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Original Article

Serum amyloid-a may be an early marker in diagnosis of preterm premature rupture of membrane and chorioamnionitis

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Abstract

Aim: To determine the efficiency of serum amyloid-A (SAA) in predicting preterm premature rupture of membrane (PPROM) and chorioamnionitis in risky cases.

Study Design: The study consists of 20 women 26-37 weeks of pregnancy who had PPROM and 20 pregnant women without water breaks. Levels of SAA were determined in maternal venous and umbilical cord blood.

Results: SAA values in cord blood and venous blood of mothers with PPROM were higher than in the control group (p<0,05). SAA values in the patients with clinical chorioamnionitis (n=9) were significantly higher (p<0,05) than both PPROM patients without chorioamnionitis (n=11) and the control group (n=20).

Conclusion: In women at risk for PPROM in whom diagnosis cannot be established, blood SSA levels can be used as a marker. Increased SAA values in pregnant women with PPROM who were conservatively treated were considered a marker for chorioamnionitis.

Keywords: Premature rupture of membrane; chorioamnionitis; serum Amiloid A

INTRODUCTION

Premature rupture of membrane (PROM) is a condition that occurs in pregnancy when there is rupture chorioamniotic membranes more than one hour before the onset of labor (1, 2). It is called preterm premature rupture of membrane (PPROM) when it occurs before 37 weeks gestation and term premature rupture of membrane when it occurs after 37 weeks gestation. In addition, it is called prolonged premature rupture of membrane when the latent period between rupture of membrane and onset of labor exceeds 24 hours (2-4)

PROM occurs in 8–10% of all pregnancies and accounts for approximately one-third of preterm labors and 18–20% of perinatal deaths (5-7). Fetal membranes function as a barrier to ascending infections. The pregnant woman and the fetus can experience various problems, particularly infection, when the membrane is ruptured. Common problems include increased prematurity, perinatal infection, oligohydramnios, and perinatal morbidity and mortality associated with fetal pulmonary hypoplasia(8).

In PPROM, the risk of perinatal mortality is increased four times and the risk of neonatal morbidity is increased three times (6, 9, 10). Neonatal complications resulting from prematurity include respiratory distress syndrome, necrotizing enterocolitis, intraventricular hemorrhage, bronchopulmonary dysplasia, premature retinopathy, patent ductus arteriosus, and neonatal sepsis. The frequency of such complications increases as gestation age decreases (11).

The incidence of intra-amniotic infection is 41% in PROM before 27 weeks gestation, 15% between 28 and 36 weeks, and 2% at term. Chorioamnionitis, prolonged PROM, multiple vaginal examinations, early gestation stage, and serious oligohydramnios are associated with prolonged labor and nulliparity cases (6). The risk of infection increases when the latent period is prolonged.

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Chorioamnionitis and decollement placenta are observed in 13-60% of PPROM patients . In those cases, because of infection, postpartum bleeding and placenta retention (12%) are higher (7,12,13).

PROM remains obscure in many ways, even though it is a significant cause of maternal and neonatal morbidity and mortality. There are no fixed evaluation and treatment criteria, and the treatment plan is based on the patient (14-16).

Serum amyloid-A (SAA) is an acute phase reactant that increases approximately 1000 times more than basal level in infectious diseases. The level of SAA rises and peaks early, particularly in viral and bacterial infections. It returns to normal when inflammatory stimulus stops (17-20).

The aim of this study was to investigate the efficiency of SAA in predicting PPROM and chorioamnionitis in at-risk patients by testing SAA levels in pregnant women with PPROM and PPROM with chorioamnionitis.

MATERIAL and METHODS

Our study was designed as a prospective cohort study. This study was conducted with 20 women 26–37 weeks pregnant who had PPROM and 20 pregnant women without water breaks but with another obstetric indication. All patients presented at the Department of Obstetrics and Gynecology of Ondokuz Mayis University Medical Faculty. The local ethics committee approved the study (TAEK/2010-151). Subjects have given their written informed consent. Patient information forms for women in both groups recorded demographic, reproductive, laboratory and ultrasonographic data, presence of chorioamnionitis findings, gestational week, time of membrane rupture, and delivery method.

The diagnosis of PPROM was made by observing accumulated amniotic fluid in the posterior fornix during sterile speculum examination and then using the Amnisure® ROM test (Amnisure International LLC, Cambridge, MA) in those suspected of having PPROM (21, 22). Gestation of each patient was evaluated, and then, either follow-up or delivery was planned. Patients with any infectious, autoimmune, or chronic systemic diseases were excluded from the study. Delivery indications of patients in the control group were generally preterm and preeclampsia.

To make a clinical chorioamnionitis diagnosis, maternal temperature had to be 380 C or higher and fetal tachycardia (160 pulse/min) and maternal tachycardia (100 pulse/min) had to be present. Maternal white blood cell count had to be higher than 15,000/µl, and at least the two of the findings, uterine sensitivity and fetid vaginal discharge, had to be present (23).

Venous blood was taken from mothers within an hour after delivery. Cord blood was taken with an injector at the end of the placenta. Blood samples were centrifuged (Shimadzu UV160A, SNo: 28006648, Japan) at 3000 rpm for 10 min and the serum stored at -800 C.

Concentrations of SAA in serum were measured using commercially available, solid-phase enzyme-linked immune-sorbent assay (ELISA) kits (Human SAA, Immunoassay Kit, KHA0012, Invitrogen Co., Camarillo, CA). Enzymatic reactions were quantified in an automatic microplate photometer. SAA levels were expressed as µg/ml. The lower detection limit was 4 ng/ml. The mean interassay coefficient of variation (CV) percent and intra-assay CV percent were 7.4% and 4.6%, respectively. All assays were conducted according to the manufacturer's instructions. Samples that showed higher concentrations were diluted and measured twice.

Data were analyzed using the statistical analysis software SPSS 15.0. Data were presented in mean ± standard deviation. The groups were compared using a Mann-Whitney U-test. Data were correlated using a Spearman correlation test. The significance level for all tests was $p < 0.05$.

RESULTS

The average age of pregnant women with PPROM was 27.2±5,2 and for women in the control group, 30.3±5,4 ($p < 0.05$). The gravida median value of women with PPROM and in the control group both were 2 ($p > 0.05$). The median value of abort numbers in women with PPROM and in the control group both were 0 ($p > 0.05$). The mean white blood cell count of women with PPROM was 12.130/ml and of those in the control group was 11.735/ml ($p > 0.05$). Therefore, there was no significant difference in the mean of gravida, aborts, and white blood cell count between women with PPROM and those in the control group ($p > 0.05$) (Table 1).

The mean SAA value of women with PPROM was 80 µg/ml; the mean SAA value of women in the control group was 10 µg/ml, indicating that the values of those with PPROM were much higher than of those in the control group ($p < 0.05$). When SAA values of cord blood were compared, cord blood from mothers with PPROM had a mean SAA value of 84 µg/ml, and blood from those in the control group had a mean SAA value of 7.4 µg/ml. Again, this indicated that SAA values in cord blood from those with PPROM were much higher than from those in the control group ($p < 0.05$) (Table 2) (Figure 1).

All women found to have chorioamnionitis were also in the PPROM group. The mean SAA value of those with chorioamnionitis was 107 µg/ml, and it was 21 µg/ml for those without. Therefore, SAA values in patients diagnosed with clinical chorioamnionitis ($n = 9$) were significantly higher ($p < 0.01$) than both the PPROM patients without chorioamnionitis ($n = 11$) and the control group ($n = 20$).

SAA values in cord blood from mothers with chorioamnionitis and in cord blood from mothers without were compared. Mean SAA values in cord blood for those with chorioamnionitis were 115 µg/ml and for those without, 26 µg/ml, indicating that SAA values in cord blood for mothers with chorioamnionitis were significantly higher than values in those without ($p < 0.01$) (Table 3).

Table 1. Clinical findings of patients			
	PPROM pregnant (n = 20) Mean ± SD	Control pregnant (n = 20) Mean ± SD	p
Age	27.2 ±5,2	30.3±4,4	0.049
Gravida	2 (1-6) *	2 (1-7) *	0.944
Aborts	0 (0-3) *	0 (0-3) *	0.521
WBC	12.130 ± 3.786	11.735 ± 3.230	0.797
*Median (min-max)			

Table 2. Comparison of the SAA levels of PPROM pregnant with control pregnant groups			
		SAA (µg/ml)	p
PPROM pregnant	(n=20)	80 ± 44	p<0.05
Control pregnant	(n=20)	10 ± 7.2	
PPROM umbilical cord	(n=20)	84 ± 40	p<0.05
Control umbilical cord	(n=20)	7.4 ±3.0	

Table 3. SAA levels of pregnant with/without chorioamnionitis			
		SAA (µg/ml)	p
Positive- pregnant	(n=9)	107 ± 8.4	p<0.001
Negative- pregnant	(n=31)	21 ± 4.5	
Positive-umbilical cord	(n=9)	115 ± 12	p<0.001
Negative umbilical cord	(n=31)	26 ± 32	

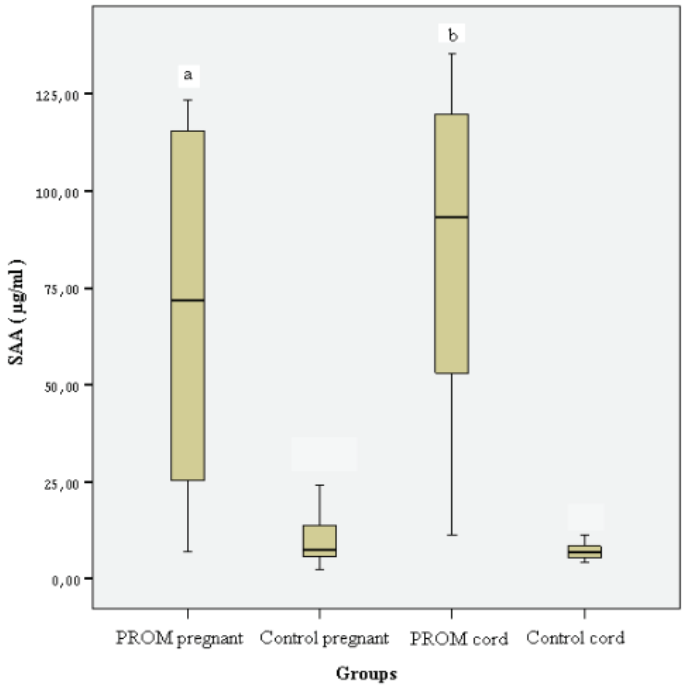

Figure 1. Comparison of the levels of SAA within the groups

DISCUSSION

No study has yet compared SAA values between pregnant women with PROM and a control group. Early diagnose of infection in patients with PPROM will allow earlier intervention to prevent morbidity and mortality, particularly neonatal mortality (24).

In a study of PROM patients, de Villiers et al. took blood samples and cord blood from patients in different peripartum periods. They observed that SAA values of women immediately after delivery at 38 weeks gestation were higher than those measured 24 hours after delivery and that SAA values for healthy women measured immediately after and 24 hours after delivery were the similar with C-reactive protein (CRP). They also found a significant difference between maternal and neonatal SAA levels (25).

Cicarelli et al. showed that the maximum increase in SAA levels 24 hours postpartum in women with PROM who had given birth at approximately 39 weeks gestation was higher than it was for CRP. In Cicarelli's study, the SAA and CRP concentrations in maternal blood were significantly higher than in cord blood, agreeing with results of the study by de Villiers et al. [26]. Both Cicarelli and de Villiers reported difficulties with using SAA and CRP as markers of maternal postpartum infection. SAA levels were much lower in cord blood than in maternal blood, indicating that those proteins had no placental transmission during delivery. Therefore, the increase of SAA and CRP in newborns might be a result of infection, trauma, or disrupted placental barrier(26).

Laurenti et al. found that SAA concentrations in healthy newborns were similar to those in normal adults (27). In the current study, there was no significant difference in the SAA levels of maternal and cord blood, either in the PROM group or in the control group. Mean SAA values in the blood of women with PPROM and in their cord blood

were both higher than levels in the control group. For patients with chorioamnionitis, SAA levels in cord blood were higher than in either PPROM patients or the control group. SAA levels were increased in the blood of mothers with chorioamnionitis and in their cord blood. This increase in SAA levels in cord blood might be an indicator of transplacental SAA transmission.

Cicarelli et al. stated that the body's defense system might have increased SAA in the mother's womb in response to the effects of instruments and the trauma of delivery and that it was difficult to use the postpartum increase at 24 hours to identify maternal infection (25). However, this proposed explanation does not account for why SAA levels were higher in chorioamnionitis patients than in PROM patients or control group patients.

Both de Villiers et al. and Cicarelli et al. selected patients with term PROM and their study groups were smaller than those in the current study were. They did not state whether their patients had clinical, subclinical, or histological chorioamnionitis, so it is not possible to evaluate from their results whether SAA could be used to diagnose maternal infection.

Laurenti et al. specified that SAA levels were not affected by patients' ages, gestational ages, or method of delivery (27). In the current study, SAA levels did not correlate with gestational ages.

PROM might lead to infection; infection is in the etiopathogenesis of PROM. The literature focuses on CRP in diagnosing chorioamnionitis. In the current study, increases in CRP and SAA concentrations were observed to be simultaneous. It has been reported that SAA levels are more effective in diagnosing and following up early infection than are CRP levels (26). In viral infections, CRP levels remain normal, but SAA levels increase in both viral and bacterial infections (28).

In PPROM patients in the current study, increased SAA levels in both maternal blood and cord blood suggested that they might have had subclinical chorioamnionitis that was undetected. This is because SAA values are higher in the maternal blood and cord blood of chorioamnionitis patients compared to patients with PPROM. SAA levels were higher in women with PPROM than in those without PPROM, indicating that SAA levels of patients at risk for PPROM can be used to predict onset of PPROM.

Limitations of the current study include the small number of patients included and that SAA values in maternal and cord blood were measured only once. Therefore, future studies should take multiple serum samples that include the whole peripartum period and should use a larger number of patients to make peripartum evaluations of PROM. Similarly, comparison of various acute phase reactants with SAA protein levels, particularly in women histologically diagnosed with chorioamnionitis, will help to determine whether measuring SAA levels is an efficient way to diagnose PPROM and chorioamnionitis.

CONCLUSION

In conclusion, the current study showed that detecting high SAA levels as an acute phase reactant in the blood of pregnant women could help diagnose PROM in those at risk for it that had suspicious physical examination findings. Increased SAA values in women with PPROM who are conservatively treated appear to be a precursor parameter for chorioamnionitis..

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