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Gebeliğin İntrahepatik Kolestazında Serum Midkine Düzeyinin Değerlendirilmesi: Üçüncü Basamak Bir Merkezde Vaka-Kontrol Çalışması

Evaluation of serum midkine level in intrahepatic cholestasis of pregnancy: a case-control study in a tertiary center

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ÖZ

Amaç: Gebeliğin intrahepatik kolestazı (ICP) tanısı alan kadınlarda midkine düzeylerini araştırmak.

Gereç ve Yöntem: Bu çalışma 40'ı ICP (çalışma grubu) ve 72'si kontrol grubu olmak üzere toplam 112 gebe üzerinde yapıldı. İki grup demografik özellikler, obstetrik veriler, laboratuvar parametreleri ve serum midkine düzeyleri açısından karşılaştırıldı. Ayrıca, çalışma grubunda midkine düzeyi ile aspartat aminotransferaz (AST), alanin aminotransferaz (ALT) ve serum safra asidi (SBA) değerleri arasında bir korelasyon analizi yapıldı.

Bulgular: Midkine değeri çalışma grubunda 0.495 ng/ml ve kontrol grubunda 0.275 ng/ml olarak tespit edildi. Midkine düzeyi kolestazlı hastalarda istatistiksel olarak anlamlı derecede yüksekti ($p<0.001$). Midkine düzeyi ile SBA veya transaminaz değerleri arasında korelasyon bulunmadı. ROC analizine göre, ICP için midkine kesme değeri 0,345 ng/ml alındığında; özgüllük: %70; duyarlılık: %70 bulundu.

Sonuç: Kolestazlı gebelerde serum midkine düzeyi daha yüksek bulundu, ancak maternal midkine düzeyi ile SBA düzeyi arasında korelasyon bulunmadı.

Anahtar Kelimeler: Serum Safra Asidi, Kolestaz, ICP, Karaciğer Enzimleri, Midkine

ABSTRACT

Aim: To investigate midkine levels in women diagnosed with intrahepatic cholestasis of pregnancy (ICP).

Materials and Method: This study was conducted with 112 pregnant women, 40 of whom had ICP (study group) and 72 were in the control group. The two groups were compared regarding demographic characteristics, obstetric data, laboratory parameters, and serum midkine levels. In addition, a correlation analysis was undertaken between the midkine level and aspartate aminotransferase (AST), alanine aminotransferase (ALT), and serum bile acid (SBA) values in the study group.

Results: The midkine value was determined to be 0.495 ng/ml in the study group and 0.275 ng/ml in the control group. It was statistically significantly higher in the patients with cholestasis ($p<0.001$). No correlation was found between the midkine level and the SBA or transaminase values. According to the receiver operating characteristic analysis, the midkine cut-off value for ICP was 0.345 ng/ml (specificity: 70%; sensitivity: 70%).

Conclusion: The serum midkine level was found to be higher in pregnant women with cholestasis, but there was no significant relationship between the maternal midkine level and the SBA level.

Keywords: Serum Bile Acid, Cholestasis, ICP, Liver Enzymes, Midkine

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INTRODUCTION

Intrahepatic cholestasis of pregnancy (ICP) is the most common liver disease in pregnancy. It usually presents in the form of itching that starts in the last trimester of pregnancy. It is characterized by increased liver enzymes and increased serum bile acid (SBA≥ 10 µmol/L) in laboratory tests (1). In addition to many factors such as environmental effects, hormonal changes, and genetic variations, the activation of inflammatory cells and uptake of proinflammatory cytokines into the liver also plays a role in the pathogenesis of ICP (2,3). ICP is associated with adverse pregnancy outcomes. Furthermore, many hepatobiliary diseases and hepatocellular carcinoma have been reported to be more common in women with a history of cholestasis during pregnancy (4). So, knowing the underlying pathology in cholestasis patients can shed light on liver diseases that may occur in the future.

Midkine was first identified in 1988, being defined as the product of the retinoic acid-responsive gene involved in embryogenesis (5). Midkine is a heparin-binding secreted growth factor protein that plays an important role in cell growth and angiogenesis (6). It promotes the migration of macrophages and neutrophils, which is crucial for inflammation. In addition, midkine suppresses the development of regulatory T cells, and therefore it may be a molecular target for treating or preventing inflammatory diseases (7).

In the present study, we aimed to investigate midkine levels in women diagnosed with ICP and evaluate its correlation with the SBA level.

MATERIALS AND METHOD

This prospective study was conducted with a total of 112 participants. Pregnant women diagnosed with ICP according to the diagnostic criteria (study group) (n = 40) were compared with a control group of gestational age-matched pregnant women without any identified risk factors (n = 72). Written informed consent was obtained from all the participants. Approval for the study was obtained from the local ethics committee (E2-21-725), and the study was conducted in accordance with the ethical standards and principles of the Declaration of Helsinki. The two groups were compared in terms of demographic characteristics, obstetric data, laboratory parameters, and midkine levels. In addition, a correlation analysis was performed between midkine and the aspartate aminotransferase (AST), alanine aminotransferase (ALT), and SBA values in the study group.

Patient selection

The diagnostic criteria for ICP were defined as follows: unexplained pruritus develops during pregnancy and abnormal liver function tests and/or bile acids are elevated, both of which resolve after delivery (8). While evaluating all the cases, viral hepatitis serology screening and abdominal ultrasonography were undertaken to rule out other liver diseases. Patients with chronic liver or skin disease, allergic diseases, symptomatic cholelithiasis, elevated liver enzymes after viral hepatitis, preeclampsia, or acute fatty liver of pregnancy were excluded from the study.

Biological samples and analyses

Venous blood samples were obtained from each participant by venipuncture at the gestational week of diagnosis time. Blood samples were collected from all participants at a similar gestational week before delivery. Immediately after the blood sample was taken, it was centrifuged at 3,000 g for 15 minutes. Plasma was aliquoted in plastic tubes and stored at -80 °C until the analysis day. Midkine level measurements were made using the enzyme-linked immunosorbent assay method (Human Midkine Elisa Kit, Bioassay Technology Laboratory) following the manufacturer's instructions for the use of the commercial kit. The researcher who performed the tests evaluated the results of clinical data in a blinded manner.

Statistical analysis

Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS v. 22, IBM SPSS Statistics for Windows, Version 22.0 Armonk, NY: IBM Corp.). Visual and analytical methods (Kolmogorov-Smirnov test) were used to determine whether the variables were normally distributed. Descriptive statistics were presented as median and interquartile range values for non-normally distributed variables. Since continuous variables were not normally distributed, the Mann-Whitney U-test was conducted to compare the median values between the groups. A correlation analysis was undertaken using Spearman's rho test. The receiver operating characteristic (ROC) curve was constructed to evaluate the performance of the midkine in women diagnosed with ICP. A two-tailed p-value of <0.05 was considered statistically significant.

RESULTS

A total of 112 pregnant women were included in the study (40 patients with ICP in the study group and 72 controls). When the

study and control groups were examined in terms of demographics and obstetrics, they were found to be similar (p> 0.05). The AST and ALT values were significantly higher in the study group (p <0.001). The mean midkine value was 0.275 ng/ml in the control group and 0.495 ng/ml in the study group. The midkine value was statistically significantly higher in the patients with ICP (p <0.001) (Table 1).

Table 1: Comparison of demographic, obstetric, and laboratory parameters of the groups

	Control group (n = 72)		Study group (n = 40)		p
	Median	IQR	Median	IQR	
Age (year)	**29**	**10.75**	**28**	**7**	**0.959**
Body mass index (kg/m2)	**29.3**	**7.63**	**26.9**	**6.15**	**0.216**
Gravity	**2**	**3**	**2**	**2**	**0.095**
Parity	**1**	**2**	**0**	**2**	**0.156**
Miscarriage	**0**	**1**	**0**	**0**	**0.227**
Gestational age at diagnosis (weeks)	**36**	**7.75**	**36**	**2.75**	**0.959**
Estimated fetal weight (g)	**2492**	**945**	**2594**	**598**	**0.597**
Single deepest vertical pocket (mm)	**52**	**16**	**50**	**14.75**	**0.595**
AST (U/L)	**16**	**4.8**	**59**	**44**	**<0.001**
ALT (U/L)	**14**	**6.2**	**75**	**68**	**<0.001**
Serum Midkine level (ng/ml)	**0.275**	**0.21**	**0.495**	**0.51**	**<0.001**

Mann-Whitney U

IQR: Interquartile range, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

The correlations between the midkine, SBA, AST, and ALT values were examined. Although there was a positive significant correlation between AST and ALT, no correlation was observed between midkine and SBA, or between these two parameters and AST or ALT (Table 2).

Table 2: Non-parametric correlations between midkine, SBA, AST, and ALT

		Midkine	SBA	AST	ALT
Midkine	Correlation coefficient	1.000	-.066	.093	.043
	Sig. (two-tailed)	-	.688	.568	.790
SBA	Correlation coefficient	-.066	1.000	.242	.216
	Sig. (two-tailed)	.688	-	.133	.181
AST	Correlation coefficient	.093	.242	1.000	.912**
	Sig. (two-tailed)	.568	.133	-	.000
ALT	Correlation coefficient	.043	.216	.912**	1.000
	Sig. (two-tailed)	.790	.181	.000	-

**Correlation significant at the 0.01 level (two-tailed). Spearman's rho

SBA: Serum bile acid, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase,

n the receiver operating characteristic (ROC) analysis, at a cut-off of 0.345 ng/ml, midkine had specificity and sensitivity values of 70% and 70%, respectively for ICP (Table 3).

	Cut-off	AUC	P	95% CI	Sensitivity	Specificity
Midkine	0.345	77.3	<0.001	0.686-0.86	70%	70%

AUC: Area under the curve, CI: Confidence interval

DISCUSSION

In this study, we investigated the maternal serum midkine level in a group of patients with ICP and compared the results with a control group with uncomplicated pregnancies. We found that when compared to the healthy control group, the midkine levels were statistically significantly higher in the patients with ICP, which develops on an inflammatory basis and is the most common liver disease in pregnancy. In our ROC analysis to determine the increased risk of ICP, we determined the cut-off value as 0.345 ng/ml, at which specificity and sensitivity were 70% and 70%, respectively. When we examined the correlation of the midkine level with SBA, AST, and ALT, we found no significant correlation.

Midkine is a biochemical marker found in small amounts in the plasma of healthy individuals and is detected at elevated levels in some inflammatory and malignant conditions (9). Previous studies on cholestasis and inflammation have shown that ICP is an inflammatory process. In addition, they claim that there is a relationship between inflammation markers and the severity of the disease (10,11). Increasing bile acids are considered to cause inflammation and stimulate the secretion of proinflammatory mediators through their direct effects on hepatocytes (12). In the present study, although we found higher serum midkine levels in the study group, we did not find a correlation between serum midkine levels and SBA.

ICP is characterized by itching and elevated serum bile acid concentrations, which typically develop in the late second and/or third trimester and resolve rapidly after delivery (8,13). Although the incidence of ICP varies depending on the regional and ethnic structure, it has been reported to range from 0.5 to 1.5% in Europe (14). Increased bile acids may pass through the placenta and accumulate in the fetus and amniotic fluid (15). Pregnant women with cholestasis have an increased risk of intrauterine death, meconium-containing amniotic fluid, premature birth (spontaneous and iatrogenic), and neonatal respiratory distress syndrome (16,17). In a meta-analysis, adverse perinatal outcomes were observed more frequently as serum bile acid increased (16). In a similar systematic review, fetal

death rates were found to be 0.4, 0.3, and 6.8% in pregnant women with total bile acid concentrations of <40 micromole/L, 40-99 micromole/L, and ≥100 micromole/L, respectively (18). It is considered that increased bile acids lead to fetal death by causing fetal arrhythmia and sudden vasospasm in placental chorionic vessels (19). Fortunately, since fetal death usually occurs in the last weeks of pregnancy, planning the timing of delivery according to bile acid concentration reduces negative perinatal outcomes (16,18). Fetal death was not observed in any of our patients because delivery was scheduled in accordance with current treatment protocols in our clinic. Since we had no cases with adverse perinatal outcomes, we evaluated a correlation between serum midkine levels and SBA to examine the correlation with disease severity. In the correlation analysis, there was no correlation between the midkine level and SBA. Therefore, we were not able to establish a relationship between the maternal serum midkine level and the severity of ICP.

According to previous studies, the risk of liver, biliary tract, and pancreatic diseases and hepatobiliary cancer is increased in women with a history of ICP during pregnancy (4). Although the serum midkine level may be elevated in many cancer cases, such as esophagus, stomach, bladder, and lung, the most investigated type of cancer is hepatocellular carcinoma (2,22). In a cohort study conducted in Sweden evaluating 125,281 women who gave birth, liver cancer risk was 3.61 times higher and bile cancer 2.62 times higher in women with a history of cholestasis during pregnancy (23). In other studies, it was shown that an increased midkine level could be used as a tumor marker even in the early stages of hepatocellular carcinoma (24,25). In a study in which hepatectomy was performed on mice, liver regeneration was found to be slower in those whose midkine gene was suppressed (26). In another study, it was reported that midkine secretion increased in liver cells damaged by cadmium and reduced this tissue damage (27). Based on these results, we can refer to a potential relationship between liver damage and repair and midkine levels. Long-term cohort studies are needed to establish a relationship between the risk of hepatobiliary disease or cancer and high midkine levels in pregnant women.

ICP, the most common liver disease of pregnancy, is associated with adverse perinatal outcomes. In addition, women with a history of ICP in pregnancy have an increased risk of liver disease and cancer later in life. Therefore, understanding the mechanism underlying ICP pathology may shed light on many diseases. We believe that future studies may elucidate this hypothesis thanks to this study in which we have shown an association between ICP and elevated serum midkine levels. However, in this study, we could not show a correlation between serum midkine level and disease severity.

To the best of our knowledge, this is the first study to investigate the midkine level in pregnant women with cholestasis, and therefore we consider that our results will make a significant contribution to the literature. However, this study also had certain limitations, including the small number of patients and the absence of perinatal and long-term maternal outcomes. In this context, there is a need for randomized controlled studies with a large number of participants.

Conclusion

We found a higher serum midkine level in pregnant women with cholestasis. There was no correlation between the midkine level and SBA or transaminases. When the cut-off value of midkine was taken as 0.345 ng/ml for the prediction of ICP, specificity and sensitivity were determined as 70% and 70%, respectively.

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