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Serum adropin and nitric oxide levels in missed abortus cases

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Ethics Committee Approval
The approval is obtained from Ethics Committee of Kafkas University Medical Faculty (date: 25.05.2016, Verdict No: 2016/6).
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Abstract

Background/Aim: Missed abortus is an emergency obstetric pathology, defined as intrauterine fetal viability loss before the 20th week of pregnancy. It is known that there is a correlation between endothelium dysfunction, neovascularization, hemodynamic regulation and adropin and nitric oxide levels. In this study, it is intended to research the possible roles of adropin and nitric levels in etiopathogenesis.

Methods: In this case-control study, a total of fifty-nine volunteers, including healthy pregnant women and missed abortion cases, were included in the study. They were divided into 2 groups, as healthy individuals (Group 1, n=29), and those diagnosed with missed abortus (Group 2, n=30). Group 2 patients were followed weekly until β-HCG values fell below 5 ng/ml after termination. Serum adropin and nitric oxide levels were measured in all participants and those diagnosed with missed abortion when negative β-HCG value was obtained after termination.

Results: Serum adropin and nitric oxide levels were significantly low in the missed abortus group (P=0.03) compared to healthy pregnant women, and in the missed abortus group compared to those whose β-HCG fell below 5 ng/ml after termination (P=0.02), while nitric oxide levels were similar in the latter comparison (P=0.38). Regarding age, body mass index, obstetric parameters, and other biochemical parameters, the two groups were similar (P>0.05 for each).

Conclusion: This is the first research which evaluates adropin levels in missed abortus cases. Lower adropin and nitric oxide levels in missed abortus cases, and their increase after post-termination show that they may be playing a role in abortus etiopathogenesis.

Keywords: Missed abortus, Adropin, Nitric oxide

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Introduction

Missed abortus is an emergency with intrauterine fetal viability loss and diagnosed with no fetal cardiac activity in ultrasonography. Clinically, 12-15% of the noticed pregnancies are concluded with abortus between the 4th and 20th weeks. According to the World Health Organization, the disposal of all embryo, fetus, and their counterparts out of the uterine cavity before the 20th week of pregnancy and/or the newborn weighing less than five hundred grams is called abortus [1]. Besides vaginal bleeding, missed abortus cases have an important place in terms of obstetric practice due to maternal obstetric complications. The treatment of missed abortus is emptying the uterus surgically or medically. In pathological examination, hemorrhage into decidua basalis and necrotic changes with degenerations on placental villi can be seen. Fetal, maternal, and paternal causes are in the etiopathology. Malformations, and chromosomal anomalies in the fetus, and immunological causes, endocrine defects, uterine anomalies, drug use, environmental factors and trauma in the mother are among the causes [2]. Besides these known factors, vascular endothelial damage may cause ischemic damage progression by preventing the implantation of fetus to the endometrium or preventing the development of the fetus by inhibiting placental functions, therefore any situation that causes vasoconstriction may result with missed abortus.

Adropin is a peptide hormone composed of seventy-six amino acids. First defined by Kumar et al. [3] in 2008, it plays a role in protecting endothelial functions, vascular hemostasis, and neovascularization. It has a half-life of 3 to 30 minutes. Its effect on the endothelium is first studied by Lovren et al. [4] in 2010 and results attesting these hypotheses were obtained. In the study of Dong Lin et al. on hypertensive diseases complicating pregnancy, serum adropin levels were low in this group and its determination in the early days of the pregnancy allowed the detection of risky patients [5].

Nitric Oxide (NO) has a noticeably short half-life (2-30 seconds) due to its free radical structure, remarkably high affinity, a low molecular weight, and is reactively secreted. Beginning with smooth muscle relaxation, it plays major roles in physiologic and pathophysiologic pathways. With its vasodilator effect, it increases local blood flow, regulating blood pressure and protecting endothelium. Independent of receptors, it can diffuse easily through the membranes. With all its properties, NO is an ideal messenger [6,7].

In the light of this information, we think that in addition to their protective nature on endothelial structure and function, due to their angiogenetic, smooth muscle relaxant and vasodilator effects, both adropin and nitric oxide may play a role in the implantation and growing of fetus, perfusion of placenta and continuation of it functions. Thus, they may be involved in the etiopathogenesis of missed abortus.

Materials and methods

This case-control study was conducted in July 2016 - October 2016 in Kafkas University Medical Faculty Training and Research Hospital, Gynecology & Obstetrics Clinic. It was started after the approval of Kafkas University Medical Faculty Ethics Committee was obtained, dated 25.05.2016 (Decision No: 6). The patients were informed about the study, and they all signed informed consent forms before the start.

Twenty-nine healthy pregnant women under 20 weeks of gestation (Group 1) with no complaints or ailments and 30 patients (Group 2) diagnosed with Missed Abortus for the first time were included in the study (n_{total}=59). Missed abortion cases in Group 2 were terminated and followed weekly until the β-HCG value fell below <5 ng/ml. Serum adropin and nitric oxide levels were researched for healthy pregnant women, missed abortus cases whose β-HCG did and did not decrease below 5 ng/ml.

People with abortus history, cigarette-alcohol consumption, teratogen drug use, endocrinologic-hematologic diseases, uterine anomalies, a space-occupying lesion in the uterus, trauma history, cases with infection findings, systemic diseases like DM and HT and the ones in the missed abortus group with poor general condition and DIC were excluded from the study. Demographic data (age, gravida, parity, abortus and curettage), BMI, pregnancy weeks of the cases were noted. All cases' complete blood count parameters (hemoglobin, hematocrit, white blood cells, platelet), fasting blood sugar, TSH, Free T3, Free T4, ALT, AST, Urea, Creatinine, Prothrombin Time (seconds) and INR, and Active Partial Thromboplastin Time levels were evaluated.

Collection of blood samples

Five milliliters of fasting venous blood samples were obtained from the cases in the morning between 08:00-10:00. Hormonal and biochemical measurements obtained from the venous blood samples were studied on the same day. Also, to prevent the disintegration of peptides, the serums were centrifuged, stored in -80°C in tubes washed with aprotinin until analyzed.

Adropin and NO level measurement procedure

On the study day, after being melted in the room temperature, samples were analyzed in Fırat University Hospital Medical Biochemistry Laboratory using the ELISA procedure with ready-made commercial kits (Human Adropin AD ELISA Kit, EASTBIOPHARM CO LTD, Inc. Code:ck-e90267 and Human Nitric Oxide NO ELISA Kit, EASTBIOPHARM CO.LTD, Inc. Code: CK-E11333) per the instructions of the manufacturer. The reference ranges for adropin and nitric oxide were 5-1000ng/L and 2-600 μmol/L, respectively.

Statistical analysis

SPSS 20.0 package program was used for statistical analyses. Continuous variables were expressed as median (25-75 percentage) and standard deviation according to their distribution. Independent variables were compared with T-Test and Mann Whitney U Test, while Wilcoxon Test was used to compare dependent variables. The relationship between studied parameters was assessed with Pearson and Spearman correlation analysis. In power analysis, a sample size of 59 individuals, including 29 controls and 30 patients, had α=0.05, 1- β=0.85 and d=0.8. A *P*-value of <0.05 was considered statistically significant.

Results

Twenty-nine healthy pregnant women before the 20th gestational week with no complaints (Group 1) and thirty cases who were diagnosed with missed abortus for the first time (Group 2) were included in the study, with a total of fifty-nine volunteers. The missed abortus cases underwent dilation and curettage, and were followed up until their β-HCG levels fell below 5 ng/ml. In these patients, when β-HCG levels became negative, their physiology and metabolism is likely similar to those of non-pregnant women.

Demographic and obstetric attributes of groups

No significant differences were found between the groups in terms of age (*P*=0.92), gravida (*P*=0.14), parity (*P*=0.48), number of living children (*P*=0.20) and body mass index (*P*=0.06) (Table 1).

Laboratory parameters

The hemoglobin (*P*=0.72), hematocrit (*P*=0.26), thrombocyte (*P*=0.10), leucocyte (*P*=0.31), fasting blood sugar (*P*=0.20), ALT (*P*=0.11), AST (*P*=0.10), Urea (*P*=0.85), Creatinine (*P*=0.24), TSH (*P*=0.73), Free T4 (*P*=0.06), Free T3 (*P*=0.08), Prothrombin Time second (PTZsec) (*P*=0.32), Prothrombin Time INR (PTZinr) (*P*=0.66), Active Partial Thromboplastin Time (aPTT) (*P*=0.12) levels were similar between groups 1 and 2 (Table 2).

Table 1: Demographic and obstetric features of the groups

Variable	Healthy Pregnant (Group 1) (n=29)	Missed Abortus (Group 2) (n=30)	*P*-value
Age	27.9 (6.1)	27.8 (6.1)	0.92
Gravida	2.3 (1.6)	2.9 (1.8)	0.14
Parity	0.9 (1.2)	1.6 (1.3)	0.48
Number of living children	0.8 (0.9)	1.5 (1.1)	0.20
Body Mass Index	24 (3.5)	25.2 (4.5)	0.06

Median (standard deviation), Statistical significance P<0.05

Table 2: Biochemical features of healthy pregnant women and missed abortion cases

Variable	Healthy Pregnant (Group 1) (n=29)	Missed Abortus (Group 2) (n=30)	*P*-value
Leukocyte (10^3)	7.5 (2.1)	8.9 (2.5)	0.31
Hemoglobin (g/dL)	12.8 (1.3)	12.9 (1.1)	0.72
Hematocrit (%)	38.1 (3.5)	39.1 (3.4)	0.26
Thrombocyte (10^3)	253 (60.3)	285 (85.4)	0.10
ALT (U/L)	18.3 (8.9)	14 (8.8)	0.11
AST (U/L)	17.9 (3.4)	16.5 (3.3)	0.10
Glucose (mg/dL)	90.6 (16.2)	96.7 (19.6)	0.20
Urea (mg/dL)	18.4 (5.8)	18.7 (5.7)	0.85
Creatinine (mg/dL)	0.6 (0.1)	0.6 (0.1)	0.24
TSH (IU/mL)	2.1 (1.4)	1.6 (0.1)	0.73
Free T3 (ng/dL)	3.1 (0.9)	3.7 (1.4)	0.08
Free T4 (ng/dL)	0.1 (0.2)	0.9 (0.1)	0.06
PTZsec (sec)	12.1 (1.1)	12.4 (1.4)	0.32
PTZinr (sec)	0.1 (0.1)	0.1 (0.1)	0.66
aPTT (sec)	99.9 (5.9)	103.5 (10.8)	0.12

Median (standard deviation), Statistical significance P<0.05

Comparison of adropin and nitric oxide levels

Adropin (*P*=0.03) and NO (*P*=0.04) levels were significantly lower in missed abortus cases compared to healthy pregnant women (Table 3).

Comparison of adropin and NO levels among missed abortion cases, between patients whose β-HCG values did and did not fall below 5 ng/ml after termination

The adropin and NO levels of missed abortus patients were lower than those whose β-HCG levels fell below 5 ng/ml after termination. The difference between adropin levels was significant (p=0.02), while that between nitric oxide levels was not (p=0.38) (Table 4).

Table 3: Adropin and NO levels in healthy pregnant women and missed abortus cases

Variable	Healthy Pregnant (Group 1) (n=29)			Missed Abortus (Group 2) (n=30)			*P*- value
	Median	IQR 25%	IQR 75%	Median	IQR 25%	IQR 75%	
Adropin (ng/L)	71.1	53.2	160.4	61.8	35.1	79.3	0.03
NO (μmol/L)	84.1	59.5	225.7	60.6	41.8	86.7	0.04

IQR: Inter quartile range

Table 4: Adropin and NO levels in cases of missed abortion and missed abortion cases where β-HCG blood value fell below 5 ng/ml after termination.

Variable	Group 2 Missed abortion (n=30)			Group 2 Missed abortion cases β-HCG blood value falls below <5 ng/ml after termination (n=30)			*P*-value
	Median	IQR 25%	IQR 75%	Median	IQR 25%	IQR 75%	
Adropin (ng/L)	61.8	35.1	79.3	124.8	40.3	175.5	0.02
NO (μmol/L)	60.6	41.8	86.7	76.9	40.4	172.1	0.38

Discussion

Even though there is intrauterine fetal viability loss in missed abortus cases, just like the other abortus types, because of the lack of cervical dilatation, expired fetal material may not be completely expelled. This may lead to serious metabolic complications of the mother, including coagulation defects and bleeding. The most lethal complication is disseminated intravascular coagulation. The risk of DIC is directly associated with gestational age and the time passed after the death of the fetus. Today, numerous factors have been defined about the etiopathogenesis of missed abortus, but it is still researched as an up-to-date topic.

Adropin, a newly discovered peptide, is spotted in liver, brain, kidney, heart, pancreas, muscle, vascular endothelial cells, and human umbilical vein endothelial cells [4, 8, 9-11]. It plays a role in preserving endothelial function, vascular hemostasis, and neovascularization. Primarily starting with relaxing the smooth muscles, NO affects by increasing local blood flow, regulating systemic blood pressure, and preserving endothelium with its powerful vasodilator effect [6].

It is known that endothelial nitric oxide synthase (eNOS) enzyme plays a role in the maintenance of vascular physiology and placental vascularization [12]. Adropin upregulates the expression of eNOS (VEGFR2) with the -phosphatidylinositol3-kinase-AKt pathway, increasing endothelial NO production, thus supporting the endothelial function, and protecting perfusion and angiogenesis [4].

In a study on the effect of adropin on the endothelium with Balb/c mice by Lovren et al. [4], high proliferation in endothelial cells and conversion to capillary tube form were seen with exogenous administration of adropin. The results show that adropin has a protective and integrity-providing role in the endothelium. Again, in patients with Type 2 diabetes mellitus with known endothelial dysfunction, low levels of adropin were found, which showed a relationship between adropin and endothelial dysfunction [13]. In the study of Dong Lin et al., serum adropin levels were low in patients with hypertensive diseases that complicate pregnancy [5]. As is known, hypertension is related to endothelial dysfunction and placental vascular dysfunction is one of the causes of preterm labor [14]. Studies show that eNOS plays a pivotal role in placental vascular function [15]. Low levels of Adropin in the circulation and thus low NO production can cause placental dysfunction and preterm labor [11].

By the result of our study, it can be said that pregnancies end with missed abortus due to significantly low levels of adropin and NO, endothelial dysfunction, vascularization defects and perfusion defects secondary to vasoconstriction. The fact that adropin and NO levels are increased as β-HCG becomes negative after termination in missed abortus cases supports our idea. Low levels of adropin and NO in missed abortion cases may play a role in etiopathogenesis, but the significance in the difference of adropin levels show that adropin may be even more crucial in this mechanism compared to NO.

In a study by Paradisi et al. [16], compared to healthy pregnant women, serum NO concentrations were higher in the non-pregnant control and lower in the missed abortus group. The low NO levels detected in missed abortus have been reported cause vasoconstriction in the uterine vascular bed and abortus due to decidual platelet aggregation activation. Also, in our study, the NO levels were significantly lower in the missed abortus group compared to healthy pregnant women. These results are parallel with the studies in the literature.

Missed abortus is important in obstetrics practice due to its frequent occurrence. Although many factors have been defined in the etiopathogenesis, comprehensive studies are needed to figure out the yet unknown causes to prevent maternal and fetal losses. Contrary to the findings of our study, a significant increase in maternal adropin levels was detected in a study conducted in cases with severe intrauterine growth retardation. The authors explained this by the probability that fetal development may have occurred despite existing endothelial dysfunction, and the compensatory regulatory feedback mechanism to meet the energy need [17].

Limitations

The sparse sample size, self-reporting of maternal-paternal chromosomal disease history, and lack of exclusion of genetic and histopathologic investigation in abortus material after the termination are the factors that limit our study.

Conclusion

It was not fully revealed in our recent findings whether low levels of adropin and NO in the missed abortus group is associated with endothelial dysfunction, or a result of an unknown reason pathology endothelial dysfunction, vasospasm, insufficient perfusion. Further research is needed for elucidation.

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