6 (4289)[d]	65.7 (7.5)	6 (4)

Abbreviations: SD: Standard Deviation; Pred: Prednisone; CPA= Cyclophosphamide; Csa: Cyclosporine A; Pulse MP: Pulse Methyl Prednisolone; Pred AD: Prednisone Alternating Days; BSA: Body Surface Area; E.O.D: Every Other Day; UACR: Urinary Albumin Creatinine Ratio
Statistics: [a]p<0.001 vs. other group (Kruskall Wallis test), [b]p=0.01 vs. other group (Kruskall Wallis test), [c]p<0.001vs. SSNS (Mann Whitney), [d]p<0.001 vs. other group (Anova)

Table 1: Demographic characteristics of the study subjects.

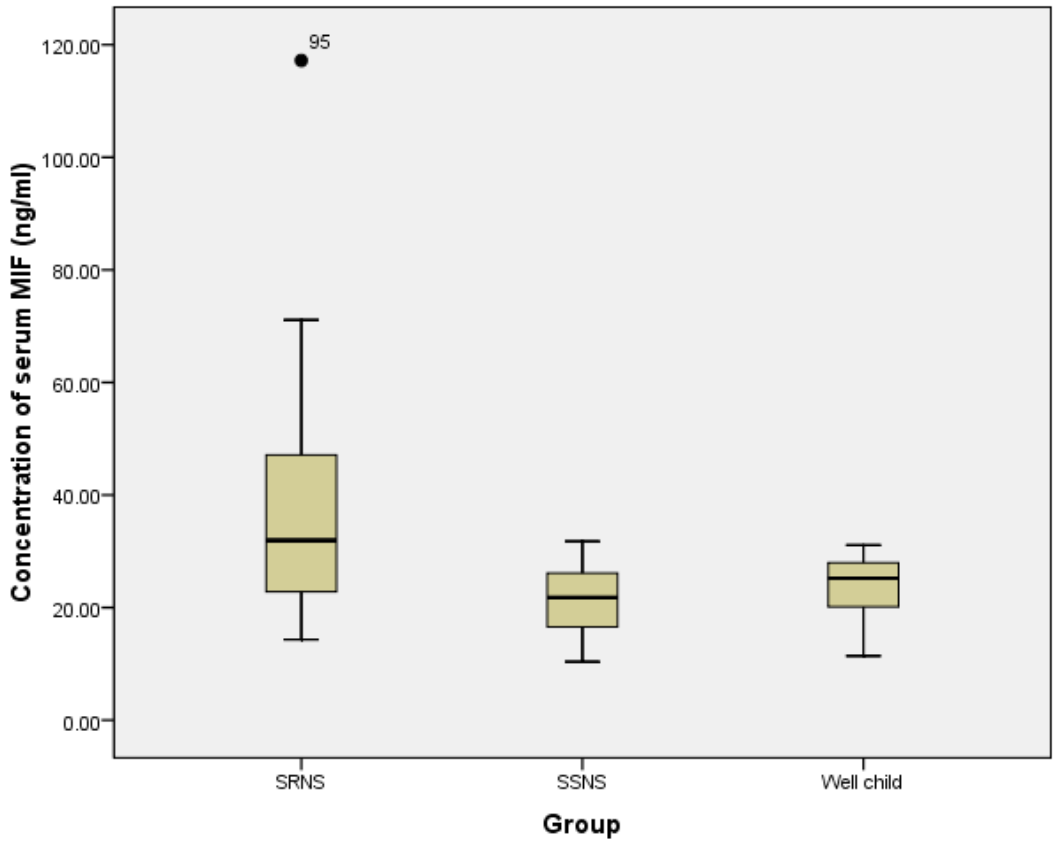


Figure 2: Box plot concentration of serum MIF between group. p<0.001 between SRNS and SSNS; p=0.12 between SSNS and well child; p=0.001 between SRNS and well child (Mann Whitney test).

Variable	SRNS with elevated serum MIF n=20	Control n=79	P
Boys Girls	15 5	49 30	0,278
Systolic hypertension Normotension	8 12	15 64	0,047
Diastolic hypertension Normotension	9 11	12 67	0,004
Higher Ang II Low Ang II	5 15	52 27	0,001

Abbreviations: Ang II: plasma angiotensin II

Table 2: Bivariate analysis factors related to higher MIF serum levels.

Model	Coefficient	S.E	P	OR	95% CI
Constant	-4.101	1.007	0.017		
Diastolic hypertension	1.188	0.581	0.041	3.282	1.052-10.242
Higher Ang II	1.540	0.585	0.009	4.665	1.481-14.691

Abbreviations: Ang II: Plasma Angiotensin II; SE: Standard Error; OR: Odds Ratio; 95% CI; 95% Confidence Interval

Table 3: Final logistic regression models for higher MIF serum in SRNS.

has a protective effect on blood-vessel damage [19]. In the pre-dialysis context, for example, factors such as inflammation can aggravate atherosclerosis in chronic kidney disease [20]. Thus, managing SRNS patients only by resolving proteinuria problems is inadequate. Other factors need to be considered, such as atherosclerosis, by improving the MIF serum balance and managing hypertension.

This research is the first study (to our knowledge) that looked for correlations between serum MIF and plasma angiotensin II in SRNS patients with GFR upper than 90 ml/min/1.73 m^2. Although in this study, we did not find patients with renal function below than 90 ml/min/1.73 m^2, it is very important to prevent SRNS patients' progressing to more serious levels of chronic kidney disease. The balancing of serum MIF concentration and the management of hypertension do have an impact on this disease. The main hope for the effective treatment of SRNS lies with newer drugs and novel treatments (such as anti-MIF antibodies and antagonist MIF). Nevertheless, further information about the currently used options is of interest to those dealing with

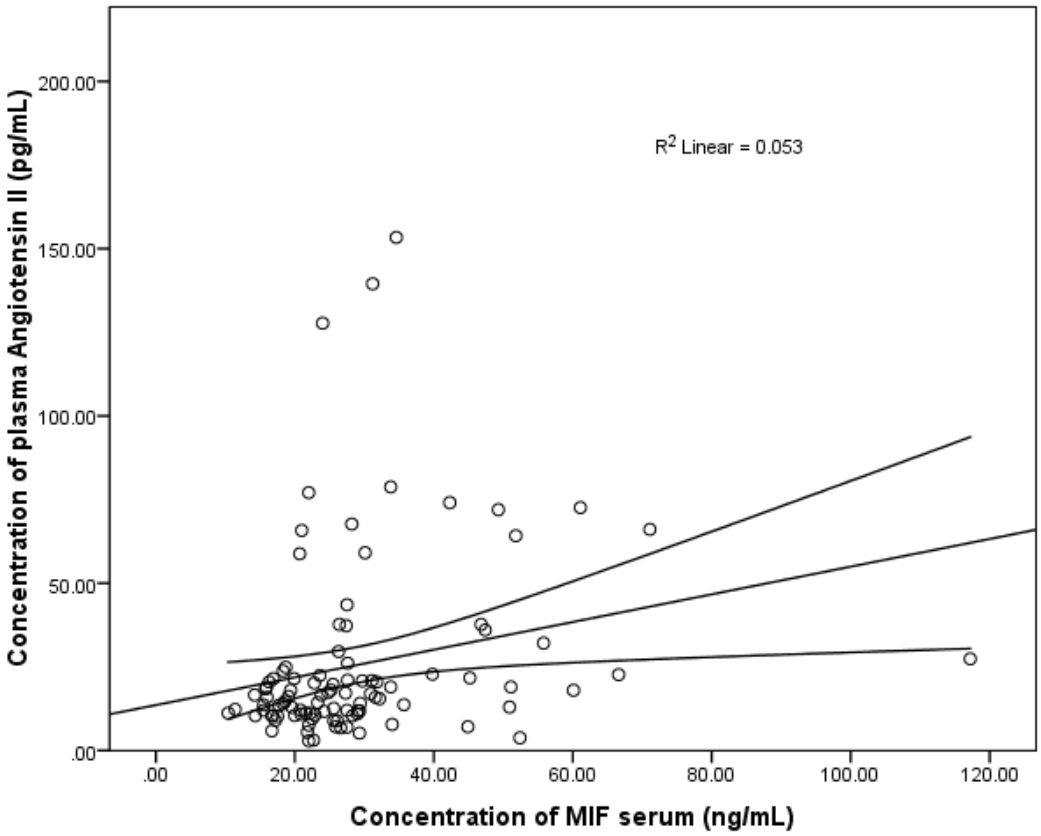


Figure 3: Correlation between MIF serum and plasma angiotensin II.

these questions today. The main limitations of the current study were it's a cross-sectional design and selection bias, which could exclude other morbid patients.

Conclusions

In conclusion, our data showed higher serum MIF levels in SRNS. These associated with diastolic blood pressure and plasma angiotensin II levels. Despite the huge threshold dose of steroid at inclusion among the SRNS subjects, higher MIF concentrations obscure the effects of steroid therapy.

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