Chitotriosidase Activity in Normal Pregnancies and in Abortions

Normal ve Aborte Eden Gebeliklerde Kitotriosidaz Aktivitesi

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ABSTRACT Objective: The aim of this study was to gather data on the importance of chitotriosidase in the prediction of abortion by comparing maternal serum chitotriosidase activity in normal pregnancies and in abortions. **Material and Methods:** This study was designed as a prospective case-control study and included 142 pregnancies; 81 ended with abortion before 10 weeks of gestation, and 61 normal pregnancies. Five women in the spontaneous abortion group and one woman in the normal pregnancy group were excluded from the study. Peripheral blood samples were obtained from all pregnant women. Chitotriosidase activity was studied in these samples using the fluorometric method. **Results:** Chitotriosidase activity was significantly higher in the spontaneous abortion group compared to the control group ($p<0.01$). A threshold of 46 nmol/mL/hour for maternal chitotriosidase activity yielded 53.9% sensitivity and 85% specificity. There was no significant difference between the type of abortion and chitotriosidase activity ($p>0.05$). **Conclusion:** Activated macrophages may play a role in the pathogenesis of abortion. Further studies are warranted on this subject. This is the first study evaluating macrophage activation in spontaneous abortion.

Key Words: Chitotriosidase; abortion, spontaneous

ÖZET Amaç: Çalışmamızda normal ve abortus tanısı almış gebelerde maternal serum kitotriosidaz aktivitesini karşılaştırarak abortus öngörüsünde kitotriosidazın önemi hakkında bilgi edinilmesi amaçlandı. **Gereç ve Yöntemler:** Prospektif vaka-kontrol çalışması olarak planlandı. Çalışma 10. gebelik haftasının altında olan 81 spontan abortus olgusu ve 61 normal gebe olmak üzere, toplam 142 gebelik olgusu üzerinde yapıldı. Spontan abortus grubundan beş gebe ve sağlıklı gebe grubundan bir gebe çalışma dışı bırakıldı. Bu gebelerden maternal periferik kan örneği alındı. Bu örneklerden fluorometri yöntemi ile kitotriosidaz aktivitesi belirlendi. **Bulgular:** Spontan abortus grubunda maternal serum kitotriosidaz aktivitesi, kontrol grubuna göre anlamlı şekilde yüksek bulundu ($p<0,001$). Maternal serum kitotriosidaz aktivitesi için 46 nmol/mL/saat eşik değerinin sensivitesi %53,9 ve spesifitesi %85 olarak saptandı. Abortus tipi ile kitotriosidaz aktivitesi arasında anlamlı bir fark bulunmadı ($p>0,05$). **Sonuç:** Abortus patogenezinde aktive makrofajların rolü olabilir. Bu konuda ileri çalışmalara ihtiyaç vardır. Bu çalışma, spontan abortuslarda makrofaj aktivasyonunu değerlendiren ilk çalışmadır.

Anahtar Kelimeler: Kitotriosidaz; abortus, spontan

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Chitin is the most abundant polysaccharide in the world following cellulose. Chitotriosidase (CHIT) is an enzyme of the chitinase class, which can hydrolyze chitin and various artificial substrates. The human CHIT gene is located on 1q31-q32, and contains a sequence of 20 kb, consisting of 12 exons.[1] CHIT enzymes are synthesized selectively in re-

sponse to specific stimuli by activated macrophages and polymorphonuclear neutrophils (PMN). The main source of serum CHIT activity is PMN.[2-5] Although the properties of the CHIT enzyme have been described in detail, the physiological functions of the enzyme are not yet known.[6] Macrophages synthesize excessive amounts of CHIT and secrete it in pathological conditions causing macrophage activation, and this eventually leads to a high serum CHIT activity.

CHIT is considered as the serum biochemical marker for macrophage activation, and it is elevated in metabolic disorders such as Gaucher's disease.[7-11] Furthermore, serum CHIT activity increases moderately in Niemann-Pick Type A and in patients with lipid storage diseases, such as GM1-gangliosidosis, Niemann-Pick Type C, and acid lipase deficiency.[2,12-14] In addition, moderate increases have been found in clinical conditions such as sarcoidosis, atherosclerosis, neurological diseases, and parasitic diseases.[15-20]

Abortion is defined as the loss of pregnancy before the fetus is viable.[21] Twelve to fifteen percent of clinically defined pregnancies and 60% of all pregnancies result in abortion. More than 80% of abortions occur before 12 weeks, and termination of pregnancy prior to this time is defined as early pregnancy loss. An abortion after 12 weeks is less common, and known as late pregnancy loss.[22] Hormonal, thrombotic, genetic, infectious, autoimmune, and environmental factors may contribute to abortion.[23] Although most pregnancy losses in the first trimester are due to sporadic chromosomal defects; gestational age, and histological and genetic abnormalities may provide prognostic information for future pregnancies.[21]

Macrophages originating from the mesenchyme of the placental villi, known as Hofbauer cells, and monocytes exist in the blood stream.[24] Serum CHIT activity is thought to change in the fetal-placental developmental abnormality due to the activation of these cells during the inflammatory systemic response. In this study, serum CHIT levels, which serve as an indicator of macrophage activity, were compared between women with normal pregnancies and women with spontaneous abortions.

MATERIAL AND METHODS

PATIENT SELECTION

Our study was approved by the Ethics Committee and informed consents were obtained from all participants. Sixty-one women with normal pregnancies and 81 women with spontaneous abortion under 10 weeks, admitted to the Etimesgut Military Hospital Clinic of Obstetrics and Gynecology and Etlik Zubeyde Hanım Women's Health Training and Research Hospital Outpatient Clinic between November 2011 and October 2012 were included in this study. Four pregnant women with systemic diseases and one pregnant woman who smoked more than five cigarettes a day in the spontaneous abortion group were excluded from the study. In addition, one twin-pregnant patient in the healthy pregnancy group was excluded from the study.

An abortion is called spontaneous due to natural start and termination of pregnancy. Gestational age was calculated according to the last menstrual date or crown-to-rump length (CRL) on the first trimester ultrasonography. Pregnant women with any systemic diseases, multiparity, women who smoked more than five cigarettes per day, and anembryonic pregnancies (blighted ovum) were excluded from the study. There were no abortions or any other pregnancy complications in the control group.

BLOOD SAMPLES AND BIOCHEMICAL ANALYSIS

Peripheral venous blood samples of each pregnant woman were collected into vacutainer tubes. After the blood samples were centrifuged at 2500 rpm/min at 4°C for 10 minutes, the serum was isolated from the blood sample. The serums were stored at -80°C until analysis. The CHIT examination method was performed based on the identification method described by Hollak et al.[2] Briefly, CHIT activity was measured by incubating 5 µL of serum with 100 µL of 22 µmol/L 4-methylumbelliferyl β-D-N,N',N''- triacetylchitotrioside (Sigma Chemical, St. Louis, MO, USA) substrate in Mcll-

vain phosphate-citrate buffer, pH 5.2, for 1 h at 37°C. The reaction was terminated by adding 120 µl of 0.5 mol/L Na2CO3–NaHCO3 buffer, pH 10.7, and the fluorescence of 4-methylumbelliferone was measured with a fluorometer with excitation set at 355 nm and emission at 460 nm (Titertek, Huntsville, AL, USA). Following the methods of Artieda et al., CHIT activity was expressed as nanomoles of substrate hydrolyzed per hour per milliliter of incubated serum.[16] Serum CHIT activity was measured by duplication, and the coefficient of variation was less than 5% in all cases.

STATISTICAL ANALYSIS

The data were analyzed using the SPSS version 18.0 for Windows software program (SPSS Inc., Chicago, Illinois, USA) and tested for normal distribution with Kolmogorov-Smirnov test. T-test, Chi-square test or the Mann-Whitney U test were used for comparison of the two groups, where appropriate. The ROC analysis was performed to calculate cutoff values, sensitivity, and specificity. Finally, cutoff points were calculated by acquiring the best Youden's index. The index is defined as sensitivity+specificity -1, where sensitivity and specificity are calculated as proportions. p values <0.05 were considered statistically significant.

RESULTS

Clinical characteristics and maternal serum CHIT concentrations of healthy pregnant women and pregnant women with a diagnosis of spontaneous abortion are shown in Table 1.

There was no significant difference between two groups in terms of maternal age, parity, gravidity, or gestational age while obtaining blood samples (p>0.05). There was a history of recurrent pregnancy loss in 22.6% of the spontaneous abortion group.

CHIT maternal serum concentration was significantly higher in the spontaneous abortion group (p<0.001). CHIT activities according to abortion type and vaginal bleeding status in pregnant women with spontaneous abortion are shown in Table 2; no significant difference was found (p>0.05).

TABLE 1: Characteristics and enzyme levels in healthy pregnancies and abortions.

	Control (n=60)	Spontaneous abortion (n=76)	p
Age	27.9±4.9	28.4±6.6	0.068
Gravidity	2.07±1.1	2.53±1.7	0.064
Parity	0.78±0.8	0.83±0.9	0.766
Number of abortions	0.17±0.40	0.80±1.4	0.001
Abortion history*	10 (17%)	25 (33%)	0.032
A history of recurrent pregnancy loss**	0 (0%)	17 (22.6%)	<0.001
Pregnancy week	6.98±1.27	6.68±1.25	0.150
Maternal serum chitotriosidase concentrations (nmol/mL per h)†	23 (0-94)	50.5 (0-419)	<0.001

* Patients with a history of abortion

** Patients with pregnancy loss ≥ 2

† Median (min-max)

TABLE 2: Enzyme levels according to abortion type and bleeding status.

Abortion type	Missed (n=58)	Incomplete (n=18)	p
CHIT nmol/mL per h*	51.0 (3-419)	45.5 (0-194)	0.219
Vaginal bleeding	Yes	No	
CHIT nmol/mL per h*	50.5 (3-419)	46.0 (0-208)	0.193

CHIT: Chitotriosidase

*Median (min-max).

The ROC area under the curve (AUC) was 0.759 and p<0.0001, therefore a sum score of the classifier at 48 nmol/mL per hour was chosen as the optimal cutoff, as it had the highest Youden's index (J) of 0.389. At this cutoff value, the sensitivity was 53.9% and the specificity was 85% (Figure 1).

DISCUSSION

In this study, the serum CHIT level, which served as an indicator of macrophage activation, was measured to determine the role of macrophage activation in the pathogenesis of spontaneous abortion, and to characterize the role of CHIT in predicting abortion. CHIT activity was found higher in women with a diagnosis of spontaneous abortion less than 10 weeks. The results showed that abortion was associated with maternal serum

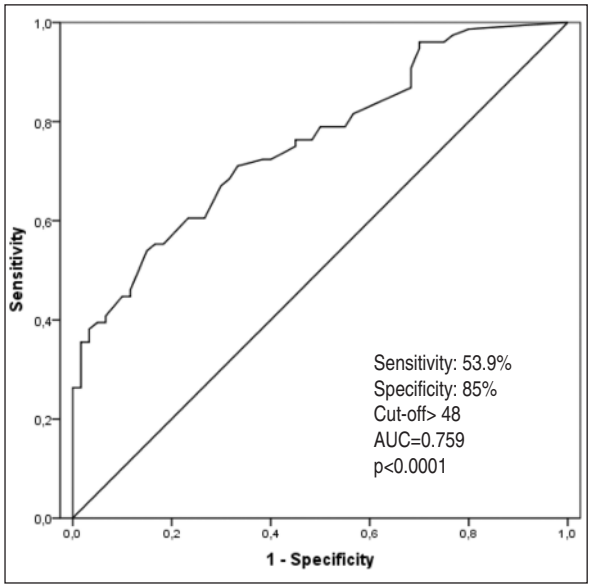

FIGURE 1: Receiver operating curve analysis showed the best cut-off value for chitotriosidase was 48 nmol/mL.
AUC: Area under the curve.

CHIT activity. This study is the first one in the literature evaluating maternal serum CHIT activity as a biomarker of abortion.

CHIT is an indicator of activated macrophages and secreted mainly by activated macrophages.[7] Van Eijk et al. reported that neutrophils and macrophages are the source of CHIT, and it is secreted as a result of stimulation by the granulocyte-macrophage colony-stimulating factor.[5] This secretion is thought to occur as a result of a defect in the fetal-placental development process in spontaneous abortion. Fetal-placental development occurs simultaneously.[25] Fetal developmental abnormalities are associated with the fetal part of the placenta.[26] The uterus recognizes the developmental defect of the placenta and evacuates the products of conception, thus leading to spontaneous abortion.[27]

Chorionic villi play an important role in the normal development of the placenta. Chorionic villi are functional units of the placenta and provide oxygen and nutrients to the fetus, and also work as a discharge unit.[28] Chorionic villi begin to form in the first trimester with the implantation of cytotrophoblasts into the decidua. Nevertheless, in the first trimester, macrophage migration occurs in the implantation side in the decidua and in the region adjacent to trophoblasts.[29] The apoptotic cells in this region are removed by macrophages and natural killer cells. Thus, decidua is made available for trophoblast invasion and physiological implantation. However, if excessive macrophage migration occurs, trophoblast invasion is impaired.[30] Recognition of abnormal placental development and the beginning of abortion can be local, systemic or both.[27]

This is the first study that evaluates macrophage activation in spontaneous abortion. The release of pro-inflammatory cytokines causes oxidative damage, and may result in functional and structural alterations. Therefore, vascular integrity, tonus, and coagulation are affected.[31] The fibrosis, in almost all disease forms, is the final common pathway leading to end-stage organ failure.[32] Haque et al. performed histopathological examination of spontaneous abortions, and found stromal fibrosis in 83% of the cases.[33] The stromal fibrosis in the terminal villi may be the result of impaired placental circulation caused by regression following intrauterine fetal death or various underlying factors.[34] Fibrosis reduces the number of vessels in the villi and causes abnormal placental development. Thus, release of cytokines and enzymes play a role in the pathogenesis of spontaneous abortion.

The prediction of spontaneous abortion may allow for the implementation of medical precautions, as well as taking timely psychological measures. Depression following abortion is encountered in more than 20% of women, and the prevalence is even higher in patients sustaining repeated abortions.[35] If a pregnancy ends with abortion, the perception of abortion gains a critical place in the long-term health status of the women and their willingness for a new pregnancy. In the present study, maternal serum CHIT activity as the indicator of activated macrophages was higher in spontaneous abortions.

Furthermore, Madazli et al. found significantly higher CHIT activity in the maternal and umbilical cord blood in pre-eclamptic women compared to the control group, and they suggested macrophage activation in pre-eclamptic women.[36] Alanbay et al.

found higher plasma CHIT enzyme levels in patients with endometriosis compared to the control group.[37]

CHIT activity was assessed in the current study regardless of the etiology of spontaneous abortion, and thus, CHIT activity in relation to specific types of abortion could not be evaluated. In addition, this study is the first of its kind to evaluate CHIT activity as a biomarker for spontaneous abortion. Studies evaluating CHIT enzyme activity before spontaneous abortion are needed to use chitotriosidase enzyme activity as a predictor of spontaneous abortion.

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