

Paraoxonase 1 Gene Polymorphism (rs662) and Increased Risk of Idiopathic Foetal Growth Restriction

NILESH CHANDRA¹, MOHIT MEHNDIRATTA², ASHOK KUMAR TRIPATHI³, KIRAN GULERIA⁴, BASU DEV BANERJEE⁵

ABSTRACT

Introduction: Idiopathic Foetal Growth Restriction (IFGR) is a major health challenge. One of the proposed mechanisms for IFGR is oxidative stress. Paraoxonase (PON) enzyme protects Low Density Lipoprotein (LDL) from oxidation. Paraoxonase 1 (PON1) gene polymorphism causes a decrease in PON activity.

Aim: The present study aimed at exploring possible association of PON1 gene Q192R (rs662) polymorphism and expression with development of IFGR.

Materials and Methods: A cross-sectional case-control study was conducted from December 2011 to March 2013. 75 unrelated IFGR neonates and their mothers were recruited as cases with controls. PON1 polymorphism was detected by Polymerase Chain Reaction-Restriction Fragment length Polymorphism

(PCR-RFLP). PON1 expression and activity were assessed by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and spectrophotometrically, respectively. The genotypes were compared using one-way ANOVA. True Fold change was used to assess change in gene expression. Chi-square test and t-test were used for the comparison of biochemical data in the different groups.

Results: The QR and RR genotype of rs662 in mothers and neonates were found to be associated with increased incidence of IFGR. Decreased PON1 expression and activity was observed in affected mothers.

Conclusion: Incidence of IFGR is higher in foetus and mothers with unfavourable PON1 rs662 polymorphism and may be used to identify pregnancies at greater risk for FGR.

Keywords: Gene expression, Neonatal weight, Paraoxonase1-Q192R, Prevalence

INTRODUCTION

Perinatal mortality is a major challenge for health services in the developing world and Foetal Growth Restriction (FGR) has long been accepted as one of the leading causes [1]. Although occurrence of FGR is seen in the developed world as well, the rate of incidence (about 10%) [2] is quite alarming in the developing world and India accounts for 43.6% of FGR among developing nations [3]. The ill effects of FGR are not limited to the perinatal period and infancy, rather, the sequelae persists in adulthood and pre-disposes the individual to increased incidence of several non-communicable disorders in later stages of life, including cardiovascular disease, diabetes mellitus and metabolic syndrome [4-7].

Various factors predispose to FGR, which can be categorized based on their origin. Maternal morbidity, addictions and nutritional status (both before and during pregnancy) affect the foetal development in utero. Foetal structural and chromosomal anomalies, infections and multiple pregnancies have adverse effect on intrauterine growth. Placental abnormalities also restrict the growth of the foetus. However, about 40% of FGR are still categorized as 'idiopathic' [8]. Paraoxonase 1 (PON1) is a High Density Lipoprotein (HDL) bound enzyme, the major contributor to the anti-oxidant action of HDL that can regulate the levels of Oxidative Stress (OS) [9] through the reduction of oxidized Low Density Lipoprotein (LDL) [10]. Chen D et al., hypothesized that PON1 activity, which is well known for its antioxidant property towards LDL, when decreased, could be related to vascular endothelial damage, placental insufficiency and thrombosis and thus, could lead to adverse pregnancy outcomes [11].

Variation of paraoxonase activity among individuals related to PON1 gene polymorphism is quite prominent. Among the several polymorphic variants of the gene, the most commonly encountered types are the Q192R variant, the L55M variant (both in the coding regions) and the C108T variant (in the promoter region) [12]. Q192R polymorphism has been shown to be strongly correlated with

variation in paraoxonase activity. Moreover, Q192R genotype and paraoxonase activity have shown significance in the prediction of coronary artery disease [13]. No significant literature is available on association of PON1 polymorphism with Idiopathic FGR (IFGR), although studies with unfavourable pregnancy outcomes have been conducted [14]. The infant and maternal PON1-192R allele have been shown to be associated with preterm delivery in studies conducted in Chinese population and British gravida women [11,15].

Interestingly, a host of non-communicable disorders, which have been found to be associated with FGR, have recently been shown to also be associated with PON1 polymorphism. Diabetic nephropathy [16], metabolic syndrome [17] and coronary artery disease [18] are all more pronounced and comparatively more frequent in individuals with unfavourable genotype for PON1. This association has been found to persist across all regions in different ethnic groups (Caucasian and Asian) with strong statistical significance [16-18].

The biochemical data related to paraoxonase enzyme activity has already been published as part of assessment of oxidative stress in the same group of patients in relation to IFGR [19]. In view of above, this study was designed to explore the relationship of foetal growth restriction with PON1 gene polymorphism and expression in IFGR neonates and their mothers.

MATERIALS AND METHODS

The study was designed as a cross-sectional case-control study and was conducted in the Department of Biochemistry in association with the Department of Obstetrics and Gynaecology, University College of Medical Sciences and Guru Teg Bahadur Hospital, New Delhi, from December 2011 to March 2013. Ethical clearance was obtained from the Institutional Ethical Committee-Human Research. Informed written consent was taken from each subject before recruitment. In the present study, we analyzed PON1 Q192R (rs662) polymorphism and estimated mRNA expression in whole blood and

PON1 activity in serum of IFGR neonates and their mothers, along with controls.

Subjects

A total of 75 unrelated live births and their mothers were included (referred subsequently as IFGR neonates and IFGR mothers) with 75 matched controls.

For cases, pregnancies between 37-40 weeks with neonatal birth weight <10th percentile for that gestational age were selected. Mothers were in the age group of 18-35 years, had a pre-pregnancy Body Mass Index (BMI) of 19-26 kg/m² and were singleton pregnancies. Exclusion criteria included; parity >4, chronic maternal diseases (including hypertension, diabetes mellitus, renal disease, liver disease, heart disease and moderate/severe anaemia), antepartum haemorrhage-abruptio placentae, placenta previa, pre-eclampsia, eclampsia, Anti Phospho-Lipid Antibody (APLA) syndrome and known parental genetic disease, history of substance abuse (smoking, alcohol and drugs), evidence of urinary tract infection or any intra-uterine infection, gross placental abnormality, or any known cause of foetal growth restriction in history, examination and investigations. Cases with ultrasonologically detected anomalies in foetus were also excluded.

Information was collected and recorded for each enrolled subject for mode of delivery, neonatal gestational age and anthropometry (birth weight, length and circumference of mid arm, head and chest). At the time of delivery, the attending obstetrician also carried out physical examination as well as measurement of weight of the placenta. Physical examination included inspection of maternal and foetal surfaces of placenta as well as the site of cord insertion for presence or absence of any calcifications, membrane, any obvious abnormality and any retroplacental clots. Presence or absence of all cotyledons and number of vessels in the cord were also examined. Any abnormality was ground for exclusion, as mentioned in exclusion criteria and deliveries with only normal appearing placenta were included in the study.

Women with low risk pregnancies between 37-40 weeks of gestation, with normal neonates based on gestational age and anthropometric measurements (birth weight, length and circumference of mid-arm, head and chest) were recruited as controls. Other inclusion and exclusion criteria were same as those for cases.

Haemoglobin level, blood group, VDRL test (Venereal Disease Research Laboratory), VCT (Voluntary Counseling and Testing for HIV), GCT (Glucose Challenge Test), urine (routine/microscopy and culture) and obstetrical ultrasound were carried out for all recruited subjects. Special investigations, including APLA (Antiphospholipid antibodies), TORCH (Toxoplasmosis, Rubella, Cytomegalovirus, Herpes Simplex Virus-2, and other infections) and targeted obstetrical scan were carried out for subjects.

Sample Collection

Total of 5 mL each of maternal venous blood and cord blood was collected {2 mL in Ethylenediaminetetraacetic acid (EDTA) vacutainer and 3 mL in plain vacutainer}. For genomic study, 500 µL whole blood was kept separately at 4°-8°C and DNA was isolated within 2 weeks of collection. For mRNA isolation, 350 µL of whole blood was diluted with equal volume of Nuclease Free Water (NFW) and subsequently mixed with TRIzol LS (Invitrogen) in a ratio of 1:3 and stored at -80°C. RNA extraction was carried out within a month of sample collection. Serum was collected and kept at -80°C in aliquots for estimation of the different parameters till further use.

DNA Extraction, Quantification and Genotyping Analysis

Genomic DNA was extracted from whole blood by commercially available kit (Quick-g DNA™ MiniPrep, Zymo Research) as per the instructions provided by the manufacturer. The extracted DNA was

quantified by taking optical density (OD) of the sample at 260nm by spectrophotometer (Thermo Scientific NanoDrop 2000c). The purity of DNA was ascertained by taking ratio of ODs at 260 and 280nm.

PON1 Genotyping

PCR amplification was done using thermo cycler (Eppendorf Mastercycler Gradient-5331). Briefly, 50 ng of DNA was amplified in a 20µL reaction mixture containing 15 pM of each of the PON1 primers, 1.25 mM MgCl₂, 200 µM Deoxynucleotide Triphosphates (dNTPs), 2µL 10X PCR buffer (10X buffer containing 500 mM KCl, 100 mM Tris-HCl, pH 9.0), and 1 U Taq DNA polymerase (All reagents from MBI Fermentas). The PCR protocol included an initial incubation at temperature of 94°C (5 min) followed by 35 cycles of amplification (denaturation for 30 s at 94°C, annealing for 30 s at 60°C and extension for 30 s at 72°C). A final 10 min extension step was done at 72°C. The primer sequences used are given in [Table/ Fig-1].

The final PCR product from amplification of PON1 was subjected to digestion by BspPI Restriction Endonuclease (RE) enzyme (Thermo

For PON1:	F: 5'-TATTGTTGCTGTGGGACCTGAG-3'
	R: 5'-CACGCTAAACCCAAATACATCTC-3'

[Table/Fig-1]: Primer sequences for PON1 genotyping (rs662). The size before treatment was 99bp and size after treatment with RE 66bp and 33bp. Reference: Lakshmy R et al., [29].

PON1:
Forward Primer: 5'-TATTGTTGCTGTGGGACCTGAG-3'
Reverse Primer: 5'-CCACAGATATGTTATCCACG-3'.
GAPDH:
Forward Primer: 5'-CCAAGGTCATCCATGACAACCTTTGGT-3'
Reverse Primer: 5'-TGTTGAAGTCAGAGGAGACCACCTG-3'

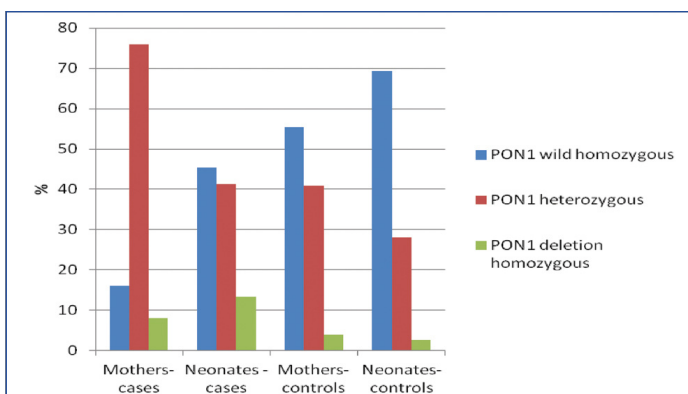
[Table/Fig-2]: Primer sequences for PON1 qPCR. Reference: Parker-Katirae L et al., [30].

Scientific). A 10µL of PCR reaction mixture was mixed with 2 U of BspPI RE enzyme, 2 µL of 10X Buffer Tango and 18 µL of NFW. The mixture was incubated at 55°C for 16 hours. The enzyme was then inactivated by incubation at 80°C for 20 min. The digested product was visualized after electrophoresis in ethidium bromide (EtBr)-stained 2.5% agarose gel on gel documentation system (UVI-TEC).

Analysis of Gene Expression

Expression of the genes was assessed by RT-PCR/chemical assays. Only representative samples (15 samples) in each group, viz., cases and controls, were processed to establish correlation between PON1 mRNA expression and development of IFGR. Total RNA was extracted from peripheral blood kept in EDTA anticoagulant with TRIzol LS (Invitrogen, Carlsbad, CA). The extracted RNA was found to have an OD 280/260 ratio between 1.8 and 2.0. 500 ng of RNA was mixed with Oligo-dT and Random Hexamer (from Sigma Aldrich), and incubated at 65°C for 5 minutes followed by incubation on ice till addition of next set of reagents. The final mixture of 20 µL, which was used for synthesizing cDNA, contained 4 µL of 5X Reaction Buffer (MBI Fermentas), 40U of Ribolock® (MBI Fermentas), 200 U of RevertAid® (MBI Fermentas) and 200µM each of dNTPs. The mixture was incubated at 25°C for 10 min, 45°C for 60 minutes, followed by 70°C for 10 minutes in a thermocycler (Eppendorf Mastercycler Gradient-5331). The diluted solution (1:4 dilution with NFW) was then used as template sample for qPCR. The reaction mixture contained 3 µL of template cDNA, 5 µL of Pyrostart Fast PCR Mix (MBI Fermentas), 1 µL of Syto 9 dye (50 µM) and 15 pM each of forward and reverse primers [Table/Fig-2].

The PCR protocol included an initial activation temperature of 95°C (1 min) followed by 35 cycles of amplification (15s at 95°C, 15s at 54.8°C and 30s at 72°C). The fluorescence was acquired at 72°C.



[Table/Fig-3]: Prevalence of PON1 Q192R genotypes in IFGR materno-foetal dyads with controls. The prevalence of various genotypic variants in each subject group is represented in the figure as percentages, where the bar represents the percentage of subjects in the depicted genotype.

The results were analysed as described below. Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) gene was used as an internal control. Differences in threshold cycle (ΔC_t) values for PON1 and housekeeping gene from qPCR were calculated for both cases and controls, which were then used to derive the difference of ΔC_t between cases and controls ($\Delta\Delta C_t$) values for PON1 mRNA expression in foetus and mothers. Gene expression normalization (using GAPDH gene) was done by determining delta Ct, where ΔC_t = average C_t (target) – average C_t (normalizer). Then the difference of mean C_t values between test and control was determined, $\Delta\Delta C_t$ = ΔC_t (control) – ΔC_t (test). Determination of fold change (FC) was based on $FC = \text{Primer Efficiency}^{\Delta\Delta C_t}$. True fold change was represented by the following: if $FC > 1$, true fold change = FC; if $FC < 1$, true fold change = $-1/FC$.

Biochemical PON1 Activity

Paraoxonase enzyme activity was estimated following the conversion of paraoxon to p-nitro phenol. The 1 mL assay mixture contained 10mM Tris HCl, 1 M NaCl and 2mM $CaCl_2$. 0.025 mL of serum was present in the assay mixture. Paraoxon was added at a concentration of 1.5mM of final mixture. The rate of formation of p-nitro phenol was monitored by taking readings at every consecutive minute for 5 min at 405 nm using spectrophotometer (Shimadzu UV-2450). Extinction coefficient ($17,000 \text{ M}^{-1} \text{ cm}^{-1}$) was used to calculate the enzyme activity and it was expressed as nmol min⁻¹ mL⁻¹ of serum [20].

STATISTICAL ANALYSIS

Statistical analysis was done by SPSS software version 20.0. For comparisons of demographic and biochemical data, student t-test and Chi-square test were used. The genotypes were compared using one-way ANOVA. Binary logistic was used to find out the Odds Ratio (OR) for IFGR amongst the different genotypes. The QQ genotype was considered as reference. A p-value < 0.05 was taken as Statistically significant.

True Fold change was used to assess change in gene expression. Finally, the linear correlation of maternal and foetal PON1 with respect to foetal weight was estimated using SPSS software. One-way ANOVA with linear trend was applied to find the mean trend of maternal paraoxonase activity with RR, QR and QQ genotypes. Since no previous study has been done for PON1 in IFGR mother-neonate dyads, power of study for the selected sample size could not be determined.

RESULTS

Gene Polymorphism

The prevalence of Q192R polymorphic variants of PON1 gene is shown in [Table/Fig-3] for the four study groups namely, mothers

of IFGR neonates, control mothers, IFGR neonates and control neonates. In mothers of IFGR neonates, the frequency of RR and QR genotype as well as R allele was found to be significantly higher as compared to controls [Table/Fig-4a]. Presence of RR and QR genotype was found to be significantly associated with the incidence of IFGR (OR = 7, p-value=0.013 and OR=6.435, p-value <0.001 respectively).

Similarly, in IFGR neonates, the frequency of RR and QR genotype as well as R allele was found to be significantly higher as compared to controls. Presence of RR and QR genotype was found to be significantly associated with the incidence of IFGR (OR=7.64, p-value=0.012 and OR=2.258, p-value=0.023 respectively) [Table/Fig-4b].

Groups Genotypes	Mothers (cases)	Mothers (controls)	Odds Ratio	95% CI	p-value
QQ	12 (16%)	41 (54.7%)	1	Ref	Ref
QR	57 (76%)	31 (41.3%)	6.435	2.96–13.98	<0.001*
RR	6 (8%)	3 (4%)	7.00	1.52 – 32.33	0.013*
Allele Frequency					
Q	0.54	0.753			
R	0.46	0.247			

[Table/Fig-4]: a) Odds ratio of PON1 Q192R genotypes in mothers.

Groups Genotypes	Neonates (cases)	Neonates (controls)	Odds Ratio	95% CI	p-value
QQ	34 (45.3%)	52 (69.3%)	1	Ref	Ref
QR	31 (41.3%)	21 (28%)	2.258	1.118–4.558	0.023*
RR	10 (13.3%)	2 (2.67%)	7.647	1.577–37.071	0.012*
Allele Frequency					
Q	0.66	0.833			
R	0.34	0.167			

[Table/Fig-5]: (b) Odds ratio of PON1 Q192R genotypes in IFGR babies.

Tables show the prevalence of various genotypes in mothers (4a) and in neonates (4b). Also, shown is the odds ratio (using Chi square test) of incidence of IFGR with the specific genotype. Allele frequency for each allele is shown for both mothers and neonates of both groups separately. *p<0.05 was considered to be significant.

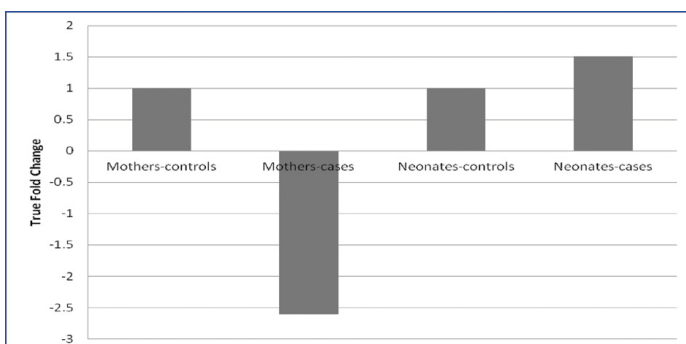
Gene Expression

Reverse Transcription- quantitative (Real Time) Polymerase Chain Reaction, which was used for estimating the expression levels of PON1 mRNA in the blood samples of the study subjects, showed differing results in mothers and neonates. Change in ΔC_t value ($\Delta\Delta C_t$) was negative in mothers (-1.38), which indicated a decrease in expression of mRNA in mothers giving birth to IFGR neonates, while a positive $\Delta\Delta C_t$ in foetus (0.59) is indicative of increase in expression of mRNA in IFGR neonates. To find out the degree of change in expression, True Fold Change was calculated.

As can be seen from [Table/Fig-5], there was decrease in mRNA levels of the PON1 gene in cases of mothers as compared to controls, and the decrease was 2.6 times that of the control group. However, the foetus group showed increased PON1 mRNA expression in the cases group when compared with the control group, and the increase was 1.5 times more.

Biochemical Activity

PON1 levels [Table/Fig-6] in mothers giving birth to IFGR babies was about 30% less than the values found in the control group of mothers (118 vs 147.77 mol min⁻¹ mL⁻¹ of serum), and this difference was found to be statistically significant (p<0.05). However, similar results were not found in the foetal plasma samples. The PON1 enzymatic activity that was the calculated in the IFGR babies was found to be 127% higher than control group (123.49 vs 54.74 mol min⁻¹



[Table/Fig-5]: True Fold Change in mothers and neonates for PON1 mRNA expression. Shown here is the difference in expression of PON1 in mothers (cases) and neonates (cases) as compared with controls. A decrease in expression is seen in mothers, while expression in neonates was found to be increased.

Groups	PON serum enzyme activity	S.D.
Mothers- controls	147.77	77.83
Mothers- cases	118	45.54
Neonates- controls	54.74	28.92
Neonates- cases	123.49	68.26

[Table/Fig-6]: Shown here are the levels of enzymatic PON1 activity in the various groups. PON1 enzymatic activity was found to be decreased in mothers of IFGR neonates, while the values were found to be increased in the IFGR neonates. The differences in both mothers and neonates were found to be statistically significant ($p < 0.01$ for both groups).

mL⁻¹ of serum), and this difference was also found to be statistically significant ($p < 0.001$).

Correlation Analyses

Correlation analysis was carried out between foetal weight and serum PON1 levels to determine the trend of change of foetal weight with this parameter and whether, the change was statistically significant or not. There was no statistically significant correlation of maternal PON1 levels with foetal weight ($r = 0.068$; $p = 0.4$) but significant negative correlation of foetal levels of PON1 with foetal weight ($r = -0.195$; $p = 0.016$) was observed.

On statistical analysis, the difference in activity of PON1 was found to correlate significantly with the genotype (R allele resulting in lower activity) in mothers giving birth to IFGR neonates (p -value = 0.042). Although, the pattern of alteration of activity in relation to genotypes was similar in the other three groups, the correlation was statistically not significant (p -value: mothers (control) = 0.1; IFGR neonates = 0.07; neonates (control) = 0.387).

DISCUSSION

The aim of this study was to examine the relationship of IFGR with PON1 gene polymorphism and expression in both neonates and mothers. Our results showed that PON1 Q192R polymorphism is associated with IFGR. We also found a significant impact of PON1 expression in mothers on foetal weight, an important risk marker for IFGR. Considering the distinct ethnic feature of the Indian population, the high prevalence of IFGR in them and the absence of any report on PON1 gene polymorphism in Indian IFGR patients, the findings of this report are of considerable importance.

Genotype Prevalence

In this study, the prevalence of the PON1 Q192R genotype was found to be as 62% (QQ), 34.7% (QR) and 3.3% (RR) in the mother and foetus controls. In a separate study, different distributions of genotypes were found in different populations. The distribution of genotypes in Finnish and American Caucasian population was found to be 50-52% (QQ), 41-44% (QR) and 4-9% (RR) [21]. Thus, the prevalence in our study was similar to American Caucasian and European population. However, the frequencies in Indian population in same study were reported as 47% (QQ), 40% (QR) and 13% (RR)

[21], which were quite different from the other Asian populations. The reported frequencies of PON1 Q192R genotypes in Japanese, Chinese, Malay and American Blacks population were 15-24% (QQ), 35-50% (QR) and 33-42% (RR) [21]. The results of genotype frequency in our study are quite different from the previous reports [21]. This difference is probably because of difference in case selection; while the previous study included subjects from both North and South India, subjects in our study were drawn only from North Indian population, in and around Delhi. The difference is therefore expected, since ethnic variation between North and South Indian population has already been established [22].

In mothers of IFGR neonates, the prevalence of QR genotype was found to be 76% and that of RR genotype was 8%. Mothers having R allele were found to have higher odds' ratio of development of IFGR [Table/Fig-4a]. High prevalence of QR and RR genotype were also associated with low level of PON1 expression and activity. This low level of PON1 activity arising due to genetic polymorphism may be indirectly responsible for development of IFGR. In the neonates also, the presence of R allele was found to be associated more commonly with development of IFGR. No significant report is available regarding PON1 polymorphism in IFGR materno-foetal dyads to compare the result.

Gene Expression

The variation in PON1 expression in the study subjects was estimated by measuring the mRNA levels. The levels between cases and controls were compared for materno-foetal dyads. A decrease in expression levels was found in mothers giving birth to IFGR babies as compared to controls. This lower expression corroborates with the low plasma PON1 activity in mothers of IFGR babies. This is consistent with the accepted knowledge that inflammatory state gives rise to increased Oxidative Stress (OS) and inflammatory state causes decrease in levels of PON1 [23,24] and hints at the possibility that an underlying inflammatory state may be playing a role in IFGR along with the decreased antioxidant capacity of the subjects.

Biochemical Activity

Decreased PON1 levels in the maternal serum can also lead to placental insufficiency. Chen D et al., have postulated that the milieu of increased OS and decreased PON1 levels may lead to formation of microatheromas in the placental microvasculature, leading to blockage of these vessels due to increased oxidation of LDL [11]. This, in turn, will give rise to placental insufficiency leading to IFGR. However, in IFGR babies, an increase in PON1 expression levels was found when compared with controls. This finding can be explained by the influence of adipose content of the body on PON1 levels. Adipose stores in the body are inversely related to the serum PON1 levels; lower the adipose stores of the body, more will be the PON1 levels in the serum [25]. Thus, in FGR neonates, where the body stores of adipose tissue is lower when compared with the controls, the higher PON1 levels in the serum are expected.

While the PON1 activity in IFGR babies was found to be significantly increased, it was found to be significantly decreased in their mothers, as shown in [Table/Fig-6]. This finding is in agreement with the results obtained by the expression study (RT-PCR) and helps to re-establish the presence of higher levels of PON1 enzyme in the IFGR neonates, which may be caused by decreased adipose stores of the body, as discussed previously [25].

Paraoxonase enzyme has been shown to be an important antioxidant in the serum and most of the esterase activity of HDL is ascribed to the PON1 enzyme attached to HDL [26]. It has been definitively shown that the reduction of oxidized LDL is a result of the arylesterase activity of PON1. Also known is the fact that oxidized LDL is an important precursor to plaque formation, which eventually leads to clotting and clogging of vessels. It has been postulated that decreased PON1 activity in mothers may promote blockage

of placental vessels, thus, inducing Ischemia-Reperfusion type of injury in addition to the decreased antioxidant capacity of the body, thus, leading to increased OS and amplification of the effects of its decreased activity [27].

In this study, the activity of PON1 in plasma was significantly decreased in mothers giving birth to IFGR babies. PON1 activity however, was not found to correlate significantly with foetal weight. Similarly, a study involving Korean women with preterm delivery also showed no significant difference in serum PON1 levels [28]. The levels of PON1 activity in the IFGR babies themselves were found to be conversely elevated. PON1 enzymatic activity and foetal weight was found to be negatively correlated ($r = -0.195$, $p = 0.016$). Foetal PON1 enzymatic activity has shown significant increase with increase in OS and decrease in foetal weight.

LIMITATION

The extrapolation of any conclusions from the present study may be insufficient on account of small sample size.

CONCLUSION

In conclusion, the results of the present study demonstrate that pregnant females with mutant variants of Q192R polymorphism associated with low PON1 expression and activity in mothers are at a risk of giving birth to IFGR neonates. Further large, multiethnic studies should be performed to confirm the role of PON1 gene polymorphism on susceptibility to IFGR neonates in pregnant females.

ACKNOWLEDGEMENTS

The authors are thankful to the Indian Council for Medical Research, New Delhi and University College of Medical Sciences, Delhi for providing support in the form of Post Graduate thesis grant and intramural research grant respectively for completion of this work.

REFERENCES

- [1] Wolfe HM, Gross TL. Increased risk to the growth retarded fetus. In: Gross TM, Sokol RJ. (eds). Intrauterine Growth Retardation: a practical approach. Chicago: Year Book Medical Publishers; 1989. Pp.11.
- [2] Campbell S, Thomas A. Ultrasonic measurement of the fetal head to abdomen circumference ratio in the assessment of growth retardation. Br J Obstet and Gynaecol:1977;84(3):165-74.
- [3] Qadir M, Bhutta ZA. Low birth weight in developing countries. In Small for Gestational Age, Karger Publishers, 2009;13:148-62.
- [4] Strauss RS. Adult functional outcome of those born small for gestational age: twenty-six-year follow up of 1970 British birth cohort. JAMA. 2000;283(5):625-32.
- [5] Lundgren EM, Tuvemo T. Effects of being born small for gestational age on long-term intellectual performance. In Best Practice and Research Clinical Endocrinology & Metabolism. 2008;22(3):477-88.
- [6] Chan PYL, Morris JM, Leslie GL, Kelly PJ, Gallery ED. The long term effects of prematurity and intrauterine growth restriction on cardiovascular, renal and metabolic functions. Int J Pediatr. 2010;2010:280402
- [7] Barker DJ. Mothers, Babies and Disease in Later Life. London: British Medical Journal Publishing; 1994.
- [8] Wang X, Zuckerman B, Pearson C, Kaufman G, Chen C, Wang G, et al. Maternal cigarette smoking, metabolic gene polymorphism, and infant birth weight. JAMA. 2002;287(2):195-202.
- [9] Watson AD, Berliner JA, Hama SY, La Du BN, Fault KF, Fogelman AM, et al. Protective effect of high-density lipoprotein associated paraoxonase—inhibition of the biological activity of minimally oxidised low-density lipoprotein. J Clin Invest. 1995;96(6):2882.
- [10] Durrington PN, Mackness B, Mackness MI. Paraoxonase and atherosclerosis. Arterioscler, Thromb, Vasc Biol. 2001;21(4):473-80.
- [11] Chen D, Hu Y, Chen C, Yang F, Fang Z, Wang L, et al. Polymorphism of the paraoxonase gene and risk of preterm delivery. Epidemiology. 2004;15(4):466-70.
- [12] Infante-Rivard C. Genetic association between single nucleotide polymorphisms in the paraoxonase 1 gene and small-for-gestational-age birth in related and unrelated subjects. Am J Epidemiol. 2010;171(9):999-1006.
- [13] Jarvik GP, Hatsukami TS, Carlson C, Richter RJ, Jampsa R, Brophy VH, et al. Paraoxonase activity, but not haplotype utilizing the linkage disequilibrium structure, predicts vascular disease. Arterioscler, Thromb, Vasc Biol. 2003;23(8):1465-71.
- [14] Mogarekar MR, Rojekar MV. Harbingers of neonatal birth weight: The PON1 arylesterase and lactonase activities. Turkish Journal of Biochemistry/Turk Biyokimya Dergisi. 2014;39(1):25-29.
- [15] Lawlor DA, Gaunt TR, Hinks LJ, Davey Smith G, Timpson N, Day IN, Ebrahim S. The association of the PON1 Q192R polymorphism with complications and outcomes of pregnancy: findings from the British Women's Heart and Health cohort study. Paediatr Perinat Epidemiol. 2006;20(3):244-50.
- [16] Wang J, Yang MM, Rong SS, Ng TK, Li YB, Liu XM. Association of paraoxonase gene polymorphisms with diabetic nephropathy and retinopathy. Mole Med Rep. 2013;8(6):1845-51.
- [17] Kordi-Tamandani DM, Hashemi M, Sharifi N, Kaykhaei MA, Torkamanzei A. Association between paraoxonase-1 gene polymorphisms and risk of metabolic syndrome. Mol Biol Rep. 2012;39(2):937-43.
- [18] Liu T, Zhang X, Zhang J, Liang Z, Cai W, Huang M, et al. Association between PON1 rs662 polymorphism and coronary artery disease. Eur J Clin Nutr. 2014;68(9):1029-35.
- [19] Chandra N, Mehndiratta M, Banerjee BD, Guleria K, Tripathi AK. Idiopathic fetal growth restriction: repercussion of modulation in oxidative stress. Indian J Clin Biochem. 2016;31(1):30-37.
- [20] Bansal S, Chawla D, Banerjee BD, Madhu SV, Tripathi AK. Association of RAGE gene polymorphism with circulating AGEs level and paraoxonase activity in relation to macro-vascular complications in Indian type 2 diabetes mellitus patients. Gene. 2013;526(2):325-30.
- [21] Mohamed Ali S, Chia SE. Interethnic variability of plasma paraoxonase (PON1) activity towards organophosphates and PON1 polymorphisms among Asian populations—a short review. Ind Health. 2008;46(4):309-17.
- [22] Majumdar PP, Mukherjee BN. Genetic diversity and affinities among Indian population: an overview. In Human population genetics 1993 (pp. 255-275). Springer US. Pp. 255.
- [23] Van Lenten BJ, Hama SY, de Beer FC, Stafforini DM, McIntyre TM, Prescott SM, et al. Anti-inflammatory HDL becomes pro-inflammatory during the acute phase response. J Clin Invest. 1995;96(6):2758-67.
- [24] Feingold KR, Memon RA, Moser AH, Grunfeld C. Paraoxonase activity in the serum and hepatic mRNA levels decrease during the acute phase response. Atherosclerosis. 1998;139(2):307-15.
- [25] Koncsos P, Seres I, Harangi M, Illyes I, Jozsa L, Gonczi F, et al. Human paraoxonase-1 activity in childhood obesity and its relation to leptin and adiponectin levels. Pediatric Research. 2010;67(3):309-13.
- [26] Litvinov D, Mahini H, Garelnabi M. Antioxidant and anti-inflammatory role of paraoxonase 1: implication in arteriosclerosis diseases. N Am J Med Sci. 2012;4(11):523-32.
- [27] Scifres CM, Nelson DM. Intrauterine growth restriction, human placental development and trophoblast cell death. J Physiol. 2009;587(14):3453-58.
- [28] Lee BE, Park H, Park EA, Gwak H, Ha EH, Pang MG, et al. Paraoxonase 1 gene and glutathione S-transferase μ 1 gene interaction with preterm delivery in Korean women. Am J Obstet Gynecol. 2010;203(6):569-e1.
- [29] Lakshmy R, Ahmad D, Abraham RA, Sharma M, Vempalala K, Das S, et al. Paraoxonase gene Q192R & L55M polymorphisms in Indians with acute myocardial infarction & association with oxidized low density lipoprotein. Indian Journal of Medical Research. 2010;131(4):522-29.
- [30] Parker-Katiraei L, Bousiaki E, Monk D, Moore GE, Nakabayashi K, Scherer SW. Dynamic variation in allele-specific gene expression of Paraoxonase-1 in murine and human tissues. Human Molecular Genetics. 2008;17(21):3263-70.

PARTICULARS OF CONTRIBUTORS:

1. Senior Demonstrator, Department of Biochemistry, University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi, India.
2. Associate Professor, Department of Biochemistry, University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi, India.
3. Ex-Professor, Department of Biochemistry, University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi, India.
4. Professor, Department of Obstetrics and Gynaecology, University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi, India.
5. Professor, Department of Biochemistry, University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Mohit Mehndiratta,
Associate Professor, Department of Biochemistry, University College of Medical Sciences and Guru Teg Bahadur Hospital,
Delhi-110095, India.
E-mail: dnmohitucms@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: May 18, 2017

Date of Peer Review: Jun 26, 2017

Date of Acceptance: Sep 26, 2017

Date of Publishing: Nov 01, 2017