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A study of prolidase in mothers, their newborn babies and in non-pregnant controls

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Abstract The prolidase activity in serum and in erythrocytes was measured in 28 healthy mothers and in the cord blood of their newborn babies in using a modified Chinard method. 45 healthy non-pregnant women aged between 15–36 years formed a control group. Biochemical parameters (CK, BUN, C-peptid, AFP, Uric acid) were also measured. The serum and erythrocyte prolidase activities in maternal blood were 45.8±13.4 U/L and 37.8±2.7 U/g Hb respectively. There was no significant difference in the enzyme activities between pregnant women and the control group (p>0.05). However serum and erythrocyte prolidase activity in cord blood (20.3±8.2 U/L and 31.6±7.3 U/g Hb) was significantly different when compared with control group (53.4± 14.7 U/L in serum and 42.3±10.3 U/g Hb in erythrocyte, p<0.001). There was a significant correlation between maternal and cord blood serum enzyme activity (r: 0.76 p<0.01). This correlation was also shown in erythrocyte prolidase activities of both groups (r: 0.49, p<0.05). Cord blood prolidase activity was positively correlated with birth weight (r: 0.89, p<0.01). Prolidase activity in cord blood was low even though collagen turnover is increased in fetal growth.

Keywords Prolidase · Cord blood · Maternal blood

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Introduction

Connective tissue protein collagen comprise one-third of total body protein. Prolidase (E.C: 3.4.13.9) is an iminodipeptidase which can catalyse the rapid hydrolysis of the peptide bond involving the imino nitrogen of proline or hydroxyproline [3, 7, 12]. Because of the high proportion of the iminoacids in collagen (25% Pro and Hyp together), this enzyme plays an important role in its degradation and its activity might be correlated with the rate of collagen degradation [10]. Since the collagen turnover rate is expected to be high during fetal growth, the level of prolidase activity may reflect fetal maturation.

The presence of this enzyme in various tissues has been demonstrated [9]. However there is no report concerning prolidase activity in maternal and cord blood. A recent study has shown prolidase activity in amniotic fluid and its activity level was positively correlated with the body weight and lecithin levels in mature newborns [7].

We investigated the serum and erythrocyte prolidase activities of healthy pregnant women and their new-born. In addition CK, BUN, c-peptide, AFP and Uric acid were measured in the same groups. These values were compared with a control group of healty non-pregnant women.

Material and method

We studied maternal 28 healthy mothers and their newborn and 45 non-pregnant control women aged between 15–36 at Gaziantep University, Faculty of Medicine, Department of Obstetrics and Gynecology. 5 ml samples of venous blood were placed in a centrifuge tube and spun for 5 min at 2000 rpm. Biochemical parameters were measured on the same day. For hemolysate, venous blood was collected into heparinized tubes. After centrifugation at 2000 rpm for three min, plasma was separeted. Erythrocytes were washed three times with saline (0.9%). 2.5 mM $MnCl_2$ was used as a hemolysing reagent. The hemolysate and serum were stored at –20°C.

Serum prolidase assay:Serum was diluted 40 fold with 2.5 mmole/L Mn^{++}, 40 mmole/L Trizma HCl buffer (pH : 8) and

preincubated at 37°C for 2 h. The reaction mixture contaning 30 mmole/L substrate (gly-pro), 40 mmole/L trizma HCl buffer (pH: 8) and 100 µl of preincubated serum in 1 ml was incubated at 37°C for 30 min. The incubation reaction was then stopped by adding 0.5 ml of 20% TCA (trichloroacetic acid) solution. The supernatant was used for measurement of proline which was formed by prolidase. Proline was measured by the Chinard method [4, 5, 11]. For the erythrocyte prolidase assay we used same procedure but no preincubation. The hemolysate was diluted 200 fold with 2.5 mmole/L $MnCl_2$ and preincubated at 37°C for 2 h.

One unit of enzyme activity split 1 mmole of substrate in 1 h. The enzyme activity was given as U/L. One unit of haemolysate enzyme activity split 1 µm substrate in 1 min. All reagent were of analytical grade and obtained from Sigma (St Louis, USA) and Merck (Darmstadt, Germany).

Statistical evaluation

Data is given as means±SD. Statistical differences between groups were tested by two-tailed Student's t tests. Two tailed p values lower than 0.05 were considered significant. Figures were drawn as multiple-variable graphs with box -and-whisker plots and dots. Statistical analysis and scatter diagrams of the groups were performed with Med-Calc and SSPS software statistics programs.

Results

All prolidase activities are presented in Table 1. Maternal serum and erythrocyte prolidase activity were respectively 45.8±13.4 U/L and 37.8±2.7 U/g Hb and in the control group 53.4±14.7 U/L and 42.3±10.3 U/g Hb respectively.

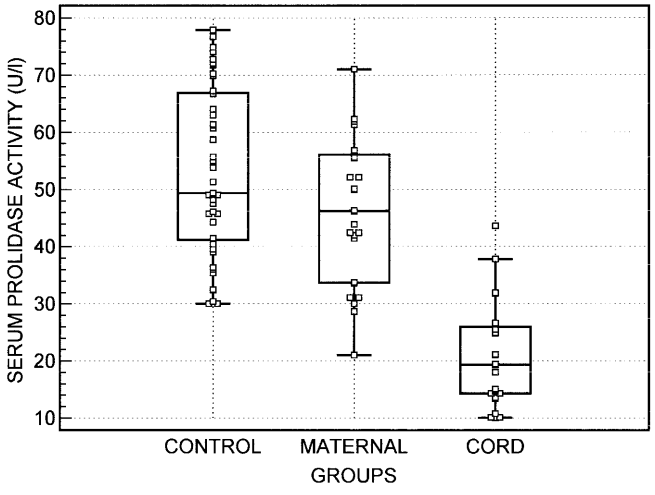

Fig. 1 Serum prolidase activity. The central box represents the values between the 25th and 75th percentiles. The line across the box represents the median value

The difference between the two groups was not significant ($p>0.05$). However serum and erythrocyte prolidase activity in cord blood were 20.3±8.2 U/L and 31.6± 7.3 U/g Hb respectively, which was significantly lower than in the control group (53.4±14.7 U/L and 42.3± 10.3 U/g Hb $p<0.001$). Distributions of serum and erythrocyte prolidase activity in the control, maternal and cord blood groups are shown in Fig. 1, 2. The central box represents the values between the 25th and 75th percentile. The line across the box represents the median.

The serum CK, BUN, C-peptide, AFP and uric acid were also determined in all samples. The correlation coefficients (r) between these biochemical parameters and serum prolidase activity is shown in Table 2. The only significant correlation was between control blood prolidase and AFP.

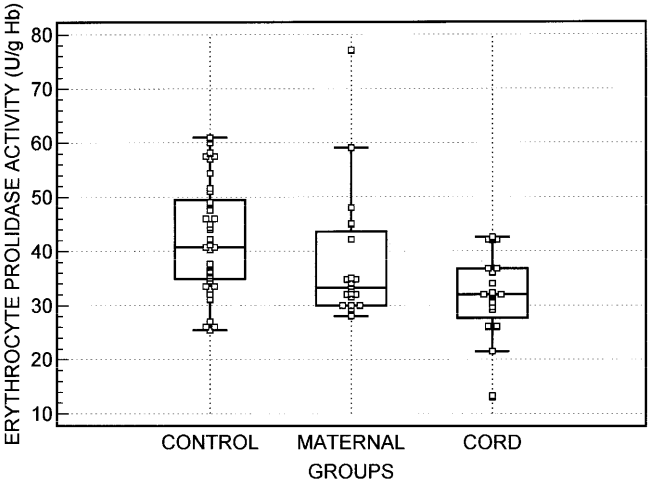

Fig. 2 Erythrocyte prolidase activity. The central box represents the values between the 25th to 75th percentiles. The line across the box represents the median value

Table 2 Correlation coefficient (*r*) between serum prolidase activity and various biochemical measurements

Prolidase	Control	Maternal blood	Cord blood
Prolidase-CK	–0.063	0.049	–0.074
Prolidase-BUN	–0.309	–0.197	–0.372
Prolidase-C peptid	0.321	–0.149	0.418
Prolidase-AFP	–0.384*	0.199	–0.108
Prolidase-Uric acid	0.063	0.031	–0.289

* $p<0.05$
CK creatinine kinase, *BUN* blood urea nitrogen, *AFP* α-fetoprotein

Table 1 Maternal, cord blood and non-pregnant control serum and erythrocyte prolidase activities

	Non-pregnant control ($\bar{X}$±SD)	Maternal ($\bar{X}$±SD)	Cord blood ($\bar{X}$±SD)
Serum prolidase activity (U/L)	53.4±14.7 (*n*=45)	45.8±13.4 (*n*=28)	20.3±8.2* (*n*=28)
Erythrocyte prolidase activity (U/g Hb)	42.3±10.3 (*n*=45)	37.8±2.7 (*n*=28)	31.6±7.3** (*n*=28)

* $p=0.0001$
** $p=0.0002$

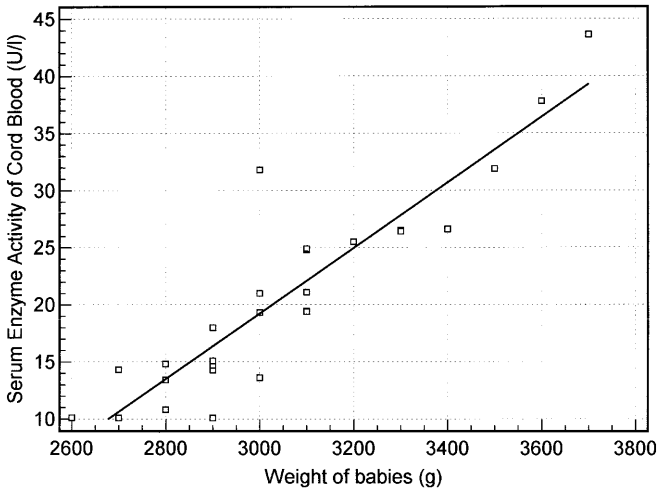

Fig. 3 Correlation between serum prolidase activity in cord blood and birth weight, *r*: 0.89 and $p<0.01$

There was a positive correlation between maternal and cord blood prolidase activity. However, prolidase activity in cord serum was positively correlated with birth weight (*r*: 0.89, $p<0.01$, Fig. 3).

Discussion

Prolidase is a highly specific peptidase. It is the only enzyme known to catalyse hydrolysis of compounds in which the sensitive peptide bond involves the imino nitrogen of proline or hydroxyproline and plays an important role in conservation of proline [8]. The conformational restrictions imposed by proline in a peptide chain appear to imply important structural or biological functions as can be deduced from their often remarkably high degree of conservation as found in many proteins and peptides, especially cytokines, growth factors, G-protein coupled receptors and neuroactive peptides [14]. Thus the enzyme has functional role and hereditary prolidase deficiency, the multisystemic disorder is characterised by a wide spectrum of clinical manifestations including skin ulcers, mental retardation and susceptibility to infections [2, 6]. The enzyme is expressed in all human tissues and cells [13]. Gürdol et al have shown prolidase activity in amniotic fluid [7].

Maternal erythrocyte prolidase activity (37.8±2.7 U/g Hb) was lower than in the control group (42.3±10.3 U/g Hb). But the difference did not reach statistical significance ($p>0.05$). Equally serum prolidase activity in maternal blood (45.8±13.4 U/L) was lower than in the control group (53.4±14.7 U/L, $p>0.05$). But serum and erythrocyte prolidase activities of cord blood were significantly lower than in the control group (20.3±8.2 U/L and 31.6±7.3 U/g Hb, $p<0.001$ Table 1 and Fig. 1, 2).

The prolidase activity of serum and erythrocte as well as some other biochemical parameters (CK, BUN, c-peptide, AFP and Uric acid) of a maternal and baby cord blood taken during the birth were investigated. The results compared with those taken from a control group. Only significant correlation between control prolidase activity and AFP was found (*r*: –0.384, $p<0.05$).

In this study cord blood serum prolidase activity was positively correlated with the birth weight (*r*: 0.89, $p<0.01$, Fig. 3). Gürdol et al had observed a positive correlation between amniotic fluid prolidase and birth weight [7]. In another study, there was no correlation between cord serum and amniotic fluid growth hormone (GH) levels [1]. Prolidase activity is known to be positively correlated with the turnover rate of collagen. It was concluded that prolidase activity reflects fetal growth.

Further studies are needed to establish whether prolidase activity of this enzyme might be used for the dedection of congenital anomalies involving growth or bone disorders in the first and second trimesters of pregnancy.

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