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ROLE OF MATERNAL ERYTHROCYTE ARGINASE ACTIVITY IN PREGNANCY – A PILOT STUDY

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Abstract:
Introduction: Arginase is an urea cycle enzyme which catalyzes the cleavage of L arginine to L- ornithine and urea. It is expressed in liver, erythrocyte, brain, kidney, mammary gland and intestine. The arginase activity detected in nonhepatic tissues that lack a complete set of urea cycle enzymes is thought to provide ornithine, the biosynthetic precursor of proline, an important constituent of collagen, and the polyamines, which are important for cell proliferation.
Aim and objectives: In the current study arginase level in maternal erythrocytes were determined to ascertain any possible role in pregnancy.
Study design: The study group comprised of total 45 subjects including twenty non – pregnant women (mean age 31.0 ± 6.0 years) and twenty five pregnant women (mean age 29.6 ± 6.1 years) of gestational age between 28 – 38 weeks.
Results: We found a significant increase in the level of maternal erythrocyte arginase (p 0.05) in pregnant women when compared to non – pregnant women.
Conclusion: Our study suggests that the increased maternal erythrocyte arginase activity may have a role in fetal growth and development.
Keywords : Arginase, Ornithine, Non-pregnant women, Pregnancy, Erythrocyte, polyamine, proline

Introduction

Arginase (L- Agginine amidino hydrolase, E,C 3.5.3.1) is an urea cycle enzyme which catalyzes the cleavage of L arginine to L- ornithine and urea. Arginase exists in two isoforms. Arginase-1 is a cytosolic protein, expressed primarily in the liver and to some extent in the erythrocytes. Hepatic arginase-1 activity serves urea synthesis and nitrogen homoeostasis.Arginase-2 is a mitochondrial protein, expressed in many extrahepatic tissues, such as brain, spinal cord, kidney, small intestine and mammary gland, but not in mature erythrocytes [1-4].

Arginase activity detected in non-hepatic tissues that lack a complete set of urea cycle enzymes is thought to provide ornithine, the biosynthetic precursor of proline, an important constituent of collagen and the polyamines, which are important for cell proliferation. Erythrocyte arginase activity is one of the sources for ornithine present in plasma. The availability of ornithine may be important for peripheral tissues such as cartilage and bone, since these tissues have low or no arginase activity [5]. Different studies have revealed that the requirement of rapidly dividing tissues for enhanced polyamine biosynthesis is apparently met by increased arginase activity, for eg, in gastric cancers, breast cancer, colorectal carcinoma [6 – 8]. In lactating mammary gland arginase activity rises to about 25% of that found in liver in order to supply the proline required for milk protein biosynthesis [9]. Experimental evidences suggest that, myometrial arginase activity increases ~ 25-fold during pregnancy to supply the rapidly growing fetus with polyamines to facilitate cell proliferation [10].

In our survey of literature regarding the erythrocyte arginase in pregnancy, no previous data was available .Hence; the current study was taken up to find out the probable role of erythrocyte arginase in pregnancy.

Materials and Methods

The study group consisted of total 45 subjects which included twenty non – pregnant women (mean age 31.0

± 6.0 years) and twenty five pregnant women (mean age 29.6 ± 6.1 years) of gestational age between 28 – 38 weeks .Subjects with history of coronary heart disease, hypertension, renal disease, diabetes mellitus, gestational diabetes mellitus, pre-eclampsia or any systemic disease were excluded from the study. The study was approved by the institutional ethical committee and informed consent was obtained from all the subjects involved in the study.

Collection of samples: 5 ml of venous blood was collected from the anticubital vein under aseptic conditions from each subject. 2ml of blood was added to the EDTA bottles for the separation of RBCs for the arginase estimation and 3ml to the plain bottles for separation of serum. The samples were then subjected to centrifugation for 3000g for 10 minutes within 2hrs of collection.

Preparation of RBC suspension: From the EDTA added blood the plasma was removed and the RBCs were washed with saline. For this they were mixed with about 10 ml of 0.9% saline and centrifuged. Supernatant was removed and the process was repeated for three times. Then the RBCs were suspended in saline to get 50% of RBC suspension.

Serum separated from the plain tube sample was used for the estimation of urea.

Determination of arginase activity: Arginase activity is measured colorimetrically. L- arginine is cleaved by arginase into urea and ornithine. The amount of urea formed by the action of arginase is measured by Berthlot reaction. One unit enzyme activity is the amount of enzyme required to produce1 µmol urea per minutes at 37° C [11.] Arginase activity is expressed as units per gm hemoglobin.

Results

Statistical analysis was performed using the statistical package for social sciences (SPSS 11.5). Independent student's't'test was used to compare mean values between the groups. The results are expressed as mean ± standard deviation (SD) in pregnant and non- pregnant women. Probability less than 0.05 was considered statistically significant.

A significant increase is observed in the level of maternal erythrocyte arginase (p 0.05) in pregnant women when compared to non – pregnant women. There is a significant decrease in urea level in pregnant women when compared to non- pregnant women.

Discussion

In intact erythrocytes, arginase catalyses the hydrolytic cleavage of the guinidino group of arginine, a semi essential amino acid to urea and ornithine. Since ornithine transcarbamoylase is absent in erythrocyte they cannot have functioning urea cycle. Urea formed in the erythrocyte comes out and accounts for one percent of blood urea. Several investigators have suggested that ornithine is an important precursor for proline, a critical constituent of proteins, e.g., collagen, in peripheral tissues and polyamines which are essential for cell proliferation. Peripheral tissues have relatively high levels of ornithine aminotranferase and proline -5-carboxylate reductase, the enzymes that sequentially convert ornithine to proline, but they have relatively low levels of arginase. Hence, an enzymatic complementarity exists between red blood cells and peripheral tissues with regard to the conversion of arginine to proline; i.e., red blood cells convert arginine to ornithine, and release into plasma. Peripheral tissues take up this ornithine and convert it sequentially to pyrroline -5- carboxylate and proline. The arginase activity present in RBC may be important also during healing process, where red blood cells within a fibrin clot can act as a metabolic source of ornithine [2, 6.]

Growing fetus requires polyamines for cell proliferation and differentiation and proline for bone growth (13). Fetal growth and development are dependent on the adequate provision of substrates from the maternal circulation. Red blood cells act as source of amino acids from one site to another and they have a role in

interorgan amino acid transport[13]

Investigators have suggested that there are various sources such as myometrium, placenta and plasma which supply ornithine required for the fetal growth and development. Experimental evidence suggests that, myometrial arginase activity increases ~ 25-fold during pregnancy to supply the rapidly growing fetus with polyamines to facilitate cell proliferation. [10]. Elevated arginase activity observed in the placental villi in the early gestational period may be responsible for proliferation of trophoblasts by increasing polyamines production. These results suggest that the l-arginine-ornithine-polyamine pathways play a role in placental growth and development [14]

A Study conducted in rats suggests that plasma arginase activity is increased in late pregnancy as well as during lactation, but not reflected on the circulating urea level. The arginase activity changes have been related to known changes in feeding and nitrogen handling pattern as well as hormonal variation[15].

In the present we found that erythrocyte arginase activity increased significantly in pregnant women. There is significant decrease in the serum urea level in pregnant women due to hemodilution. This suggests that the increased maternal erythrocyte arginase activity may be an additional source of ornithine required for fetal growth and development. However, there are few limitations. Number of sample used for the study is less. Another limitation is that arginase activity has been studied only in the third trimester

Conclusion

Erythrocyte arginase activity increases in the third trimester of pregnancy .This suggests that it has a role in providing ornithine for fetal growth and development. To the best of our knowledge, this study is first of its kind. Investigation of erythrocyte arginase activity in all trimesters of pregnancy is required to correlate the increase in activity with progressive fetal growth.

Table 1: Levels of hemoglobin, serum urea and erythrocyte arginase in Non –pregnant women and pregnant women

Parameters	Non –pregnant women (n= 20)	Pregnant women (n= 25)	Significance (p value)
Hemoglobin (gm/dl) (mean± SD)	12.2 ± 0.56	10.56 ± 0.9	0.001
Serum urea (mg/dl) (mean± SD)	32.16 ± 3.12	22.0 ± 0.56	0.001
Erythrocyte arginase (µmol/gm Hb) (mean± SD)	6.13 ± 0.68	17.92± 3.2	0.0001

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