Assessment of serum amyloid A levels in preeclamptic women and healthy pregnant women

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Abstract

Objective. *The link between preeclampsia and inflammation has directed the research to the investigation of inflammatory markers in preeclampsia. The present study aimed to evaluate serum amyloid A (SAA) levels in preeclamptic and healthy pregnant women detecting the relationship between the preeclampsia and SAA levels.* ***Methods.*** *Twenty preeclamptic women and eighteen pregnant women without any medical history, which constitute the control group, having between 35th to 41st gestational weeks, were included in the present study. For the analysis of SAA levels, maternal blood samples were collected within two hours before caesarean section, and the umbilical cord blood samples were collected at birth. SAA levels were measured using the enzyme linked immunosorbent assay method.* ***Results.*** *No significant difference was observed between the preeclamptic women and healthy pregnant women in terms of SAA levels (286.46 ng/mL versus 83.24 ng/mL; p> .144, median values). Furthermore, the same results were found in the umbilical cord blood SAA levels between the babies born from preeclamptic women and those born from healthy pregnant women (7.66 ng/mL versus 7.84 ng/mL; p= .725, median values).* ***Conclusions.*** *An elevated plasma level of SAA in healthy and preeclamptic women should be considered pathologic, and in this respect, the response of relationship between the preeclampsia and SAA levels could be caused by an inflammatory condition other than preeclampsia.*

Keywords: *preeclampsia, serum amyloid A, caesarean section*

Introduction

Hypertension is among the most common medical disorders during pregnancy and the rate of hypertension ranges from 5% to 6% for all types of pregnancy. Hypertension together with hemorrhage and infection is responsible for the majority of maternal morbidity and mortality during pregnancy[1]. Furthermore, hypertension is responsible for 16% of maternal mortalities in developed countries[2].

The etiopathogenesis of preeclampsia has not been elucidated until present. However, it is known that preeclampsia is characterized by an excessive maternal inflammatory response. Both preeclampsia and cardiovascular diseases are associated with inflammation and share common risk factors such as obesity[3]. In other studies, different results have been reported for the levels of C-reactive protein (CRP), which is known to be an inflammatory marker and acute phase reactant, in preeclamptic women[4-7]. Another inflammatory marker, serum amyloid A (SAA), is a precursor protein leading to the formation of AA fibrils in systemic AA amyloidosis and plays an important role in the development of systemic AA amyloidosis[8]. Serum levels of SAA increase considerably in response to viral and bacterial infections, inflammation, tumor growth and physical stress[9]. Acute phase SAA levels in serum may increase up to 1000-fold and may reach up to the level of 500-1000 μg/mL[10]. SAA is synthesized by hepatocytes in response to inflammatory cytokines[11]. It has been reported that SAA is also produced by fibroblasts, synovial cells, macrophages and adipocytes[12]. To our knowledge, a limited number of studies evaluated the SAA levels in pregnant women with preeclampsia and conflicting results were reported[4,5]. The present study aimed to evaluate SAA levels in preeclamptic and healthy pregnant women, detecting the relationship between the preeclampsia and SAA levels.

Methods

Twenty preeclamptic women and eighteen healthy pregnant women without any medical

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history which constitute the control group were admitted to the Department of Obstetrics and Gynecological Diseases, Ondokuz Mayıs University Medical Faculty, between August 01 2009 and October 15 2009 and included in the present study. All women were between 35th to 41st gestational weeks. Written informed consent was obtained and the study was approved by the Ethical Committee of Ondokuz Mayıs University Medical Faculty.

A preeclampsia diagnosis was established if a blood pressure of >140/90 mmHg was confirmed by two measurements performed at 6 hours intervals and in the presence of excess amount of protein (>300 mg/L) in a 24 hours urine specimen. Severe preeclampsia was defined as follows: a blood pressure level of >160/110 mmHg, proteinuria (>5 g/24 h), oliguria (<500mL/24 h), vision disorders, headache, epigastric pain, increase in the level of serum creatinine, thrombocytopenia (<100000/mm^3), liver dysfunctions and/or abnormal peripheral smear, pulmonary edema, and intrauterine growth retardation together with abnormal umbilical artery doppler findings or oligohydramnios.

Socio-demographic characteristics of the women and their reproductive and medical data (hemoglobin (Hb), hematocrit (Htc), white blood cell (WBC), neutrophil and platelet (Plt) counts, glucose, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels) were assigned in the patients' record. The data obtained from the ultrasound examinations of the fetus and blood gas tests, which were routinely performed immediately after birth, were also assigned.

Women who had multiple pregnancies, fetal anomalies, diabetes mellitus, chronic hypertension, autoimmune disease, early membrane rupture, infection, systemic disease and those in active labor were excluded from the study. Since labor may induce an inflammatory response, and thereby may affect the results, women who gave birth through elective caesarean section were selected. The indications for caesarian section were preeclampsia, repeated caesarean section, abnormal fetal presentation and cephalopelvic disproportion.

As the time elapsed between blood collection and caesarean section operation may affect the results; 8 mL of blood was obtained from women within two hours before caesarean section for the analysis of SAA levels, and 8 mL of blood was obtained from the placental side of the umbilical cord after cutting the umbilical cord at birth for the analysis of umbilical cord blood SAA levels. The collected blood was centrifuged at a speed of 4500 rpm for 15 minutes, and the serum was stored at -80°C until processing. The serum was diluted twice before the analysis. The measurements of SAA levels were performed using the Invitrogen Human SAA enzyme linked immunosorbent assay (ELISA) kit (Invitrogen Corporation, Camarillo, CA, USA). The detection limit of the assay was established at 4ng/mL.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS 15.0 integrated version) was used. Continuous variables were expressed as mean ± standard deviation and percentages. The Shapiro-Wilk Test was used to test that the population is normally distributed. Further, we performed Mann-Whitney U test for the unpaired comparison of the groups. A p value of < .05 was considered statistically significant.

Table 1 Demographic characteristics of preeclamptic women and healthy pregnant women

Characteristics	Preeclampsia Group (n=20)	Control Group (n=18)	p
Maternal age	28.5±6.2	27.5±5.3	0.639
Gestational age	36.5±1.2	38.6±0.9	0.0006
Nulliparous	9 (45)	8 (45)	-
Multiparous	11 (55)	10 (55)	-

Data are presented as mean ± standard deviation or n (%), where appropriate

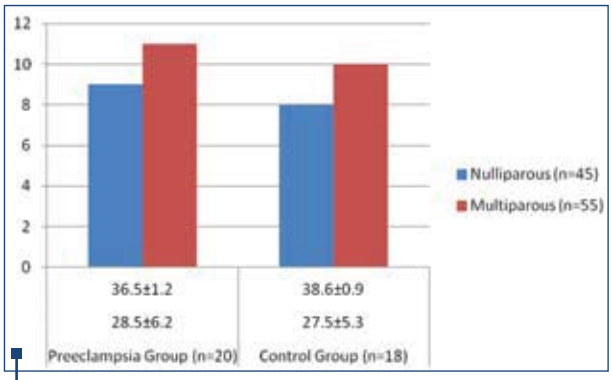

Figure 1. Characteristics of preeclampsia and control groups

Results

In the present study, 20 pregnant women with preeclampsia comprising the preeclampsia group and 18 women with normal pregnancy comprising the control group were analyzed. Of the pregnant women in the preeclampsia group, four were diagnosed with mild preeclampsia, 13 were diagnosed with severe preeclampsia and the remaining three were diagnosed with HELLP syndrome, in which "H" is for hemolysis (breakage of red blood cells), "EL" for elevated liver enzymes, and "LP" for low Plt count. Demographic characteristics of the patients are presented in Table 1.

The mean maternal age was 27.5±5.3 years in the control group and 28.5±6.2 years in the preeclampsia group. The mean gestational age was 38.6±0.9 weeks in the control group and 36.5±1.2 weeks in the preeclampsia group, respectively (Figure 1). While no significant difference was found between the groups in terms of maternal age (p= .639), the mean gestational age of the preeclampsia group was lower than control group (p= .0006).

No statistically differences were found between the groups in terms of the Hb and Htc levels, WBC, Plt, and neutrophil counts (Table 2). Moreover, there were no significant differences between the groups with respect to the levels of glucose, AST, ALT, BUN and creatinine (Table 3).

The median SAA level was 286.46 ng/mL in preeclamptic women and 83.24 ng/mL in healthy pregnant women. No significant difference was observed between SAA levels of women with or without preeclampsia, p> .144 (Figures 2 and 3).

The median SAA levels measured from the umbilical cord blood of babies born from preeclamptic women and women in the control group were 7.66 ng/mL and 7.84 ng/mL, respectively. No significant difference was found in the umbilical cord SAA levels between the babies born to preeclamptic women and those born from healthy pregnant women (p=.725).

Discussion

In the present study, we investigated the SAA levels in both preeclamptic and healthy pregnant women, and the relationship between the preeclampsia and SAA levels. However, no significant difference was noted in the SAA levels of preeclamptic women and healthy pregnant women.

In the study of Gatt and colleagues[13], SAA was shown in vitro to have modulatory effects on human neutrophil functions. Using recombinant SAA, Badolato et al.[14] demonstrated

Table 2 Hematological parameters of preeclamptic women and healthy pregnant women

Parameters	Preeclampsia Group (Mean±SD)	Control Group (Mean±SD)	p
Hb	12.1±1.36	11.9±1.19	0.303
Htc	35.8±3.44	35.2±3.36	0.771
Plt	183.9±82.25	189.6±35.47	0.060
Leukocyte	11765±3617.39	11033.3±3569.14	0.837
Neutrophil	9100±3953.67	8155.5±3138.04	0.693

Hb: hemoglobin, Htc: hematocrit, plt: platelet, SD: standard deviation

Table 3 Comparison of the laboratory values of preeclamptic women and healthy pregnant women

Parameters	Preeclampsia Group (Mean±SD)	Control Group (Mean±SD)	p
Glucose	97.7±17.54	84.3±14.93	0.05
AST	35±37.31	17.9±5.04	0.069
ALT	22.7±20.68	11.7±3.55	0.202
BUN	10.6±±4.01	12.7±16.61	0.349
Creatinine	0.62±0.76	0.53±0.077	0.11

AST: aspartate aminotransferase, ALT: alanine aminotransferase, BUN: blood urea nitrogen, SD: standard deviation

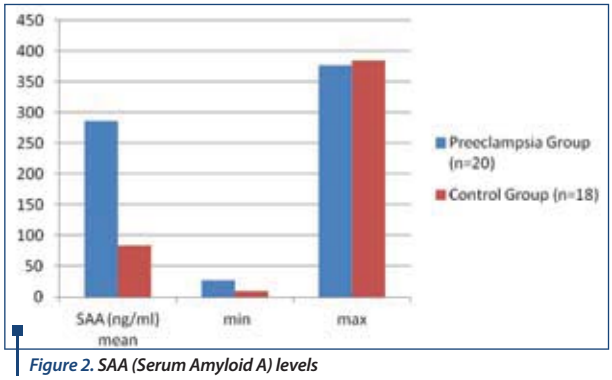

Figure 2. SAA (Serum Amyloid A) levels

that recombinant SAA induced the migration, adhesion and infiltrations of monocytes and polymorfonuclear leukocytes and functioned as a chemoattractant. In another study of Peristeris et al.[15], the inhibitory effects of SAA on the leukocytes were demonstrated. Shainkin-Kestenbaum et al.[16] showed that SAA increased the prostaglandin-I2 release from endothelium in bovine aortic endothelial cell culture. All these findings have pointed out modulatory role for SAA in the course of inflammation.

De Villiers et al.[17] analyzed CRP and SAA values during pregnancy and postpartum period and determined that SAA levels, in parallel to CRP levels, increased upon the end of labor. Cicarelli et al.[18] aimed to predict the development of postpartum maternal infections and measured the SAA levels, together with many inflammation mediators and acute phase reactants in maternal and cord blood during and after delivery. They reported that an increase was observed in the SAA levels of women after the delivery; however, such an increase did not occur in the umbilical cord blood of the babies. They determined that this increase did not differ according to the type of delivery and concluded that SAA alone could not be used as an inflammatory marker.

To our knowledge, there are a limited number of studies on SAA levels in pregnant women with preeclampsia, and different conflicting results have been reported[4,5]. In a pilot study of Engin-Ustun et al.[4], in which SAA levels of normal pregnant and preeclamptic women were evaluated, the SAA levels of preeclamptic women were found to be significantly higher than that of normal pregnant women. They reported that SAA appeared to be a marker of inflammation in preeclampsia.

Kristensen et al.[5] showed that plasma levels of SAA were evaluated in pregnant women with and without preeclampsia and non-pregnant women, and no significant increase was reported in the SAA levels of pregnant women with preeclampsia as compared to those without preeclampsia and non-pregnant women. In addition, they suggested that an elevated plasma level of SAA in healthy and preeclamptic pregnant women should be considered pathologic because it may be caused by an inflammatory condition other than preeclampsia. Although the SAA levels of preeclamptic women were increased compared to control group, no statistically difference was found between the two groups in the present study. Our study

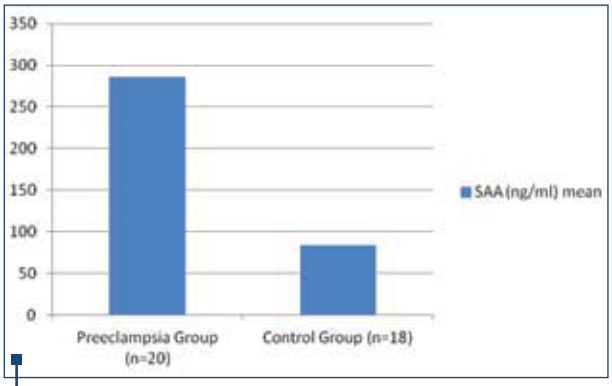

Figure 3. SAA (Serum Amyloid A) average levels in preeclampsia and control groups

showed similar results with the one reported by Kristensen et al[5]. Only a considerable difference was noted between the maternal blood and umbilical cord blood SAA levels both in the preeclampsia and control. This was suggestive taking into consideration the lack of transplacental transfer of SAA. De Villiers et al.[17] reported significant differences between maternal and neonatal SAA levels, and suggested that there was no transplacental transfer of SAA during labor. Moreover, Cicarelli et al.[18] reported significantly higher SAA levels in maternal serum than that in newborns, suggesting the lack of transplacental transfer of SAA.

Conclusions

Our data sustain the limited number of studies investigating the SAA levels in both preeclamptic and healthy pregnant women, in which it was hard to reach a consensus regarding the association between SAA levels and preeclampsia. Taken in consideration that an elevated plasma level of SAA in healthy and preeclamptic women should be considered pathologic, we believe that the response of relationship between the preeclampsia and SAA levels could be caused by an inflammatory condition other than preeclampsia. ■

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