CYSTEINE PROTEASE INHIBITOR LEVEL IN PREGNANCY AND PREGNANCY ASSOCIATED DISORDERS

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SUMMARY

In the present study total cysteine protease inhibitor levels (CPI) have been estimated in the maternal blood during normal and abnormal pregnancy. We observed that during normal pregnancy the CPI level increases as the pregnancy advances. However, in cases of placenta previa, accidental haemorrhage, pre-eclamptic toxaemia and threatened abortion the CPI levels decreased ($P<0.05$) and increases in case of ecalmpsia ($P<0.05$) and puerperium as compared to their corresponding period of the normal pregnancy.

INTRODUCTION

Proteolytic processes in living systems are under biological control at the level of protein synthesis or mediated through protein inhibitors. The cysteine protease inhibitor (CPI) levels in health and diseases is poorly understood. Earlier reported an inhibitor of cysteine protease such as ficin, papain and cathepsin B, H and L which occur in serum, urine and other body fluids (Barrett and Salvesen 1986; Sasaki et al 1977; 1981; Assfalg et al. 1992).

It is known that pregnancy is associated with substantial alterations in the concentration of total protein and of specific proteins (Minakata et al, 1983). The concentrations of ceruloplasmin, transferrin and fibringogen increases while that of albumin, haptoglobin and

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immunoglobulin decreases (Minakata et al. 1983; Song et al. 1970). As regard to protease inhibitors the levels of d_1 protease inhibitor increases and d_2 M remains unchanged in pregnant women (Minakata et al. 1983). The serum d-cysteine protease inhibitor (d_1 CPI) levels in female patients were not significantly different from the healthy women (Minakata et al. 1983). Although there are several reports on dCPI and d_2 M in different trimesters of pregnancy (Adam et al. 1985; Barrett and Salvesen 1986; Minakata et al. 1983), but to our knowledge still there are no reports of CPI level in pregnancy associated diseases. In this study we examined the CPI level in pregnant women associated with placenta previa, accidental haemorrhage, pre-eclamptic toxaemia, threatened abortion or eclampsia and compared with that of normal pregnant women.

MATERIALS AND METHODS

Samples

Blood was collected from 50 pregnant women associated with various diseases aged 16 to 42 years, 45 healthy pregnant women aged 20 - 40 years and 15 healthy non-pregnant women from out-patients of the Department of Obstetrics and Gynaecology of J.N. Medical College, Aligarh Muslim University, Aligarh, India. Collected sera were stored at -20°C until used.

Inhibitor assay

The assay of cysteine protease inhibitor activity was followed as described by Udaka and Hayashi (1965). Briefly, an equal volume of activating agent (0.05 M cysteine and 0.02 M EDTA, pH 8.0) was added to 0.2 ml (40 ug) of papain (SD. Fine Chem., India) and incubated for 10 min at 37°C. To the reaction mixture 0.2 ml with 10 mM phosphate buffer, pH 8.0 and incubated further for 30 min at 37°C. Then added 1.0 ml of 2% (w/v) casein (Difco Lab. USA) and incubated for 30 min at 37°C. Reaction was stopped by the addition of 1.0 ml of 10% (w/v) trichloroacetic acid and centrifuged after 15 min at 300xg for 10 min. In the filtrate the protein concentration was determined by Lowry's method (Lowry et al. 1951). The inhibition levels were expressed as CPI unit/ml of serum. Student's test was performed to analyse the level of statistical significance of the difference of these value.

RESULTS AND DISCUSSION

The CPI levels of 15 healthy women showing an average value of 0.489 (SD+0.016) units/ml was considered control value. A comparison of this value with the three trimesters of pregnancy shows that the level of the inhibitor increases gradually with increase in the gestation period. In the third trimester the CPI level reaches the mean value of 0.756 units/ml which was 54% higher than the control value (Fig. 1).

In case of pregnant women associated with placenta previa, accidental haemorrhage, pre-eclamptic toxaemia or threatened abortion the CPI level was significantly low ($P<0.05$) however, it was significantly high ($P<0.05$) in case of eclampsia as compared to the normal pregnant women (Table I).

TABLE I
CPI LEVELS IN PREGNANCY ASSOCIATED DISORDERS (Unit/ml).

Weeks of gestation	Placenta previa	Accidental haemorrhage	Pre-eclamptic toxaemia	Threatened abortion	Eclampsia
6		-	-	0 095	-
10	-	-		0.143	-
12		-	-	0.095	-
				0.162	
				0.171	
				0.171	
18	-	-	-	0.095	
				0.100	
20		-	-	0.095	-
				0.120	
26		-	0.467	-	-
28	-	-	0.475	-	0 800
0.867					
30	0.467	0.467	0.500	-	0.733
	0.500				
	0.525		0.665		
31		0.593	-	-	-
		0.667			
32	0.380		0.593	-	-
	0.500				
34	0 520	-	-	-	0.760
					0 847
					0.927
36	0.580	0.500	0.595	-	0.855
		0.515			
		0.620			
37	0.613	-	0.500	-	
			0.595		
38	0.573	0.573	0.573	-	0.847
40	0 547	0.500	0.620	-	0.900
		0.495			0.927
		0.590			
Mean	0.520	0.552	0.558	0.124	0.846
SD ±	0.065	0.065	0.067	0.033	0.065

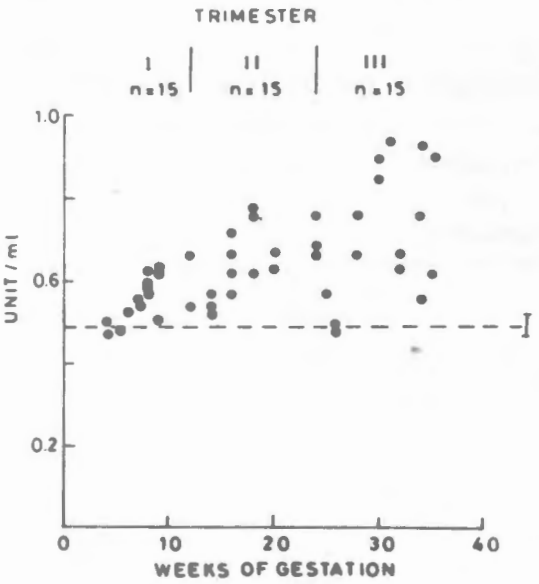

Fig. 1. CPI levels changing with advancement of weeks of gestation in normal pregnancy. The CPI level of normal control women is shown by broken line and the bar represents the standard deviation.

Our observation that the CPI level in normal pregnant women increases with advancement of gestation period is consistent with findings of Adam et al. (1985), Minakata et al. (1983). Therefore, suggests that this inhibitor is physiologically essential in normal pregnancy. In pregnant women associated with placenta previa, accidental haemorrhage, pre-eclamptic toxaemia or threatened abortion we observed decreased level of CPI. This may be due to either a failure of the physiological mechanisms which leads to decrease in the inhibitor level or inhibitors becoming the substrate for the enzyme that it inhibits (Carrell and Travis 1985). In eclampsia the higher level of CPI observed may be due to the excess production of the inhibitor leading to inbalance of the protease inhibitor ratio. However, Minakata et al. (1983) reported that the CPI levels in patients with myoma of uterus, endometritis, cervical cancer, ovarian cyst and ovarian cancer were not significantly different from the normal healthy control men.

The exact role of this inhibitor is unknown so far (Tokaji 1971; Hayashi 1962; Minakata et al. 1983). Probably, the increase in CPI level noticed in normal pregnancy may be due to the protective mechanisms against some harmful proteases secreted in pregnancy or against microbial proteases produced by infection due to lowering of immunoglobulin in pregnancy (Song et al. 1970).

In the present study, we confirm that increase of CPI level is essential for mother and foetus.

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