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ORIGINAL ARTICLE

Comparative analysis of ACTH and oxytocinase plasma concentration during pregnancy

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Abstract

INTRODUCTION: Normal function of the hypotalamo-adrenal axis is important for the regulation of feto-maternal intrauterine homeostasis including immunomodulating activity and may influence the timing of parturition. ACTH is produced during pregnancy by mother pituitary gland and by trophoblast cells, which are the place of oxytocinase production. Oxytocinase is basically secreted by syncytiotrophoblast cells and rises progressively during pregnancy until the labor. Oxytocinase may play a role as one of the main factors suppressing uterine contractions, controlling the vascular resistance and the volume of the retroplacental blood pool.

MATERIAL AND METHODS: The study group consisted of 102 patients with pregnancy following infertility treatment. The assessment was provided longitudinally in six selected groups progressively according to the gestational age. Immunoassay was used to measure ACTH plasma concentration. Oxytocinase plasma activity was established using l-cystine-di-β-naphthylamide as a substrate.

RESULTS: Significantly increased oxytocinase plasma level was identified during pregnancy, progressive rise was observed beginning with the first trimester of pregnancy, until the labor. ACTH plasma concentration was observed to be at comparable level in the first and second trimester of pregnancy, while sudden statistically significant increase of ACTH plasma level was noted beginning with 28th week of gestation.

CONCLUSIONS: The assessment of ACTH and oxytocinase plasma concentration might be useful for the evaluation of pregnancy development.

Introduction

Epilepsy is a common disease. In the USA abThe efficacy of hormonal therapy treatment depends on the metabolism of hormones and their effectors status, and is represented intermediately by their blood serum level. Each hormone is decomposed by enzymes controlled by metabolic pathways. In sum, enzymes are responsible for hormones effectiveness. The alteration of enzyme level influences the concentration of hormone, the effect depends on the hormone-enzyme and their metabolic environment balance. This phenomenon is demonstrated by, for example, the oxytocinase-oxytocin system. The level of biologically active oxytocin is regulated by oxytocinase activity (serum aminopeptidase activity) [18]. Oxytocinase may be also involved in gonadotropin releasing hormone (GnRH) degradation. Hypothalamus is responsible for the regulation of neuro-endocrine system through hypota-

lamo-adrenal axis (HPA) and hypothalamo-gonadal axis. The former secretes ACTH in response to stress stimuli, and the latter, regulates the fertility. In both of them steroid hormones are finally produced. ACTH is produced during pregnancy by mother pituitary gland and by trophoblast cells.

Normal function of the HPA axis is important for the regulation of feto-maternal intrauterine homestasis. It may influence the timing of parturition. The source of CRH growth during pregnancy are both pituitary cells and placental cells. Although it was previously suggested that the pregnancy ACTH growth results from CRH secretion, no correlation between CRH levels and answer of maternal as well as fetal HPA axis was identified [9,30,32]. The ACTH and CRH presence in placental microenvironment is directly involved in progesterone regulation in trophoblast cells [12]. Since the blockade of progesterone is one of the mechanism possibly involved in initiation of labor, the labor results from the immune tolerance change. The ACTH and CRH might influence the immune regulation during pregnancy. Progesterone, necessary for normal pregnancy development accumulates during pregnancy and generally stimulates the conversion of Th0 to Th2 cells [33]. 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 [8,13]. ACTH appears to be a basic factor responsible for the bi-directional communication between neuroendocrine and immune system.

Oxytocinase (cystine amino peptidase – CAP), which was originally recognized during pregnancy in blood serum as an enzymatic activity capable of cleaving synthetic substrates such as L-cystine-di-β-naphthylamide, is a member of mammalian zinc containing the family of aminopeptidases [17,18,27]. In maternal serum a soluble form of CAP can be detected due to the deletion of cytoplasmic and hydrophobic transmembrane domains of native form. Oxytocinase is basically secreted by syncytiotrophoblast cells and rises progressively during pregnancy until the labor [14,29]. The membrane form of oxytocinase was identified also in skeletal muscles, heart, decidua, Graffian follicle, brain and human umbilical vein endothelial cells [38]. CAP is responsible for degradation of the following peptide hormones: vasopresin (AVP), oxytocin (OT), angiotensin III (ANG III) and it may regulate the level of these peptide hormones in fetal and maternal serum. This enzyme may play a role as one of the main factors suppressing uterine contraction, controlling the vascular resistance and the volume of the retroplacental blood pool.

The aim of our study was to evaluate the concentration of blood ACTH and oxitocinase serum activity in order to establish the role of these factors in the materno-fetal homeostasis during pregnancy.

Materials and Methods

Group of patients

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. The 102 patients in this study were randomly selected from 250 women treated because of infertility at the Gynecology and Infertility Department of the Jagiellonian University between March 2003 and December 2004. The mean age of all patients was 30.3 (± 4.6) years, the average gestational age during the labor was 38.4 (± 2.0) weeks, average newborns birth weight 2890.3 (± 468 g), average newborns length 52.5cm (± 3.7), Apgar 9.6 (± 0.8), K- index of newborns maturity 11.5 (± 1.0) and parity 1.9 (± 1.2). The patients were observed for the whole course of pregnancy. The assessment was provided longitudinally six times during the gestation in following groups: group I – pregnant women between 7th and 9th week of gestation, group II – between 15th and 17th week of pregnancy, group III – between 18th and 20th , group IV – between 28th and 30th , group V – between 32nd and 33rd and group VI – between 36th and 37th weeks of gestation.

Hormone assays

ACTH concentration and oxytocinase serum level were established totally in 612 plasma samples. It was assessed in whole blood samples, collected approximetly 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 [17]. 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 [16,22,24].

Statistical analysis

Statistical calculations were performed using 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 CAP1 and CAP 2 serum level and ACTH concentration was performed in a group of 102 pregnant women. The assessment was provided longitudinally in all selected groups (from group I to group VI) (*Table 1.*)

Table 1: ACTH and oxytocinase (CAP1 and CAP2) concentration according to progressive rise of gestational age of pregnant women.

Groups (gestational age)	ACTH (pg/ml) serum level +SD (range)	CAP1 (µmol/l/min) serum level +SD (range)	CAP2 (µmol/l/min) serum level +SD (range)
Group I – 7÷9 weeks	12.1 ± 5.8	0.59 ± 0.2*	1.35 ± 0.3#
(n=102)	(5 ÷ 2.4)	(0.3 ÷ 1.7)	(0.1 ÷ 2.3)
Group II – 15÷17 weeks	11.3 ± 4.8	1.21 ± 0.5*	1.8 ± 0.4#
(n=102)	(5 ÷ 23.7)	(0.4 ÷ 3.4)	(1.1 ÷ 3.4)
Group III – 18÷20 weeks	11.8 ± 5.0	1.61 ± 0.7*	2.11 ± 0.5#
(n=102)	(4.9 ÷ 29.3)	(0.4 ÷ 5.0)	(1.1 ÷ 4.5)
Group IV – 28÷30 weeks	15.1 ± 5.3	3.41 ± 1.4*	3.45 ± 1.6#
(n=102)	(5.4 ÷ 29.3)	(1.3 ÷ 7.9)	(2.0 ÷ 6.5)
Group V – 32÷33 weeks	19.3 ± 8.9	4.9 ± 2.5*	4.5 ± 1.9#
(n=102)	(5.4 ÷ 51)	(1.3 ÷ 14)	(2 ÷12.6)
Group VI – 36÷37 weeks	22.9 ± 1.0	7.5 ± 2.3*	6.2 ± 1.8#
(n=102)	(5.4 ÷ 52.3)	(3.2 ÷ 14.9)	(3.4 ÷12.6)

The differences statistically significant p<0.01; n-number of patients
* The differences statistically significant p<0.01; n-number of patients

High statistically significant correlation was found between the CAP1 and CAP2 serum levels (r=0.95, p=0.0001). No correlation was observed between ACTH serum concentration and the levels of CAP1 and CAP2, respectively r=0.18 and r=0.11). Enzymes levels rose progressively with the gestational age and the differences between each groups were statistically significant (p<0.0001).

The level of ACTH was at comparable levels in I, II, III groups (gestational age between 7th and 20th weeks of pregnancy). Beginning with 28th week of gestation ACTH concentration started to rise, the difference between 7th–20th (groups I, II, III) and 28th week of gestation (group IV) was statistically significant (p=0.001). Progressive increase of ACTH maintained until the labor, and the difference between group IV and VI was statistically significant (p=0.001), while the growth of ACTH serum level between 30rd and 33th weeks of gestation was not statistically significant, however the tendency of ACTH rise was observed.

The comparative analysis between ACTH and oxytocinase (CAP1 and CAP2) serum levels before and after 28th week of gestation was also performed. *Figure 1 and 2* demonstrate the results of ACTH and CAP1 and CAP2 levels comparison.

Discussion

In the present study, significantly increased oxytocinase level was identified during pregnancy, with a progressive growth from early pregnancy until the labor. ACTH plasma concentration was observed to be at comparable level in the first and second trimesters of pregnancy, while sudden statistically significant increase of ACTH plasma level was noted beginning 28th week of gestation.

Maternal pituitary ACTH secretion and plasma ACTH levels rise during pregnancy. Additional secretion of ACTH from placenta takes place during pregnancy. Maximum plasma concentration of ACTH was

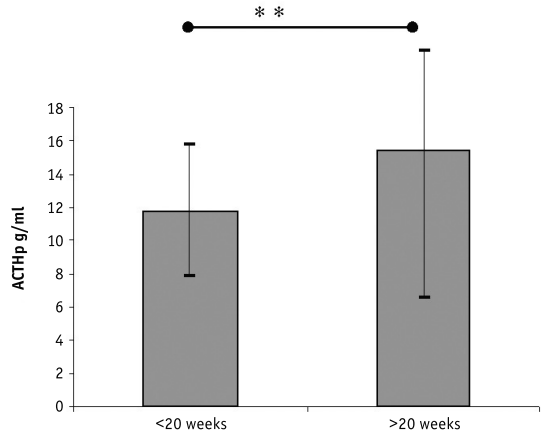

Figure 1. Presents the ACTH plasma concentration changes according to growth of gestational age (**p<0.01)

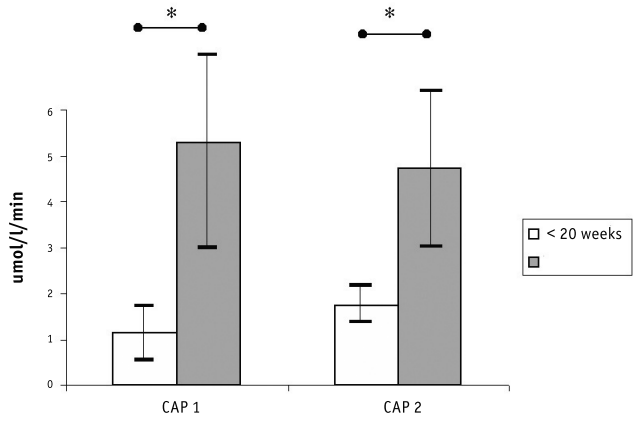

Figure 2. Demonstrates the changes of oxytocinase serum activity during pregnancy (*p<0.001).

reported in 34th week of gestation with a significant fall in late pregnancy [26]. Our study does not confirm this observation, since the ACTH plasma concentration starts to increase beginning 28th week of gestation and progressively rise until the labor. Similarly to our study, progressively the growing ACTH level with the development of gestation was shown by Jeschke et al [12]. The highest ACTH level identified in pregnancy was noted during the labor [30,31]. ACTH plasma concentration was not associated with the progression of the labor, although concomitant increase of Cortisol serum level was observed [2]. No differences were noticed among ACTH plasma concentrations between patients receiving or not receiving mifepristone therapy during labor at term [2]. ACTH seems to modulate the endocrine function of throphoblast cells downregulating progesteron production in vitro [12].

The pituitary gland responds to stressful stimuli by rapidly increasing the secretion of adrenocorticotropin, which in turn increases the secretion of cortisol. Since placental clock was suggested to determine the length of gestation, maternal plasma level might be used as an indicator of these events increasing until the labor (cortisol, ACTH, CRH) [4]. To confirm the link between increasing labor stress and the growing ACTH serum level standardized CPT (cold pressor test) was performed in two groups of pregnant women, fearful and fearless ones. Women with fear of labor were characterized by reduced pain tolerance both before and after labor. ACTH, cortisol and beta endorphin did not differ between both groups, however ACTH baseline levels during pregnancy were significantly higher than those after pregnancy in both groups (with or without fear of labor) [34]. 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 the ACTH serum level was higher in women with induced labor in comparison to labor with spontaneous beginning. The ACTH level observed during pregnancy seems to be independent from CRH increasing concentration because ACTH levels are during pregnancy within the range of no pregnant subject, despite extremely elevated maternal CRH. [30,31].

The highest concentration of ACTH observed in induced labor receiving oxytocin treatment might result from the modulation of anterior pituitary secretion by oxytocin as a central maternal regulation of the maternal hypothalamo pituitary axis [30]. The differences of ACTH maternal plasma level observed in our study between 28th and 32nd week of gestation might indicate that labor associated stress but also other factors seem to be responsible for the ACTH increase. The rise in the ACTH level observed immediately before normal time of the onset of labor might result from immunological activity changes necessary to initiation of spontaneous labor at term. The neuroendocrine hormone ACTH alters immune responses and may be a factor in mediating stress-induced immunomodulation [35]. Wermerskirchen et al. reports that ACTH modulates in vitro IL-2 secretion from activated splenic lymphocytes (Cytotoxic T Lymphocytes Leukemia cells CTLL-2) and indicates two-directional action (suppression and activation) according to the concentration changes [36]. Serum Il-2 receptor level was also reported to rise during pregnancy [10]. It was also noticed that ACTH modulates macrophages function [8], and macrophages changes in decidua were identified during spontaneous labor (Sindram 2004). HPA axis is also involved in the pathogenesis of treatment-resistant depression, in which this patient presents disturbance of the activation of lymphocytes function [25]. Immunomodulating activity is also characteristic for oxitocinase [27]. Statistically significantly higher oxytocinase serum level was found a few days before induced labor in comparison to spontaneous labor in previous studies [21,37]. Spontaneous labor requires the termination of maternal immunological tolerance to fetal antigens, and the activation of cytotoxic immune response [11,37]. The ACTH growing level seems to result from these changes in maternal immune response.

Preeclampsia seems to be secondary to anomaly of the the uterine spiral arteries invasions by cytotrophoblast cells and finnaly results in hypertension [1,28]. One of biological effects of oxytocinase activity is the regulation of placental blood flow, what was confirmed by simultaneous assessment of CAP serum level and Doppler ultrasound examination [16,22]. In our previous research CAP1 and CAP2 serum decreased levels in patients with preeclampsia were confirmed [6,21]. Among other vasomotor factors like NO, ET-1 (endothelin-1), CAP1 seems to appear as an important factor regulating the placental blood flow. Elevated ACTH level was identified in the umbilical artery in pregnancies complicated by preeclampsia or IUGR [5,28].

In conclusion, the assessment of ACTH and CAP might be useful for the evaluation of pregnancy development.

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