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ORIGINAL ARTICLE

Plasma Protein Thiols, Malondialdehyde, Phosphodiesterase and RBC Acetylcholinesterase in Patients with Intrauterine Growth Restriction

***PRABHU K, #KUMAR P, °BHAT P, *RAO A, *MOHAN S, *SHARMA S**

ABSTRACT

Introduction: Intrauterine growth restriction (IUGR) is a term which is used to describe a condition in which the foetus is smaller than expected, for the number of weeks of pregnancy. One of the main reasons for this condition is that the foetus is not receiving the necessary nutrients, blood and oxygen which are needed for the growth and development of its organs and tissues. Nitric oxide (NO) which acts through cyclic GMP (cGMP), plays an important role in the pathophysiology of the vascular system. So, phosphodiesterase (PDE) activity may play a role in pre-eclampsia and endothelial dysfunction. Another compound that maintains the vascular tone is acetylcholine (Ach). It exerts its effect at neuromuscular junctions and is involved with muscle contraction. The oxidant status of the cell modulates angiogenesis, which is critical for embryonic growth.

Aim: To estimate plasma phosphodiesterase, protein thiols, malondialdehyde (MDA) and RBC acetylcholinesterase (AchE) in normal pregnant women who were in their 28-36th weeks of gestation and to compare their respective levels of these parameters in pregnant women who were diagnosed with IUGR in the same time period of their gestation.

Results and Discussion: We found a significant decrease in PDE ($p < 0.05$) and a marginal decrease in AchE activities in patients with IUGR as compared to the controls. Both protein thiols and MDA were found to be marginally elevated in IUGR patients, thus indicating an increased turnover of Reactive Oxygen Species (ROS). Our study shows that the pathophysiology of IUGR is multifactorial and a large scale study in this matter is required to further substantiate our findings.

Key Messages: The foetoplacental circulation plays a key role in the growth and the development of the foetus. Both acetylcholinesterase and phosphodiesterase may play key roles in the growth and development of a foetus. If a definitive marker can be found in this regard, it will be of immense help to mankind. Any attempt to find such markers is worth rewarding.

Key words: Intrauterine growth restriction; Acetylcholinesterase; Antioxidants; Phosphodiesterase

*Department of Biochemistry, #Department of Reproductive Medicine, Kasturba Medical College Manipal, Manipal University, India.

°Department of Obstetrics and Gynecology, TMA Pai Rotary Hospital, Manipal University, Udupi.
2571927, Ph: 0820 2922326 (O)

Corresponding author:
Dr.Krishnananda Prabhu, Associate Professor, Department of Biochemistry Kastutrba Medical College, Manipal, Manipal University, India -576104.
E mail: krishnakunj2000@yahoo.com
Fax: 910820 2571927

Intrauterine growth restriction (IUGR) is a term which is used to describe a condition in which the foetus is smaller than expected for the number of weeks of pregnancy. A foetus with IUGR often has an estimated foetal weight which is less than the 10th percentile. There are several causes for IUGR which can be maternal, genetic, foetal, placental, etc. In all, one of the main reasons is that the foetus is not receiving the necessary nutrients, blood and oxygen which are needed for the growth and development of its organs and tissues. This abnormality prevents its cells and tissues from growing or it causes the cells to decrease in size.

Several factors are important in the maintenance of the vascular tone in the placenta. Nitric oxide plays an important role in several physiological and pathological processes, especially in the pathophysiology of the vascular system. Nitric oxide is thought to cause dilation of the placental vasculature, which is important for the blood supply of the developing foetus [1]. Nitric oxide acts through the second messenger cGMP. Cyclic nucleotide accumulation has been implicated in the dilatation of the intact tonic spiral arteries of the uterus and in the relaxation of the myometrium [2],[3]. So, PDE activity which is responsible for the cleaving and inactivation of these cyclic nucleotides may play a role in pre-eclampsia and endothelial dysfunction [4],[5].

Another compound that maintains the vascular tone is acetylcholine. It exerts its effect at neuromuscular junctions and is involved with muscle contraction. Acetylcholine and butyrylcholine are involved in many non cholinergic functions such as cell proliferation, cellular adhesions, fertility, foetal growth, apoptosis, etc [6],[7],[8]. Human studies have shown that pyridostigmine (cholinesterase inhibitor) which is given along with infertility treatment, resulted in higher levels of growth hormone and growth factors in the follicular fluid, which resulted in higher rates of ovulation and a higher rate of pregnancy [9]. Accidental exposure to pesticides (containing cholinesterase inhibitors) in a selected population resulted in a decreased incidence of low birth weight babies and a decreased incidence of abortions as compared to unexposed females in the same population, thus further indicating the role for acetylcholine in foetal growth and development [10].

Reactive oxygen species affect multiple physiological processes from oocyte maturation to fertilization, embryo development and pregnancy. The oxidant status of the cell modulates angiogenesis, which is critical for follicular growth, corpus luteum formation, endometrial differentiation and embryonic growth. The serial measurement of oxidative stress biomarkers in longitudinal studies may help to delineate the aetiology of some of the disorders in female reproduction such as preeclampsia, preterm labour, intrauterine growth retardation, etc [11],[12],[13],[14],[15]. It has been reported that during pregnancy which is complicated with IUGR, malondialdehyde (MDA) concentration in the amniotic fluid was found to be almost three times more than that in normal pregnancy.

So, we sought to estimate plasma PDE, protein thiols, MDA and RBC AchE in normal pregnant women who were in their 28-36th weeks of gestation and to compare their respective levels of these parameters in pregnant women who were diagnosed with IUGR in the same time period of their gestation.

Materials and Methods

The Institutional Ethics Committee gave the clearance to carry out this study. Seventeen pregnant women with IUGR (ultrasoundconfirmed), in the 28th-36th weeks of gestation, visiting Kasturba Medical College Hospital, Manipal and at Udupi TMA Pai Hospital in Karnataka, were the test subjects. Ten pregnant women, in the 28th-36th weeks of gestation with no complications, served as the controls.

Specimen

2ml of blood was collected in EDTA vacutainers.

Haemoglobin was estimated in 20 µl of the whole blood by Drabkin's method.

The remaining blood in the vacutainer was centrifuged to separate the blood cells and plasma and the plasma was used for the estimation of protein thiols, malondialdehyde and phosphodiesterase. The seperated blood cells were washed thrice with saline and then 100µl of the washed cells were haemolysed by diluting with distilled water (1: 50). 20µl of the haemolysate was used for the assay of acetylcholinesterase.

Acetylcholinesterase (AchE) assay

The enzyme activity was typically measured by using acetylthiocholine ester as a substrate.

Acetylthiocholine ⟶ thiocholine + acetate

Thiocholine + dithiobisnitrobenzoate ⟶ 5-thio-2—nitrobenzoate (yellow colour)

The catalytic activity was measured by following the increase in the intensity of the yellow colour spectrophotometrically at 412 nm (mec: 1.361x mmol $^{-1}$x mm $^{-1}$). The assay was carried out at 37^{o}C. The values of the acetylcholinesterase activity were expressed in IU/gr/ltr of haemoglobin [16].

Phosphodiesterase Assay

The hydrolysis of p-nitrophenyl phosphate results in a yellow colour due to the liberation of p-nitrophenylate at alkaline pH, which was read at 400nm at 37^{o}C (mec: 17, 600 Mol $^{-1}$x cm $^{-1}$) [17].

Protein Thiols

Ellman's reagent or dithiobisnitrobenzoate (DTNB) readily undergoes a thiol-disulphide interchange reaction in the presence of free thiol. The 2- nitro 5 - thiobenzoate di-anion has a relatively intense absorbance at 412nm (mec: 13600 M-1 cm-1), which can be used to assess thiols [18].

Malondialdehyde

MDA levels were estimated by the thiobarbituric acid (TBA) reaction. TBA reacts with MDA to form an MDA-TBA_2 complex which is pink coloured. The pink coloured complex was brought to room temperature and was measured spectrophotometrically at 532nm (mec: 1.56 x 10 ^{5} Mol $^{-1}$x cm $^{-1}$) [19],[20].

Results

Our studies have shown a significantly decreased activity of PDE ($p < 0.05$) in the IUGR cases as compared to that of the healthy controls [Table. 1] . We found increased plasma protein thiols and increased plasma MDA levels, both indicating an increased turnover of free radicals [Table. 1].

(Table/Fig 1) Comparison of variables between normal pregnant women and patients with IUGR of same gestational period.

PARAMETER	CONTROL (n = 10)	IUGR (n = 17)	P value *
Acetylcholinesterase (IU/gr/l of Hb)	33 (20, 33.56)	31.2 (9, 55.5)	0.802
Phosphodiesterase (µmol/l)	29.6 (17.1, 38.1)	7.7 (0, 21)	0.011
MDA (µmol/L)	4.68 (3.7, 6.2)	5.44 (3.4, 9.9)	0.940
Protein Thiol (µmol/l)	293.63 (245.7, 376.9)	369 (303.8, 860.6)	0.075

***All test results were statistically analyzed by non parametric Mann Whitney Test.**
† MDA = Molndialdehyde

Discussion

Both menstruation and pregnancy are inflammatory conditions that cause a degree of physiological ischaemia-reperfusion [21]. The contractility of the uterine smooth muscle is essential for the cyclic shedding of the endometrial lining and also for the expulsion of the foetus during parturition [22].

Acetylcholine is a neurotransmitter which is involved in muscle contraction and in the maintaining of the vascular muscle tone. This compound is hydrolyzed by AchE which may be an indication of the activity of the neurotransmitter. As mentioned above, acetylcholine and butyrylcholine are involved in many non cholinergic functions and they may have a role in foetal growth and development [5],[6],[7],[8],[9]. Our studies have shown a decrease in the AchE activity in IUGR patients

as compared to normal pregnant cases, thus indicating its probable role in the female reproductive system and foetal growth.

Isoforms of the PDE family are shown to be involved in smooth muscle contraction and inflammation. PDE4 inhibitors have been shown to prevent inflammation-induced preterm delivery by countering hypoxaemic foetoplacental vasoconstriction, thus improving foetal oxygenation and nutrition [23].

The inactivation of cGMP is brought about by the enzyme, PDE. The activity of PDE may be an indication of the amount of available active cGMP and therefore, it can relate indirectly to the nitric oxide activity and therefore, to vascular maintenance.

Our studies have shown a significantly decreased activity of PDE ($p < 0.05$) in the IUGR cases as compared to the healthy controls [Table. 1], which probably indicate an increased turnover, leading to decreased cGMP levels which are required to maintain foetoplacental vasculature which leads to IUGR.

Pregnancy itself may predispose to increased oxidative stress, mostly because of the increased oxygen requirements and mitochondria-rich placenta [24] . Studies have shown that during advanced pregnancy, a derangement of the oxidative balance can lead to improper activation of inflammatory changes, thus triggering premature labour as well as other complications [25],[26]. We found increased plasma protein thiols and increased plasma MDA levels, both indicating an increased turnover of free radicals [Table. 1].

Our study shows that the pathophysiology of IUGR is multifactorial and that a large scale study in this matter is required to further substantiate our findings.

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