Arginase Activity and Lecithin/Sphingomyelin (L/S) Ratio in the Amniotic Fluid of Pregnant Women

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Abstract Arginase activity is important in polyamines and nitric oxide production which are required for the normal growth of placenta and embryo. A considerable arginase activity is observed in amniotic fluid in women at the end of pregnancy. Lecithin to sphingomyelin (L/S) ratio is widely used in order to assess fetal lung immaturity and prevention of neonatal respiratory distress syndrome, the major cause of neonatal morbidity and mortality. The purpose of our study was to determine if there is a relationship between arginase activity and L/S ratio in amniotic fluid. The study included 170 pregnant women, 18–43 years old, with normal and pathological pregnancy. The arginase activity was measured on the basis of the determination of the amount of liberated ornithine from arginine as substrate. The L/S ratio was done by using a thin layer chromatography. Increased level of arginase activity correlates with the fetal lung maturity. Arginase activity and L/S values may be useful biochemical data, for intrauterine baby maturity.

Keywords Amniotic fluid · Arginase activity · Lecithin/sphingomyelin (L/S) ratio · Women

Introduction

Arginase, (L-arginine amidinohydrolase, EC 3.5.3.1), as part of the urea cycle, detoxifies human body from ammonia. Arginase activity has been detected in a number of nonhepatic tissues that lack a complete urea cycle [1–4]. It is believed that arginase activity is more important in production of ornithine, subsequently leading to the production of other biologically relevant molecules such as polyamines (spermine and spermidine), proline or glutamate, and nitric oxide (NO) [5–7]. Proline is the major component of many structural proteins, especially collagen and therefore is important amino acid for tissues growth [5–8]. Both, proline and ornithine are a main precursor of polyamines, spermine and spermidine, required for the normal growth of placenta end embryo [1, 6, 7, 9]. Nitric oxide is synthesized from L-arginine by the enzyme NO synthase (NOS) and this process is dependent on the availability of L-arginine. In this regard, the L-arginine catabolism via the arginase pathway can act as an endogenous negative control system to regulate overall NO production [4, 10–12].

Besides its fundamental role in the hepatic urea cycle, arginase, is an S-phase enzyme in a human cell [13]. Also, arginase is expressed the immune system. Arginase I is constitutively expressed in polymorpho nuclear neutrophils and is liberated during inflammation. Arginase activity has very important function during pregnancy in the insurance of materno-fetal tolerance; arginase-mediated T cell hyporesponsiveness is involved in the suppression of the maternal anti-fetal immune response [14, 15]. A considerable

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arginase activity is observed in amniotic fluid in women at the end of pregnancy [16].

Amniotic fluid is the protective liquid that surrounds the unborn baby while it is in the mother's uterus. This body fluid contains phospholipids, including phosphatidylcholine (lecithin), sphingomyelin, phosphatidylinositol and phosphatidylglycerol (PG), cholesterol, and a variety of proteins. They altogether compose surfactant, which is secreted by the fetal lungs into the amniotic fluid. Surfactant is required to decrease surface tension within the alveoli thereby preventing alveolar collapse of the lung at the end of expiration [15–19]. The amount of these substances in the amniotic fluid changes towards the end of gestation in a manner related to fetal lung maturity [16, 20]. The lecithin/sphingomyelin (L/S) ratio is a biochemical test for measuring the maturity of baby's lungs. This test gives a unique ability to determine whether a baby will be able to breathe after delivery, or whether it will need the aid of a respirator.

Neonatal respiratory distress syndrome (RDS), or hyaline membrane disease, remains a major cause of neonatal morbidity and mortality. RDS is primarily a disease of premature infants with low birth weight, and is related to advanced maternal age, maternal diabetes and hypertension [20].

An important prerequisite for the management of high risk pregnancies, and to prevent the risk of RDS as an accurate prediction of fetal lung maturity. Determination of the L/S ratio is by far the most widely used and accepted method to assess for fetal lung immaturity [21, 22].

It is based on the original observations that lecithin (but not sphingomyelin) in the fetal pulmonary secretions increases after 32–33 weeks' gestation and that neonatal RDS was uncommon after the L/S ratio reached 2.0, which occurs around 35 weeks in uncomplicated pregnancies. This cutoff has remained an accepted threshold for the determination of fetal pulmonary maturity. Infants who are born in the late preterm period (35–37 weeks' gestation) are at increased risk of respiratory morbidity compared with infants born at term, [16, 19, 20].

The probability of neonatal RDS using both gestational age and the L/S ratio may aid in clinical decision making concerning the timing of delivery [20].

Our idea was to access the arginase activity in amniotic fluid, taking into account the very importance of this enzyme for the cellular growth and development of the fetus, and to compare with the values of L/S ratio, which is accepted as a sign of the fetal maturity.

The obtained results point out that the arginase activity in amniotic fluid samples increases parallel with the

increasing the values of L/S ratios and may serve as an important prerequisite for the management of high risk pregnancies as the accurate prediction of fetal lung maturity.

Materials and Methods

The study included pregnant 170 pregnant women with both normal and pathological pregnancy, 18–43 years old, who have been hospitalized at the Clinic for Obstetric and Gynecology in Clinical Center, Faculty of Medicine, University of Nis, Serbia. In 77 women included in this study complications such as diabetes, hypertension or eclampsia were observed.

The amniotic fluid samples were obtained using amniocentesis. Amniocentesis has been performed between 15 and 42 week of gestation, using ultrasound as a guide for needle placement. A small amount of amniotic fluid was withdrawn and thrown away, prior to the sample collection. The hemorrhagic samples have not been used for this study.

Samples of amniotic fluid have been centrifuged at 3,000 rpm/min, in order to form precipitates that were later discarded. Only the upper layer of liquid was stored in refrigerator at -20°C . The samples were used for arginase activity and L/S ratio analyses. Lecithin/sphingomyelin ratio was estimated by using a thin layer chromatography assay kit (Helena laboratory, Beaumont, Texas, USA). The L/S ratio of 0–1.5 points out to immature fetal lungs, the L/S ratio from 1.6 to 1.9 is considered to be borderline and, the L/S ratio over the 2.1 represents fetal lung maturity [21–23]. L/S ratio, over the 2.1 represents the values that may aid in clinical decision making concerning the timing of delivery and a mature fetal lung.

The arginase activity was measured with spectrophotometric method by Porembska and Kedra [24] on the basis of the determination of the amount of liberated ornithine from arginine as substrate.

Parallel, with the performance the biochemical examinations, the clinical observation for the fetal maturity, a priori gestational age were done.

The study was approved by detailed information of every enrolled pregnant mother and their written consent was obtained prior to sample collection.

Statistics

The obtained results were statistically analyzed using Student's *t* test applying the SPSS computer statistical program. All the results are presented as mean $\pm$ SD.

Results

Our results show that L/S ratio increases during the pregnancy. The highest values were found in the amniotic fluid obtained from the pregnant women during the third trimester of pregnancy (Fig. 1).

Arginase activity in the amniotic fluid increases simultaneously with the increase of L/S ratio. The initial increase in arginase activity, compared to the control, is observed in immature embryos when L/S ratio is lower than 1.5. During the process of maturation increase in arginase activity correlates with an increase in L/S values. Arginase activity is found to be the highest when L/S ratio was found to be 2.0 or higher (Fig. 2, 3).

Discussion

An important prerequisite for the management of high-risk pregnancies and for the prevention of the RDS is accurate prediction of fetal lung maturity. Determination of the L/S

Fig. 1 Values of L/S ratio in amniotic fluid samples during the pregnancy

Fig. 2 Arginase activity in amniotic fluid during the pregnancy (U/l) in comparison with L/S ratios

Fig. 3 The dynamic of arginase activity in comparison with L/S ratio in amniotic fluid during the pregnancy. Increased level of arginase activity correlates with the newborn maturity

ratio is by far the most widely used and accepted method to assess for fetal lung immaturity, [18, 20, 21]. Possible sources of enzymes found in amniotic fluid are mother's blood and placenta [25, 26].

Arginase activity has been detected in a number of nonhepatic tissues that lack a complete urea cycle. The reaction is thought to provide a source of ornithine, the biosynthetic precursor of polyamines and the proline which are essential for cell proliferation and collagen production control as well as for growth and development of placenta and embryo [1, 5, 7, 9, 25, 26].

Wu et al. [7] have demonstrated that arginase activity was significantly reduced at mid-gestation but significantly enhanced at term gestation, [7, 11]. A considerable arginase activity is observed in amniotic fluid in women at the end of pregnancy. [27]. The function of the high arginase activity is appropriate in placental and fetal growth and development, [5]. Myometrial arginase activity increases 25-fold during pregnancy, and is significantly higher in uterine muscle tissue from pregnant animals to supply the rapidly growing fetus with polyamines which are important for placental and fetal growth and development [5]. Arginase activity is also found to be increased in plasma of pregnant and lactating female rats [25]. Our present results in amniotic fluid are in agreement with the literature data.

Polyamines (putrescine, spermidine, and spermine) are essential for placental growth and angiogenesis. Arginine and proline are the major sources of ornithine for placental polyamine production in pigs. Results of the recent studies conducted in animals show that proline is a major substrate for polyamine synthesis in the placenta. Both porcine and ovine placentas have a high capacity for proline catabolism and polyamine production. In addition, amniotic fluid contains enzymes that convert proline into ornithine, which is delivered through the circulation to placental tissues. There is exquisite metabolic coordination among highest

rates of polyamine synthesis and concentrations in placenta during gestation, as well as fetal growth [6, 7].

The availability of intracellular arginine is a rate-limiting factor in NO production [10, 28]. NO have many important biological functions; the best known is the regulation of vascular tone [29]. The ability of exogenous NO to promote uterine relaxation is well documented. NO donors have been shown to cause uterine relaxation, and to inhibit contractions in vivo in humans [29]. NO is considered to be an important local mediator that suppresses uterine contractility in rats and rabbits during pregnancy until term [30]. Human myometrium in both the pregnant and non-pregnant state contains inducible nitric oxide synthase cells (iNOS-containing cells). Their expression changes during gestation and prior to labor [11]. iNOS expression in the myocytes of human myometrium declines towards term of labor and may participate in the regulation of uterine activity during human pregnancy [29, 30].

Because of the fact that arginase shares L-arginine as a common substrate with NO synthase (NOS), NO production depends on the availability of L-arginine to NOS [4]. In this regard, the L-arginine catabolism via the arginase pathway can act as an endogenous negative control system to regulate overall NO production, [11].

The presented results show that arginase activity increases simultaneously with the values of L/S ratio with the progression of pregnancy. The activity of arginase increases with both, the increasing the values of L/S ratios and with the progressing of pregnancy. We showed the higher arginase activity found in mature amniotic fluids (during the last pregnancy trimester) when L/S ratio is found to be 2.1 and more. Higher arginase activity might be implicated in myometrial contractions occurrence and the beginning of labor at the end of pregnancy. The detection of arginase activity in the amniotic fluid of second and third trimester indicates a physiological role of this enzyme in human pregnancy.

Conclusion

The detection of arginase activity in the amniotic fluid of second and third trimester indicates a physiological role of this enzyme in human pregnancy. Arginase activity increases simultaneously with the values of L/S ratio with the progression of pregnancy.

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