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ORIGINAL ARTICLE

Changes of maternal ACTH and oxytocinase plasma concentrations during the first trimester of spontaneous abortion

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Abstract

INTRODUCTION: The labor at term finishes normal pregnancy. Both labor at term and first trimester spontaneous abortion are connected with increasing cytotoxic immune response within decidua. Th1 cytokines including IL-2 and INF-gamma are able to exert an effect on HPA axis and result in ACTH secretion. Oxytocinase serum level during pregnancy rises with the fetal devlopment and arrest of oxytocinase serum growth might indicate the its development impairment, what might result in spontaneous abortion.

MATERIAL AND METHODS: The study group consisted of 27 patients with clinical symptoms of missed abortion. A control group consisted of 89 pregnant women, who were successfully treated because of infertility. Immunoassay was used to measure ACTH plasma concentration. Oxytocinase plasma activity was established using l-cystine-di-β-naphthylamide as a substrate.

RESULTS: In the present study, significant increase in ACTH plasma concentration was observed during first trimester of spontaneous abortion. These patients were not characterized by significant increase of oxytocinase plasma level.

CONCLUSIONS: The observed ACTH rise during spontaneous abortion might be also related to the alterations at the maternal-fetal interface and the response of HPA axis to the growing cytotoxic activity.

Introduction

Normal implantation is enabled by the interaction between the embryo and various components of the decidua and require the synchronization between embryonic development and endometrial maturity. The impairment of these phenomenon during the early pregnancy might lead to spontaneous abortion [23]. Abortion is defined as a spontaneous loss of pregnancy before the fetus is sufficiently developed to survive outside the mother [1]. The development of diagnostic techniques, especially transvaginal ultrasound, have anticipated and increased the diagnosis of the first trimester non-viable pregnancies. This entity is thought to occur in 10–20% of clinically manifested pregnancies [28].

Notwithstanding the cause of the abortion, this process is connected with growing mothers' cytotoxic immune response. Genetic anomalies (50–60%), hormonal abnormalities as well as maternal factors such as antiphospholipid antibodies or uterine abnormalities as a cause of spontaneous abortion is linked with alterations among the number and activity of cytotoxic immune cells within decidua basalis and decidua parietalis [29].

Th1 cytokines including IL-2 and INF-gamma are able to exert an effect on HPA axis and result in ACTH secretion [33]. ACTH is produced during pregnancy by mother pituitary gland and by trophoblast cells. ACTH in not only the neuroendorcine hormone, but is also produced by immune cells (lymphocytes and macrophages), so ACTH receptor is detected on various immune cells [3,10,33,34]. ACTH appears to be a basic factor responsible for the bi-directional communication between neuroendocrine and immune system. Recently pronounced rise of ACTH was noticed beginning 28th week of pregnancy [13] until the labor. ACTH rise was observed during the labor and the decrease was seen just after the expulsion [25].

Oxytocinase (cystine amino peptidase – CAP) is a member of mammalian zinc containing the family of aminopeptidases [11,16,18,36]. In maternal serum a soluble form of CAP can be detected. It rises progressively during pregnancy until the labor [16–18,22,24]. Oxytocinase is basically secreted by syncytiotrophoblast cells [22]. The oxytocinase was also found in skeletal muscles, heart, decidua, Graffian follicle, brain and human umbilical vein endothelial cells, but the level of this enzyme was markedly lower than those found in pregnant women's serum [11,24]. Oxytocinase serum level during pregnancy rises with the fetal devlopment and arrest of oxytocinase serum growth might indicate the its development impairment, what might result in spontaneous abortion [16].

The aim of our study was to evaluate the concentration of plasma ACTH and oxitocinase activity in the first trimester spontaneous abortion in order to establish the role of these factors in the maternal-fetal homeostasis during early pregnancy.

Materials and methods

Human subject

27 women were recruited randomly from 95 patients referred with symptoms of threatened abortion (vaginal bleeding, lower abdominal pain) admitted to the Gynecology and Infertility Clinic of the Jagiellonian University Hospital, Krakow between March 2004 and December 2004. The presence of first-trimester early pregnancy was confirmed by transvaginal ultrsound examination and serum beta human chorionic gonadotropins (β-HCG) level (ranging from 565 to 11 700 U/l) at least twice.

Women with recurrent miscarriage (RM, defined as the occurrence of three or more clinically detectable pregnancy losses) [32], and blighted ovum, pregnancy following fertility treatments (ovulation induction, in vitro fertilization) were excluded from our consideration. Patients in our study were not treated by any hormonal therapy during the 6 months preceding the entry into the study. All obtained tissue samples were histopathologically verified using the classical hematoxylin and eosin staining technique after fixation in formalin and the presence of fetal tissue was confirmed. A control group consisted of 89 pregnant women, who were successfully treated because of infertility in Gynecology and Infertility Clinic and were observed for the whole course of pregnancy until the labor at term at the same time as the group of patients with spontaneous abortion. In all cases patients'consent was obtained. The approval for the research program from the Ethical Committee of the Jagiellonian University in Krakow: KBET/379/13/2003 was also granted.

Hormone assays

ACTH plasma concentration and oxytocinase serum level were established in 232 plasma samples. It was assessed in whole blood samples, collected approximetly at o'clock 9 in the morning in silicon-coated glass tubes containing EDTA as an anticoagulant, and were centrifuged immediately in a refrigerated centrifuge. All samples were frozen at –20 °C until the ACTH analysis was performed. Immunoassay was used to measure ACTH (Immulite 2000 ACTH, DPC Ltd – United States).

The CAP plasma activity was evaluated using Tuppy and Nesvadba method, modified by Klimek [15]. The assessment of oxytocinase in two pH levels using the same substrate (l-cystine-di-β-naphthylamide) results in obtaining two peeks of aminopeptidase's activity (CAP1 – pH 7.9; and CAP2 – pH 6.7). The detailed method of CAP estimation was described in previous studies [13–18].

Statistical analysis

Statistical calculations were performed using a Statistica computer program (StatSoft, Poland). The normal distribution of value of ACTH and CAP was checked by means of the Shapiro-Wilk test. Mann-Whitney U test was applied to compare the differences between parametric data. A value of $p<0.05$ was considered as significant

Results

The analysis of oxytocinase activity and ACTH concentration was performed in all patients included in the study during the early pregnancy. The gestation was every time diagnosed using transvaginal ultrasound examination (presence of normal gestational sac) and by elevated serum beta human chorionic gonadotropins, between 4th and 5th week from the last menstrual period. The second assessment was established during the presence of clinical symptoms of missed abortion: abdominal cramps and bleeding from vagina. These symptoms occurred naturally without interference. The diagnosis of missed abortion was established during the gynecological examination, transvaginal ultrasound examination, and the lack of beta human chorionic gonadotropins serum growth. Additionally, during these two periods of gestation the plasma concentration of ACTH and oxytocinase activity were assessed (*Table 1*).

A comparative analysis of ACTH plasma level and oxytocinase activity (CAP1, CAP2) during early pregnancy between the first trimester of spontaneous abortion and normal pregnancy (delivered at term) was per-

Table 1. ACTH and oxytocinase (CAP1 and CAP2) plasma concentrations during first trimester spontaneous abortion

Variables	Early pregnancy	Missed abortion	p- value
Average ACTH plasma concentration (pg/ml) (n=27)	11.18 (±4.88)	20.99 (±14.69)	0.035
Average CAP1 plasma level (μmol/l/min) (n=27)	0.604 (±0.20)	0.606 (±0.16)	NS
Average CAP1 plasma level (μmol/l/min) (n=27)	1.37 (±0.30)	1.41 (±0.26)	NS

n – number of patients, NS – no statically significant

Table 2. ACTH and oxytocinase (CAP1 and CAP2) plasma concentrations during normal pregnancy – control group.

Variables	4th and 5th week	6th and 10th week	p- value
Average ACTH plasma concentration (pg/ml) (n=89)	11.68 (±6.1)	10.84 (±4.4)	NS
Average CAP1 plasma level (μmol/l/min) (n=89)	0.62 (±0.18)	1.28 (±1.28)	0.001
Average CAP1 plasma level (μmol/l/min) (n=89)	1.36 (±0.25)	1.88 (±0.29)	0.001

n – number of patients, NS – no statically significant

formed. ACTH plasma level and oxytocinase activity were established respectively in two groups from control. The first group included women between 4th and 5th week of pregnancy and the second group comprised women between 6th and 10th week of pregnancy.

Discussion

In the present study, significant increase in ACTH plasma concentration was observed during first trimester of spontaneous abortion. These patients were not characterized by significant increase of oxytocinase plasma level.

Interactions between hormonal and immunological systems take place through HPA axis and play relevant role in reproduction. The oxytocinase serum level is directly related to the development of pregnancy. In our previous reports the arrested rise of oxytocinase preceded spontaneous abortions and preterm delivery [2,14,16,19,20,35]. In the current study no changes in oxytocinase level between 5th and 10th week of pregnancy in the group of women with spontaneous abortion were observed in comparison to the control group in which the oxytocinase serum level grew statistically significantly during the first trimester of pregnancy. This confirms the former observation, that oxytocianse is a marker of normal pregnancy development [16,18].

The labor at term finishes normal pregnancy. Both labor at term and first trimester spontaneous abortion are connected with increasing cytotoxic immune response within deciduas [5,6,34]. The highest ACTH level identified in pregnancy was noted during the labor [25]. In our previous study ACTH level was growing beginning with 28th week of gestation. ACTH plasma concentration was not associated with the progression of the labor [13]. Contrary to this observation Ochedalski et al. reported that maternal pituitary response to stress factors grew during delivery and the ACTH plasma concentration raised. The rise of ACTH serum level was higher in women with induced labor in comparison to labor with spontaneous beginning [21,25,31]. HPA axis is stimulated not only by stress, HPA axis seems to be the place where the exchange of information between hormonal and immunological system occur. ACTH appears to be a basic factor responsible for the bi-directional communication between neuroendocrine and immune system. The growth of Il-2, Il-1, Il-6 and INF-gamma might induce profound effects on hypothalamic pituitary axis [12,33,34]. Spontaneous abortion is related to the growth of cytotoxic activity of immune system [29]. The growth of dNK cells was observed in spontaneous abortion. Cytotoxic T cells are activated by Th1 cells and cause miscarriage [8]. Also the drop of CD4+CD25+ receptors was observed during commenced spontaneous abortion in comparison to normal pregnancy [9,30]. CD4+CD25+ lymphocytes strongly suppress cytotoxic cells. IL-6 is the cytokine able to inhibit CD4+CD25+ cells, the concentration of IL-6 rises during the proliferation of trophoblast [27]. It was shown that IL-6 together with IL-4 and IL-7 induced hCG release [7]. IL-6 influences the HPA axis.

The homeostasis of maternal-fetal interface which determines the normal development of pregnancy is maintained by the interactions of cytokines. The ACTH level rise in our study was observed in the group of patients with spontaneous abortion in comparison to the control group. The alterations of other pituitary hormones were reported within decidua in patients with recurrent miscarriages. The lack of expression of endometrial prolactin concentration within decidua during the 'implantation window' was reported in cases of repeated miscarriages [4]. The ACTH rise dur-

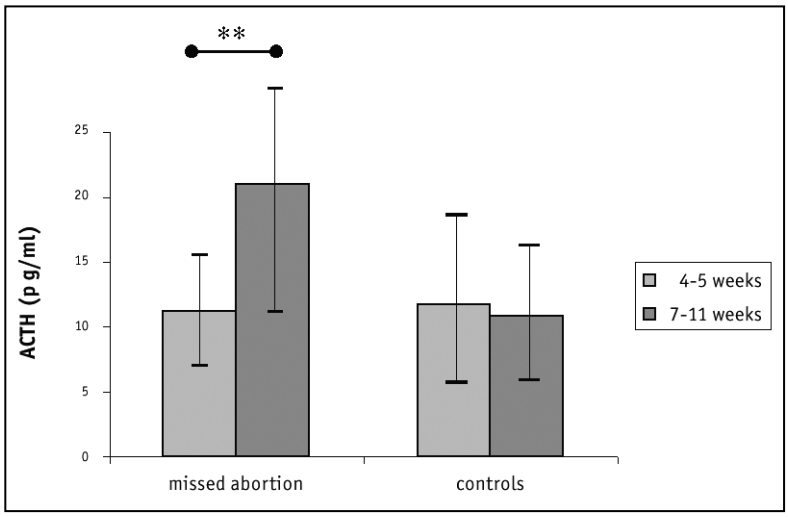

Figure 1. The comparative analysis of ACTH plasma concentration in the first trimester spontaneous abortion and in early normal pregnancy, delivered at term (** p=0.035)..

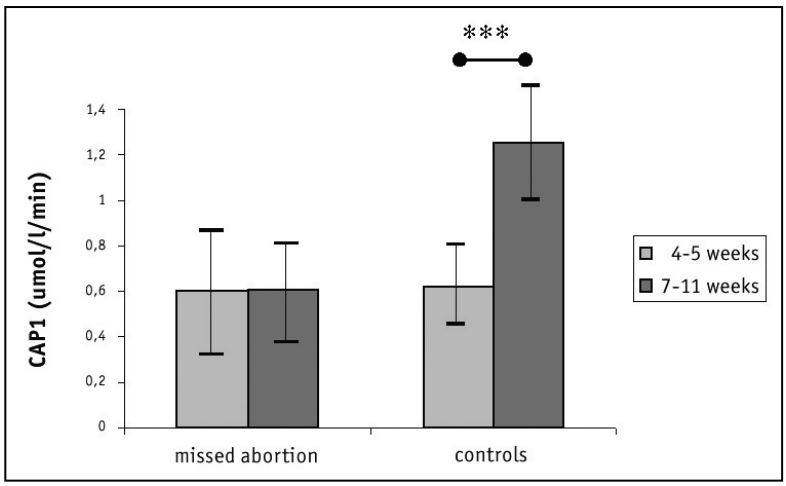

Figure 2. The lack of the rise of CAP1 plasma level in the first trimester of spontaneous abortion in comparison to a significant growth of CAP1 serum level in normal early pregnancy delivered at term (*** p=0.001)

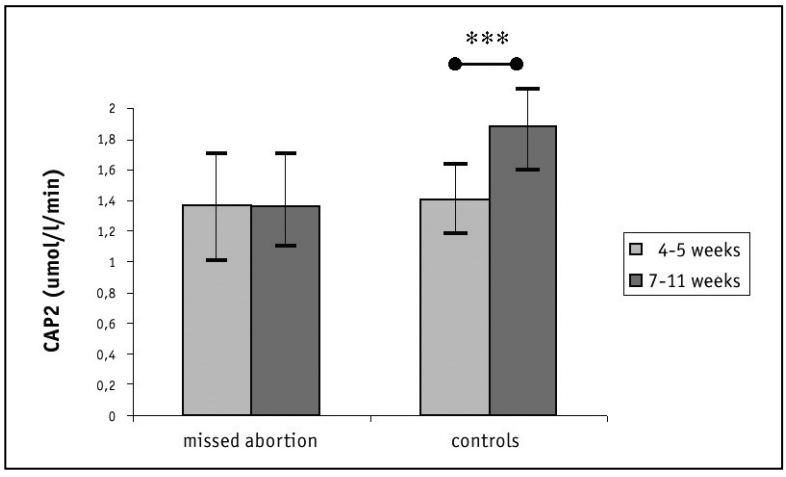

Figure 3. The lack of CAP2 plasma level in the first trimester of spontaneous abortion in comparison to a significant growth of CAP1 serum level in normal early pregnancy delivered at term (*** p=0.001).

ing spontaneous abortion was accompanied by growing cytotoxic activity and alterations in interaction between trophoblast and decidua.

Conclusions

The observed ACTH rise during spontaneous abortion might be also related to the alterations at the maternal-fetal interface and the response of HPA axis to the growing cytotoxic activity.

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