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Original Article

Obstetrics and Gynaecology Section

Comparison of Placental Vitamin D Receptor Target Gene Expression in Idiopathic Foetal Growth Restriction Pregnancy and Gestational Age-matched Healthy Controls: A Case-control Study

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ABSTRACT

Introduction: Foetal Growth Restriction (FGR) is a common pregnancy complication that affects up to 10% of pregnancies and is associated with significant morbidity and mortality in the perinatal period and infancy. Despite extensive investigations, a definite etiology cannot be found in up to 70% of cases of FGR, which are termed idiopathic. One of the causative factors responsible for idiopathic FGR could be altered placental expression of Vitamin D Receptor (VDR) genes.

Aim: To estimate placental VDR target gene expression in pregnancies affected by idiopathic FGR and to compare it with gestational age-matched healthy pregnant women.

Materials and Methods: A case-control study was conducted in the Department of Obstetrics and Gynaecology, University College of Medical Sciences and GTB Hospital, New Delhi, India, from November 2019 to October 2021. All pregnant women ≥28 weeks gestation with FGR were evaluated to determine the etiology. Those with a negative work-up were defined as having idiopathic FGR (cases, n=30). Gestational age-matched healthy pregnant women were enrolled as controls (n=30). A maternal blood sample for Vitamin D levels was taken before delivery. After delivery, a small portion of the placenta was collected for Ribonucleic Acid (RNA) extraction to study the relative gene expression of VDR and the downstream target gene in the VDR pathway, Transforming Growth Factor Beta 3 (TGFβ3). Statistical tests used in the study included the Independent t-test and Analysis of Variance (ANOVA) for quantitative variables and the Chi-square test or Fisher's exact test for qualitative variables. The Pearson's correlation coefficient was used to correlate maternal Vitamin D level (ng/mL) with VDR messenger RNA (mRNA) expression fold change and TGFβ3 mRNA expression fold change. Univariate logistic regression was used to calculate the odds ratio. A p-value of <0.05 was considered significant. The software used for statistical analysis was Statistical Package for Social Sciences (SPSS) software version 21.0.

Results: Both groups were comparable in demographic characteristics such as age, socio-economic status, parity and Body Mass Index (BMI). The mean value of maternal Vitamin D levels in idiopathic FGR was 15.73±7.65, compared to 20.01±5.27 in controls. The majority of the patients in our study had Vitamin D deficiency (<20 ng/mL), with 70% of cases and 60% of controls. The mean VDR mRNA Δct value in cases was higher compared to controls (5.09±0.86 vs. 4.53±1.03, p-value=0.027), implying reduced VDR mRNA gene expression in cases. The mean TGFβ3 mRNA Δct value was 8.11±1.81 in cases and 6.73±1.64 in controls (p-value=0.003), suggesting reduced TGFβ3 mRNA gene expression in cases. Maternal Vitamin D levels were not found to have any correlation with VDR mRNA and TGFβ3 mRNA expression (correlation coefficients 0.008 and 0.194, respectively).

Conclusion: Women with idiopathic FGR had reduced placental VDR and TGFβ3 mRNA expression. However, no correlation was found with maternal Vitamin D levels, suggesting defects in the placental VDR pathway as an etiological factor in FGR.

Keywords: Gene expression, Placenta, Transforming growth factor beta 3, Vitamin D deficiency

INTRODUCTION

The FGR is defined as the inability of a foetus to maintain expected growth, with an estimated foetal weight or actual birth weight below the 10th percentile for gestational age, along with evidence of placental dysfunction in the form of oligohydramnios or abnormal Doppler studies. FGR is a common pregnancy complication that affects up to 10% of pregnancies and is associated with significant morbidity and mortality in the perinatal period and infancy [1]. Furthermore, FGR has potential lifelong consequences for an infant, including impaired neuropsychological development, reduced intelligence quotient and a greater risk of chronic adulthood diseases like hypertension, ischaemic heart disease and diabetes [2]. It is important to establish the etiology of FGR because of the high incidence of recurrence; however, despite extensive investigations, a definite etiology cannot be found in up to 70% of cases of FGR, which are termed idiopathic [3].

Foetoplacental growth requires tight regulation of cell cycle gene expression. Vitamin D is a secosteroid hormone with pleiotropic effects that extend far beyond calcium homeostasis and bone metabolism. These effects include cellular proliferation, differentiation and immune response modulation [4]. Vitamin D exerts its physiological effects by binding to its nuclear receptor, VDR. VDR can mediate both transcriptional and non transcriptional effects. In the transcriptional pathway, 1,25(OH)2D-bound VDR heterodimerises with a partner receptor, Retinoid X Receptor (RXR). This VDR-RXR complex binds to the vitamin D Response Element (VDRE) and further regulates the transcription of vitamin D target genes. The non transcriptional pathway involves the calcitriol-VDR complex binding with caveolae, stimulating numerous signaling cascades such as protein kinase C and mitogen-activated protein kinase, which play important roles in cellular proliferation, differentiation, invasion and apoptosis, thereby highlighting the modulatory effects of vitamin D [2]. Altered VDR

expression is associated with several health concerns, but the role of VDR and vitamin D signaling in pregnancy is poorly understood [2].

Vitamin D plays an important role in maintaining the health of both the mother and foetus during pregnancy. A deficiency of vitamin D has been linked to several complications associated with placental insufficiency, including FGR [5]. Sufficient vitamin D intake during pregnancy has been shown to reduce these complications and has beneficial effects on foetal birth weight [6]. Therefore, optimal maternal 25-hydroxy levels are believed to be beneficial to the health of both the mother and foetus. However, no consensus has been reached regarding the biological actions of vitamin D in perinatal outcomes [7]. The placenta expresses the VDR, which is involved in vitamin D signaling, suggesting that vitamin D functions in a tissue-specific manner at the foetal-maternal interface. Reduced placental expression of VDR may be a contributing factor to the pathology of idiopathic FGR-affected pregnancies [2].

Nguyen TP et al., reported reduced VDR expression and decreased mRNA and protein expression of TGFβ3 in FGR placentae [3]. Reduced TGFβ3 may cause changes in VDR signaling, which may directly or indirectly contribute to placental insufficiency.

Despite low maternal vitamin D levels, not all foetuses are growth-restricted. Similarly, even with normal vitamin D levels, some foetuses may be growth-restricted. Therefore, the authors proposed that alterations in the genes involved in the vitamin D metabolic pathway in the placenta may be the causative factor responsible for idiopathic FGR.

If this hypothesis is correct, methods for detecting placental VDR expression may have clinical utility in treating FGR. Appropriate therapeutic interventions may be explored in the future once we determine the etiology of idiopathic FGR. Further clarification of the biological mechanisms by which placental vitamin D metabolism contributes to FGR will enhance our understanding of the molecular mechanisms underlying FGR and may also provide novel approaches to intervention. With this hypothesis and background, the present study was conducted with the aim of estimating placental VDR target gene expression in idiopathic FGR and comparing it with gestational age-matched low-risk pregnancies.

MATERIALS AND METHODS

The present case-control study was conducted in the Department of Obstetrics and Gynaecology, University College of Medical Sciences and GTB Hospital, New Delhi, India, from November 2019 to October 2021. The present study was conducted in accordance with the ethical standards of the Institutional Ethical Committee (IEC-HR/2019/41/75, dated 16/10/2019). Written informed consent was obtained from all the study participants.

Sample size: During the study period, 250 patients with FGR were recruited. After an elaborate work-up that included a detailed history, examination and blood investigations, no etiology could be found in 83 patients and they were labeled as having Idiopathic FGR. A total of 53 patients refused to participate; hence, 30 patients were recruited as cases.

Inclusion criteria: All pregnant women between 20 years and 34 years of age (spontaneous conception, singleton), ≥28 weeks of gestation with Ultrasound (USG)-diagnosed FGR, defined as Estimated Foetal Weight (EFW) <10th percentile for that gestational age, accompanied by one or more of the following features: abnormal umbilical artery Doppler flow velocimetry defined as Umbilical Artery Pulsatility Index (UA-PI) >95th centile or Cerebroplacental Ratio (CPR) <5th centile, or oligohydramnios defined as Amniotic Fluid Index (AFI) <5, were considered for the study. Severe FGR was defined as EFW <3rd percentile for that gestational age.

Exclusion criteria: Pregnant women with hypertensive disorders of pregnancy, diabetes mellitus, chronic hypertension, a history of chronic medical disorders (renal disease, jaundice, epilepsy), multifoetal pregnancy, prolonged Premature Rupture of Membranes (PROM), smoking, alcohol and drug abuse, or placental abruption were excluded. Additionally, if any of the following investigations were abnormal: Oral Glucose Tolerance Test (OGTT), Venereal Disease Research Laboratory (VDRL), Toxoplasmosis, Rubella, Cytomegalovirus, Herpes simplex virus- Immunoglobulin M (TORCH IgM), liver function tests, kidney function tests, or Antiphospholipid Antibody (APLA) work-up (including lupus anticoagulant and anticardiolipin antibodies), or if a foetal anomaly was detected on Ultrasonography (USG), those patients were also excluded.

Gestational age-matched pregnant women with no obstetrical complications until delivery were enrolled as controls.

Study Procedure

Patients were managed according to the hospital protocol for FGR. A 5 mL maternal blood sample was taken prior to delivery for the measurement of vitamin D (25OH) levels for both cases and controls using radioimmunoassay. After delivery, the placentas of all the participants were grossly examined and a small portion of the maternal side of the placenta was stored in phosphate buffer solution at -80°C for RNA extraction to study the relative gene expression of VDR and TGFβ3 in the VDR pathway. The placenta was stored in 10% formalin and sent for histopathological analysis. Total RNA was isolated using TRIzol reagent (Thermo Scientific TRIzol reagent as per the manufacturer's instructions). The RNA concentration and purity were estimated using NanoDrop™. RNA quality was assessed by the Agilent 2100 Bioanalyser. The authors selected the target gene (TGFβ3) in the VDR pathway based on a similar study conducted earlier [3] with a similar sample profile. TGFβ3 was further validated by real-time RT-PCR.

Reverse transcription was carried out using the USA Verso cDNA synthesis kit on the isolated RNA sample. Reverse transcription was conducted in a thermal cycler at 42°C for 30 minutes and 92°C for two minutes (to inactivate the reverse transcriptase).

Reverse transcription and real time Polymerase Chain Reaction (PCR) assay: Real-time PCR was performed in a mixture containing 10 μL of SsoFast™ EvaGreen® supermix with forward and reverse primers (1 μL each), cDNA (1 μL) and 9 μL of nuclease-free water. The PCR reaction was carried out for VDR and TGFβ3 and the melt curve was analysed at 55°C to 95°C with an increment of 0.5° in the thermocycler. Primers for each gene were synthesised by SahaGene (Biological Research India), with the sequences provided in the table below. Each sample was run in duplicates. The relative gene expression (fold change) levels were determined by the comparative ΔΔCt method after normalisation to the expression level of Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH).

Primer sequence **5′→3′**

VDR	Forward Reverse	ATCTGGGAAATGGGCTCGG TTCTGTCCCCTGCTTCTCTCTC
TGFβ3	Forward Reverse	AGACCTCACAGAAGAGCACC CCATTGCCTCCATCCCTGAA
GAPDH	Forward Reverse	CCAAGGTCATCCATGACAACTTTGGT TGTTGAAGTCAGAGGAGACCACCTG

The placenta of all patients was sent for histopathological examination. At the time of delivery, the birth weight of the baby was recorded. Neonatal outcomes were noted in terms of Neonatal Intensive Care Unit (NICU) admission, length of NICU stay (number of days), sepsis, neonatal morbidity and mortality.

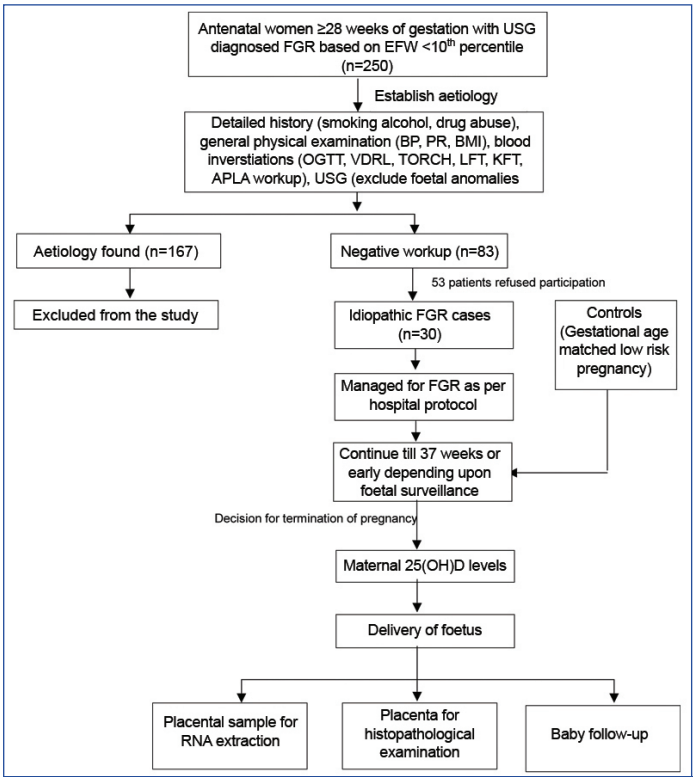
STATISTICAL ANALYSIS

Quantitative variables were compared using an independent t-test (for two groups) and ANOVA to assess the association of quantitative variables with vitamin D levels. Qualitative variables were compared using the Chi-square test or Fisher's exact test. The Pearson's

correlation coefficient was used to evaluate the correlation between maternal vitamin D levels (ng/mL) and the fold change in VDR mRNA expression, as well as, the fold change in TGFβ3 mRNA expression. Univariate logistic regression was employed to calculate the odds ratio for VDR mRNA ΔCt values and TGFβ3 mRNA ΔCt values. A p-value of less than 0.05 was considered significant. The software used for statistical analysis was SPSS software version 21.0.

RESULTS

The overall incidence of idiopathic FGR in the present study was 33.2% [Table/Fig-1]. Both groups were comparable concerning demographic characteristics such as age, socio-economic status, parity and BMI [Table/Fig-2].



[Table/Fig-1]: Study flowchart.
n=cases (Idiopathic FGR), N=controls (healthy gestational age matched low risk pregnancy)

Demographic characteristics	Cases (n=30)	Controls (n=30)	p-value
Age (years) (mean±SD)	24.30±3.41	23.13±2.15	0.119
Socio-economic status, n (%)			
Lower/Lower middle	14 (46.67)	14 (46.67)	1.000
Upper lower	16 (53.33)	16 (53.33)	
Parity, n (%)			
Nullipara	(46.7)	17 (56.7)	0.597
Multipara	16 (53.3)	13 (43.3)	
Mean BMI (kg/m^2)	21.96±1.01	22.69±0.89	0.004*
Education level, n (%)			
Illiterate	6 (20)	4 (13.33)	0.952
Primary school	12 (40)	11 (36.67)	
Middle school	6 (20)	7 (23.3)	
High school	6 (20)	8 (26.67)	

[Table/Fig-2]: Demographic profile of cases and controls.
BMI: Body mass index; Independent t-test, Fisher's exact test used; *The p-value <0.05 was considered statistically significant

The mean birth weight in the FGR group was 2.08±0.28 kg, which was significantly lower compared to 2.71±0.15 kg in the controls (p-value <0.001). The majority of patients delivered vaginally. A caesarean section was necessary for seven patients in the FGR group: one patient at 30 weeks 0 days had severe FGR with Absent End-diastolic Flow (AEDF) and a non reassuring Non Stress Test (NST), while another patient at 36 weeks 2 days presented with breech presentation, anhydramnios and absent foetal movements. Additionally, five patients developed foetal distress and underwent Lower Segment Caesarean Section (LSCS). In the control group, the indications for instrumentation were poor maternal bearing efforts and foetal distress in the second stage [Table/Fig-3].

Delivery and neonatal outcomes	Cases n=30	Controls n=30	p-value
Gestational age at delivery (weeks) (mean±SD)	36.57±2.00	39.00±0.15	0.021*
Birth weight (kg) (mean±SD)	2.08±0.28	2.71±0.15	<0.0001*
Mode of delivery, n (%)			
Normal vaginal delivery	23 (76.67)	28 (93.3)	0.005*
Instrumental delivery	0	2 (6.7)	
Caesarean section	7 (23.33)	0	
NICU admission	22 (73.33)	8 (26.67)	0.0003*
Duration of NICU stay (days) (days) (mean±SD)	3.2±2.455	0.97±1.752	0.0002*
Neonatal morbidity	22 (73.33)	8 (26.67)	0.0003*
Sepsis	4 (13.33)	0	0.112
Neonatal mortality	1 (3.33)	0	1

[Table/Fig-3]: Delivery details and neonatal outcomes amongst cases and controls.
NICU: Neonatal intensive care unit; Independent t-test, Fisher's exact test, Chi-square test used

All the pregnancies affected by FGR could be continued to term with regular foetal surveillance, except in seven cases where pregnancies were terminated at 34 weeks due to the development of AEDF in the foetuses. Among the cases, 22 babies experienced neonatal morbidities: nine developed Respiratory Distress Syndrome (RDS), four had sepsis and hypoglycaemia and jaundice were observed in 13 and 18 cases, respectively; three developed hypothermia and were admitted to the Neonatal Intensive Care Unit (NICU), compared to eight babies in the control group who required NICU admission. One baby in the FGR group died in the NICU due to sepsis leading to multiple organ involvement [Table/Fig-3].

The mean placental weight in the FGR group was 420±23.76 g, which was significantly less than 459.33±38.05 g in the control group (p-value <0.0001) [Table/Fig-4].

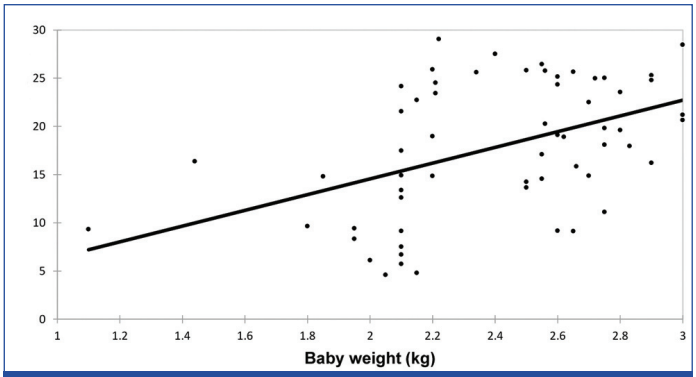
Histopathology	Cases n (%)	Controls n (%)
Placental weight (grams) (Mean±SD)	420±23.76	459.33±38.05
Normal	11 (36.67)	24 (80)
Villous infraction	15 (50)	4 (13.33)
Placental vascular thrombosis	6 (20)	0
Villous fibrosis	5 (16.67)	2 (6.67)
Perivillous/villous fibrin deposition	9 (30)	3 (10)
Villitis	4 (13.33)	1 (3.33)
Avascular/hypovascular villi	8 (26.67)	0

[Table/Fig-4]: Placental histopathology among cases and controls.

The mean maternal vitamin D levels in idiopathic FGR cases were 15.73±7.65 ng/mL compared to 20.01±5.27 ng/mL in controls (p-value=0.014). Approximately 21 (70%) of cases and 18 (60%) of controls had vitamin D deficiency.

When correlating neonatal birth weight with maternal vitamin D levels, the Pearson's correlation coefficient was found to be 0.456, suggesting a positive correlation between neonatal birth weight and maternal vitamin D levels. This correlation was statistically significant with a p-value of 0.0002 [Table/Fig-5].

The mean VDR mRNA Δct value in cases was 5.09±0.86, compared to 4.53±1.03 in controls (p-value=0.027) [Table/Fig-6]. The higher Δct value implies reduced VDR mRNA expression; therefore, VDR mRNA expression was lower in cases compared to controls in our



[Table/Fig-5]: Correlation of baby birthweight (kg) with maternal vitamin D level (ng/mL).

Variables	Cases (Mean±SD)	Controls (Mean±SD)	p-value
VDR mRNA Δct value	5.09±0.86	4.53±1.03	0.027*
TGFβ3 mRNA Δct value	8.11±1.81	6.73±1.64	0.003*

[Table/Fig-6]: Vitamin D Receptor (VDR) mRNA distribution in cases and controls. *Independent test

study. The odds ratio was 1.915, suggesting that an increase of one unit in the VDR mRNA Δct value implies a decrease in VDR mRNA expression, which increases the risk of the foetus being affected by FGR by twofold.

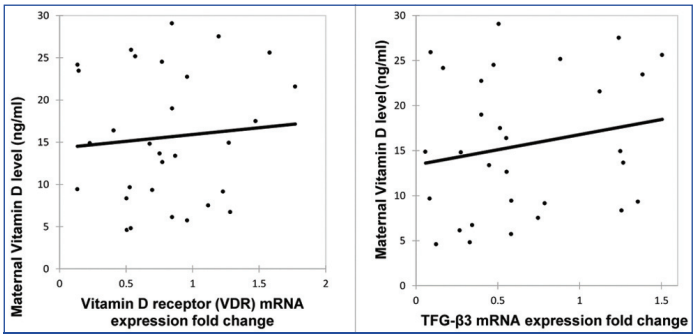
The mean TGFβ3 mRNA Δct value was 8.11±1.81 in cases, compared to 6.73±1.64 in controls (p-value=0.003), implying significantly reduced TGFβ3 mRNA gene expression in placental tissue in cases compared to controls [Table/Fig-6]. The odds ratio was 0.642, suggesting that an increase of one unit in the TGFβ3 mRNA Δct value implies a decrease in TGFβ3 mRNA expression and the risk of the foetus being diagnosed with FGR increases by one fold.

In the comparison of VDR mRNA expression between cases and controls, 73.33% of cases had downregulation of gene expression, whereas 26.67% had upregulation of gene expression. Similar findings for TGFβ3 mRNA expression were observed between cases and controls [Table/Fig-7].

Gene expression	Frequency (n)	Percentage (%)
Vitamin D receptor mRNA expression		
Downregulation (<1)	22	73.33%
Upregulation (≥1)	8	26.67%
TGFβ3 mRNA expression		
Downregulation (<1)	22	73.33%
Upregulation (≥1)	8	26.67%

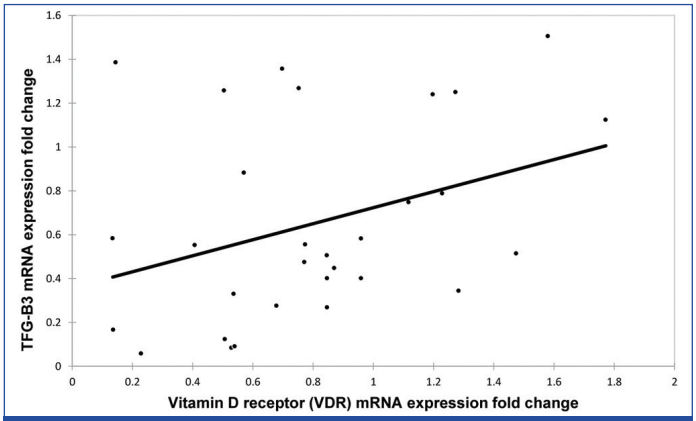
[Table/Fig-7]: Vitamin D Receptor (VDR) mRNA expression and TGFβ3 mRNA expression in cases vs controls.

When correlating maternal vitamin D levels with VDR mRNA expression and TGFβ3 mRNA expression using the Pearson's correlation coefficient, no significant correlation was found, with correlation coefficients of 0.008 and 0.194, respectively [Table/Fig-8].



[Table/Fig-8]: Correlation of maternal Vitamin D level (ng/mL) with: a) Vitamin D Receptor (VDR) mRNA expression fold change; b) TFG-β3 mRNA expression fold change.

However, when correlating VDR mRNA expression with TGFβ3 mRNA expression, the Pearson's correlation coefficient was found to be 0.348, indicating a weak positive correlation. This correlation was not statistically significant, with a p-value of 0.060 [Table/Fig-9].



[Table/Fig-9]: Correlation of Vitamin D Receptor (VDR) mRNA expression (fold change) with TFG-β3 mRNA expression (fold change).

DISCUSSION

The overall prevalence of idiopathic FGR cases in the present study was 33.2%. The mean maternal vitamin D levels were significantly lower in cases compared to controls. The majority of patients in our study had vitamin D deficiency (<20 ng/mL), with 70% of cases and 60% of controls. The rest of the patients were vitamin D insufficient (20-29 ng/mL).

Goswami R et al., measured serum 25(OH) vitamin D in apparently healthy subjects and showed that significant hypovitaminosis D was present in up to 90 percent of them [8]. Further studies from different parts of our country have pointed towards widespread vitamin D deficiency in Asian Indians of all age groups residing in rural and urban areas [9].

There was a positive correlation between neonatal birth weight and maternal vitamin D levels in the current study. Robinson CJ et al., and Fernando M et al., reported a positive correlation between maternal 25(OH)D and neonatal birth weight [5,10]. However, Eggemoen AR et al., conducted a longitudinal, multi-ethnic population-based study and measured maternal 25(OH) vitamin D levels [11]. They found that 51% of pregnant women in early pregnancy were vitamin D deficient, but there was no independent relation between maternal vitamin D levels and any of the neonatal anthropometric measures, including birth weight, abdominal circumference and head circumference.

In the present study, all the mothers had low serum vitamin D levels, including the controls, but not all the foetuses were growth-restricted. Therefore, low maternal vitamin D levels alone might not be responsible for growth-restricted foetuses. There may be other mechanisms operational within the placenta itself that lead to FGR. One such proposed mechanism is the placental VDR pathway.

The VDR mRNA expression was decreased in idiopathic FGR compared to controls in the present study. This finding was similar to a study by Nguyen TP et al., who reported a decrease in placental VDR mRNA expression in cases and suggested that decreased placental VDR expression alters the expression of cell-cycle genes in FGR placentae [3].

Nguyen TP et al., studied VDR mRNA and protein expression in placental samples of women with idiopathic FGR and controls using PCR, immunohistochemistry and immunoblotting [7]. They found significantly decreased VDR mRNA expression (p-value=0.0005) in women affected by idiopathic FGR and concluded that VDR is an important regulator of trophoblast functions; decreased VDR expression in the placenta may be a cause or a consequence of the pathophysiological defects observed in FGR-affected placentae.

They also demonstrated that when VDR mRNA was reduced following siRNA treatment, an increased syncytium formation was

observed compared to cells that were treated with non targeted siRNA control. These observations suggested that VDR inactivation influenced larger syncytium formation. They further demonstrated that reduced VDR also significantly increased apoptosis when measured using TP53 mRNA as a marker of apoptosis.

Therefore, a decrease in placental VDR expression may impair its actions and limit the beneficial effects of maternal vitamin D in the regulation of foetoplacental growth, thus playing a role in the pathophysiology of idiopathic FGR.

Gene TGFβ3 is one of the downstream target genes of VDR. In the present study, TGFβ3 mRNA gene expression was found to be reduced in placental tissue obtained from pregnancies affected by idiopathic FGR compared to controls.

Nguyen TP et al., reported that TGFβ3 mRNA expression was significantly reduced by 46% in cases compared to controls [3]. Additionally, the protein expression of TGFβ3 showed a significant reduction of 64% in FGR placentas compared to controls.

Decreased TGFβ3, caused by changes in VDR signaling, may directly or indirectly contribute to placental insufficiency by causing uncontrolled cell proliferation. This, in turn, may lead to impaired ion and nutrient exchange, as well as decreased synthesis of hormones required for foetoplacental growth.

We found no correlation between maternal vitamin D levels and VDR mRNA or TGFβ3 mRNA expression, but we did find a weak positive correlation between VDR mRNA expression and TGFβ3 mRNA expression. This suggests that placental expression of the VDR pathway is independent of maternal vitamin D levels. Therefore, even if vitamin D levels are low, the normal expression of VDR pathway genes, as seen in controls, may mean that foetuses are not affected.

Our study is supported by the findings of Young BE et al., who found that placental VDR expression was not significantly related to maternal 25(OH)D and the negative association approached significance (p-value=0.080), while a positive association was observed with neonatal 1,25(OH)2D (p-value=0.006) [12]. They concluded that placental VDR may play a role in transplacental calcium transfer and foetal bone development. All these findings suggest that there is a definite and significant relationship between placental VDR and TGFβ3 mRNA expression, as well as, the likelihood of idiopathic FGR.

Limitation(s)

The limitations of the present study include a small sample size. The authors collected the placental tissue at the time of delivery, which did not allow us to infer the dynamic changes of VDR throughout pregnancy. Additionally, the authors did not study placental vitamin D levels, so they could not analyse the correlation between maternal and placental vitamin D levels.

CONCLUSION(S)

The association of VDR mRNA expression and TGFβ3 mRNA expression with idiopathic FGR supports the hypothesis that reduced TGFβ3 contributes to the pathogenesis of idiopathic FGR. The present study provides evidence that the placental expression of the VDR pathway is independent of maternal vitamin D levels. Therefore, further intervention strategies that target the placenta might alleviate altered foetal growth by improving placental vitamin D metabolism. The establishment of the pathogenesis of idiopathic FGR may open new avenues for the early prediction of FGR, leading to adverse neonatal outcomes and thereby allowing for timely interventions to prevent such outcomes.

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Authors' contribution: RA: Conceived and designed the study, developed the data collection instruments, performed the statistical analysis and wrote first version of the manuscript. PS: Participated in data collection, PS, BDB and PG: Participated in data interpretation and analysis. PS: Designed the study and drafted the report. BDB: Carried out the measurement of various biochemical markers used in the study. PG: Carried out the placental histopatholgical examination. RA, RS, PS and PG: Critically reviewed, revised and approved the final manuscript.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Aug 03, 2024
- Manual Googling: Nov 30, 2024
- iThenticate Software: Dec 02, 2024 (18%)

ETYMOLOGY: Author Origin

EMENDATIONS: 6

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

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