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Increased Serum Met-enkephalin Level in Patients with Intrahepatic Cholestasis of Pregnancy

Gebeliğin İntrahepatik Kolestazı Olan Hastalarda Artmış Serum Met-enkefalin Seviyesi

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Abstract

Objective: To evaluate met-enkephalin levels in women with intrahepatic cholestasis of pregnancy (ICP) by comparison to healthy controls and to compare the pregnancy outcomes.

Method: This cross-sectional study was conducted in 43 pregnant women with ICP and 40 randomly selected healthy pregnant women, who formed the control group. Serum met-enkephalin concentrations were measured using an enzyme-linked immunosorbent assay. The patients' age, body mass index (BMI), gestational week, fasting serum bile acid, serum parameters, birth week, and APGAR scores at 1st and 5th minutes were recorded.

Results: Maternal age, BMI and gestational age at blood sampling were similar between the two groups. Considering the pregnancy outcomes, birth week was significantly lower in the ICP group than in the control group (37 vs. 38 weeks, respectively; p<0.001). The median met-enkephalin level was significantly higher in the ICP group compared to the control group (273 vs. 121.5, respectively; p<0.001). A significant negative correlation was observed between met-enkephalin level and birth week (r=-0.33; p=0.002). The area under the curve was 91% for the cut-off point 145 of met-enkephalin (p<0.001). The specificity and sensitivity were 77.5% and 79%, respectively. A significant effect of met-enkephalin on the diagnosis of cholestasis was observed (odds ratio: 1.01, p<0.001, 95% confidence interval: 1.007-1.02)

Conclusion: Serum met-enkephalin levels were increased in patients with gestational cholestasis compared to healthy controls. Based on this, opioid receptor antagonists may be a promising treatment alternative.

Keywords: Bile acids, cholestasis, opioid, pregnancy, pruritus

Öz

Amaç: Gebeliğin intrahepatik kolestazı (GİK) olan gebelerde serum met-enkefalin düzeylerini sağlıklı kontrollerle karşılaştırarak değerlendirmek ve gebelik sonuçlarını karşılaştırmaktır.

Yöntem: Bu kesitsel çalışma, GİK 43 gebe ve kontrol grubunu oluşturan rastgele seçilmiş 40 sağlıklı gebe ile yürütüldü. Serum met-enkefalin konsantrasyonları, enzime bağlı immünosorbent testi kullanılarak ölçüldü. Hastaların yaşı, vücut kitle indeksi (VKİ), gebelik haftası, açlık serum safra asidi, serum parametreleri, doğum haftası, 1. ve 5. dakika APGAR skorları kaydedildi.

Bulgular: Anne yaşı, VKİ ve gebelik yaşı iki grup arasında benzerdi. Gebelik sonuçları dikkate alındığında, doğum haftası GİK grubunda kontrol grubuna göre anlamlı olarak daha düşüktü (sırasıyla 37 ve 38 hafta; p<0,001). Medyan met-enkefalin düzeyi, kontrol grubuna kıyasla kolestaz grubunda anlamlı olarak daha yüksekti (sırasıyla 273'e 121,5; p<0,001). Met-enkefalin düzeyi ile doğum haftası arasında anlamlı negatif korelasyon gözlendi (r=-0,33; p=0,002). Met-enkefalinin kesme noktası 145 için eğri altında kalan alan %91 idi (p<0,001). Özgüllük ve duyarlılık sırasıyla %77,5 ve %79 idi. Met-enkefalin düzeyinin kolestaz tanısı koymada anlamlı etkisi gözlendi (olasılık oranı: 1,01, p<0,001, %95 güven aralığı: 1,007-1,02).

Sonuç: Serum Met-enkefalin düzeyi gebelik kolestazı olan hastalarda sağlıklı kontrollere göre anlamlı olarak artmış izlenmiştir. Buna dayanarak, Met-enkefalin gestasyonel kolestaz tanısında yeni bir belirteç olarak düşünülebilir ve ayrıca opioid reseptör antagonistleri umut verici bir tedavi alternatifi olabilir.

Anahtar kelimeler: Gebelik, kaşıntı, kolestaz, opioid, safra asitleri

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Introduction

Intrahepatic cholestasis of pregnancy (ICP) is defined as the presence of gestational pruritus along with increased serum bile acids and aminotransferase levels, which are observed through the second or third trimester of pregnancy (1). The incidence of ICP is reported as 0.1-2% in all pregnant women worldwide, and it can be complicated by an increased risk of preterm labor, fetal distress, meconium-stained amniotic fluid, fetal bradycardia, and sudden fetal demise (2,3). A variety of factors such as genetic, ethnic, deteriorations in the hepatocellular transport cycles, hormonal factors, and environmental contributors are accused in the pathogenesis of the disease (4).

Since it may complicate pregnancies, there are still no reliable markers to predict the risk of ICP. The maternal serum bile acid level above 40 µmol/L is the most commonly preferred marker used in the prediction of obstetrical outcomes so far (5). Aside from bile acid levels, pregnancy-associated plasma protein-A (PAPP-A), aspartate aminotransferase (AST) to platelet ratio, and oxidative markers, and inflammatory molecules have been investigated to predict early onset of ICP (4-6).

The etiology of ICP is still unclear and it was reported that increased levels of endogenous opioid met-enkephalin could cause cholestasis in the general population (7). The pentapeptide met-enkephalin is known as an inhibitory growth factor that has functions in cell development, tissue renewal, wound healing, angiogenesis, and inhibition of DNA synthesis in fetal cells (8). Chronic exposure to met-enkephalin could also cause detrimental effects on pregnancy outcomes and it could harm brain, kidney, liver, and lung functions (8). To date, no studies have evaluated the relationship between met-enkephalin levels and pregnancies complicated with ICP.

This study aimed to evaluate met-enkephalin levels in women with ICP by comparison to healthy controls.

Materials and Methods

This cross-sectional study was carried out in 83 pregnant women between March 2021 and September 2021 at a tertiary referral center. University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital Ethics Committee's approval was obtained before the study was undertaken (2021/171). Helsinki guidelines regarding ethical considerations on human subjects were followed. Informed consent was obtained from all of the participants.

Maternal demographic and clinical features, laboratory parameters, serum met-enkephalin levels, and delivery outcomes of the participants were recorded. All patients were evaluated with a complete obstetric examination and transabdominal ultrasound.

For the study group, the diagnosis of ICP in a pregnant woman was based on the clinical presentation of widespread itching involving palms and soles without rash, accompanied by either elevated serum liver enzymes and/or elevated serum TBA levels (>10 µmol/L) in the absence of other possible etiologies (9). Any patients with hematological or liver disorders, viral hepatitis, autoimmune diseases, malignancy, dermatological pathology, medications affecting liver enzymes and bile acids, risk factors for preeclampsia or gestational diabetes, and patients with a history of poor obstetric outcomes were excluded.

Age, gestational week and body mass index (BMI)- matched healthy pregnant women were included as healthy controls. All patients with complete clinical follow-up data were recruited into the study.

Met-enkephalin Measurement

Venous blood samples were obtained from the antecubital vein of the patients after overnight fasting period. The samples were kept for 2 hours at room temperature at 2-8 °C before centrifugation for 15 min at 1000×g. The supernatant was stored at -80 °C until use. The serum concentration of met-enkephalin was evaluated by the enzyme-linked immunosorbent assay (ELISA) technique using an ELISA kit (Cat no: E-EL-0020) purchased from Elabscience (USA) following the manufacturer's instructions and were expressed in pg/mL. The detection range was 125.00-8000 pg/mL for met-enkephalin and coefficient of variation was <10%.

Statistical Analysis

Statistical analysis was performed using the IBM SPSS version 22.0 software (IBM Corp., Armonk, NY, USA). The Shapiro Wilk test was used to evaluate the distribution of the continuous data. Descriptive data were expressed in median and interquartile ranges. The Mann-Whitney U test was used to compare the quantitative data of control and ICP groups. The Spearman correlation analysis was performed to assess the relationship between met-enkephalin levels and age, BMI, bile acid level, and birth week.

The optimal cut-off value of the met-enkephalin to detect ICP was determined using receiver operating characteristic (ROC) curves and Youden's index. A logistic regression

analysis was performed to assess the adjusted effect of met-enkephalin on ICP. A value of p<0.05 was considered statistically significant.

Results

Fourty-three women with ICP and 40 healthy pregnant control were involved in the study. The median age of the patients was 30.5 years in the control group, whereas it was 29 years in the ICP group and no significant difference was observed between the two groups. As expected, serum AST, alanine aminotransferase, total bilirubin, and bile acid levels were significantly higher in the ICP group compared to the control group (p<0.05 for all comparisons). However, no significant difference was observed regarding the gestational week, BMI and GGT levels between the study and control groups. The median met-enkephalin level was significantly higher in the ICP group compared to the control group (273 pg/mL vs. 121.5 pg/mL, respectively; p<0.001).

Considering the obstetric outcomes, the birth week was significantly lower in the ICP group than in the control group (37 vs. 38 weeks, respectively; p<0.001). No significant difference was observed regarding 1st and 5th minutes APGAR scores between the study groups (Table 1).

Table 1. Comparison of control and ICP groups regarding basic characteristic, laboratory parameters and fetal outcomes

	Control (n=40)	ICP (n=43)	p-value
	Median (IQR)	Median (IQR)	
Age (years)	30.5 (6)	29 (16)	0.58
Gravidity	3 (1.75)	2 (2)	**<0.001**
Parity	1.5 (1)	0 (1)	**<0.001**
BMI (kg/m²)	27.2 (2.3)	28.9 (5.1)	0.16
Gestational week	33 (4.75)	3 3(6)	0.53
AST (IU/L)	24.5 (8)	93 (118)	**<0.001**
ALT (IU/L)	35 (10.75)	159 (192)	**<0.001**
GGT (IU/L)	29 (10.75)	3 1(8)	0.65
Total bilirubin (mg/dL)	0.55 (0.3)	1.1 (0.5)	**<0.001**
Bile acid (μmol/L)	4 (2.75)	40 (31)	**<0.001**
Birth week	38 (1)	37 (2)	**<0.001**
APGAR 1 min	7 (1.75)	7 (1)	0.55
AGGAR 5 min	9 (1.5)	9 (1)	0.48
Met-enkephalin (pg/mL)	121.5 (36)	273 (247)	**<0.001**

IQR: Interquartile range, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, BMI: Body mass index, ICP: Intrahepatic cholestasis of pregnancy

A significant positive correlation was observed between met-enkephalin level and BMI (r=0.3; p=0.005). On the other hand, a significant negative correlation was observed between met-enkephalin level and birth week (r=-0.33; p=0.002) (Table 2).

ROC analysis revealed an AUC of 91% for the cut-off point 145 pg/mL of met-enkephalin (p<0.001). The specificity and sensitivity were 77.5% and 79%, respectively (Figure 1, Table 3).

Since BMI had a significant correlation with met-enkephalin levels, we further performed a logistic regression analysis to achieve the adjusted effect of met-enkephalin. A significant effect of met-enkephalin on ICP was observed (odds ratio: 1.01, p<0.001, 95% confidence interval: 1.007-1.02) (Table 4).

Discussion

This study revealed that met-enkephalin could increase in patients with ICP compared to controls. A cut-off level above 145 pg/mL could be used in the diagnosis of ICP.

The opioid peptide levels and opioidergic neurotransmission are increased in cases with cholestasis and pruritus (10,11). Besides, increased plasma met-enkephalin level was considered as a predictor of reduced survival marker in patients with primary biliary cirrhosis (7). These findings confirmed the relationship between opioid peptides and the pruritus of cholestasis (12). In experimental cholestasis models, it was reported that met-enkephalin immunoreactivity could be observed in the liver tissue, particularly in cholangiocytes (10). Moreover, the administration of opioid receptor antagonists such as naloxone and nalmefene to patients with primary biliary cirrhosis improved plasma bilirubin levels (7). In our study, at the same line with the previously reported studies, significantly increased met-enkephalin levels were detected in patients suffering ICP compared to controls. We may speculate that met-enkephalin could be associated with pruritus and increased risk of cholestasis due to the effect on the hepatobiliary system.

Previous studies also tried to find out a marker to predict ICP in early stages of gestation. Tolunay et al. (4) evaluated AST to platelet ratio index (APRI) for that purpose. They revealed a sensitivity of 86.5% and specificity of 77.3% for an optimal cut-off value of 0.57. The authors concluded that a liver-specific enzyme to platelet ratio could be used to determine ICP from blood tests obtained in the first trimester (4). Another study evaluated- serum Tyrosine (Y), Lysine (K),

Table 2. Spearman correlation analysis of met-enkephalin and age, BMI, bile acid, and birth week

	Age (years)		BMI (kg/m²)		Bile acid (µmol/L)		Birth week	
	r	p	r	p	r	p	r	p
Met-enkephalin (pg/mL)	-0.02	0.98	0.3	**0.005**	0.61	**<0.001**	-0.33	**0.002**

BMI: Body mass index

Table 3. ROC analysis of met-enkephalin levels in prediction of ICP

	AUC	Specificity	Sensitivity	PPV	NPV	LR+	LR-	p-value	95% CI	
									Lower	Upper
Met-enkephalin (145 pg/mL)	91%	77.5%	79%	21%	77%	3.5	0.3	**<0.01**	85%	96%

ROC: Receiver operating characteristic, ICP: Intrahepatic cholestasis of pregnancy, AUC: Area under the curve, CI: Confidence interval

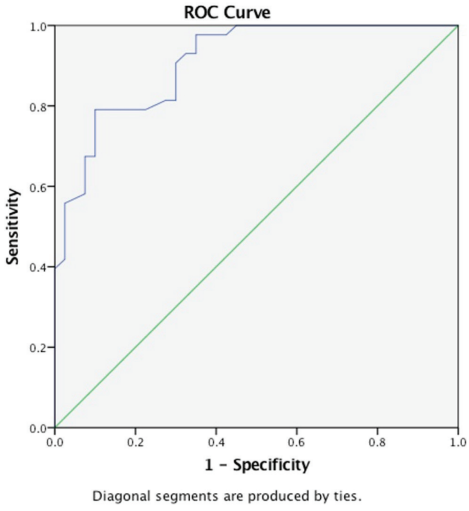

Figure 1. ROC analysis of met-encephalin levels in prediction of ICP

ROC: Receiver operating characteristic, ICP: Intrahepatic cholestasis of pregnancy

Table 4. Logistic regression analysis of met-enkephalin levels and cholestasis considering BMI levels

	B	p-value	Exp (B)	95% CI	
				Lower	Upper
BMI (kg/m²)	0.11	0.33	1.1	0.9	1.3
Met-enkephalin (pg/mL)	0.01	**<0.001**	1.01	1.007	1.02

Constant (-5.72). BMI: Body mass index, CI: Confidence interval

and Leucine (L)- YKL-40 levels in pregnant women with ICP and concluded that YKL-40 levels were significantly higher with a 40% sensitivity and 93% specificity for serum YKL-40 concentration of 84.80 ng/mL (13). The studies focusing on inflammatory and immunological theories also investigated pro-inflammatory cytokines (14,15) and found increased levels of IL-6 and IL-17 in patients with ICP (13). Moreover, the lipid peroxidation markers such as Gpx and plasma 8-iso-PGF2α level were also investigated (6). Hu et al. (6) reported that lower levels of these markers could be used in predicting the risk of perinatal complications. Regarding the studies mentioned above, inflammation and oxidative processes could be accused in ICP pathogenesis. Still, no utilization of these markers is yet available in clinical practice, and these theories are far away from explaining the pruritus seen in ICP.

Another issue of concern in ICP is poor obstetric outcomes and postnatal complications (16,17). A meta-analysis evaluating ICP and its relationship with adverse perinatal risks showed that an increased risk could be observed when bile acids were over 40 µmol/L (18). However, another study concluded that the threshold of bile acids should be 100 µmol/L to predict poor prenatal outcomes. The authors concluded that different levels could be associated with various methods in the analysis (1). The postnatal complications include sweating and fever, decreased sleep periods, jaundice, sepsis, seizures, increased muscle tone, continuous high-pitched crying, gastrointestinal dysfunction, vomiting, and sudden death (18). There was no significant difference regarding 1st and 5th minutes of APGAR scores between ICP and control groups in our study. However, the birth week was significantly less in the ICP group compared to the control group. The risk of increased fetal demise or poor obstetric outcomes could lead the obstetricians to early induce labor.

Study Limitations

The limitation of our study is its small sample size and the lack of more comprehensive follow-up data. However, this is the first study in the literature including a prospective cohort of age-matched pregnant women with and without ICP. Although the OR is too close to the upper confidence interval level; there is still a prominent significant P value. This is probably related to the significant difference in the big gap of met-enkephalin levels and the distribution

of the met-enkephalin levels at the upper border of the confidence interval in the study group. The area under curve 91% clarifies that our results are promising in the diagnosis of ICP.

Conclusion

Met-enkephalin could increase patients with ICP compared to controls, and opioid receptor antagonists may be a promising treatment alternative. However, our study findings need to be confirmed in larger populations from different ethnicities.

Ethics

Ethics Committee Approval: Ethics approval to carry out the study was provided by Clinical Research Ethics Committee of University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, (date: 15.03.2021, no: 2021.06.47, protocol :2021/171).

Informed Consent: Informed consent was obtained from all of the participants.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: S.S.Ç., Design: S.S.Ç., Data Collection or Processing: M.E., Analysis or Interpretation: M.E., Final Approval and Accountability: M.E., Drafting Manuscript: S.S.Ç., Critical Revision of Manuscript: S.S.Ç., M.E., Writing: S.S.Ç., M.E.

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