DOI: 10.4274/terh.galenos.2020.81489
J Tepecik Educ Res Hosp 2022;32(1):9-14

CLINICAL RESEARCH / KLİNİK ARAŞTIRMA

Serum Amyloid A Protein as a Potential Biomarker for Ectopic Pregnancy

Ektopik Gebelik Tanısında Potansiyel Bir Biyobelirteç Serum Amiloid A

Suna Yıldırım Karaca[1], İbrahim Gülhan[1], Giray Bozkaya[2], Mehmet Özeren[1]

[1]University of Health Sciences Turkey, İzmir Tepecik Education and Reseach Hospital, Clinic of Obstetrics and Gynecology, İzmir, Turkey
[2]University of Health Sciences Turkey, İzmir Bozyaka Education and Research Hospital, Clinic of Biochemistry, İzmir, Turkey

Cite as: Yıldırım Karaca S, Gülhan İ, Bozkaya G, Özeren M. Serum Amyloid A Protein as a Potential Biomarker for Ectopic Pregnancy. J Tepecik Educ Res Hosp 2022;32(1):9-14

Abstract

Objective: This study compared the concentrations of serum amyloid A (SAA), which is an inflammatory marker, in ectopic and intrauterine pregnancies.

Methods: This prospective case-control study was conducted at Tepecik Education and Research Hospital in İzmir, Turkey, between 2015 and 2016. It included 39 patients diagnosed with tubal ectopic pregnancies and 41 patients diagnosed with early intrauterine pregnancies. The SAA, β human chorionic gonadotropin, progesterone and C-reactive protein levels were measured in the serum samples taken from both groups. Data were presented as mean±standard deviation and a p value <0.05 was accepted as statistically significant. The distribution of the variables was measured using the Kolmogorov-Smirnov test, while the independent samples t-test and Mann-Whitney U test were used in the analysis of the quantitative independent data. The impact level and cut-off value were investigated using a receiver operating characteristic curve.

Results: SAA levels in the ectopic pregnancy patients (2.4±2.6 µg/mL) was significantly higher than in the intrauterine pregnancies (1.4±1.9 µg/mL) (p=0.008). When using the SAA cut-off value of 0.7 µg/mL for the ectopic pregnancy diagnosis, the sensitivity was 73.2% and the specificity was 63.4%.

Conclusion: The inflammation due to tubal tissue damage in an ectopic pregnancy may increase the SAA level in the maternal blood. Even though larger prospective studies are needed, our study suggests that the SAA is an important biomarker for the early diagnosis of an ectopic pregnancy.

Keywords: Serum amyloid A, biomarker, ectopic pregnancy, inflammation

Öz

Amaç: Bu çalışmanın amacı bir enflamasyon markırı olan serum amiloid A proteininin (SAA) ektopik gebelikte ve intrauterin gebelikteki konsantrasyonunu karşılaştırmaktır.

Yöntem: Bu prospektif olgu kontrol çalışması Tepecik Eğitim ve Araştırma Hastanesi, İzmir, Türkiye, 2015 ile 2016 tarihleri arasında yapıldı. Tubal ektopik gebelik tanısı alan 41 hasta ve erken intrauterin gebelik tanısı alan 41 hasta çalışmaya dahil edildi. Her iki grubun serum örneklerinden SAA, β Hcg, progesteron ve C-reaktif protein seviyelerine bakıldı. Değişkenlerin dağılımı Kolmogorov-Smirnov test ile ölçüldü. Nicel bağımsız verilerin analizinde bağımsız örneklem t-test ve Mann-Whitney U test kullanıldı. Etki düzey ve cut-off değeri ROC eğrisi ile araştırıldı. P değeri <0,05 anlamlı olarak kabul edildi.

Bulgular: Her iki grup kendi arasında karşılaştırıldığında, ektopik gebeliklerde SAA seviyesi (2,4±2,6 µg/mL) intrauterin gebeliklerden (1,4±1,9 µg/mL) anlamlı olarak yüksek saptandı (p=0,008). Ektopik gebelik tanısı için SAA'nın cut off değeri 0,7 µg/mL alındığında sensivite %73,2 spesifite %63,4 olarak saptanmıştır.

Address for Correspondence/Yazışma Adresi: Suna Yıldırım Karaca MD, İzmir Tepecik Education and Reseach Hospital, Clinic of Obstetrics and Gynecology, İzmir, Turkey
Phone: +90 507 593 02 82 **E-mail:** drsunayildirimkaraca@gmail.com
ORCID ID: orcid.org/0000-0001-8319-3052

Received/Geliş tarihi: 30.06.2020
Accepted/Kabul tarihi: 05.08.2020

Öz

Sonuç: Tubal doku hasarına bağlı oluşan enflamasyonun maternal kanda SAA protein düzeyini arttırabilir. Büyük çaplı prospektif çalışmalara ihtiyacımız olsa da, çalışmamız ektopik gebeliğin erken tanısında SAA'nın önemli bir biomarker olduğunu düşündürmektedir.

Anahtar Kelimeler: Amiloid A, biyobelirteç, dış gebelik, enflamasyon

Introduction

An ectopic pregnancy is a state in which the fertilized ovum is implanted in tissue outside the uterine cavity[1]. Its frequency has increased worldwide recently due to tubal surgeries, an increase in sexually transmitted diseases, an increase in assisted reproduction techniques and diagnostic methods that can detect an ectopic pregnancy at an earlier stage[2]. However, in 8-31% of the cases, no intrauterine or extrauterine distinction can be made at the first pregnancy examination[3]. Unfortunately, this delay in the diagnosis can lead to increased maternal mortality and morbidity.

The diagnosis of an ectopic pregnancy is made via transvaginal ultrasound and serial B b human chorionic gonadotropin (β hCG) measurements. When serial β hCG measurements are required, precise and accurate noninvasive tests are needed for an early diagnosis. For this research, we hypothesized that the concentration of serum amyloid A (SAA), an acute phase protein, may increase with the inflammation caused by tubal tissue injury in an ectopic pregnancy. The SAA is an important acute phase marker whose plasma level increases in inflammatory diseases, tissue damage, infections and cancer[4]. The SAA is a precursor protein of secondary reactive amyloidosis[5], with a size of 12 kD, containing 104 amino acids[6]. It is primarily synthesized in the hepatocytes[7], as well as in the adipocytes[8], synoviocytes[9], tumor cells[10] and first the trimester trophoblasts[11]. The SAA enhances the activity of metalloproteinases that induce decidual invasion in the trophoblasts[11]; therefore, it plays an important role in early fetal development. To our knowledge, this is the first study that demonstrates the SAA concentration in ectopic pregnancy.

Materials and Methods

This study was conducted at the Tepecik Education and Research Hospital in İzmir, Turkey between 2015 and 2016, and approval was obtained from the non-invasive Human Research Ethics Committee (protocol no: 46144, date: 12.05.2015). All the participants provided written informed consent. This study included 39 patients diagnosed with ectopic pregnancies and 41 patients with intrauterine pregnancies as the control group. The ectopic pregnancy and normal pregnancy participants were randomly sampled. Gestational ages of the participants were calculated from the last menstrual period. The sample size was calculated using G-power 3.1 statistical program. All of the systemic and obstetric were examined by the same person, so there was no difference between the observers. An ectopic pregnancy diagnosis was made when the gestational sac was seen outside the uterus via transvaginal ultrasonography. The SAA, progesterone, β hCG and C-reactive protein (CRP) levels were measured in both groups. Those patients diagnosed with a ruptured ectopic pregnancy, those diagnosed with a chronic or autoimmune disease, those who used anti-inflammatory drugs, and those who smoked were excluded from this study. Twenty-five of ectopic pregnancies were managed by medical treatment using single dose methotrexate while 14 ectopic pregnancies were managed by surgery and confirmed by pathological examination. Normal pregnancies had no complications at the end of their pregnancies.

Serum Amyloid A Measurement

The fasting blood samples taken from the patients between 9 and 12 am, and put in into 5 mL heparinized tubes at the time of admission after ultrasound scan before any medication or operation related to ectopic pregnancy. These were centrifuged at 1000 g for 15 min, and the serum samples were stored at -80 °C for a maximum of 4 weeks before SAA determination. To determine the SAA level, a double sandwich enzyme-linked immunosorbent assay (ELISA) with a highly purified monoclonal antibody against human SAA (ELISA kit; Assaypro, USA) used.

Statistical Analysis

All data were analyzed using the statistical software package Statistical Package for the Social Sciences statistics version 22. The distribution of the variables was measured using the Kolmogorov-Smirnov test. Data were expressed as mean±standard deviation The t-test and Mann-Whitney U test were used in the analysis of the quantitative independent data. The impact level and cut off value were investigated using a receiver operating characteristic (ROC) curve.

Results

A total of 80 patients were included in this study, and the ectopic pregnancy group consisted of 39 patients and intrauterine pregnancy group consisted of 41 patients. The two groups were similar in terms of age, gravida, parity, abortus, body mass index and gestational age (Table 1).

The SAA, β hCG, progesterone and CRP levels are shown in Table 2. The SAA level was found to be significantly higher (p=0.008) in the ectopic pregnancy group (2.4±2.6 µg/mL) than in the intrauterine pregnancy group (1.1±1.9 µg/mL).

Significant efficacy in the SAA value was observed in the distinction between an ectopic pregnancy and intrauterine pregnancy [area under the ROC curve (AUC)=0.669 (0.551-0.787)] (Figure 1). When the cut-off value of the SAA concentration was 0.7 µg/mL, its sensitivity was 73.2%, specificity was 63.4%, positive predictive value was 66.7% and negative predictive value was 70.3% in the diagnosis of an ectopic pregnancy (Table 3).

Significant efficacy in the progesterone value was observed in the distinction between an ectopic pregnancy and intrauterine pregnancy [AUC=0.974 (0.940-1.00)] (Figure 2). When the cut-off value of the progesterone concentration was 11 ng/mL, its sensitivity was 97.6%, specificity was 92.7%, positive predictive value was 93.0% and negative predictive value was 97.4% in the diagnosis of an ectopic pregnancy (Table 3).

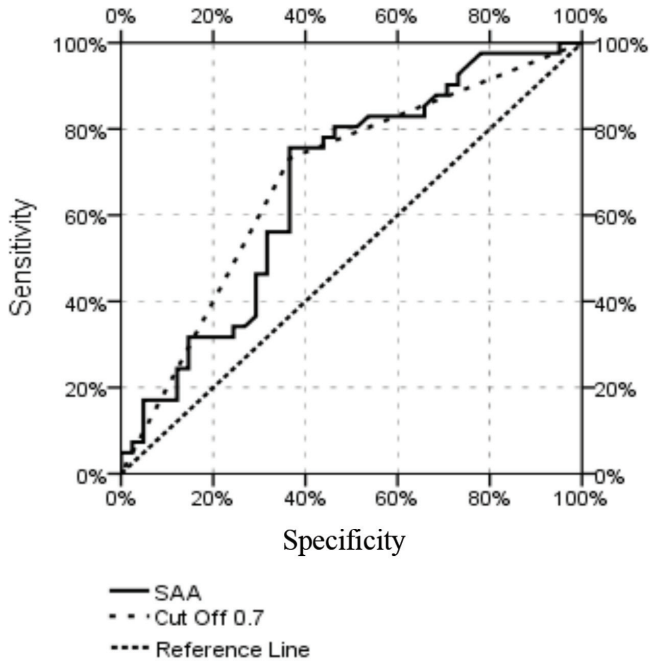

Figure 1. Receiver operating characteristic curves of the serum amyloid A (SAA) protein levels as a diagnostic test for ectopic pregnancy

Table 1. Demographic characteristics of the patients

Parameters (Mean±standard deviation)	Ectopic pregnancy n=39 (Mean±SD)	Intrauterine pregnancy n=41 (Mean±SD)	p value
Age (years)	29.5±5.4	27.2±6.6	0.208
BMI (kg/m²)	24.2±3.7	25.5±4.1	0.139
Gravida	2.2±1.3	2.0±1.1	0.351
Parity	0.3±0.5	0.7±0.8	0.215
Gestational age (weeks)	5.2±0.7	5.6±0.9	0.32

BMI: Body mass index, SD: Standard deviation

Table 2. Comparison of the SAA, progesterone, β hCG and CRP levels between the ectopic pregnancy and intrauterine pregnancy groups

Parameters	Ectopic pregnancy (Mean±SD)	Intrauterine pregnancy (Mean±SD)	p value
SAA (µg/mL)	2.4±2.6	1.4±1.9	**0.008**
Progesterone (ng/mL)	4.0±3.6	18.0±7.8	**0.0001**
β hCG (×10³)	2.9±4.7	5.5±2.9	**0.0001**
CRP (mg/L)	8.8±19	4.4±4.4	0.616
WBC (×10³)	8.1±2.2	8.1±1.9	0.886

Mann-Whitney U test.
SAA: Serum amyloid A, β hCG: β human chorionic gonadotropin, CRP: C-reactive protein, WBC: White blood cell, SD: Standard deviation

There was no difference in the CRP levels or white blood cell counts between the groups.

Discussion

The SAA is an important acute phase marker that increases in chronic inflammatory diseases, and it is synthesized primarily in the hepatocytes[7]. Additionally, it is extrahepatically synthesized in leukocytes[12], adipocytes[8], synoviocytes[9], tumor cells[10] and first trimester trophoblasts[11]. The SAA increases mRNA expression and the activity of metalloproteinases (MMP-2 and MMP-9), which can lead to the decidual invasion of extravillous trophoblasts[13]. Kovacevic et al.[11] also revealed SAA in trophoblasts at 10-12 weeks of gestation and found that it plays an important role in early fetal development.

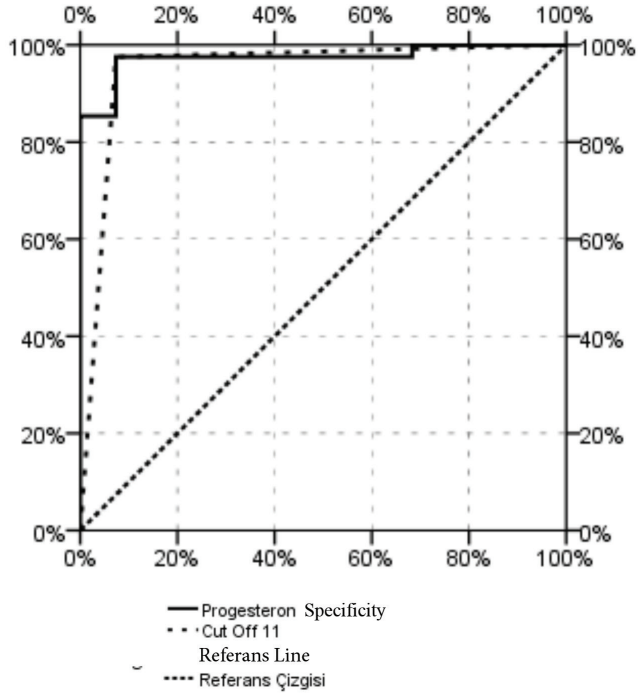

Figure 2. Receiver operating characteristic curves of the progesterone levels as diagnostic tests for ectopic pregnancy

Table 3. Cut-off values of the serum amyloid A and progesterone levels when using a receiver operating characteristic curve analysis in ectopic pregnancy

Parameters	Serum amyloid A	Progesterone
Cut-off value	0.7 µg/mL	11 ng/mL
Sensitivity	73.2%	97.6%
Specificity	63.4%	92.7%
PPD	66.7%	93%
NPD	70.3%	97.4%
AUC	0.669	0.974
P value	0.008	0.0001

PPD: Positive predictive value, NPD: Negative predictive value, AUC: Area under the curve

SAA concentrations are associated with the degree of tissue damage resulting from inflammation. They are also measured for evaluating the response to treatment in inflammatory diseases. Additionally, the SAA plays important roles in lipid metabolism[14], cell proliferation[15] and invasion[16], immunomodulation[17] and the induction of matrix metalloproteinases[18]. The SAA level, which is 1-4 µg/mL under normal physiological conditions, can reach up to 1000 times that in inflammatory conditions[19]. In an ectopic pregnancy, tissue damage occurs because of the implantation and invasion of trophoblasts into the tubal tissue. To detect the inflammation caused by this damage, many markers have been studied. However, our study is the first to evaluate the SAA level in the early diagnosis of ectopic pregnancy.

There are very few studies in the literature investigating the role of SAA in pregnancy. In one study comparing preeclamptic and normal pregnancies, the SAA levels were found to be significantly higher in the preeclamptic pregnancies (28.2 ng/L in the preeclampsia group and 7.8 ng/L in the control 30 group)[20]. Another study revealed that the SAA level was higher in patients with premature membrane rupture than in the control group. These studies have indicated that the SAA level increases in inflammatory pregnancy cases, such as in preeclampsia and premature membrane rupture[21]. The synthesis of SAA is largely regulated by cytokines, as well as peptide hormonal signals produced by the endothelial cells, lymphocytes, and particularly, activated monocytes and macrophages[17]. Moreover, cytokines, such as interleukin IL-1, IL-6, and tumor necrosis factor (TNF)-α, stimulate the hepatic and extrahepatic synthesis of SAA[22]. In hepatic cell culture studies, by inhibiting the combined effects of the IL-1, IL-6, and TNF-α with the blockage of IL-6, SAA mRNA stimulation prevented[23]. In a study assessing the IL-6 levels in the early diagnosis of ectopic pregnancy, the IL-6 was increased compared to that of a normal intrauterine pregnancy, while the sensitivity was 53.57% and specificity was 80% when the cut-off value was 26.48 pg/mL[23].

Our hypothesis was that the SAA concentration resulting from tubal tissue damage in an ectopic pregnancy reaches a higher value than that in a normal intrauterine pregnancy. In

our study, the SAA level was found to be significantly higher (p=0.008) in the ectopic pregnancy group (2.4±2.6 µg/mL) than in the intrauterine pregnancy group (1.1±1.9 µg/mL). Therefore, our findings support our hypothesis. When the SAA cut-off value was 0.7 µg/mL, its sensitivity was 73.2%, specificity was 63.4%, positive predictive value was 66.7% and negative predictive value was 70.3% in the diagnosis of ectopic pregnancy. When the progesterone cut-off value was 11 ng/mL in the distinction between ectopic pregnancy and intrauterine pregnancy, its sensitivity and specificity were 97% and 92.7%, respectively. Our results indicate that progesterone is a more valuable marker than the SAA in ectopic pregnancy.

Study Limitations

There are several limitations to this study. First, we could not surgically confirm all the patients diagnosed with ectopic pregnancy. The other limitation is that although we determined that the SAA concentration was high in the ectopic pregnancy group, the values in both groups were within normal physiological limits. We think that the reason for this is that ectopic pregnancies are detected before tubal rupture when tissue damage is minimal.

Conclusion

This study was the first to show the SAA level in the early diagnosis of ectopic pregnancy. We believe that the high levels of SAA in ectopic pregnancies may be an indicator of inflammation in the tubal mucosa. We believe that in the future, inflammatory markers such as SAA protein may help clinicians diagnose ectopic pregnancy at an early stage.

Ethics

Ethics Committee Approval: This study was conducted at the Tepecik Education and Research Hospital in İzmir, Turkey between 2015 and 2016, and approval was obtained from the non-invasive Human Research Ethics Committee (protocol no: 46144, date: 12.05.2015).

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.Y.K., İ.G., G.B., M.Ö., Concept: S.Y.K., İ.G., G.B., M.Ö., Design: S.Y.K., İ.G., G.B., M.Ö., Data Collection or Processing: S.Y.K., İ.G., G.B., M.Ö., Analysis or Interpretation: S.Y.K., İ.G., G.B., M.Ö., Literature Search: S.Y.K., İ.G., G.B., M.Ö., Writing: S.Y.K., İ.G., G.B., M.Ö.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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