Original Article

A COMPARISON OF CYSTEINE PEPTIDASE ACTIVITY AND THEIR INHIBITORS IN THE BLOOD SERUM OF PREGNANT WOMEN

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ABSTRACT

Aim: To determine the activity of enzymes cysteine peptidases, cathepsin B and L and their inhibitors in pregnant women in their first, second and third trimesters of pregnancy as compared to the control group of matchable non pregnant women.

Setting: Dept. of Obstetrics & Gynaecology, Regional Hospital, Legniea and First Dept. of Obstetrics & Gynaecology, Medical Academy, Wroclaw.

Patients & Methods: Forty two pregnant women (14 each) in first, second and third trimester and 14 healthy non-pregnant women in contrast group.

Results: Enzyme and their inhibitors specific activities gradually increased from the beginning to the end of the third trimester of pregnancy [for cathepsins B and L, and their inhibitors (2.7 and 2.1 folds)], respectively. The increase in activity was statistically significant in comparison to controls ($p \leq 0.0001$). The maximum increase was found in the third trimester before delivery.

Conclusion: Differences in the activities of cysteine peptidases and their inhibitors have been found in the sera of healthy pregnant women during pregnancy in comparison to non-pregnant women as a control.

KEY WORDS: Pregnancy, cysteine peptidases, cysteine protease inhibitors.

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INTRODUCTION

Many research studies have suggested a similarity between the changes occurring in the activities of proteolytic enzymes taking place during pregnancy development and the activities of these enzymes accompanying tumor development processes[1,2]. Researches in which such comparisons were made referred to the terminology trophoblast as a pseudo cancer tissue which as was demonstrated was associated with its invasion into the deeper layers of the decidual tissues. It was also shown that the implantation of the trophoblast in the decidua and the changes in development of the placenta (the trophoblast) controls the activity of the biological changes during the development of the fetus. One of the factors initiating the reaction of the trophoblast in decidual tissues is proteolytic enzymes[3]. The reactions are similar to the processes accompanying the invasion of host tissue by cancer cells[4,5]. The results of the research carried out by Nakashima and associates showed that the most intensive extra cellular secretion of proteolytic enzymes were observed between the 8th and 10th day

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of conception as well as a few days before delivery. These changes were probably associated with the development of the trophoblast invasion which occurs in the first trimester of pregnancy and the degradation of the placenta[3]. The phenomenon involving the increase of peptidase activity during pregnancy and their limited area of action leads to an assumption that the phenomenon is associated with the appearance of inhibitors, which limits the destructive actions of these peptidases[6]. In this research work, we are therefore presenting results of the cysteine peptidases activity as well as their inhibitors in the serum of pregnant women at different stages of the pregnancy as compared to the results of the serum enzyme values of non pregnant women who served as control.

MATERIALS AND METHODS

Biological materials:

One ml of serum was collected from pregnant women in the department of Gynecology and Obstetrics of the Regional Hospital in Legnica and in the First Department of Gynecology and Obstetrics of the Medical Academy in Wroclaw, the year 1999 and 2000.

Samples of blood serum were collected from patients who were earlier divided into groups comprising of 14 each at various stage of gestation. The samples were frozen at -20°C. With respect to the stage of pregnancy development the groups are:

1) Women in the first trimester of pregnancy (14 women).
2) Women in the second trimester of pregnancy (14 women).
3) Women in the third trimester of pregnancy (14 women).
4) Control group-healthy non pregnant women (14 women).

Determination of cathepsin B and L activities:

Cathepsin B activity was measured according to Barrett et al[7]. Fluorescence was measured in a Luminescence Spectrometer, Perkin Elmer LS 50 B at 370 nm excitation and 440 nm emission wavelengths using fluorescent substrate Z-Aeg-Arg-AMC for cathepsin B and Z-Phe-Arg-AMC for cathepsin L. Fluorescence readings of the sample assays were standardized with the reaction product 7-AMC (7-amino-4-methylcoumarin)[8]. 1 mEU of activity was defined as the quantity releasing 1 nM of 7-AMC.

Inhibitory activity against papain and cathepsin B and L:

This inhibitory activity was referred to as total cysteine peptidase inhibitor activity (CPI), the method described by Heidtman et al[9]. One inhibitory unit against papain or cathepsin B and L represents the amount of the inhibitor that totally inhibits one activity unit of papain or cathepsin B and L in the assay. This amount is determined by extrapolation of the titration curve to zero papain and cathepsin B and L activities.

Inactivatio of a-macrogloblin:

a-macroglobulin (α-M) was inactivated by incubation at 37°C and pH 7.5 for 2 h. and the activity of CPI was determined in temperature CPI^{80} and $CPI^{37.10}$ $\triangle$CPI: Difference between CPI^{80} and $CPI^{37,}$ the results were presented as complex (latent) forms of inhibitors $\triangle DCPI = CPI^{80} - CPI^{37}$

Statistical analysis:

The data were expressed as the mean values ± SD. Walloon's rank test were used. The 0.05 level of probability was assumed as significant.

RESULTS

The results of cysteine peptidases, cathepsins B and L activities and their inhibitors are presented in (Fig 1-A). It was found that the cathepsins B and L and their inhibitors activities increased statistically significant in cases of pregnancy than the controls ($p \leq 0.0001$). The cysteine peptidase activity was in the sera of women in the first trimester of pregnancy 15.8 to 27.7 mEU / ml in the third trimester in comparison to controls (10.4 mEU / ml). The activity of cysteine peptidase was increased 2.7 folds in comparison to controls. The activity of cysteine protease inhibitors was also increased in the first trimester of pregnancy from 82, 7 to 147.0 mEU / ml in the third trimester of pregnancy in comparison to controls (69.2 mEU /

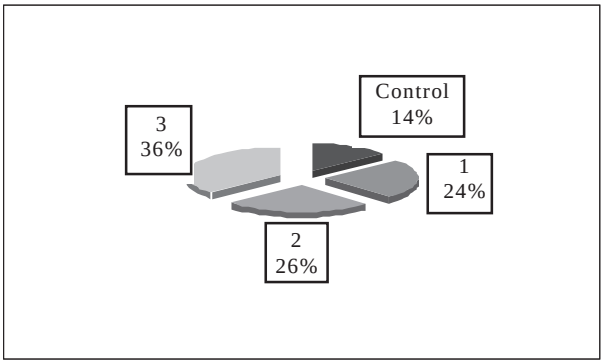
ml sera). The activity of the endogenous inhibitors was increased 2.1 folds before the delivery (Fig 1-B). Fig 2 shows that the complex form CPI was decreased from 40.2 mEU/ ml in the sera of women in the first trimester of pregnancy to 20.7 mEU/ ml sera in the third trimester in comparison to controls ($p \leq 0.0001$), thus, The CPI and complex form decreased 2.0-fold in the women sera during the various duration of pregnancy. The △CPI complex form significantly increased after the first trimester of pregnancy ($p<0.0001$) in comparison to third trimester of pregnancy (Fig 2, B and D).

DISCUSSION

It was observed that proteolytic enzymes especially metalloproteinase activators of plasminogen or cysteine end peptidases play a very important role in the interaction between the developing placenta and the decidua[1,2]. From the information obtained so far, it becomes clear that the development of the placenta is

Fig.1: Cysteine peptidases (cathepsins B and L) & their inhibitors activities in women sera during pregnancy developments
1. Women in the first trimester of pregnancy
2. Women in the second trimester of pregnancy
3. Women in the third trimester of pregnancy
4. Control group (this group comprises of serum collected from women who were healthy, non pregnant, whose blood samples were collected for routine tests).

A) Cysteine peptidases activity



B) Cysteine protease inhibitors activity

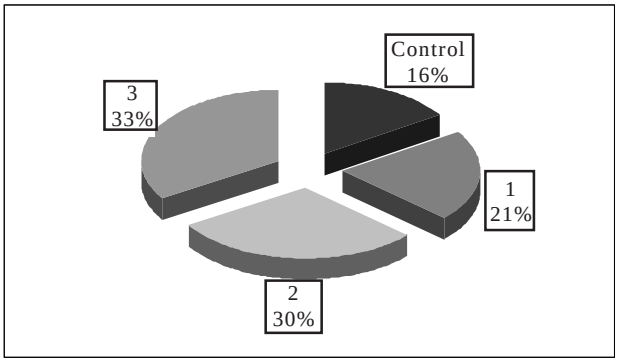
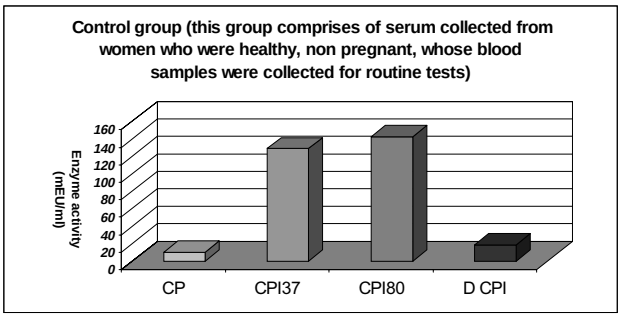
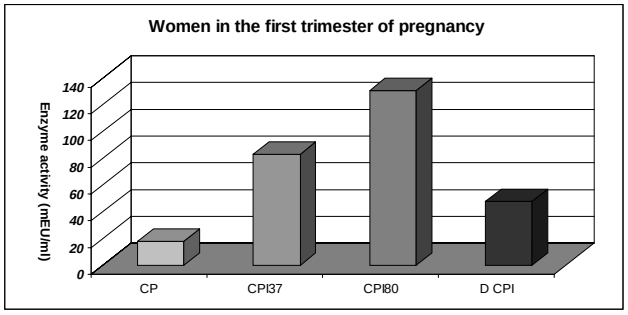


Fig. 2: Activity of cysteine peptidases and their inhibitors in the blood serum of pregnant women

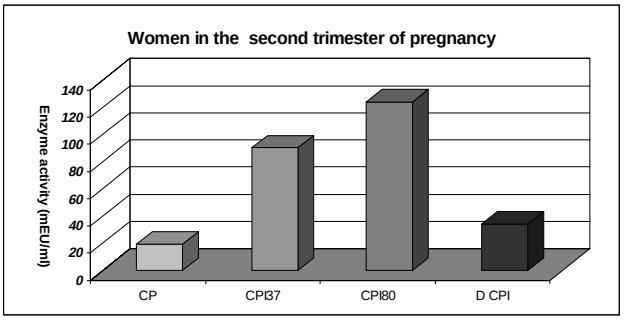
[A]



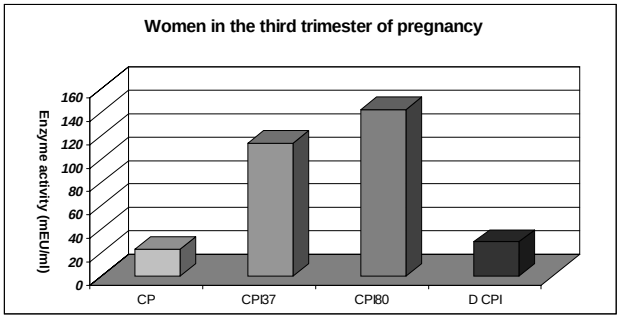
[B]



[C]



[D]



accompanied by an intensive increase in specific proteolytic enzymes whose activities are controlled by specific autogenic inhibitors. In pregnant women, they are released by the placenta in which active biologic substances controlling biologic processes. It was noticed that these processes are controlled by specific proteins produced in the placenta[11,12]. Cysteine peptidase activity and its inhibitors in the serum of pregnant women were increased in pregnancy in comparison to controls ($p \leq 0.0001$). Sol-Church et al.[13] showed that in human placenta, critical functions of the placental expressed cathepsins (PECs) are probably performed by broader specificity proteases such as cathepsins B and L. Divya et al.[14] observed the specific activity of cathepsin L in first trimester placenta to be significantly higher as compared to the term placenta. Gao et al.[15] show that amniotic fluid enhanced cathepsin B (19 micrograms protein; $p < 0.01$). Proteinases are found inside cells in normal physiological conditions, playing a variety of digestive and processing roles to maintain the normal cellular metabolism[16]. The main *in vivo* (during gestation) function of lysosomal cysteine proteinases is the degradation of proteins. Proteins are degraded in lysosomes non-selectively, and the resulting endproducts, dipeptides and amino acid diffuse through the lysosomal membrane and are re-used in protein biosynthesis.

CONCLUSION

We observed that the activities of enzymes were increased significantly in parallel during the pregnancy progression to regulate the expression of cysteine peptidases and the activities of enzymes regulated to normal after delivery, while in the cancer invasion cells the inhibitors can not regulate the over expression of the cysteine peptidases levels[17,18]. We thought that there are different mechanisms occurring in case of pregnancy in comparison with cancer cells.

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