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Unraveling the Complex Etiology of Intrauterine Growth Restriction: An Exploratory Factor Analysis Approach

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ABSTRACT

OBJECTIVE: To identify latent constructs within maternal clinical, biochemical, and molecular data using exploratory factor analysis (EFA) to evaluate their relationship with Intrauterine Growth Restriction (IUGR).

METHODOLOGY: A case-control study was conducted among 150 pregnant women at tertiary care hospitals in Lahore, Pakistan, from 15/04/2024 to 10/12/2024. Clinical, anthropometric parameters, and serum levels of oxidative stress, angiogenic, and inflammatory markers were evaluated. EFA was applied in RStudio to extract latent factors and reduce dimensionality. Correlation, regression, and model fit indices (CFI, TLI) were used to identify significant contributors to IUGR.

RESULTS: The study revealed five significant factors: maternal clinical and biochemical indicators; oxidative stress and inflammation; reproductive and obstetric history; vitamin cofactors; and antioxidants by EFA. The highest positive correlation with IUGR was found between oxidative stress and inflammation ($p < 0.001$), followed by maternal clinical indicators ($p < 0.001$). A preventive function was shown by the substantial negative correlation ($p < 0.001$) between antioxidants and IUGR. The robustness of the factor model was confirmed by the model fit indices (CFI = 0.92, TLI = 0.89).

CONCLUSION: The inflammatory and oxidative stress pathways are fundamental etiology of IUGR, while maternal clinical health and antioxidant significantly influence fetal growth. The targeted screening of these domains may improve early detection and risk stratification in pregnancies complicated by IUGR.

KEYWORDS: Intrauterine Growth Restriction, IUGR, FGR, Factor Analysis, Oxidative stress, Biomarkers, EFA.

INTRODUCTION

Intrauterine growth restriction (IUGR) is one of the global health concerns that leads to perinatal morbidity and mortality[1]. IUGR is characterized by a fetus not reaching its full growth due to inadequate nutrient and oxygen supply, while some fetuses are constitutionally small[1]. Other than the perinatal period, IUGR increases the risk of chronic diseases such as cardiovascular disease, diabetes, and chronic kidney disease in adults[2]. The prevalence of IUGR is notably high in developing countries, affecting approximately 23% of pregnancies[1]. The highest burden of IUGR complications is borne by Asia, with Pakistan ranking 3rd among the affected nations that contribute to around 20 to 24% of live births with complications and mortality[3].

The factors of IUGR or fetal growth restriction (FGR) are categorized into three groups: maternal, fetal, and placental factors[4]. FGR is indicated when birth weight is below the 10th percentile for gestational age. FGR is largely caused by maternal diseases such as cellular stress, malnourishment, hypertension, diabetes, infections, and anomalies of the placenta, like infarctions and malfunction[5]. The disease is made worse by prenatal abnormalities and genetic problems[6]. Environmental exposures, poor prenatal care, and socioeconomic variables exacerbate these risks[7]. Although our understanding of FGR has increased due to advances in obstetric care, current diagnostic methods remain insufficient[7]. Ultrasound is the gold standard for FGR identification, but due to its limitations, around 50% of cases remain undiagnosed[9]. Fetal heart rate monitoring and Doppler ultrasound are useful tools for evaluating fetal health. Still, a large percentage of FGR patients remain unnoticed, which raises the risk of stillbirth and premature birth[8].

Developing successful preventive and therapeutic methods requires an understanding of the intricate relationships among maternal, fetal, and placental variables. This study aims to identify early detection methods by investigating IUGR contributors, including maternal factors, biochemical parameters, inflammatory biomarkers, antioxidant enzyme profiles, and growth factors. Biomarkers were identified by integrating molecular, biochemical and clinical datasets. Findings from this study will enhance understanding of endothelial dysfunction's impact on fetal development, guiding therapeutic strategies to improve perinatal outcomes.

METHODOLOGY

Study design

A case-control study was conducted at tertiary care hospitals in Lahore, Pakistan (Ethical approval No. Ref-IMBB/BBBC/24/364-B) from 15/04/2024 to 10/12/2024 following the principles of the Declaration of Helsinki. The sample size was calculated based on an incidence rate of 5%.

Inclusion and exclusion criteria

This study included pregnant women with singleton pregnancies who delivered at tertiary care hospitals in Lahore, Pakistan. To ensure diagnostic accuracy and avoid challenges associated with extreme prematurity, only women in their third trimester (31 to 40 weeks of gestation) and able to provide informed consent were included in this study. Women with known congenital anomalies, chromosomal abnormalities, or multifetal gestations and not able to provide informed consent were excluded. Further, to isolate the effects of IUGR, participants with pre-existing comorbidities, including diabetes, chronic hypertension, and a history of smoking, were excluded from the study.

Participants selection

A total of 300 subjects were assessed for eligibility. Before the start of the research, informed consent was obtained, and the purpose and procedure were also explained. Two hundred and sixty participants provided informed consent to participate. However, 106 participants were disqualified because they met the exclusion criteria, and another 4 later refused to participate. A total of 150 participants, including 75 diagnosed with intrauterine growth restriction (IUGR) and 75 age-matched healthy females aged 18–40 years in the third trimester (31–40 weeks) of pregnancy, were enrolled in the study through non-random convenience sampling (**Figure 1**). Groups were primarily matched by maternal age (18–40 years) and gestational age (31–40 weeks) to ensure a consistent physiological window during the third trimester. All participants were recruited from the same tertiary care hospital in Lahore, further minimizing demographic and environmental variance between the two cohorts.

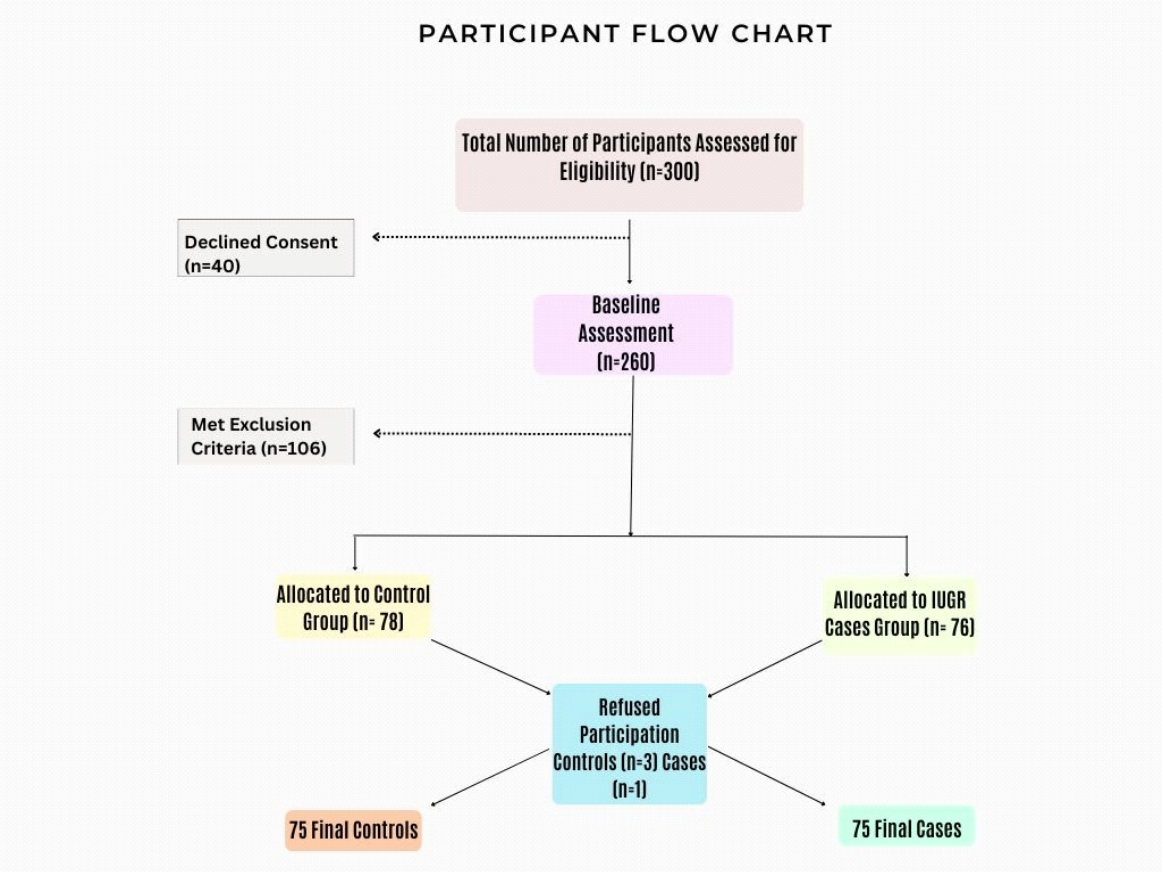

Figure 1: The selection of participants throughout the study is shown.

Sample size

The sample size was determined using the standard estimation formula based on an incidence rate of 5% as reported by **Aftab et al.**[10] With a 95% confidence level (Z = 1.96) and a 5% margin of error, the minimum required sample size was calculated as 73. Formulas are given below. To account for potential data loss and ensure robust analysis, we enrolled 75 participants per group, for a total of 150 subjects. This sample size is adequate for Exploratory Factor Analysis (EFA), as factor stability is primarily driven by the strength of the factor structure and high communalities rather than by a strict participant-to-variable ratio (**MacCallum et al., 1999**).

$$n = \frac{Z^2 P(1-P)}{(d)^2}$$

$$n = \frac{1.96^2 \; X \; 0.05(1-0.05)}{(0.05)^2}$$

Anthropometric and Clinical Assessment

Maternal anthropometric data, including pre-pregnancy weight, height, and Body Mass Index (BMI; kg/m²), were retrieved from medical records. Clinical assessment encompassed maternal and obstetric history (age, age at conception, gravidity, parity, and previous IUGR or abortions) alongside socio-economic status. IUGR was specifically defined as an estimated fetal weight below the 10th percentile for gestational age. Gestational age was strictly verified using the last menstrual period (LMP) and confirmed by ultrasonography between 31 and 40 weeks. Hemodynamic and laboratory profiles, including blood pressure, complete blood counts, and

renal, liver, and coagulation function tests, were extracted from hospital records. Fetal growth was monitored via Doppler-derived umbilical artery pulsatility indices (PI) and staged using WHO centile charts.

Biochemical Assessments

Enzymatic assays for Glutathione (GSH), Catalase (CAT), Superoxide Dismutase (SOD), Glutathione Reductase (GR), Glutathione Peroxidase (GPx), and Nitric Oxide (NO) were quantified via UV-Vis spectrophotometry, recording colorimetric changes at wavelengths between 412 nm and 560 nm. Homocysteine was uniquely measured using fluorometry (Em/Ex = 658/708 nm). To ensure high reproducibility and precision, all spectrophotometric measurements were performed in triplicate, maintaining a calculated intra-assay coefficient of variation (CV) of less than 5%.

Biomarker Assessments:

Ten milliliters of venous blood was collected using aseptic techniques, with 5 mL processed in non-coated serum-separator tubes and 5 mL in EDTA tubes. The 8-hydroxy-2-deoxyguanosine (8-OHdG) (Catalog No. ab201734), asymmetric dimethylarginine (ADMA) (Catalog No. ALX-850-323-KI01), Vitamin D (25(OH)) (Catalog No. ab213966), Vitamin B6 (Catalog No. EA0132Hu), Vitamin B9/Folic Acid (Cat.No. E-EL-0009), Vitamin B12 (Catalog No. ABIN6960490), and homocysteine (Catalog No. ab228559) kits were purchased from Abcam (UK), Enzo Life Sciences (USA), Elabscience (China), and FineTest (China). All biomarker concentrations were quantified strictly according to the manufacturers' standardized protocols. The optical density (OD) for each assay was measured by UV-Vis spectrophotometry at the wavelengths specified by the manufacturers. ELISA assays were performed in duplicate, maintaining intra-assay coefficients of variation (CV) below 8% and inter-assay CV below 10%, ensuring high precision and reliability across the 150 clinical samples.

Gene Expression:

Expression analysis was performed on genes involved in angiogenesis (*sFlt-1, PlGF,* and *VEGFA*) and those involved in inflammation and apoptosis (*IL-6, NFκB*, and *TNF-α*). Total RNA was extracted from isolated peripheral blood mononuclear cells (PBMCs) within three hours of collection using the Thermo Scientific GeneJET RNA Purification Kit (Cat. No. K0731), with purity verified by a Nanodrop One (A260/A280 ratio of 1.8–2.0). The primers used in this study are listed in **Table I**. Genomic DNA was removed using DNase I treatment, and 1 µg of purified RNA was reverse-transcribed into cDNA using the RevertAid First Strand cDNA Synthesis Kit with random hexamer primers. Quantitative PCR (qPCR) was performed using SYBR Green Master Mix (Thermo, USA) on a Real-Time PCR system with 40 cycles of denaturation (94°C for 5s), annealing (60°C for 30s), and extension (72°C for 30s). Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method, normalized to the geometric mean of three housekeeping genes (*GAPDH, -actin*, and *18S rRNA*) to ensure high normalization accuracy. To ensure high technical precision and reproducibility, all Real-Time PCR reactions for mRNA expression were performed in triplicate as specified in our protocol.

Statistical Analysis

After data collection, the variables were categorized into nominal groups, including oxidative stress markers, angiogenic growth factors, and inflammatory markers, and exploratory factor analysis (EFA) was conducted to identify underlying factors that explain the correlation among a set of observed variables. Factor analysis was evaluated using several diagnostic tests in the RStudio software (version 2024.09.1).

Pre-EFA diagnostics

Before performing the factor analysis, the dataset was evaluated for suitability through three primary diagnostic tests. The Kaiser-Meyer-Olkin (KMO) test was utilized to assess sampling adequacy. A KMO value of 0.87 was obtained, which is considered "good" for stable factor extraction. Bartlett's Test of Sphericity was performed to assess whether the variables were sufficiently intercorrelated. The determinant of the R-matrix was calculated to assess multicollinearity. A value of 0.000853 was achieved, exceeding the threshold of 0.00001, thereby confirming the absence of problematic multicollinearity.

Exploratory factor analysis (EFA)

EFA is a statistical method used to identify the key factors contributing to IUGR and to clarify the relative strength of their associations. Factor extraction was performed using principal component analysis (PCA) to reduce the dimensionality of the dataset and identify components that capture the maximum variance among the observed variables. The appropriate number of factors (five) was determined through visual and statistical inspection of the Scree plot, and eigenvalue analysis, which was further verified using parallel analysis **(Figure 2)**. Parallel analysis indicated that five components should be retained, as the observed eigenvalues for the first five components exceeded those derived from random data. This finding supports the adequacy of the five-component solution used in the study. The Oblimin method of rotation was applied. This oblique rotation was selected to account for the complex, real-world physiological interdependence between biomarkers associated with IUGR.

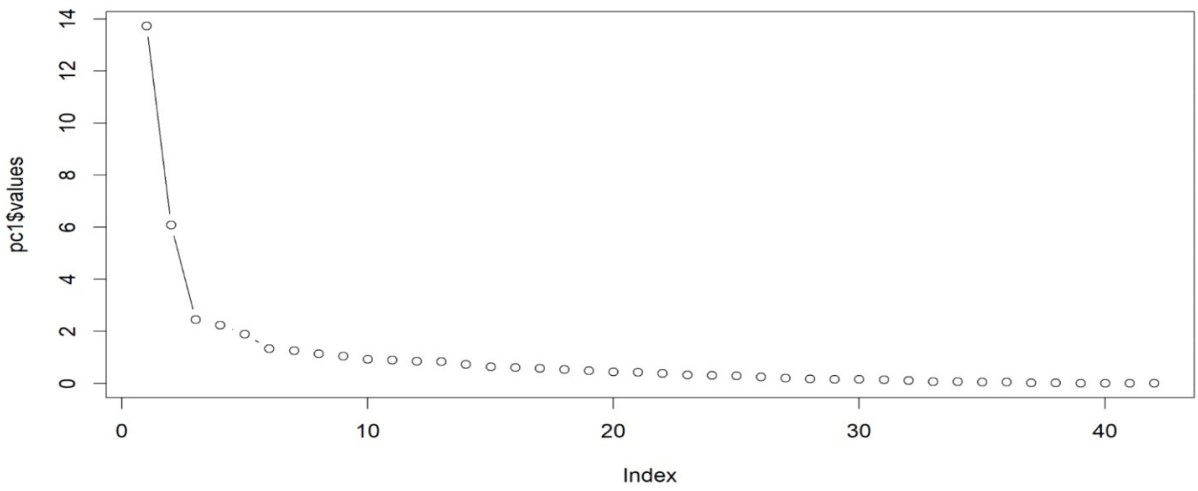

Figure 2: Scree plot of component eigenvalues.

Following the exploratory factor analysis, confirmatory factor analysis (CFA) was conducted to validate the factor structure. The five-factor model derived from EFA was specified, and structural relationships between the latent constructs and intrauterine growth restriction (IUGR) were examined using path analysis.

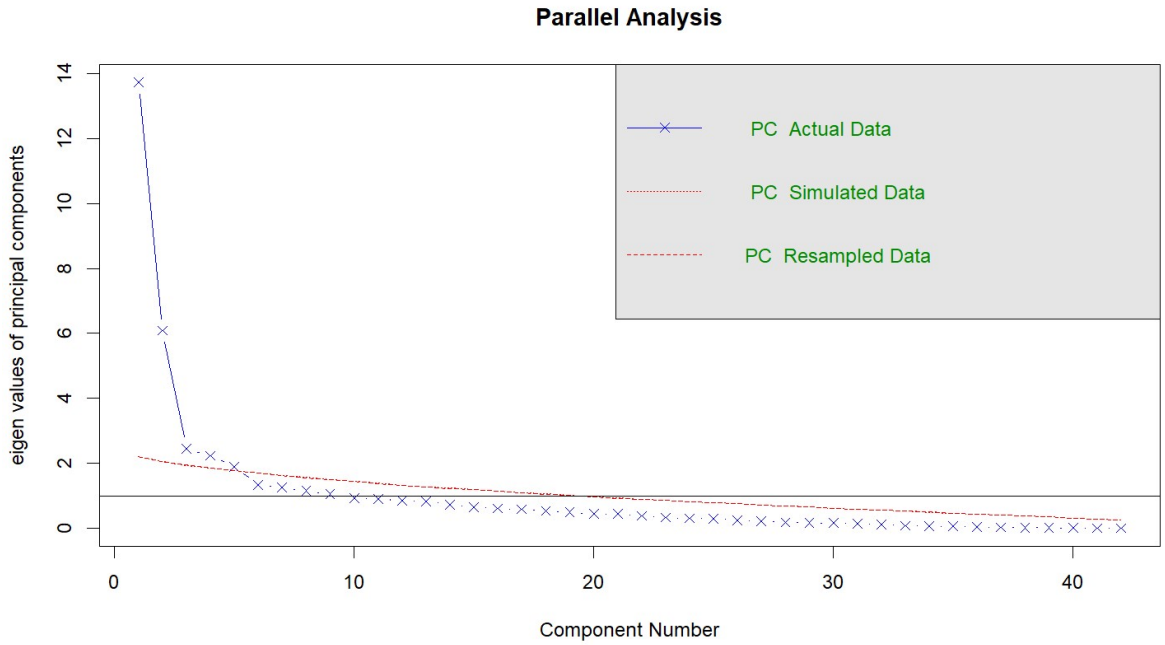

Figure 3: Parallel Analysis for Extraction of Components.

A factor loading threshold of 0.30 was applied to identify meaningful variable-factor associations. Loadings of 0.30 or greater were considered significant and used to interpret the factor structure. The robustness of the model was confirmed by strong fit indices, including a Comparative Fit Index (CFI) of 0.92 and a Tucker-Lewis Index (TLI) of 0.89 **(Figure 3).**

RESULTS

The measured variables should exhibit sufficient correlations, which is a fundamental premise of EFA. As a result, the correlation matrix was verified by utilizing Bartlett's Test of Sphericity. The result was highly significant ($\chi^2 = 7475.099$, $p < .001$), indicating that significant inter-correlations exist among the variables, thereby justifying the use of EFA for this dataset. Multicollinearity can pose a significant issue in EFA by inflating the relationships between variables. To assess this, the determinant of the R-matrix was calculated. The determinant value was 0.000853, exceeding the threshold (0.00001), thereby meeting the assumption for factor analysis. The KMO test was employed to assess sampling adequacy. The overall value of KMO was 0.87, indicating good sampling adequacy. R-matrix and KMO results prove that the datasets are well-suited for proceeding with factor analysis. The scree plot was used to determine the number of factors. A scatter plot displays the eigenvalues associated with the extracted factors in descending order to visually assess which factors account for the most variability in the data **(Figure 2).**

Factor 1, identified as maternal clinical and biochemical indicators, accounted for variables associated with complete blood count and liver and renal function markers, showing high loadings for APTT (0.92), BMI (0.91), platelets (0.90), creatinine (0.88), and ALT (0.86). This factor appears to represent overall clinical and physical health. The corresponding eigenvalues and the percentage of variance they explain indicate the extent of variance contributed by this factor. Factor 1 accounts for 33% of the total variance. Factor 2 identifies as inflammation and oxidative stress, encompassing biomarkers related to inflammatory and oxidative stress responses, with strong loadings for MDA (0.88), NFκB (0.79), TNFα (0.78), and IL6 (0.77). While some variables, like PIGF (-0.73) and VEGF (-0.63), showed negative loadings, indicating complex interactions within the domain. The corresponding eigenvalues and percentage of variance for this factor indicate that Factor 2 accounts for 14% of the variance.

Factor 3 represents the reproductive and obstetric history, which is defined by high loading of gravidity (0.97), abortion history (0.80), and previous IUGR cases (0.77) that indicate the strong interconnections among the total number of pregnancies, past pregnancy losses, and prior occurrences of IUGR. The parity (0.56) also contributed to this factor, but with a lower loading, suggesting that the live birth outcomes are less significant in defining a woman's pregnancy history. Factor 4 highlights the importance of vitamin cofactors related to nutritional health, with significant loadings for Vitamin E (0.76), NO (0.76), and Vitamin D (0.72), indicating the role of vitamins and micronutrients in pregnancy outcomes, accounting for 6% of total variance. Factor 5 mentioned the antioxidants associated with enzymatic and non-enzymatic mechanisms, with high loadings of GPx (0.79), Vitamin B9 (0.67), and CAT (0.58), which depict mechanisms for regulating oxidative stress. For Factor 2 (Inflammation and Oxidative Stress Markers), the negative loading for PIGF (-0.73), VEGF (-0.63), and Vitamin B12 (-0.52) suggest opposing trends within Factor 1. PIGF (-0.73) biomarker may decrease when inflammatory markers such as MDA, TNF, and IL6 increase, indicating it acts as a counterbalance. VEGF (-0.63), associated with angiogenesis, appears to inversely correlate with inflammatory markers, suggesting that VEGF decreases as inflammation rises. Vitamin B12 (-0.52) is involved in lowering homocysteine levels, and its negative loading suggests that lower inflammation is associated with higher Vitamin B12 levels.

Table I: Factor analysis

Factors	Items	Loadings	Eigenvalues	Total Variance (%)
Factor 1 (Maternal Clinical and Biochemical Indicators)	APTT	0.92	13.74	0.33 (33%)
	BMI	0.91		
	Platelets	0.90		
	Creatinine	0.88		
	PI	0.87		
	ALT	0.86		
	Urea	0.86		
	TLC	0.81		
	ALP	0.78		
	Hb	0.73		
	AST	0.64		
	SBP	0.63		
	PT	0.60		
Factor 2 (Inflammation and Oxidative Stress)	MDA	0.88	6.08	0.14 (14%)
	NF-κB	0.79		
	TNF-α	0.78		
	sFlt-1	0.78		
	IL6	0.77		
	PIGF	- 0.73		
	VEGF	- 0.63		
	DBP	0.59		
	Homocysteine	0.58		
	8OHdG	0.52		
	Vit. B12	- 0.52		
	Gestational Age	- 0.37		
	SOD	- 0.34		
	GRx	- 0.34		
Factor 3 (Reproductive and Obstetric History)	Gravidity	0.97	2.45	0.07 (6%)
	Abortion	0.80		
	Previous IUGR	0.77		
	Parity	0.56		
Factor 4 (Vitamin co-factors)	Vit. E	0.76	2.23	0.07 (5%)
	NO	0.76		
	Vit. D	0.72		
	Vit. C	0.33		
Factor 5 (Antioxidants)	GPx	0.79	1.90	0.05 (5%)
	Vit. B9	0.67		
	CAT	0.58		
	Vit. B6	0.55		
	GSH	0.50		

Table I presents the factor analysis, which revealed five domains of maternal and biochemical indicators. With significant loading from APTT, BMI, platelets, creatinine, and liver function indicators. Factor 1 accounted for 33% of the total variation. In addition to inflammatory markers such as MDA, NF-κB, TNF-α, and IL-6, and negative correlations with PIGF and VEGF, factor 2 accounted for 14% of the variance. Gravidity, abortion history, and prior IUGR were the main drivers of factor 3, which explained 6% of the variance. Each of factors 4 and 5, which included different vitamins (E, D, C, B6, B9) and enzymes (antioxidant enzymes), accounted for 5% of the variance. These factors highlight the complexity of maternal health and its effects on the course of pregnancy.

Table II: Factor Component Correlation Matrix

	Factor 1	**Factor 2**	**Factor 3**	**Factor 4**	**Factor 5**
Factor 1	1.00	0.31	0.20	0.13	-0.20
Factor 2	0.31	1.00	0.25	-0.22	-0.10
Factor 3	0.20	0.25	1.00	-0.09	-0.05
Factor 4	0.13	-0.22	-0.09	1.00	0.00
Factor 5	-0.20	-0.10	-0.05	0.00	1.00

The factor correlation matrix shows the relationships between the detected factors **(Figure 3)**. There may be some overlap between maternal health status, inflammatory processes, and reproductive history. Factor 1 shows a moderately positive association with Factor 2 ($r = 0.31$) and a lesser positive relationship with Factor 3 ($r = 0.20$). A weak association ($r = 0.25$) between Factors 2 and 3 suggested a potential link between inflammatory indicators and prior obstetric experiences. Factors 4 and 5 show low or negative correlations and were largely independent of other factors. These findings suggest that these variables related to oxidative stress and nutrition function relatively differently from the more general maternal clinical and inflammatory profiles.

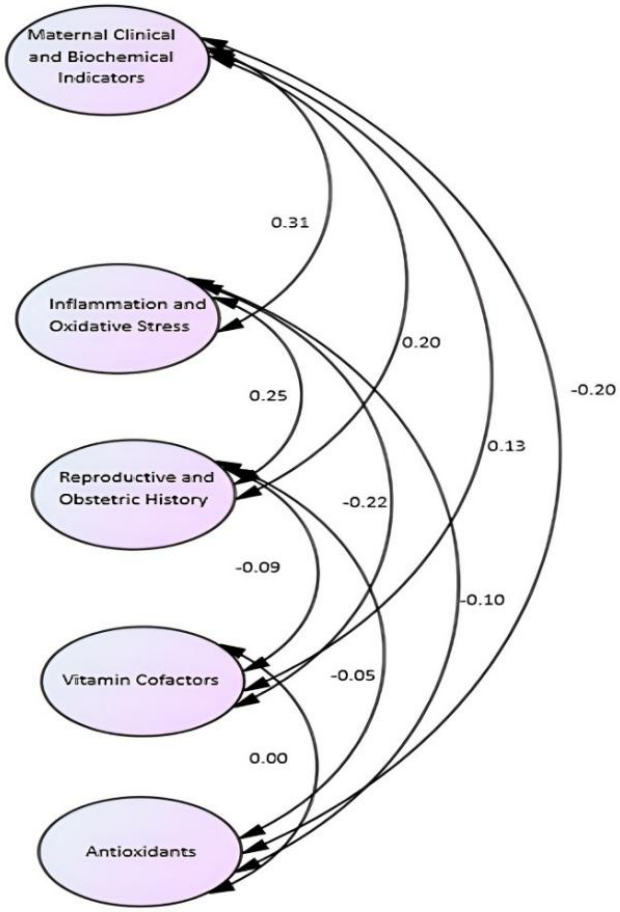

Figure 4: This figure illustrates the inter-factor correlation among five factors.

The extracted factors showed significant inter-factor correlations in the inter-factor correlation analysis **(Figure 4)**. Factor 1 indicates a moderate positive correlation with factor 2 (r = 0.31), suggesting an association between several metrics, such as BMI and markers of inflammation. Factors 1 and 3 showed a weak positive correlation (r = 0.20). In contrast, Factor 3 showed a weak negative correlation with Factor 5 (r = -0.20), suggesting that higher antioxidant levels may be inversely associated with clinical parameters. Factor 2 shows a weak positive correlation with Factor 3 (r = 0.25), suggesting that inflammation may be associated with adverse pregnancy outcomes. Factor 2 showed an inverse correlation with Factor 4 (r = -0.22), underscoring the protective effects of vitamins against inflammation. Factors 2 and 5 showed a minimal negative correlation (r = -0.10). The relationships among the other factors were generally weak, with minimal associations between Factor 4 and Factor 3 (r = -0.09) and between Factor 5 and Factor 3 (r = -0.05), while the association between Factor 4 and Factor 5 was uncorrelated (r = 0.00). These findings suggest that few factors, such as inflammation and pregnancy history, exhibit moderate interplay, whereas others, like nutritional status and oxidative stress defences, operate independently in this dataset.

Communalities

The communalities of the variables were examined to assess the proportion of variance explained by the extracted factors. Most variables demonstrated adequate communalities, indicating that the factor solution accounted for a substantial amount of their variance. Specifically, several

variables such as creatinine (0.936), alanine aminotransferase (0.809), aspartate aminotransferase (0.894), alkaline phosphatase (0.974), prothrombin time (0.964), activated partial thromboplastin time (0.946), platelet indices (0.867), and total leukocyte count (0.923) exhibited very high communalities, suggesting strong representation in the factor structure.

Moderate communalities were observed for variables including systolic blood pressure (0.717), hemoglobin (0.758), tumor necrosis factor (0.867), interleukin-6 (0.789), nuclear factor kappa B (0.800), malondialdehyde (0.696), nitric oxide (0.602), and vitamin E (0.626), indicating acceptable contribution to the extracted factors. However, a few variables showed relatively low communalities, such as socioeconomic status (0.082), gestational age (0.175), glutathione reductase (0.192), asymmetric dimethylarginine (0.162), and vitamin C (0.223). However, variables with lower communalities were retained due to their established clinical relevance, despite weaker statistical representation in the factor model. Overall, the majority of variables met the acceptable threshold for communalities (≥ 0.50), supporting the adequacy of the factor extraction.

Confirmatory Factor Analysis

Figure 5 illustrates a Fitted model of maternal factors and IUGR. The model identified five latent constructs. This model includes maternal clinical and biochemical markers, reproductive and obstetric history, vitamin cofactors, antioxidants, oxidative stress (OS), and inflammation. The best predictors of IUGR were OS and inflammation ($\beta = 0.423$, $p < 0.001$), suggesting a clear correlation between elevated oxidative and inflammatory markers and an increased risk of IUGR. In addition, there were statistically significant, albeit weaker, positive correlations between IUGR and maternal clinical, biochemical, reproductive, and obstetric histories ($\beta = 0.084$ and $\beta = 0.052$, $p < 0.001$). On the other hand, vitamin cofactors had a negative but non-significant relation with IUGR ($\beta = –0.012$, $p = 0.218$). Antioxidants had a substantial negative relationship ($\beta = –0.056$, $p < 0.001$). These results demonstrate how intricately maternal, physiological and dietary variables interact to affect fetal growth.

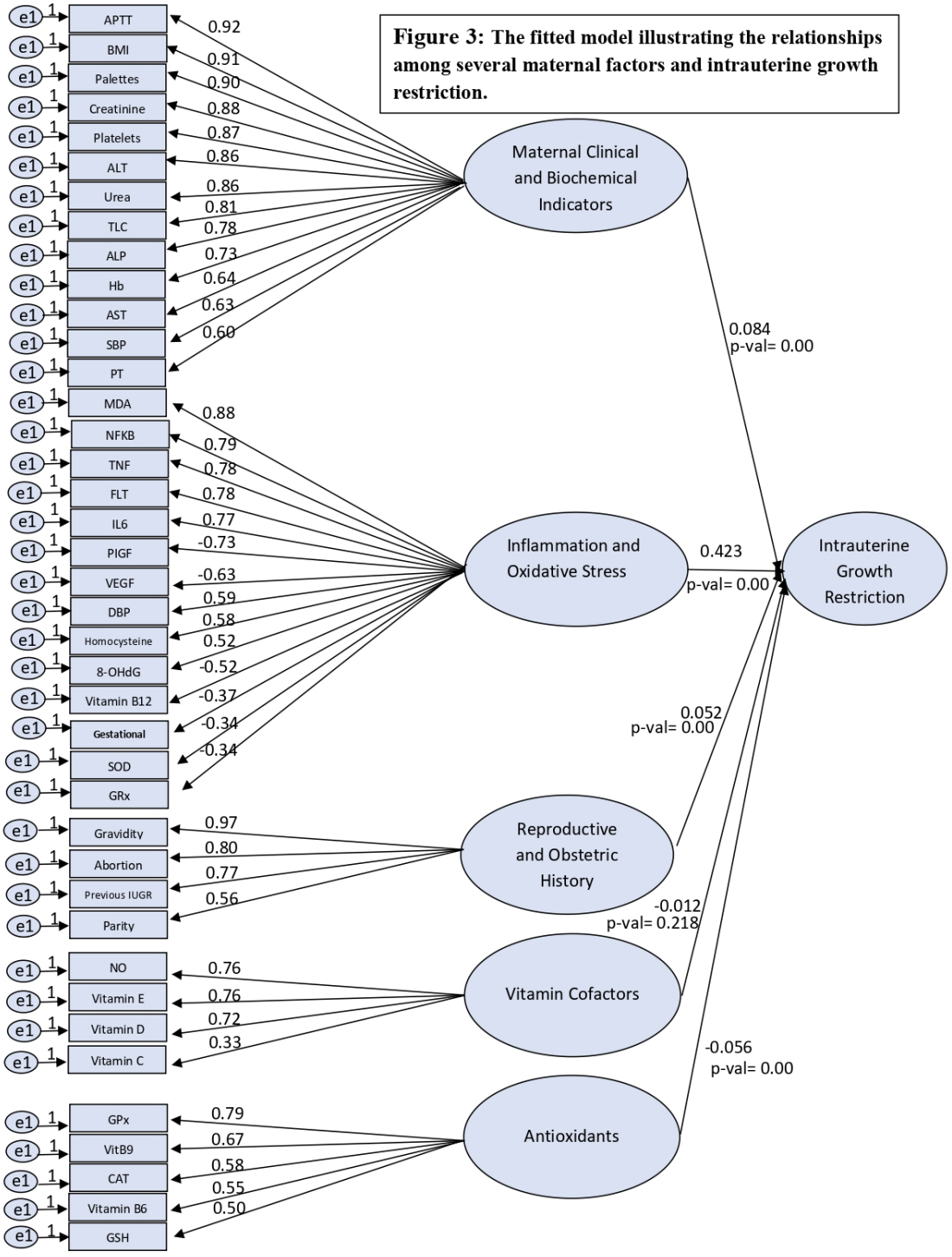

Figure 5: The fitted model illustrating the relationships among several maternal factors and intrauterine growth restriction.

Cross-loading variables

The cross-loading matrix demonstrates that the majority of indicators load highest on their respective latent constructs, thereby supporting adequate discriminant validity at the indicator level. Specifically, variables associated with clinical and biochemical indicators (e.g., BMI, creatinine, ALT, platelets) showed strong loadings on the first component, while inflammatory and oxidative stress markers (e.g., TNF, IL-6, NFKB, MDA) loaded predominantly on the second component. Reproductive and obstetric variables (e.g., gravidity, abortion, previous IUGR) were clearly associated with the third component, and antioxidant/vitamin-related variables loaded on distinct components, reflecting conceptual coherence **(Table III)**.

Additionally, a few indicators demonstrated relatively lower loadings, consistent with their lower communalities. However, these variables were retained due to their established clinical relevance and contribution to the theoretical framework of the study. Importantly, the overall pattern of loadings indicates minimal cross-loadings that do not compromise the integrity of the factor structure. Therefore, the inclusion of cross-loading analysis further strengthens the measurement model and confirms that the constructs are sufficiently distinct while maintaining clinical interpretability.

Table III: Cross-loading variables

Variable	TC1	TC2	TC3	TC5	TC4	Assigned Factor
Gravidity			0.972			TC3
Parity			0.557			TC3
Abortion			0.802			TC3
PreviousIUGR			0.766			TC3
BMI	0.91					TC1
SBP	0.627					TC1
DBP		0.589				TC2
PI	0.867					TC1
Hb	0.728					TC1
TLC	0.805					TC1
Urea	0.863					TC1
Creat	0.882					TC1
ALT	0.863					TC1
AST	0.643					TC1
ALP	0.78					TC1
Platelets	0.902					TC1
PT	0.604					TC1
APTT	0.92					TC1
PIGF.FC		-0.734				TC2
FLT.FC		0.778				TC2
VEGF.FC		-0.633				TC2
TNF		0.779				TC2

IL6FC		0.772				TC2
NFKBFC		0.788				TC2
MDA		0.879				TC2
GSH				0.526		TC5
CAT				0.58		TC5
GPx				0.789		TC5
NO					0.758	TC4
VitD					0.721	TC4
VitB6				0.546		TC5
VitB9				0.674		TC5
Homocysteine		0.583				TC2
VitE					0.762	TC4

Relationship of constructs to IUGR

OS and inflammation have a significant (p-value = 0.001) effect on IUGR, with a path coefficient of 0.423. There was a significant positive correlation between maternal clinical and biochemical parameters (Path coefficient = 0.084, p-value = 0.001). Antioxidants show a significant (p-value = 0.001) negative link, indicating that better oxidative stress management may lower the risk of IUGR (Path coefficient: -0.056). However, cellular redox enzymes and vitamin cofactors showed no significant association (p-value = 0.001) with the path coefficient of -0.012. There is a significant (p-value = 0.001) but small positive correlation between the obstetric and reproductive histories (Path coefficient: 0.052) **(Table IV)**.

Table IV: Regression Analysis

Dependent Variable	Independent Variable	Estimate	p-value	CI	Impact
Intrauterine Growth Restriction	Maternal Clinical and Biochemical Indicators	0.084	<0.001	0.72 to 0.96	Strong regression
Intrauterine Growth Restriction	Inflammation and Oxidative Stress	0. 423	<0.001	0.31 to 0.54	Moderate regression
Intrauterine Growth Restriction	Reproductive and Obstetric History	0. 052	<0.001	0.02 to 0.08	Moderate regression
Intrauterine Growth Restriction	Cellular Redox Enzymes and Vitamin Cofactors	-0.012	0.218	-0.03 to 0.01	Not significant
Intrauterine Growth Restriction	Oxidative Stress Defense	-0.056	<0.001	-0.08 to -0.03	Negligible

DISCUSSION

This study used factor analysis to investigate associations among maternal clinical indicators, oxidative stress (OS), and inflammatory markers, antioxidants, and vitamin cofactors in the context of IUGR. The findings demonstrated that the "Inflammation and Oxidative Stress" factor was the most prominent feature associated with IUGR, highlighting the relationship between these markers and restricted fetal development. In line with previous research, elevated levels of OS indicators (MDA and NFκB) and pro-inflammatory cytokines (TNF-α and IL-6) were substantially linked to IUGR cases[9,11]. These observations are consistent with the increased inflammatory activity often observed in pregnancies complicated by placental insufficiency. However, **Tosun et al.**[13] reported contrasting results, finding no significant difference in maternal serum IL-6 and TNF-α levels, which may be attributed to variations in study populations and the presence of hypertensive disorders.

Additionally, a significant positive association was identified between IUGR and Factor 1 (Maternal Clinical and Biochemical Indicators). This factor, encompassing BMI, APTT, ALT, and creatinine, reflects maternal metabolic, coagulation, and renal status. **Ram et al.** [14] reported that increased maternal BMI is frequently associated with increased IUGR risk, possibly reflecting underlying metabolic dysfunction. Conversely, maternal undernutrition, as indicated by a lower BMI, has also been identified as a risk factor[15]. Regarding renal function, while some studies show no significant differences[16], others have identified elevated urea and creatinine levels in IUGR pregnancies[17-18]. Similarly, **Lorencini et al.**[19] found an association between IUGR and markers of chronic kidney disease. In our study, liver enzyme levels were generally within the normal range in IUGR pregnancies. However, some differences in AST and ALP were observed, contrasting with the **Kumari et al.**[20] study, which reported elevated liver enzymes with other adverse pregnancy outcomes such as preeclampsia.

Factor 5 (Antioxidants) showed a significant negative association with IUGR, supporting the potential protective role of these mechanisms. Lower levels of enzymatic antioxidants (GPx and CAT) were observed in mothers of IUGR infants, consistent with **Grzeszczak et al.**[21], suggesting a diminished defense against OS. The correlation between the oxidative DNA damage marker 8-OHdG and TNF-α levels further highlights this. However, **Iftikhar et al.**[22] found higher GPx levels in IUGR patients, illustrating potential variations in antioxidant responses. Factor 4 (Vitamin D, E, and NO) did not show a significant association with IUGR in our study, contrary to Chen et al.[23,] who identified a link between Vitamin D deficiency and IUGR. Furthermore, while **Abdallah et al.**[24] observed lower levels of vitamins A, E, and D in IUGR newborns25, our study did not find a significant association with maternal vitamin levels. Although nitric oxide (NO) is critical for placental vascular function[26], the "Vitamin Co-Factors" factor as a whole did not reach statistical significance in this cohort.

While our study reinforces the strong association between oxidative stress, inflammation, and IUGR, several limitations must be noted. Importantly, the cross-sectional nature of this study precludes the inference of temporal relationships or causality; therefore, we cannot conclude that these biochemical changes are the primary etiology of IUGR rather than a consequence of it. These results highlight the significance of maternal health status and the potential role of antioxidants. Future prospective studies are needed to determine if addressing these pathways through maternal health interventions can reduce IUGR incidence.

Strengths and Limitations

Factor analysis was utilized to highlight the interrelationships and the multifaceted character of

IUGR. A thorough assessment of clinical, biochemical, inflammatory, oxidative stress, and nutritional factors was a major strength of this study. However, the study has a few limitations. The cross-sectional study design makes it difficult to determine causality or to record changes in biomarkers over time, and the small sample size and sampling technique (non-random convenience sampling) may affect the generalizability of the study. Additionally, imputing values for variables below the detection limit may introduce bias or underestimation of variance.

CONCLUSION

Our study concludes that maternal clinical health and antioxidant defences play significant roles, while oxidative stress and inflammation have a large impact on the aetiology of IUGR. These findings highlight the need for targeted therapies to lower systemic inflammation and oxidative stress during pregnancy. Future research should focus on dynamic biomarkers, non-linear connections, and new therapies to improve pregnancy outcomes and IUGR.

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AUTHOR CONTRIBUTION

Iftikhar M: Conception and design of the study, data collection and interpretation, writing – original draft and finalized the manuscript.
Sehgal SKS: Helped in coordinate and analyze testing, contributed to the literature review.
Muzammil F: Formal analysis and drafted the results section of the manuscript.
Malik FA: Assisted in patient recruitment and helped in statistical analysis.
Shaukat M: Helped in conducting the experiment.
Ain MU: Helped in reference and manuscript formatting.
Sharif S: Critical review of manuscript and help in reference citation.
Khan MIU: Helped in Literature search, data collection.

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