Heparin-binding Proteins in Amniotic Fluid: Predicter for Histological Chorioamnionitis?

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Research Article

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Abstract

Background: Histological chorioamnionitis (HCA) is a serious threat to fetal health during pregnancy, which is not easily detected due to the lack of clinical symptoms, and there is still a lack of effective predictors and diagnostic indicators. Heparin-binding protein (HBP), an antimicrobial protein secreted by neutrophils, is highly valuable in predicting a variety of infectious diseases and is expected to be an effective predictor of histological chorioamnionitis.

Methods: A total of 100 full-term healthy pregnant women delivered by cesarean section (control group) and 51 randomly enrolled pregnant women (pathology group) were included in this study. Amniotic fluid was extracted at delivery for HBP concentration, and postpartum placental pathology was performed in the pathology group to clarify the diagnosis of HCA. The predictive value of HBP in amniotic fluid for HCA was determined by establishing the reference interval for HBP in normal population and the receiver operating characteristic (ROC) curve.

Results: The 95% confidence interval (95% CI) for HBP concentration in the amniotic fluid of normal pregnant women was 0- 87.08 ng/ml, with a median of 6.41 ng/ml; HBP concentration in the amniotic fluid of pregnant women with HCA was significantly higher than that of controls ($P < 0.001$) and non-HCA pregnant women ($P < 0.001$). The ROC curve showed that when the cut-off value of HBP in amniotic fluid was 82.67 ng/ml, the corresponding sensitivity, specificity, positive predictive value, negative predictive value and area under the curve (AUC) were 84.20%, 96.90%, 94.12%, 91.18% and 0.86 ($P < 0.001$, 95% CI 0.715-1.000), respectively.

Discussion: HBP in amniotic fluid is a potential, good predictor of HCA. We will further to explore its clinical application value.

Introduction

Chorioamnionitis is also known as intrauterine infection or inflammation (IUI) [1]. Chorioamnionitis can be divided into clinical and subclinical/histological Chorioamnionitis (HCA) based on clinical symptoms or laboratory examination results [2]. HCA is asymptomatic and is diagnosed by pathological examination of inflammation of placenta [3, 4] and HCA is more common than clinical Chorioamnionitis. HCA can cause a variety of neonatal complications, including preterm birth, cerebral palsy, retinopathy of prematurity, neurological abnormalities, respiratory distress syndrome, bronchopulmonary dysplasia, neonatal sepsis and neonatal death. It may cause a variety of complications in pregnant women, including severe pelvic infection, subcutaneous wound infection, preterm labor, postpartum hemorrhage, cesarean section and sepsis [5]. These complications have a serious impact on maternal and fetus health.

In general, HCA is caused by microorganisms passing through the lower genital tract, but in rare cases, they can also invade the placenta through the hematogenous route. [1, 6]. HCA is the most common diagnosis reported in placental pathology, and is generally considered to represent the presence of

intrauterine infection or "amniotic fluid infection syndrome" [6]. HCA characterized by neutrophils infiltrating and inflammation at the mother-fetal interface. At the initial stage, neutrophils infiltrate at the junction of the placental chorionic and decidua, and infiltrate into the amniotic membrane indicating a high degree of infection or inflammation [7]. In the case of amniotic cavity infection, neutrophils circulate from the mother or fetus into the chorionic or umbilical cord in the presence of multiple chemokines (interleukin-8 and granulocyte-chemotactic proteins) [8–10]. Mature neutrophils can secrete HBP, HBP is also known as Azurocidin or 37 kda cationic antimicrobial protein (Cap 37) [11]. HBP can activate the mononuclear cells and macrophages homologous serine protease, has a variety of biological functions: mainly includes significant antimicrobial activity, and regulate inflammation chemotaxis characteristics, the protein can also modify the endothelial cells, spilling blood vessels, promote white blood cells from blood capillary migrated to the infection, and can increase vascular permeability[12–14].

Previous studies have shown that HBP can assist in the diagnosis of diseases, such as respiratory and circulatory failure [15, 16], severe sepsis [17], urinary tract infection in children [18], and acute bacterial meningitis [19]. At present, interleukin-6 [20], white-blood-cell [21], cervical bacterial culture [22] and other methods in the diagnosis of HCA are defective or controversial. Therefore, we believe that HBP may be a more reliable diagnostic indicator for HCA.

Our study attempted to establish the reference interval of HBP in amniotic fluid of normal pregnant women and to explore the diagnostic value for HCA.

Methods

2.1. Study population:

This study was conducted in the Obstetrics and Gynecology Hospital of Nanjing Medical University. All participants were recruited within 12 hours before delivery, and signed informed consent to obtain their clinical and biological sample information. The Ethics Committee of the Obstetrics and Gynecology Hospital of Nanjing Medical University approved the study (2021KY012), and all methods were carried out in accordance with relevant guidelines and regulations. This study was in accordance with the Declaration of Helsinki statement, and all subjects volunteered to participate in this study.

A total of 151 Chinese pregnant women were included in this study, including 100 healthy full-terms pregnant women (Normal group) who underwent cesarean section due to scar uterus or other factors. In addition, 51 pregnant women were randomly recruited, including 7 pregnant women who delivered at 28–37 weeks of gestation and 44 pregnant women who delivered at 37–42 weeks of gestation.

Inclusion criteria:(1) Normal group: gestational age ≥ 37 weeks, single fetus, maternal age ≥ 18 years, no pregnancy complications, no underlying diseases. Women with infectious diseases, non-gestational inflammatory diseases and recent antibiotic use were excluded. (2) Pathological group: gestational age ≥ 28 weeks, maternal age ≥ 18 years, singleton. Women with infectious diseases, non-pregnancy inflammatory diseases and recent antibiotic use were excluded.

Information on age, gestational age at delivery, BMI, mode of delivery, pregnancy complications, other non-gestational diseases, and recent antibiotic use of all subjects were collected and recorded by referring to hospital medical records.

2.2. Study Design:

Participants were divided into two groups: control group (100 cases) and pathological group (51 cases). 2 ml of amniotic fluid was extracted by syringe during cesarean section, and 2 ml each of umbilical artery and umbilical vein blood was extracted from the umbilical cord after delivery of the fetus. During physiological delivery, 2 ml of amniotic fluid was withdrawn from the cervical os using a syringe after rupture of membranes and 2 ml of blood was withdrawn from the umbilical cord artery and umbilical cord vein after delivery of the fetus. All samples were measured immediately after collection at room temperature using an immuno analyzer (jet-istar 3000, Zhonghan Shengtai Biotechnology Co., Ltd.) for HBP concentration. All operations were carried out according to the manufacturer's instructions. Each sample was tested twice and the average value were used. The detection range of immuno analyzer was from 5.90 to 300.00 ng/ml.

In the pathological group, placental pathological examination was performed after the delivery of the placenta. HCA is thought to be present when neutrophils infiltrate the chorionic, amniotic, or decidua of the placenta.

2.3. Data analysis:

After anonymous processing, the data of the patients were processed and analyzed by IBM SPSS Statistics software, 23.0. Visualize data with Graph pad Prism 8 software. Results were presented as mean ± SD for continuous variables, and number (percentage) for categorical variables. HBP distribution in the control group was examined by the Kolmogorov-Smirnov test (K-S test). Comparison of HBP concentrations in amniotic fluid of normal pregnant women with those of pregnant women with HCA by non-parametric test. The optimal cut-off value of HBP in amniotic fluid for HCA diagnosis by Receiver operating curve (ROC) analysis. $P < 0.05$ was considered statistically significant.

Results

3.1. Basic population characteristics of pregnant women

A total of 151 pregnant women were recruited for the study, 100 of whom were healthy, full-term pregnant women, whom we defined as the control group and 51 randomly included pregnant women defined as the pathological group (Table 1). All pregnancies in the control group were delivered by cesarean section. Pathological group consisted of 44 women with gestational age ranging from 37 to 42 weeks and 7 women with gestational age ranging from 28 to 37 weeks. In the pathological group, 22 (43.14%) had gestational diabetes mellitus (GDM), 5 (9.80%) had preeclampsia (PE), and 4 (7.84%) had premature rupture of membranes (PROM). No participants were excluded or lost during the study. All participants'

ages, gestational age at delivery, BMI, and mode of delivery were recorded. All relevant information is presented in Table 1.

Table 1
Baseline clinical characteristics of participants

Characteristics	Control group (100 cases)	Pathological group (51 cases)
Age (years, mean ± SD)	31.78 ± 4.39	30.96 ± 3.77
Gestational weeks (mean ± SD)	38.91 ± 0.95	38.37 ± 1.94
BMI/(kg/m^2)	27.42 ± 3.31	26.52 ± 3.20
Underweight (< 18.5)	0 (0.00%)	0 (0.00%)
Normal weight (18.5–24.9)	22 (22.00%)	18 (35.29%)
Overweight (25.0-29.9)	58 (58.00%)	25 (49.02%)
Obese (> 30.0)	19 (19.00%)	6 (11.77%)
Cesarean delivery	100 (100.00%)	41 (80.39%)
Term birth	100 (100.00%)	44 (86.29%)
Gestational disease		
GDM	0 (0.00%)	22 (43.14%)
PE	0 (0.00%)	5 (9.80%)
PROM	0 (0.00%)	4 (7.84%)

3.2. HBP in amniotic fluid

Among the 100 pregnant women in control group, 45 pregnant women's HBP was lower than the detection limit, the highest value was 174.55 ng/mL, the median was 6.41 ng/ml (Fig. 1). The HBP distribution in amniotic fluid of the control group showed a positive skewed distribution with a 95% reference range of 0-87.08 ng/ml. In the pathological group, 19 pregnant women were diagnosed with HCA and 32 pregnant women were diagnosed with non-HCA. The median HBP in amniotic fluid of pregnant women with HCA was > 300 ng/ml, with the lowest value of 90.52 ng/ml, except for three pregnancies with < 5.9 ng/ml. There was a significant difference in HBP in the amniotic fluid of pregnant women with HCA and controls ($P < 0.001$), and this difference was also significant in pregnant women with HCA and non-HCA ($P < 0.001$). Among the 32 non-CA pregnant women with amniotic fluid, the median HBP was 12.10 ng/ml, of which eight had assays < 5.9 ng/ml and all but one was below 87.08 ng/ml, except for one who was 95.45 ng/ml.

3.2. HBP in umbilical artery (UA) and umbilical vein (UV).

We collected and measured HBP in umbilical artery and umbilical vein and in a total of 94 control pregnant women, 27 non-HCA pregnant women and 8 HCA pregnant women (Fig. 2, Table 2). The median HBP of umbilical artery was greater than umbilical vein and in all three groups of pregnant women, but none of them was statistically different ($P > 0.05$). In addition, there was no statistical difference in HBP in umbilical artery and umbilical vein between HCA and non-HCA pregnant women ($P > 0.05$). The ratio of HBP in umbilical artery and umbilical vein was also not statistically different ($P > 0.05$).

Table 2
Distribution of HBP in AF, UA and UV

HBP concentration/(ng/ml)	Control group	Pathological group		P
		HCA	Non-HCA	HCA vs non-HCA
AF (Median)	6.41	> 300	12.10	< 0.001
UA (Median)	12.35	16.09	24.72	0.21
UV (Median)	9.76	10.27	16.36	0.46
P (UA vs UV)	0.37	0.96	0.06	-
UA/UV (Median)	1.24	1.03	1.39	0.42

3.4. Predictive value of HBP in amniotic fluid for HCA

All 51 pregnant women in pathological group were included in ROC. The optimal cutoff value was 82.67ng/mL, area under the curve (AUC) was 0.86 ($P < 0.001$, 95% CI 0.715-1.000), Youden index was 0.81, the corresponding sensitivity was 84.20%, and specificity was 96.90% (Fig. 3). It is sufficient to prove the superior diagnostic efficacy of HBP in amniotic fluid for HCA. Comparing the reference range of HBP in amniotic fluid of pregnant women in the normal group, this range had a diagnostic sensitivity of 84.21% and a specificity of 96.88% for HCA, which is fully consistent with the diagnostic efficacy derived from the ROC curve. The corresponding positive predictive value was 94.12% and the negative predictive value was 91.18%.

Discussion

Chorioamnionitis is diagnosed clinically by fever, maternal or fetal tachycardia, uterine pressure, vaginal discharge or foul odor of amniotic fluid, and maternal leukocytosis [6, 23]. In contrast, histological chorioamnionitis is usually clinically asymptomatic, with a minimal maternal inflammatory response [24, 25], the diagnosis cannot be confirmed by routine physical signs and laboratory tests, but only by postpartum pathology of the placenta.

A recent study found significantly higher levels of interleukin 6 (IL-6), metalloproteinase matrix 8 (MMP-8) and tumor necrosis factor alpha (TNF-α) in amniotic fluid obtained vaginally in patients with HCA compared to non-HCA patients [26]. However, the diagnostic efficacy of these indicators is not good enough to be used in clinical applications. The lack of effective detection methods can easily lead to the underdiagnosis of pregnant women with HCA who do not have clinical symptoms and eventually cause serious fetal complications. Thus, we wanted to discover a valid diagnostic index for HCA.

In this study, the reference range of HBP concentration in normal maternal amniotic fluid was established, and the diagnostic value of HBP in the prediction of HCA was evaluated. Our results showed that HBP in amniotic fluid was significantly lower in normal and non-HCA pregnant women than in those with HCA. Both the reference range established by normal pregnant women and the ROC evaluation results of abnormal pregnant women had a sensitivity of 84.20% and specificity of 96.90% for predicting HCA. In addition, there was no statistical difference in HBP in umbilical artery and umbilical vein, nor was there a statistical difference in HBP between HCA and non-HCA in umbilical artery or umbilical vein. This result suggests that HBP in amniotic fluid may not be related to umbilical artery and umbilical vein.

Overall, HBP is an excellent predictor of HCA. However, 3 of the 19 pregnant women with HCA in the study had test values < 5.9 ng/mL, which is a clear departure from the test values of other pregnant women and leads us to consider whether there are some factors influencing these results. HCA, as a manifestation of placental inflammation, is usually caused by pathogens invading the amniotic cavity, but we note recent evidence that "sterile" intra-amniotic inflammation is frequently associated with HCA [6]. In the original study design, we did not consider sterile HCA, which may explain the HBP values of < 5.9 ng/ml in the three HCA pregnant women. In addition, we found only one case of non-HCA pregnant women with HBP > 82.67 ng/ml, whose value was 95.45 ng/ml. This value is relatively low compared to the value for HCA pregnant women, perhaps due to other factors affecting this value.

Current studies suggest that the secretion of HBP in the blood activates monocytes rolling along the endothelial cells, causing monocyte stagnation. Monocytes will move across the endothelial cells and migrate to the injured site after adhesion. The pro-inflammatory effect of HBP is mainly the activation of monocytes, which play an important role in antimicrobial action, and it is a potential biomarker with diagnostic properties [24]. At present, there is no study on the relationship between HBP in amniotic fluid and HCA, but there are many studies on the relationship between HBP and infection, and HBP is considered as a reliable index for predicting severe infection. A meta-analysis of HBP in predicting sepsis in critically ill patients showed that the sensitivity and specificity of HBP in the diagnosis of sepsis were 0.85 and 0.91, respectively [27]; Another study on the relationship between HBP and sepsis also found that it has high diagnostic value. In addition, the study pointed out that the secretory granules secreting HBP were first mobilized after neutrophil activation, so the rise of HBP was prior to other infection indicators (WBC, C-reactive protein, procalcitonin, lactic acid) [28]; Furthermore, elevated CSF levels of HBP can distinguish patients with acute bacterial meningitis from patients with other central nervous system infections [29]. The relationship between HBP in amniotic fluid and infection has not been studied at present, so it is necessary to further study the source of HBP in amniotic fluid.

Previous studies suggested that neutrophils in amniotic fluid originated from the mother, but subsequent experiments showed that neutrophils in amniotic fluid may also come from the fetus [30–33]. A DNA fingerprinting study on the origin of neutrophils in amniotic fluid of pregnant women with intrauterine infection pointed out that neutrophils in amniotic fluid are effective markers of inflammation, mainly from the fetus or the mother or both, because both the fetus and the mother can participate in the immune mechanism of intra amniotic infection [34]. Since there was no significant difference in HBP in the umbilical cord vessels of HCA and non-HCA pregnant women, we speculate that HBP in amniotic fluid may not be derived from the umbilical cord vessels because neutrophils infiltrate the umbilical cord and fetus only in severe chorioamnionitis [35].

The results of this study suggest the value of HBP in the diagnosis of HCA is positive, but there are some drawbacks in our study. Due to the unique nature of pregnant women and fetuses, we encountered many difficulties in obtaining samples, resulting in a limited number of subjects that could be included. Secondly, this is the first study on the value of HBP in amniotic fluid for the diagnosis of HCA and thus may not be standard in the way the sample was collected. Finally, we did not consider HCA caused by non-bacterial infections during the experimental design phase, which prevented us from fully excluding their effect. We will continue to study the diagnostic value of HBP in the group of pregnant women with PROM, which is a high risk factor for HCA or a clinical condition caused by HCA.

Conclusion

In this study, the relationship between the concentration of HBP in amniotic fluid and HCA was analyzed and studied. HBP in amniotic fluid is a good predictor of HCA, but it is still unclear where it originates or whether the HBP-secreting neutrophils are of maternal or fetal origin. HBP may solve the long-standing problem of lack of valid diagnostic indicators for HCA, which has plagued obstetricians and gynecologists. We will further explore the diagnostic value of HBP for HCA and try to explain its origin.

List Of Abbreviations

HCA, Histological chorioamnionitis; HBP, Heparin-binding protein; ROC, Receiver operating characteristic; 95% CI, 95% confidence interval; AUC, Area under the curve; K-S test, Kolmogorov-Smirnov test; UA, umbilical artery; UV, umbilical vein.

Declarations

Ethics approval and consent to participate

The Ethics Committee of the Obstetrics and Gynecology Hospital of Nanjing Medical University approved the study (2021KY012), and all methods were carried out in accordance with relevant guidelines and regulations. This study was in accordance with the Declaration of Helsinki statement, and all subjects volunteered to participate in this study.

Consent for publication

We agree with the publication of the study results.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YW designed the study, collected the specimen and subject information, analyzed the data, and was a major contributor to the writing of the manuscript. XY designed the study, collected the specimens and analyzed the data. CW collects and analyzes subject information and participates in the writing of the manuscript. TL designed the study and collected the specimens. RJ designed and directed the study, collected the specimens; HD designed and directed the study, collected subject information, and provided funding. All authors have read and approved the manuscript and ensured the authenticity of the research content.

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Figures

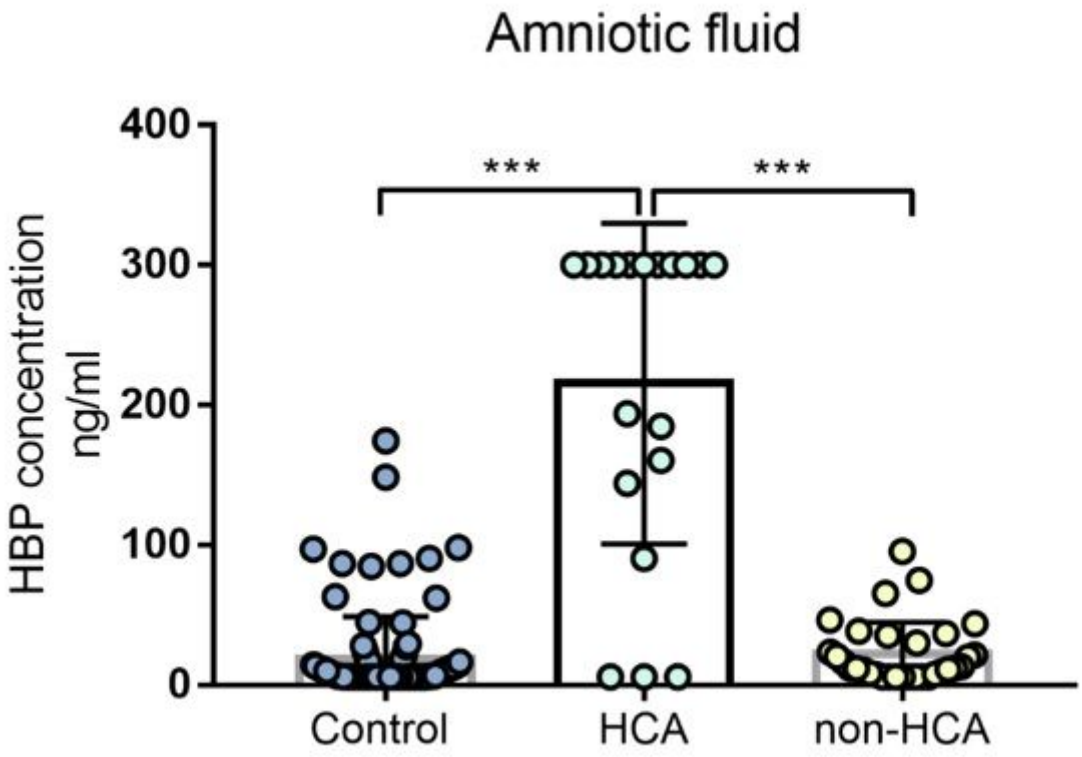

Figure 1

Amniotic fluid samples were collected from 100 control women, 19 HCA women and 32 non-HCA women. HBP was significantly higher in the HCA women than in the control ($P < 0.001$) and non-HCA groups ($P < 0.001$), while there was no statistical difference between the non-HCA and control groups. Notably, 11 of the 19 pregnant women with HCA had HBP concentrations above the detection threshold (>300 ng/ml), whereas no one in the control and non-HCA groups exceeded concentrations >300 ng/ml. (***= $P < 0.001$)

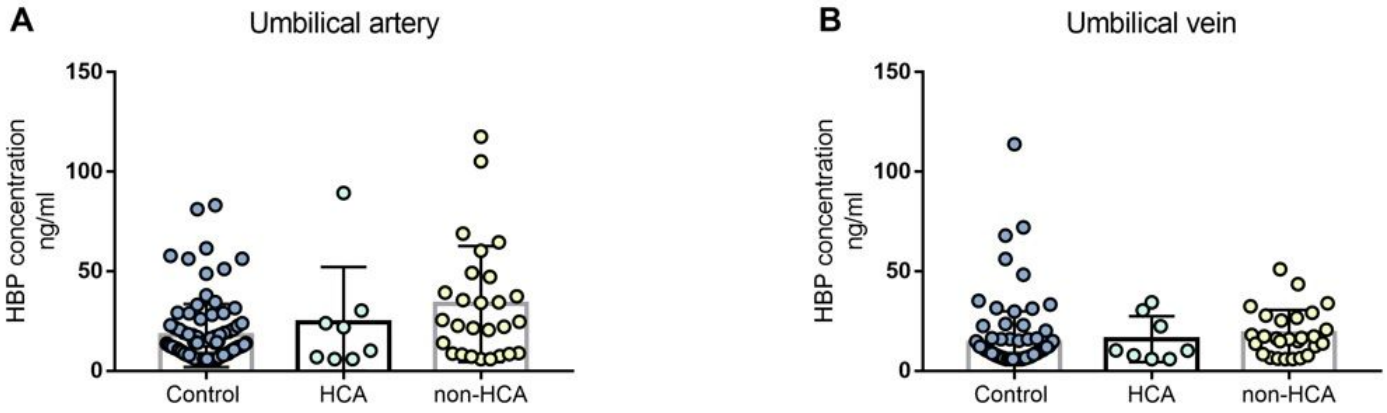

Figure 2

Concentration of HBP in umbilical artery and umbilical vein. (A) HBP concentrations in the umbilical artery did not differ significantly between the groups, but 2 concentrations over 100ng/ml were observed

in the non-HCA group. (B) HBP concentrations in umbilical cord veins were not statistically different between groups.

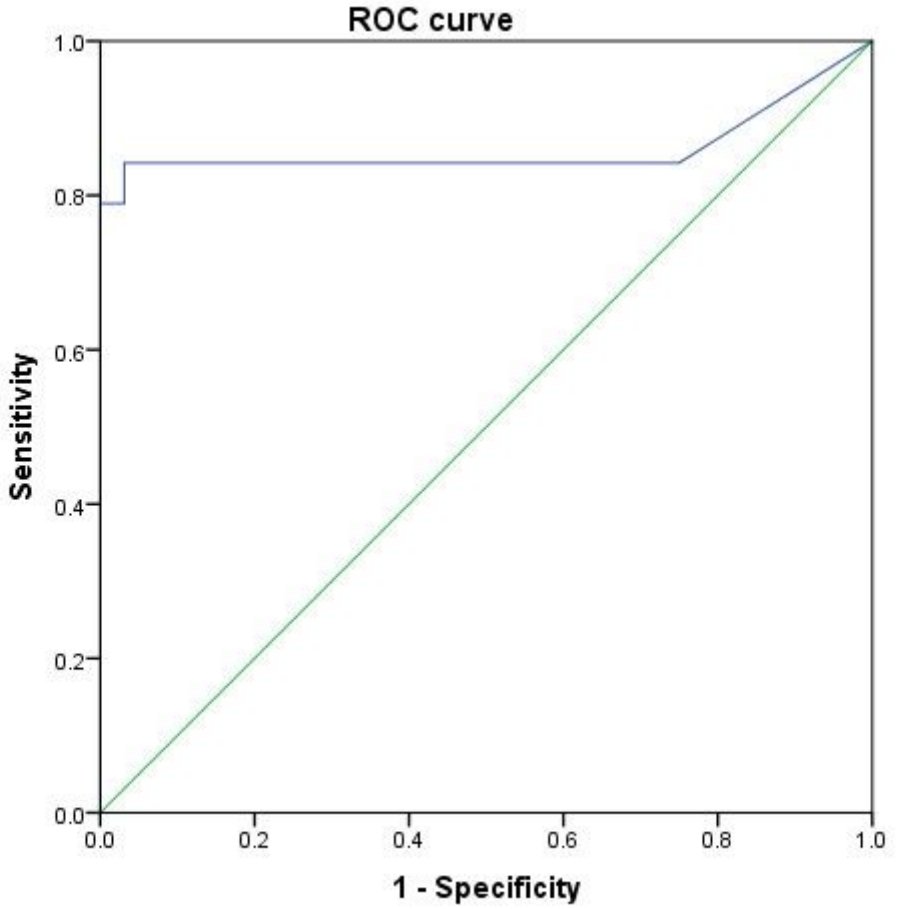

Figure 3

The ROC curve included 51 pregnant women in the pathology group with a Youden index of 0.81 and an area under the curve of 0.86 (P < 0.001, 95% CI 0.715-1.000) at a cut-off value of less than 82.67 ng/ml. The corresponding sensitivity, specificity, positive predictive value and negative predictive value for the cut-off values were 84.20%, 96.90%, 94.12% and 91.18%, respectively.