Idiopathic Fetal Growth Restriction: Repercussion of Modulation in Oxidative Stress

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Abstract Oxidative stress has been proposed as one of the causes involved in idiopathic fetal growth restriction (IFGR). However, the exact relationship between oxidative stress and IFGR is not understood. This study aimed at understanding the role of oxidative stress and antioxidant status in IFGR materno-fetal dyads and matched controls. 75 materno-fetal dyads with IFGR were enrolled with equal number of normal low risk controls. Malondialdehyde (MDA) levels were measured as marker of oxidative stress, while paraoxonase-1 (PON1) activity and total antioxidant capacity (TAC) of serum were measured as markers of antioxidant status. MDA levels were increased in both maternal and cord blood of IFGR neonates as compared to controls ($p < 0.001$). TAC of serum were found to be decreased in IFGR (both maternal and cord blood) as compared to controls ($p < 0.001$; $p < 0.05$, respectively). PON1 activity was found to be decreased in the

IFGR mothers while it was found increased in IFGR cord blood ($p < 0.01$; $p < 0.001$). IFGR is a state of increased oxidative stress. Decreased PON1 enzymatic activity in mothers is also associated with IFGR.

Keywords Idiopathic fetal growth restriction · Oxidative stress · Paraoxonase-1 · Malondialdehyde · Total antioxidant capacity

Introduction

Fetal growth restriction (FGR) has been recognized as the second most important cause for perinatal mortality [1]. FGR is seen in both developed as well as the developing world, although the incidence is more pronounced in the developing world (about 20 %) [2], with India alone recording 43.6 % of FGR birth in the developing world [3]. Besides having an increased perinatal and infant mortality, FGR neonates also show significant association with cardiovascular disease, diabetes mellitus and metabolic syndrome in later life [4–7]. Such widespread effect of FGR warrants investigation of pathophysiology of FGR to help control its incidence.

Factors which predispose to FGR are myriad. Fetal growth is a complex process controlled by many factors involving mother, placenta and fetus [8]. Key processes associated with fetal growth include availability of growth factors, regulated blood flow, adequate oxygenation and vascular remodeling. Broadly, they can be classified in four categories depending on their origin. Maternal factors include nutrition before and during pregnancy, maternal health conditions (heart disease, hypertension, kidney disease, anemia, diabetes) including infections, and life style abuse like alcohol, smoking, and drugs. Fetal factors include structural anomalies, chromosomal

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anomalies, infections and multiple pregnancies. Placental factors include placenta praevia, abruption, circumvallate placenta, infarction and mosaicism. However, despite various advances in investigative technologies in recent time, in 40 % cases, exact etiology is not known [9], and these are labeled as idiopathic FGR (IFGR).

Embryo development takes place in a relatively low oxygen environment and is highly sensitive to oxidant molecule [10], [11]. In contrast, during placentation, there is increased oxygen transfer which increases reactive oxygen species (ROS) generation. A delicate balance possibly exists between oxygen level and ROS generation during gestation period and during rapid fetal growth, when there is increased oxygen transfer to the developing fetus.

Normally the cell regulates the amount of oxidants very closely. The amount of oxidants in the cell is determined by the balance of production of oxidants versus the destruction of oxidants by antioxidants. Thus, increased oxidant production and/or decreased antioxidant function can lead to increased oxidant stress. An imbalance between ROS generation and antioxidant defence in mother and fetus may lead to development of oxidative stress [12]. Consequently, several studies have been carried out that have implicated oxidative stress in the causation of FGR. These studies have suggested oxidative stress to act by various mechanisms. Some of the proposed mechanisms of action include DNA damage [13], altered collagen turnover [14] and reduced placental growth [15].

FGR has been shown to occur as a result of the failure of various vascular developmental processes in the placenta including elongation, branching and dilation of the capillary loops and of terminal villous formation [16]. Chen et al. proposed that variation in paraoxonase-1 (PON1) activity, which is well known for its antioxidant property towards LDL, could be related to vascular endothelial damage, placental insufficiency and thrombosis and thus could lead to adverse pregnancy outcome [17]. Association of PON1 with IFGR has however not been studied in any great detail, although studies with unfavourable pregnancy outcomes have been conducted [18].

In view of above, the present study was conducted to assess the status of oxidative stress in mothers and IFGR neonates by estimating malondialdehyde (MDA) generation, total antioxidant capacity (TAC) and PON1 enzyme activity in the maternal and cord blood of IFGR cases. The study also aimed at establishing a correlation between these parameters and fetal birth weight.

Materials and Methods

The study was carried out in the Department of Biochemistry in collaboration with the Department of

Obstetrics and Gynecology, UCMS & GTB Hospital, New Delhi, from December 2011 to March 2013. Ethical clearance for the study was obtained from the institutional ethical committee.

A total of 150 unrelated live births participated as dyads (mother and neonate). 75 consecutive IFGR materno-fetal dyads (referred subsequently as IFGR mother and IFGR neonate) were recruited as cases. Each woman was interviewed to collect the relevant information on age, occupation, socio-economic status, medical history, family history, food habits, life style and health behaviour, etc. in a specially designed performa. Detailed history was recorded and clinical examination including obstetrical examination was carried out. An informed written consent was obtained from each participating woman for enrolment, and each participant was provided with a patient information sheet in either English language or Hindi language.

After delivery, information was collected and recorded in each subject for mode of delivery, placenta (weight and morphology), neonatal gestational age & anthropometry (birth weight, length and circumference of mid arm, head and chest).

Sample Collection

Total of 3 ml maternal venous/cord blood was collected in plain vacutainer. Serum was separated and kept in aliquots for estimation of the different parameters at -80°C till further use. All biochemical estimations for lipid peroxidation, antioxidant capacity and PON1 activity were carried out within 3 days of collection of the samples.

Estimation of Oxidative Stress

The oxidative stress in serum was measured by estimation of MDA levels in the plasma. MDA was measured using thiobarbituric acid method as described by Girroti et al. [19]. 3 ml of assay mixture contained 2 ml of stock solution (15 % TCA w/v, 0.375 % w/v; both prepared in 0.25 N HCl) and 1 ml of plasma. The assay mixture was heated for 5 min in boiling water bath, and after cooling the flocculent, the precipitate was removed by centrifugation at $10,000\times g$ for 10 min. The MDA concentration was calculated using extinction coefficient $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$ at 535 nm against blank (distilled water in place of plasma), and expressed as nmol ml^{-1} of plasma.

Estimation of Anti-oxidant Status

The total antioxidant capacity of serum was measured using Total Antioxidant Assay kit (Cayman's, USA) using instructions provided by the manufacturer. Under the reaction conditions used, the antioxidants in the sample cause

suppression of the absorbance at 750/405 nm to a degree which is proportional to their concentration. The capacity of the antioxidants in the sample to prevent 2, 2'-Azino-di-[3-ethylbenzthiazoline sulphonate] oxidation was compared with that of Trolox, a water-soluble tocopherol analogue, and was quantified as millimolar Trolox equivalents.

Paraoxonase-1 Enzymatic Activity

Paraoxonase enzyme activity was estimated by measuring the conversion of paraoxon to p-nitro phenol. The 1 ml assay mixture contained 10 mM TrisHCl, 1 M NaCl, 2 mM CaCl₂ and 0.025 ml of serum. Paraoxon was added at a concentration of 1.5 mM of final mixture. The rate of formation of p-nitro phenol was monitored by taking readings at every consecutive minute for 5 min using spectrophotometer (Shimadzu UV-2450). The enzyme activity was calculated using the extinction coefficient of 17,000 M⁻¹cm⁻¹ and expressed as nmol min⁻¹ ml⁻¹ of serum.

Statistical Methods

Chi square test and *t* test were used for the comparison of demographic profiles and biochemical data in the different groups. The scatter diagrams for distribution of maternal and fetal MDA, TAS, and PON1 with respect to fetal weight were drawn using SPSS software.

Results

The subject groups enrolled under the study were compared for various demographic parameters and none of the measured parameters revealed any statistically significant differences (Table 1). In case group, four women underwent caesarean section for non reassuring heart rate, where as all the control group women delivered normally. As expected, the placental weight in cases was significantly less as compared to the control group ($p < 0.001$). However, the placental morphologies in both groups were found to be grossly normal. All the anthropometric parameters of

the neonates in the case group were significantly less than control groups, which is in complete agreement with the recruitment parameters (Table 2). While birth weight in the control group was well above the cut off limits for the definition of a normal healthy baby ($3.34 \text{ kg} \pm 0.274$), the cases were similarly of lesser weight ($2.09 \text{ kg} \pm 0.16$) on account of being IFGR neonates. Similar differences were observed across all anthropometric parameters, viz., length of the neonates, the head circumference, the chest circumference and the mid-arm circumference, which were routinely measured for all delivered neonates.

Malondialdehyde (MDA), produced as a result of peroxidation of the lipids in biological membranes, was estimated as an oxidative stress marker. MDA levels in IFGR neonates and their mothers were observed to be elevated. While in mothers, the values were found to be twice that of the control group, (Fig. 1) ($p < 0.001$), in the IFGR-neonates, the values were found to be 40 % higher than the control group (Fig. 1) ($p = 0.001$). The result showed significant increase in lipid peroxidation in IFGR materno-fetal dyads.

Total Antioxidant Capacity (TAC), that represents the combined antioxidant activities of all the constituents of serum including vitamins, proteins, lipids, glutathione, uric acid, etc., was estimated as a measure of antioxidant capacity. TAC was found to be decreased in both IFGR neonates and their mothers. While in mothers, the TAC was found to be 40 % less than the values observed in the control group ($p < 0.001$), the levels in IFGR babies were observed to be 23 % less than the values found in control group ($p = 0.022$). In spite of wide variations observed in the TAC, the difference was found to be significantly decreased in IFGR materno-fetal dyads (Fig. 2).

Paraoxonase-1 (PON1) enzymatic activity in serum was measured by following hydrolysis of paraoxon. PON1 levels in mothers of IFGR neonates was about 30 % less than the values observed in the control group of mothers, and this difference was found to be statistically significant ($p = 0.005$). In contrast, more than two fold increase in the PON1 enzymatic activity in the IFGR babies was observed, and this difference was also found to be statistically significant ($p < 0.001$) (Fig. 3).

Table 1 Demographic features of mothers in the study groups; none of the measured parameters revealed any statistically significant differences

Parameter	Controls (n = 75) (mean $\pm$ SD) or %	IFGR mothers (n = 75) (mean $\pm$ SD) or %	p-value
Age (in yrs)	24.23 $\pm$ 3.12	22.93 $\pm$ 2.39	0.213
Housewives	70.5 %	81.3 %	0.118
Religion (Hindu)	73.1 %	82.7 %	0.154
Period of gestation (in weeks)	39.05 $\pm$ 1.85	38.89 $\pm$ 1.13	0.503
Parity (primipara)	52.6 %	54.7 %	0.794

Table 2 Anthropometric features of the neonates in the study groups; all the anthropometric parameters of the neonates in the case group were significantly less than control groups, which is in complete agreement with the recruitment parameters

Parameters	Controls (n = 75) (mean $\pm$ SD)	IFGR –Neonates (n = 75) (mean $\pm$ SD)	p value
Birth weight (in kg)	3.34 $\pm$ 0.274	2.09 $\pm$ 0.16	<0.001
Length (in cm)	45.14 $\pm$ 2.24	40.15 $\pm$ 1.06	<0.001
Head circumference (in cm)	34.5 $\pm$ 1.76	32.97 $\pm$ 1.49	<0.001
Chest circumference (in cm)	32.58 $\pm$ 1.91	31.34 $\pm$ 1.54	<0.001
Mid-arm circumference (in cm)	8.86 $\pm$ 0.93	8.18 $\pm$ 0.57	<0.001

Fig. 1 MDA levels in subject groups (values expressed as mean with range); * $p < 0.001$; # $p = 0.001$. MDA level in mothers of IFGR neonates was found to be twice that of the values in the control group. The MDA level in IFGR-neonates was found to be 40 % higher than the control group. (n = 75) **Fig. 2** TAC levels in subject groups (values expressed as mean with SD range): * $p < 0.002$; # $p = 0.002$. Total Antioxidant Capacity in mothers giving birth to IFGR neonates was 60 % of the values observed in the control group. TAC levels in IFGR was observed to be 77 % of the values found in control group. (n = 75)

Correlation Between Neonatal Weight and Maternal Biochemical Parameters

Correlation analysis was carried out between neonatal weight and various maternal oxidative stress parameters to determine the trend of change of fetal weight with these parameters and whether the change was statistically significant or not. There was significant and positive correlation of neonatal weight with maternal levels of TAC, and significant negative correlation of neonatal weight with maternal levels of MDA. No significant correlation was

observed between maternal PON1 activity and neonatal weight (Fig. 4).

Correlation Between Neonatal Weight and Neonatal Biochemical Parameters

Correlation analysis was carried out between neonatal weight and various neonatal biochemical parameters to determine the trend of change of neonatal weight with these parameters. There was significant and positive correlation of neonatal weight with fetal levels of TAC, and

Fig. 3 PON1 levels in subject groups (values expressed as mean with SD range); * $p = 0.005$; # $p < 0.001$. Enzymatic activity in mothers with IFGR neonates was about 30 % less than in the control group. Enzymatic activity in the IFGR neonates was observed to be 127 % higher than control group. ($n = 75$)

Fig. 4 Scatter diagram showing correlation between neonatal weight and maternal biochemical parameters. Positive correlation between neonatal weight and maternal levels of TAC, and negative correlation of neonatal weight with maternal levels of MDA were seen. There was no significant correlation of maternal PON1 activity with neonatal weight

significant negative correlation of neonatal weight with neonatal levels of MDA and PON1 (Fig. 5).

Discussion

Fetal growth restriction is of great concern to health services and more so is the idiopathic FGR (IFGR), as in

absence of any underlying cause, clinical intervention remains elusive. Recently, attempts have been made to evaluate various parameters in relation to FGR, particularly oxidative stress, since oxidative stress plays a significant role in the development of FGR. Attempts have also been made to determine the role of paraoxonase enzyme in adverse pregnancy outcomes, as decreased activity of this enzyme is actively involved in vascular endothelial

Fig. 5 Scatter diagram showing correlation between neonatal weight and maternal biochemical parameters. Positive correlation of neonatal weight with neonatal levels of TAC, and negative correlation of neonatal weight with neonatal levels of MDA and PON1 were seen

damage, placental insufficiency and thrombosis, leading to adverse pregnancy outcomes. In view of this, in the present study, we evaluated lipid peroxidation, total antioxidant capacity and PON1 enzymatic activity in idiopathic fetal growth restricted neonates and their mothers. Subsequently, we tried to extrapolate the findings to determine a relationship between the observed parameters and neonatal weight.

The results obtained by us revealed that both mothers and the neonates were having high degree of oxidative stress as evident from significant increase in lipid peroxidation and decrease in total antioxidant capacity (Figs. 1, 2). In other studies also, MDA was found to be increased in both mothers and fetus in cases of FGR neonates [20] as well as in small for gestational age neonates [21]. Previously, TAC has been shown to be decreased in FGR cases indicating decreased levels of antioxidant potential [20]. Decrease in TAC has also been noted in SGA babies [22]. Decrease in TAC in the cord blood of preterm low birth weight neonates have also been reported [23]. Correlation analysis revealed that blood level of MDA of both mother and cord blood correlated negatively with birth weight of the neonates. Malondialdehyde levels in fetal serum have previously also been shown to correlate negatively with fetal weight [24]. Total antioxidant

capacity of blood in both mother and fetus correlated positively with fetal weight, indicating significant impact of antioxidant potential on fetal birth weight, and thus, the incidence of FGR.

Not too many reports are available with regard to oxidative stress profile in IFGR. Our results are in line with a recent report by Goel et al. [12], who have reported increased level of MDA and protein carbonyl in IFGR mothers and cord blood. These authors have also reported a decrease in ferric reducing activity of plasma that represents partial antioxidant capacity of the serum. However, these authors have not carried out any correlation study with fetal weight.

Although it is not clear whether the oxidative stress is due to increased production of free radicals or decrease in the antioxidant capacity, it appears that the antioxidant defense is unable to handle increased amount of ROS being generated during pregnancy, leading to oxidative stress. The mechanism of oxidative stress-mediated FGR is yet to be elucidated; it is generally believed that increased level of ROS induces a series of signal transduction mechanisms that eventually leads to apoptosis and various pathophysiological changes in the placenta leading to FGR [25].

Paraonase-1 (PON1) enzyme activity was decreased significantly in mothers with IFGR babies. The results

obtained by us are in agreement with an early study by Açıkgöz et al., where they found decreased PON1 activity in pre-eclampsic mothers with FGR, whereas in pre-eclampsic mothers with normal babies, the level increased significantly [26]. Also, no changes in PON1 activity have been observed in mothers with preterm delivery in an early report involving Korean females [27]. Paraoxonase activity is linked to vascular endothelial damage, placental insufficiency and thrombosis [17], which may promote blockage of placental vessels, thus inducing ischemia–reperfusion type of injury, which may eventually lead to adverse pregnancy outcomes. This view is supported by the fact that genetic variants of PON1 having lesser expression of the enzyme have been shown to be associated with SGA birth [28].

In contrast to low PON1 activity in mothers, PON1 activity in cord blood of IFGR neonates was found to be increased, and showed negative correlation with birth weight. Increased mRNA expression of PON1 in the cord blood (data not shown here) further support the increased enzymatic activity observed by us. The increase in PON1 activity in cord blood is probably a compensatory response of the fetal body, but appropriate explanation for the change could not be ascertained. However, in a recent report, reduced PON1 activity has been reported in the cord blood of low birth weight neonates and these authors suggested endoplasmic reticulum stress and atherogenic changes in the placental circulation as the basis of reduced activity [18].

In conclusion, FGR per se is a state of increased oxidative stress, both in mother and the fetus, whether it is due to known risk factors or idiopathic. Additionally, PON1 activity is also associated with the incidence of IFGR, and maternal PON1 activity probably plays a more prominent role.

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Conflict of interest The authors declare that there is no conflict of interests regarding the publication of this paper.

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