Ginekol Pol. 2014, 85, 516-520

Maternal serum amyloid A levels in pregnancies complicated with preterm prelabour rupture of membranes

Poziom amyloidu A w surowicy u kobiet w ciąży powikłanej przedwczesnym pęknięciem błon płodowych

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Abstract

Objective: *The aim of the study was to investigate a possible association between maternal serum amyloid A levels (SAA) and maternal and fetal parameters in pregnancies complicated with preterm prelabor rupture of membranes (PPROM).*

Material and methods: *A total of 88 pregnant women (PPROM group, n=44 and control group, n=44) were included into this prospective case control study. Serum blood samples for SAA were obtained from both groups within 1h since the rupture of the membranes and before administration of any medicine. The samples were kept frozen at -70°C until the analysis. The recorded risk factors were: age, gravidity, parity, delivery mode, gender, fetal birth weight, APGAR scores, white blood cell count, microCRP, neutrophil/lymphocyte ratio (NLR), and maternal serum SAA levels.*

Results: *Demographic characteristics showed no statistically significant differences between the groups (p>0.05). The mode of delivery mode was cesarean section: 41% and 43.2% in the study and the control group, respectively, and this difference was statistically significant between the groups (p<0.05). Fetal parameters also showed statistically significant differences (p<0.05). There was a statistically significant difference between the groups in terms of micro CRP, NLR and SAA. SAA levels were higher in the PPROM group (p<0.005). SAA levels at a cut-off 95.63 ng/ml.*

Conclusion: *We are of the opinion that second trimester maternal serum SAA level may be a predictive marker for PPROM. However, further studies with more participants are required.*

Keywords: **preterm prelabour rupture of membranes** / **maternal** / / **serum amyloid A protein** /

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Otrzymano: **15.01.2014**
Zaakceptowano do druku: **15.03.2014**

Streszczenie

Cel pracy: *Celem badania jest ocena związku pomiędzy matczynym poziomem amyloidu A (SAA) a parametrami matczynymi i płodowymi w ciąży powikłanej przedwczesnym pęknięciem błon płodowych (PPROM).*

Materiał i metoda: *Do badania prospektywnego z grupą kontrolną włączono 88 kobiet ciężarnych (grupa z PPROM – 44 pacjentki, grupa kontrolna – 44 pacjentki). Próbki krwi do badania na SAA pobierano w ciągu godziny od pęknięcia błon płodowych i przed podaniem jakichkolwiek leków. Próbki były utrzymywane w temp.-70 C do czasu przeprowadzenia analizy. Analizowano następujące czynniki ryzyka: wiek, liczba ciąż, liczba porodów, sposób porodu, płeć płodu, masa urodzeniowa, APGAR, liczba leukocytów, wskaźnik neutrofili/limfocytów (NLR) i poziom matczynego amyloidu A w surowicy.*

Wyniki: *Czynniki demograficzne nie różniły się istotnie pomiędzy badanymi grupami (p>0,05). Odsetek porodów przez cięcie cesarskie: 41% i 43,2% odpowiednio w grupie badanej i w grupie kontrolnej różnił się istotnie (p<0,05). Parametry płodowe różniły się istotnie statystycznie pomiędzy obiema grupami (p<0,05). Znaleziono istotną statystycznie różnicę pomiędzy grupami w odniesieniu do CRP, NLR i SAA. Poziom SAA był istotnie wyższy w grupie z PPROM (p<0,005). Poziom odcięcia dla SAA wynosił 95,63ng/ml.*

Wnioski: *Wydaje się, że poziom matczynego SAA w surowicy może być markerem predykcyjnym dla PPROM. Konieczne są dalsze badania na większej grupie pacjentek.*

Słowa kluczowe: **przedwczesne pęknięcie błon płodowych** / **ciąża** / / **surowicze białko amyloidu A** /

Introduction

Preterm prelabour rupture of membranes (PPROM) is one of the most common complications of pregnancy, with 0.5-5% and 30-40% incidence for all pregnancies and preterm pregnancies, respectively [1-3]. PPROM is associated with severe complications (chorioamnionitis, premature birth, pulmonary hypoplasia, and fetal death) for both, the mother and the fetus [4, 5].

Advancement of perinatal care brought increased efforts to reduce prematurity and the risk of infection. Subclinical intrauterine infection and chorioamnionitis appear to play a significant role in the pathogenesis of PPROM [6]. Previous studies investigated the role of intrauterine infection in PPROM. For examples, Flídrová et al., reported increased concentrations of proinflammatory markers (interleukin (IL)-1beta, IL-6, tumor necrosis factor alpha and IL-8 in preterm birth and probably in PPROM [7]. Taking into consideration all of the above, in our study we also investigated the association of maternal SAA levels with other inflammatory markers. SAA is a member of apolipoproteins associated with high density lipoproteins in plasma. It is also associated with inflammatory response highly similar to erythrocyte sedimentation rate and C-reactive protein (CRP) [8, 9]. In a previous study, SAA and CRP were reported to be good predictors of histological chorioamnionitis at an earlier stage of PPROM without any clinical signs [10].

In the present study, we aimed to evaluate maternal SAA concentration in pregnancies complicated with PPROM and its association with maternal and fetal clinical and biochemical features.

Materials and methods

This prospective case control study was conducted between January 2011 and January 2012 at Dr. Zekai Tahir Burak Women's Health and Research Hospital, Department of High-Risk Pregnancy, a tertiary research and education hospital in the capital city of Turkey. This is a government-funded hospital, and most of the health services are provided free of charge.

A total of 88 pregnant women (PPROM group, n=44 and control group, n=44) were included into the study. Cases with intrauterine infection (maternal fever, significant maternal tachycardia, fetal tachycardia, uterine tenderness, cervical motion tenderness, purulent or foul-smelling amniotic fluid or vaginal discharge), maternal medical problems, fetal anomalies, and women with vaginal bleeding were excluded from the study. All subjects gave their written informed consent to the study and the protocol was approved by the Local Ethics Committee.

Patient data were collected from hospital records and patient files. The recorded risk factors included age, gravidity, parity, delivery mode, gender, fetal birth weight, APGAR scores, white blood cell count, microCRP levels, NLR and maternal SAA levels. After the initial evaluation including general, gynecological and obstetric history, vital signs, systemic examination and ultrasound examination, the study participants were hospitalized in our high-risk pregnancy department.

Gestational age (weeks) was assessed by an ultrasound examination (GE Logiq 200 PRO Ultrasound Device, USA) or according to the last menstrual period, or both. Fasting blood samples were obtained from the antecubital vein at the time of diagnosis in the PPROM group and during the regular follow-up in the control group. Serum was collected within 1h since the rupture of the membranes and before administration of any medicine and kept frozen at -70°C until the analysis.

CRP levels were determined by CRP kit (Beckman coulter, IMMAGE S/N 2528, USA), and SAA levels were determined by the nefelometric method with an enzyme-linked immunosorbent assay (Cusabio Human Serum Amyloid A (SAA) Elisa Kit,).

Table I. The demographic and clinical characteristics of the cases.

	PPROM group (n=44)	Control group (n=44)	p
Age (years)	28,02±5,65	28,5±6,41	0,572
*Gravida ≤2 >2	26(%59) 18(%41)	33(%75) 11(%25)	0,466
*Parity ≤1 >1	29(%65,9) 15(%34,1)	37(%84) 17(%16)	0,720
*Smokers (%)	6(%13,6)	5(%11,3)	0,980
Income (TL/month)	1260±576	1146±389	0,103
SAA	905.16±2652.79	72,71±100.09	0.041
MicroCRP	15,88±15,9	6,99±10,1	0,003
NLR	5,79±3,27	4,27±1,65	0,024

* Data was presented as % and p calculated by (c2) test, TL: Turkish lira, SAA: serum amyloid A

Table II. The clinical characteristics of the newborns.

	PPROM group (n=44)	Control group (n=44)	p
Birth weights (grams)	1960.68±382.18	3160.23±435.79	<0.001
Gestational age at delivery (weeks)	31,3±1,99	38,8±1.47	0,043
Delivery type Vaginal (%) C/S (%)	 26(%59) 18(%41)	 25(%56,8) 19(%43,2)	0,903
Gender Female(%) Male(%)	 17(%38,6) 34(%61,4)	 23(%52,2) 21(%47,8)	0,286
Apgar 1 4(%) 5(%) 6(%) 7(%)	1(%2,2) 2(% 2,2) 2(%4,5) 39(%88,6)	 1(%2,2) 43(%97,7)	0,220
Apgar 5 7(%) 8(%) 9(%)	3(%6,8) 1(%2,2) 40(%91)	 1(%2,2) 43(%97,8)	0,369

Apgar 1: 1.minute APGAR score, Apgar 5: 5. minute APGAR score

Table III. Odds ratio calculated by binary logistic regression method.

	β	Wald	Odds ratio	SE
MicroCRP	-,063	9,145	,939	,021
NLR	-,363	7,713	,695	,131
SAA	-,002	1,055	,998	,002

Table IV. Pearson correlation analysis between microCRP, NLR and SAA levels.

	MicroCRP		NLR	
	R	p	r	P
Serum amyloid A	-0.265	0.017	-0.334	0.023

Statistics

Mean and standard deviation (SD) were calculated for continuous variables. Normality of the variables was analyzed by Kolmogornov-Smirnov test. Chi-square (χ^2) test Student's t test and Mann Whitney U test were used to evaluate associations between categorical and continuous variables. The logistic regression method was used to find risk variables for patients by including all variables in the model and to calculate the odds ratios. Pearson correlation analysis was used to find the correlation between microCRP, NLR and SAA levels. The receiver operator characteristic (ROC) curve analysis was used to establish the cutoff values for micro CRP, NLR and SAA levels. To determine the locally appropriate cut-off point for each SAA levels, the Youden index (sensitivity + specificity − 1) was calculated and the corresponding cut-off value for the highest Youden index was considered as the optimal cut-off value. All variables were included in the backward stepwise procedure. The sample size was determined according to the results of the central limit theorem [11]. Two-sided p values were considered statistically significant at p<.05. Statistical analyses were carried out by using the statistical packages for SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

In the present study, we evaluated maternal serum SAA levels in pregnancies complicated with PPROM. We designed a prospective case control study and a total of 88 women (44/50% with PPROM) were included. Demographic features such as age, gravidity, parity, smoking and economic status showed no statistically significant differences between the groups. Gestational age and fetal birth weight of the newborns were lower in the PPROM group (p<0.05). The delivery mode, fetal gender and APGAR scores were not statistically significantly different between the groups. Maternal micro CRP levels, NLR and SAA levels were higher in the PPROM group and this difference was statistically significant between the groups. According to the logistic regression method and ROC analysis, these parameters constituted risk factors for PPROM.

The study included 88 patients (PPROM group: 44 patients and control group: 44 patients). Patient demographic and clinical data are shown in Table I.

The majority of the women were grand multiparous (n=18/41%), while the rest were nulliparous (n=26/59%). The overall mean maternal age was 28.2±5.6 years (range: 18-40 years) in the PPROM group, 28.5±6.41 (range: 17-42 years) in controls. The gestational age of the fetuses was ≤28 weeks in 24.34% of the cases, 28-32 weeks in 25%, and >32 weeks in 50.65% of the cases in the PPROM group. Mean duration of admission to the hospital since the rupture of membranes was 3.5 hours (range: 1 to 9h). There was no statistically significant difference between the period of admission and other parameters. The route of delivery was selected according to the cervical Bishop score, obstetric history, and maternal as well as fetal situation. Spontaneous vaginal delivery (SVD) is preferred at our clinic, but in an emergency situation (fetal distress, malpresentation, abnormal vaginal bleeding) we prefer to perform a CS. Of the cases in our study, 18(41%) delivered by CS, and 26(59%) by SVD in the PPROM group, and 19(43.2%) by CS and 25(56.8%) by SVD in the control group. Mean WBC levels were 15805±9967 in the PPROM group and 11514±3037 in the control group (p<0.05). Mean micro CRP levels were 15.88±15.9 cells/μL in PPROM group and 6.99±10.1 in the control group (p<0.005).NLR levels were 5.79±3.2 and 4.27 ± 1.65 U/L in the PPROM group and controls, respectively (p<0.005). There was astatistically significant difference between the groups with regard to these parameters (p<0.005; Table II). Maternal SAA levels also showed a statistically significant difference between the groups (p<0.005). SAA levels at a cut-off 95.63 ng/ml resulted in the highest Youden index.

Table III summarizes the outcomes of the logistic regression model. According to the model, micro C-RP levels, NLR and SAA levels were found to be significant risk factors in the PPROM group (p<0.001). The odds ratios for microCRP, NLR and SAA levels were 0.939, 0.695 and 0.998, respectively. Receiver operating characteristic (ROC) areas under the curves (AUC) were also evaluated for micro C-RP levels, NLR and SAA levels. According to this analysis these parameters were found to be discriminative factors for PPROM (Figure 1).

Table IV depicts the correlation between microCRP, NLR and SAA levels. We found a statistically significant correlation between these variables.

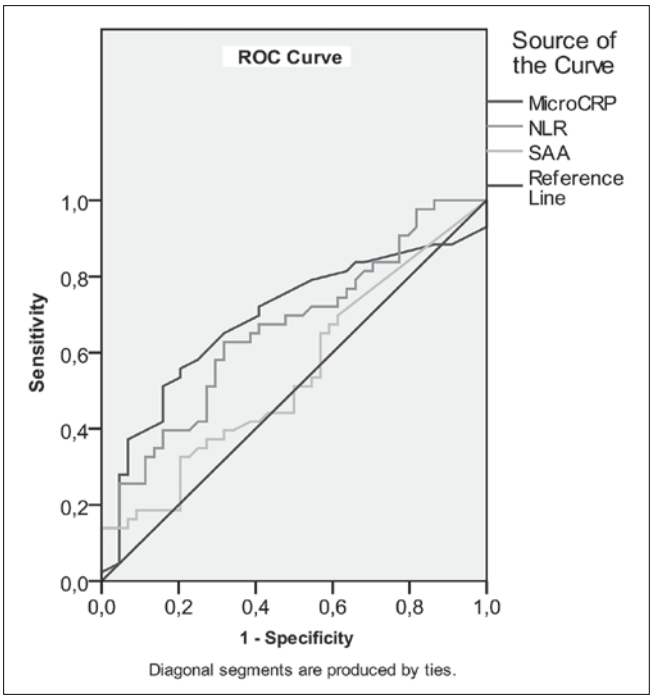

Figure 1. ROC analysis and AUC values of plasma MicroCRP, NLR and SAA levels in spontaneous preterm birth.

Discussion

Subclinical intrauterine infection has been implicated in the pathogenesis and subsequent morbidity of PPROM [12]. Goldenberg et al., [1] reported that microbial invasion of the amniotic activity may be found in 20–50% of patients with PPROM, although clinical evidence of the infection is present in as few as 12.5% of the women with positive culture results. Murtha et al., [13] reported in their study that chorioamnionitis leads to the rise of several proinflammatory cytokines in theamniotic fluid, fetal

cord blood, and maternal serum. CRP [14], IL-6 [15], procalcitonin [16], and pro-adrenomedullin and SAA [10] were among the cytokines reported to be associated with chorioamnionitis in PPROM in previous studies. Despite these studies, currently there are no reliable clinical markers to adequately indicate intra amniotic infection in PPROM. Popowski et al., [14] found that CRP was associated with clinical and histological chorioamnionitis in women with PPROM, with a specificity of >90% to predict an early onset of neonatal infection. We also found that CRP levels were higher in our PPROM group and ROC analysis showed that CRP levels may be a discriminative factor in pregnancies complicated with PPROM. NLR is a marker of infection. Terradas et al., [17] showed that NLR was a marker of infection and higher markers were independent markers of mortality in patients with bacteriemia. To the best of our knowledge, this has been the first study evaluating NLR in PPROM pregnancies. We also found that NLR was statistically significantly higher in PPROM pregnancies and this result was a predictive marker in such pregnancies.

SAA is also a proinflammatory marker to predict clinical signs of infection. Cekmez et al., [10] designed a study to evaluate maternal serum pro-adrenomedullin (pro-ADM) and SAA levels in PPROM pregnancies and its association with feto-maternal infectious morbidity. They found that both of these markers were predictive for detecting early onset of chorioamnionitis without any clinical signs in PPROM pregnancies. We also found that maternal serum SAA levels were higher in the PPROM group.

Maury et al., [18] found the measurements of SAA to be more sensitive than CRP in reflecting inflammatory activity for monitoring severity of disease and response to treatment. Lannergård et al., [9] found that SAA levels correlated significantly with CRP levels in infectious diseases. The concentrations of CRP and SAA also demonstrated a close relationship in our study. SAA levels correlated significantly with CRP levels (r=-0.265, p=0.017). It is known that neutrophilia and leukocytosis are usually detected in infectious and inflammatory diseases. Neutrophil/leukocyte ratio (NLR) has been popular in recent studies. However, to the best of our knowledge, NLR was not studied in PPROM patients previously. Romero et al., [19] investigated some hematological parameters, except NLR, of fetuses with systemic inflammatory response (SIRS). In their study, fetal blood sampling was collected in patients with PPROM and preterm labor with intact membranes. They concluded that all hematological parameters were higher in fetuses with SIRS as compared to fetuses without SIRS. Also, fetal SIRS was diagnosed more frequently in patients with PPROM than in those with preterm labor.In our study, we found that SAA levels correlated significantly with NLR in PPROM pregnancies (r=-0.334, p=0.023).

Lack of histopathological analysis of chorioamnionitis and placenta is the major limitation of our study. Both, or study and control groups generally consisted of patients in the third trimester of their pregnancies but the difference of gestational weeks of patients between the two groups was statistically significant (p=0.043).

In conclusion, we think that maternal serum micro CRP, NLR and SAA levels are useful markers to predict PPROM. However, further studies with a larger sample size are needed to reveal the role of NLR and SAA with higher specificity.

Acknowledgement

We thank to Mrs. Izabella Mrugalska for English revision.

Authors' Contribution

1. Sezen Bozkurt Köseoğlu – concept, analysis and interpretation of data.
2. Ali Irfan Guzel – corresp;onding author, concept, assumptions, study design, revised article critically.
3. Ruya Deveer – revised article critically.
4. Aytekin Tokmak – acquisition of data, analysis and interpretation of data.
5. Yaprak Engin-Ustun – revised article critically.
6. Sibel Özdas – revised article critically and edited the laboratory studies.
7. Nuri Danışman – revised article critically.

Authors' statement

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- Source of financing: **We have not conflict od interest.**

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