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Maternal serum ischemia-modified albumin as an oxidative stress biomarker in preterm pre-labor rupture of membranes

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ABSTRACT

Objectives: To evaluate the maternal serum ischemia-modified albumin (IMA) concentration as an oxidative stress biomarker in pregnancies complicated by preterm pre-labor rupture of membranes (PPROM) without maternal clinical infection and compare these results with healthy pregnancies.

Material and methods: The present cohort study included 40 pregnancies complicated by PPROM and 49 similar gestational age healthy pregnancies in the third trimester of gestation.

Maternal venous blood specimens were obtained at the day of first diagnosis. Maternal serum IMA level was assayed with an Albumin Cobalt Binding test. The subjects were followed up until delivery and perinatal outcomes were recorded.

Results: The maternal serum IMA concentrations were significantly higher in the study group (0.56 ± 0.05 absorbance units) as compared to controls (0.54 ± 0.03 absorbance units) (p = 0.020). The maternal serum IMA concentrations were not significantly correlated with the initial maternal white blood cell count (r: 0.118, p = 0.269) and C-reactive protein levels (r: 0.066, p = 0.541). The maternal serum IMA concentrations were negatively correlated with gestational age at delivery (r: –0.248, p = 0.019), birthweight (r: –0.247, p = 0.020) and Apgar scores (r: –0.200, p = 0.049; r: –0.245, p = 0.020). The threshold value of maternal serum IMA concentration above 0.55 absorbance units indicated the pregnancy complicated by PPROM by 57.5% sensitivity and 57.1% specificity (Area under curve 0.613, confidence interval 0.50–0.73).

Conclusions: The current study supported for the first time that there is an association between increased maternal serum IMA levels and the development of PPROM in the third trimester of gestation without maternal clinical infection. Elevated maternal serum IMA levels may alert the obstetrician about poor ongoing perinatal outcomes in the early phase of PPROM before increased maternal C-reactive protein and white blood cell count.

Key words: pregnancy; ischemia-modified albumin; preterm pre-labor rupture of membranes; perinatal outcome

INTRODUCTION

Preterm pre-labor rupture of membranes (PPROM) is an important issue in maternal-fetal medicine which is seen in approximately 3–4% of all pregnancies [1]. The exact pathophysiological mechanism of PPROM is not well established. It has been reported that the underlying cause can be multifactorial like infectious condition, smokers, twins, cervical insufficiency and hydroamniosis [1, 2]. Microbial invasion of the amniotic cavity (MIAC) and/or histological chorioamnionitis (CA) are shown to be the 60–70% cause of PPROM pregnancies. However, the 30–35% of them is developed in non-infectious environment [3, 4].

IMA (ischemia modified albumin) has been proposed as a relatively new molecule for ischemia and oxidative stress [5, 6]. The amino terminal end (N-terminal) of human serum albumin (HSA) binds transitional metals like copper, cobalt, and nickel in physiological status. Oxidative stress develops as a result of disturbed homeostasis between reactive oxygen

species (ROS) and the body's scavenging mechanisms [7]. ROS have various impacts on the physiological pathways and are cytotoxic for membrane phospholipids. And they contribute to cell death which is resulted from the increased membrane permeability, the disturbed membrane integrity, loss of enzyme activity, and DNA injury [8]. Oxidative free radical production increases during hypoxic environment which leads many changes in the amino terminal end (N-terminus) of HSA. These changes contribute to the decrease in the capacity of HSA binding to transition metals, especially cobalt. The new formed albumin is defined as IMA[9]. Serum IMA has been studied and shown to be increased in normal pregnancies. This change explained by the possible effect of the physiologic oxidative status of pregnancy [10]. Moreover, increased maternal IMA levels are also demonstrated in early first trimester of gestation which is linked to the hypoxic intrauterine environment during trophoblastic invasion [11].

Several researches have studied about the possible underlying mechanism of PPROM and stated that oxidative stress is associated with premature aging of fetal membranes [12–14]. We hypothesized that this elevated oxidative stress is not only apparent in intrauterine environment locally. It also leads to a systemic effect on maternal circulation in PPROM pregnancies. Thus, maternal serum IMA level which is a biomarker of oxidative stress may probably increase as a result of this process. To the best of our knowledge, maternal IMA level was examined in pregnancies complicated with preeclampsia, intrauterine growth restriction (IUGR), early pregnancy loss and gestational diabetes mellitus (GDM) before [15–18]. However, there is no reliable, well-designed study showing whether it increases in PPROM before maternal clinical infection.

Objectives

We performed a prospective cohort study to investigate the maternal serum IMA level as a biomarker of oxidative stress in pregnancies complicated by PPROM without maternal clinical infection and compare these results with healthy pregnancies.

MATERIAL AND METHODS

The current research was planned at Yuzuncu Yil University, Medical School, Obstetric department between January 2016 and July 2016. The research was approved by Local Ethics Committee. All patients gave informed written consent. The study was organized according to the Helsinki Declaration. The research consisted of 114 pregnant women, aged 18-45 years, who were examined at our tertiary clinic. The study group consisted of 54

singleton PPROM pregnancies between 24 + 0 and 36 + 6 weeks of gestation. The control group consisted of 60 gestational age matched healthy pregnancies who delivered at term. All participants were followed-up until delivery. PPROM is the leakage of amniotic fluid before onset of labor (< 37 weeks). The clinical diagnosis was done by using sterile speculum. Amnisure® test (AmniSure International LLC, Boston, Mass., USA) was made to verify the leakage of amniotic fluid, if necessary. Gestational week was calculated by using the first day of the last menstrual period. The clinician was confirmed the date by controlling early first trimester ultrasonographic measures. The PPROM patients had no maternal concomitant disorders (hypertension in pregnancy, Type 1 diabetes mellitus, thyroid disorders and gestational diabetes mellitus), fetal congenital anomalies, twin pregnancy, intrauterine growth restriction, fetal asphyxia and maternal vaginal bleeding. The pregnant women who had clinical CA and maternal clinical infection at the first examination were also excluded from the study. The control group consisted of gestational age-matched healthy pregnancies without any obstetric complications. In total, 49 healthy pregnancies and 40 pregnancies with PPROM were eligible for analysis (Fig. 1).

Maternal venous blood specimens of the study group were obtained at the first diagnosis of PPROM before any medication. These specimens were obtained at the routine prenatal care examination in the third trimester of gestation for the control group. They were centrifuged at 1500 rpm for 10 minutes and sera were stored at –80°C until the day of analysis. All materials were assayed using an Albumin Cobalt Binding test. This test was applied by adding 50 mL 0.1% cobalt (II) chloride ($CoCl_2 \cdot 6\ H_2O$) (Sigma-Aldrich Chemie GmbH Riedstrasse 2, Steinheim, Germany) to the sample. After mixing, followed by 10 minutes of incubation to allow for albumin cobalt binding, 50 mL 1.5 mg/mL dithiothreitol was added. After mixing followed by two minutes of incubation, 1.0 mL of a 0.9% sodium chloride solution was added in order to decrease the binding capacity. The blank was formed similarly with distilled water instead of dithiothreitol. A spectrophotometer was used to measure the absorbance at 470 nm. (Jenway 6315 UV/visible Scanning Spectrophotometers, United Kingdom). The results were reported in absorbance units (ABSU).

The obstetrical examination and perinatal evaluation by ultrasonography were performed for all subjects in our perinatal center. All PPROM patients were hospitalized and medicated with expectant management. Until delivery, all subjects treated with antibiotic therapy for prophylaxis (ampicillin 4 g/day) for one week. Intramuscular betamethasone injection (two 12 mg at 24 hours intervals) was used before 34 weeks of pregnancy for fetal lung maturation. Fetal reassurance was tested with non-stress test and maternal fetal

movement count, daily. The clinical CA findings like; uterine sensitivity, inflammatory vaginal leakage, maternal high temperature, maternal inflammatory markers [maternal serum C-reactive protein (CRP) and white blood cell (WBC) count] was checked until delivery. The delivery was planned with the development of clinical CA or fetal distress. The expectant management protocol followed up until 34 weeks of gestation. Delivery planning was individualized, and cesarean-section was performed only for obstetric indications.

Descriptive data were clarified with mean and standard deviation. The Student's t-test, Chi-Square test and Pearson correlation test were used where appropriate for statistical analysis. The receiver operating characteristic (ROC) analysis was performed to determine the most appropriate cut-off of maternal serum IMA concentrations. A value of $p < 0.05$ was considered statistically significant. The SPSS (IBM SPSS Statistics for Windows, Version 22.0. IBM Corp. Released 2013. Armonk, NY: IBM Corp.) calculation program was used to perform all statistical analysis.

RESULTS

The clinical characteristics, biochemical markers, obstetric and fetal outcomes of the patients were presented in Table 1. The maternal age, gravidity, parity, body mass index (BMI) and gestational week at sampling did not differ between the study and control groups. The gestational week at delivery, birthweight and Apgar scores were significantly lower in the study group as compared to controls ($p = 0.001$). The maternal serum IMA levels were significantly higher in the study group (0.56 ± 0.05 ABSU) as compared to controls (0.54 ± 0.03 ABSU) ($p = 0.020$).

The maternal serum IMA levels were not significantly correlated with the initial maternal WBC count (r: 0.118, $p = 0.269$) and CRP levels (r: 0.066, $p = 0.541$). The maternal serum IMA levels were negatively correlated with gestational week at delivery (r: –0.248, $p = 0.019$), birthweight (r: –0.247, $p = 0.020$) and Apgar scores (r: –0.200, $p = 0.049$; r: –0.245, $p = 0.020$). The threshold value of maternal serum IMA concentration above 0.55 ABSU anticipated the pregnancy complicated with PPROM by 57.5% sensitivity and 57.1% specificity [area under curve (AUC) 0.613, confidence interval (CI) 0.50–0.73] (Fig. 2).

DISCUSSION

The major finding of the current cohort study is that maternal serum IMA concentrations are increased in PPROM pregnancies without maternal clinical infection compared to healthy controls. Moreover, maternal IMA levels are negatively correlated with

gestational week at delivery, birthweight and Apgar scores. The increased IMA levels are predicted possible poor neonatal outcomes more accurately and earlier than maternal CRP and WBC count in PPROM. These results promote the theory for the first time that the development of PPROM before maternal clinical infection is associated with the elevation of maternal IMA level which is an oxidative stress biomarker in maternal systemic circulation.

PPROM is a multifactorial disease, and the processes contributing to this critical complication of gestation remain to be elucidated. The clinical nature of PPROM may vary depending on the underlying pathologies, the load of micro-organisms and the inflammatory environment they create [14, 19]. Elevated oxidative stress has been linked with several mechanisms in which lead to the development of PPROM. Oxidative stress induced injury may be the cause of inflammatory status seen in PPROM [20, 21]. However, there is still a debate on the association between elevated oxidative stress and HCA. The type of microorganisms, infectious load and the severity of HCA are the main factors that cause the occurrence of PPROM during pregnancy, each of which either independently or synergistically can create different oxidative stress profile [22].

Serum IMA concentrations was first used for a clinical biochemical marker for diagnosis of myocardial ischemia [23]. Serum IMA level has been also investigated in physiological conditions like pregnancy in which found to be elevated in the first trimester of gestation. Additionally, these levels continue to increase from the first trimester to the third trimester of pregnancy [10, 11].

In recent studies, beside the use of IMA in non-obstetric conditions, it has been investigated in complicated pregnancies like preeclampsia, IUGR to determine oxidative stress status and ischemia as contributing factors either in the mother or fetus [15, 16]. Moreover, maternal serum IMA level has been studied and proposed to predict the fetal well-being in pregnancies [24]. It is the fact that the early trophoblastic development which occurs in hypoxic environment may be a supporting mechanism for the increased maternal serum IMA concentrations in the first trimester of pregnancy [10]. Ustun et al. [15] found a positive correlation between maternal IMA concentrations and the severity of preeclampsia in which the main cause is defective trophoblastic invasion. They also suggested that the serial measurements of maternal IMA levels may be useful for monitoring pregnancies complicated with preeclampsia [15]. In another study by Ma et al. [18] showed significantly high maternal IMA levels in GDM cases. They also stated that maternal IMA levels were influenced by hyperglycemia [18]. Moreover, Ozdemir et al. [25] studied the IMA levels in recurrent miscarriage (RM) and found that there is an association between supraphysiological levels of

IMA and RM [25]. The current study analyzed maternal IMA levels, as an oxidative stress biomarker in pregnancies complicated by PPROM at the time of initial diagnosis. The maternal serum IMA value rises significantly during the third trimester of pregnancy before maternal CRP and WBC count which were markers of maternal infection, in PPROM cases. Our study also demonstrated a significant negative correlation between maternal IMA levels and gestational week at delivery, birthweight and Apgar scores in PPROM patients. Therefore, we can consider that the increased maternal IMA level which is a non-invasive method is predicted poor neonatal outcomes more accurately and earlier than maternal high CRP and WBC count in the third trimester of pregnancy among PPROM patients in the absence of clinical infection.

Despite our findings, it is noteworthy to note the limitations of the current research. First, our sample size was relatively small. Second, we did not perform a PCR analysis for verifying the MIAC and did not confirm the diagnosis of clinical CA pathologically (histological funisitis or CA). Third, we only targeted on the maternal serum IMA concentrations at the early phase of PPROM in the third trimester of pregnancy. We did not make serial measurements during the interval period of PPROM or until delivery. Thus, we could not predict about the follow-up period during gestation. And finally, our study did not include the subgroup of pregnant women complicated by preterm labor with intact membranes. Future larger studies which include this subgroup might be helpful and can confirm our results that just PPROM is related with elevated maternal serum IMA levels and higher oxidative stress.

CONCLUSIONS

The results of the current study supported for the first time that there is an association between increased maternal serum IMA levels and the development of PPROM in the third trimester of gestation before maternal clinical infection. The threshold IMA value of 0.55 ABSU had 57.5% sensitivity and 57.1% specificity. Moreover, elevated maternal serum IMA levels may alert the obstetrician about poor ongoing perinatal outcomes in the early phase of PPROM before increased maternal CRP and WBC count. We also proposed that the evaluation of possible oxidative stress biomarkers like IMA and the use of antioxidant treatments in future may be suggested as alternative management options for improving the obstetric and neonatal outcomes in PPROM cases. We believe that the results of the current research require further validation with larger follow-up studies, to confirm whether maternal

serum IMA could be considered as a potential biomarker for the development of PPROM in the absence of maternal clinical infection.

Table 1. The clinical characteristics, laboratory parameters, and perinatal outcomes of the patients

	Control group (n: 49)	**Study group (n: 40)**	**p**
Maternal age [years]	26.8 ± 5.5	28.3 ± 5.6	0.210*
BMI [kg/m^2]	27.6 ± 3.7	27.2 ± 4.2	0.675*
Gravidity	2.9 ± 1.5	3.2 ± 2.4	0.638*
Parity	1.7 ± 1.4	1.7 ± 1.6	0.730*
Gestational age at sampling [weeks]	33.0 ± 6.1	31.4 ± 3.5	0.113*
WBC [c/mm^3]	11.3 ± 3.4	11.2 ± 3.0	0.968*
CRP [mg/dL]	4.8 ± 3.7	6.1 ± 4.5	0.511*

*Student's t-test was used to compare continuous variables; BMI — body mass index; CRP — C- reactive protein; WBC — white blood cell count

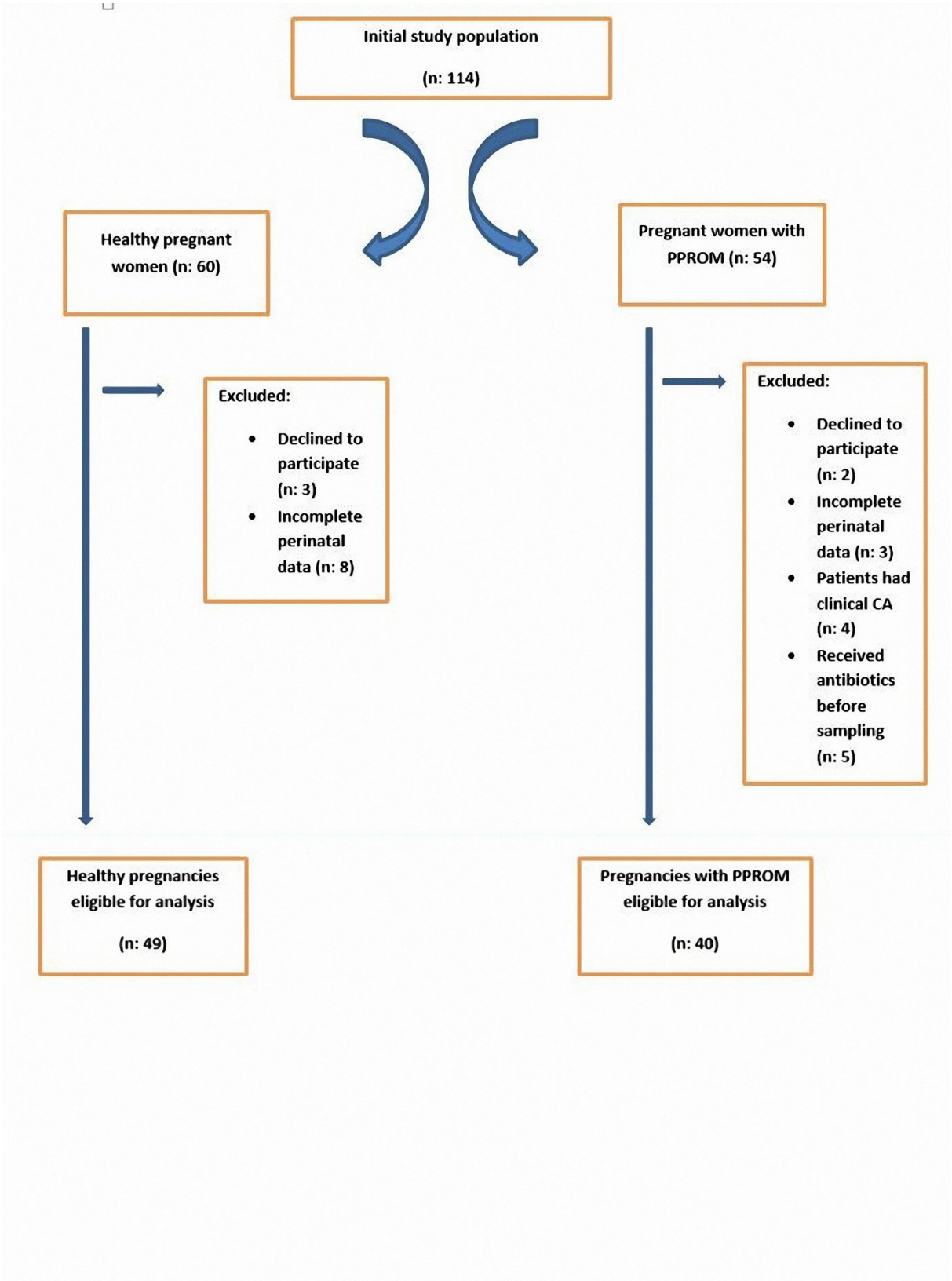

Figure 1. The flow diagram of the patients recruited in the study; PPROM — preterm pre-labor rupture of membranes

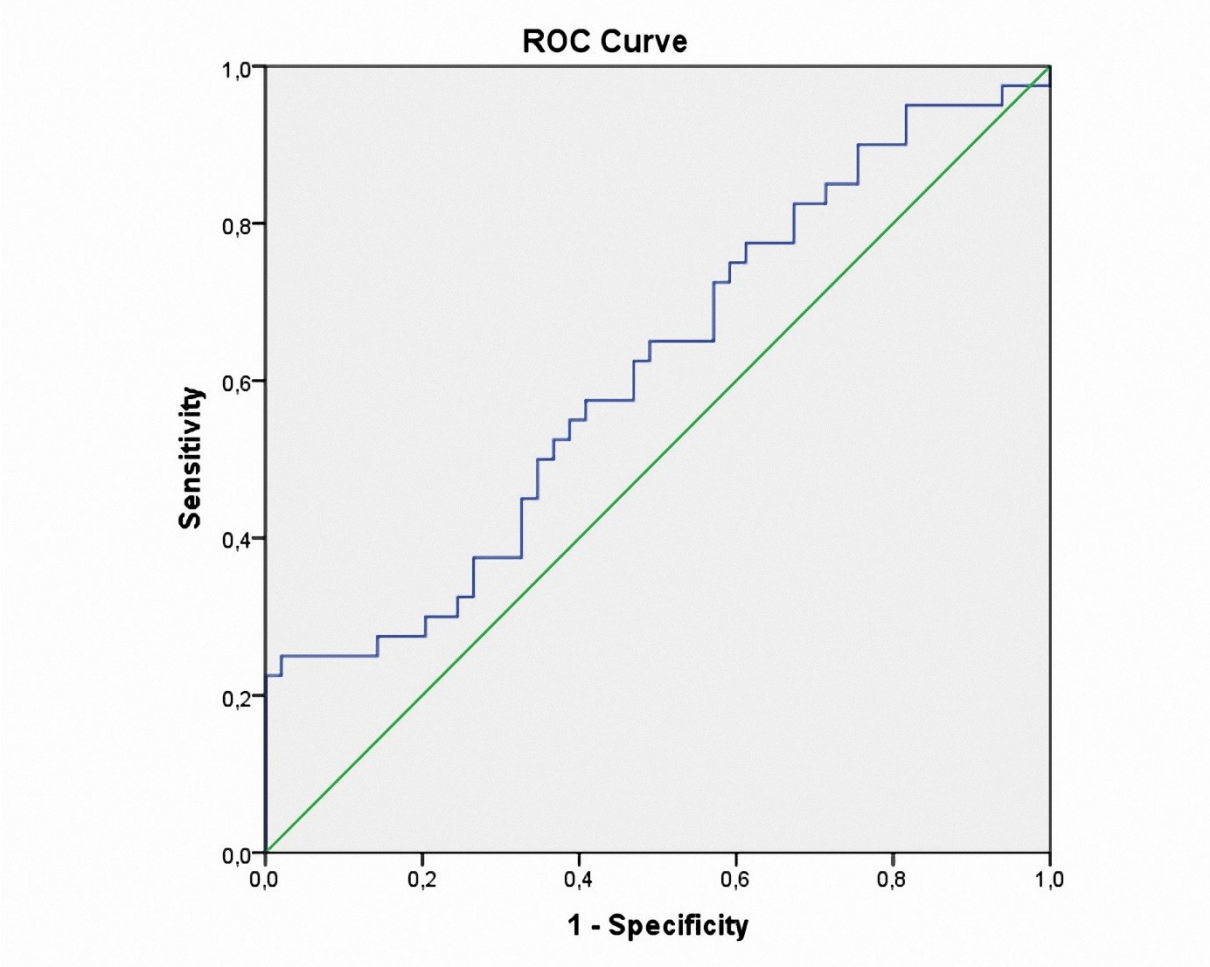

Figure 2. The receiver operating characteristic (ROC) curve analysis of maternal serum ischemia-modified albumin level anticipated pregnancies complicated by preterm pre-labor rupture of membranes

Conflict of interest

All authors declare no conflict of interest.

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