

## DETERMINATION OF MATERNAL SERUM PRO-INFLAMMATORY CYTOKINE CHANGES IN INTRAUTERINE GROWTH RESTRICTION

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**Abstract:** Background: Some fetuses were intrinsically small, registering below 10th percentile in weight for their gestational age, in accordance with their genetic growth potential. They were not growth-restricted & can be precisely characterized as small for gestational age fetuses. Factors leading to fetal growth restriction primarily encompass issues inherent to fetal-placental-maternal unit, fetal undernutrition, & intrauterine spatial constraints that hinder fetal development. Fetal growth restriction was a fetal disorder that could result in significant short-term & long-term challenges, without IUGR adversely impacting quality of life.

**Aim:** The study was to ascertain alterations in maternal serum pro-inflammatory cytokines in cases with intrauterine growth restriction.

**Methods:** This case-control study was performed in Department of Obstetrics & Gynaecology affiliated with AlImamain Alkadhimain Medical City. A total of 54 four cases with intrauterine growth restriction (Group A) & 54 controls from labor room or obstetric ward (Group B) were recruited according to study's selection criteria.

**Result:** No statistically significant differences were seen in age, BMI, gravidity, & parity among analyzed groups based on maternal characteristics. Inflammatory indicators indicated a very significant difference in ESR, Hs CRP, IL-6, & TNF- $\alpha$  across examined groups.

**Conclusion:** Pro-inflammatory cytokines, ESR, hsCRP, IL-6, & TNF- $\alpha$  levels were elevated in cases with IUGR. Consequently, it was evident that a proinflammatory condition was implicated in pathophysiology of IUGR. Further research will enable identification of particular causes of IUGR & mitigation of this inflammation, so potentially preventing onset of IUGR.

**Keywords:** Maternal, Cytokine, Intrauterine Growth Restriction.

### 1. Introduction

Normal fetal development is a tightly controlled complex process, which relies on maternal nutrition availability, placental transport capacity, fetal hormonal regulation and genetic growth potential. The maternal supply of glucose and lipid is central since deficiency and excess of both can have a negative influence on the growth of the fetus. Although long-term severe maternal malnourishment can be linked to fetal growth retardation, maternal hyperglycemia and high levels of free fatty acids are always linked to

fetal overgrowth, mediated to a great extent by fetal hyperinsulinemia and placental lipid transport changes (1).

Critical roles in cellular proliferation, differentiation, and neurodevelopment of the fetus is played by placental hormones and growth factors, especially insulin-like growth factor-1 (IGF-1), leptin, and adipokines, with the disruption playing a major role in abnormal growth paths (2). Intrauterine growth restriction (IUGR) is a pathological inability of the fetus to develop to the full extent of genetically pre-determined growth and is related to the higher perinatal morbidity, mortality, and long-term consequences, including metabolic syndrome and cardiovascular disease (3,4).

Symmetric and asymmetric forms of IUGR have been generally categorized, and the major mechanism in the latter is placental insufficiency. Chronic fetal hypoxia and nutrient deprivation are caused by impaired trophoblastic invasion, abnormal spiral artery remodeling, and reduced uteroplacental perfusion (5,6,7). There is growing evidence that inflammation is a central role in the pathogenesis of IUGR. During pregnancy, there is a highly balanced immune condition that is dominated by anti-inflammatory response; however, in IUGR, there is a change in favour of a pro-inflammatory condition (8,9).

Increased serum levels of pro-inflammatory cytokines like tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-6 (IL-6), higher erythrocyte sedimentation rate (ESR) and C-reactive protein (hs-CRP) are also consistently observed in the IUGR pregnancies (10,11,12). These mediators inhibit trophoblasts proliferation, augment the apoptotic process, interrupt placental vascular operation, and suppress the transport of amino acids and glucose to the fetus (13,14). Taken together, these results suggest that maternal serum pro-inflammatory cytokines are one of the primary factors that contribute to IUGR and can be used as a biomarker to identify the condition and risk stratify (15). Then, this is to determine changes in maternal serum pro-inflammatory cytokines in intrauterine growth restriction cases.

## 2. Method

This case-control study was conducted in the Department of Obstetrics and Gynaecology at Al-Imamain Al-Kadhmain Medical City. A total of 108 pregnant women were enrolled and divided into two groups: 54 women diagnosed with intrauterine growth restriction (IUGR) constituted the case group (Group A), and 54 pregnant women with normal fetal growth served as the control group (Group B). Participants were recruited from the labor room and obstetric wards according to predefined eligibility criteria.

Some of the cases involved pregnant women whose fetuses were estimated to have a fetal weight that was below the 10<sup>th</sup> percentile of gestational age based on ultrasonography. The controls were non pregnant women whose fetuses had not developed growth restriction. The study did not include women who had fetal developmental abnormalities or other maternal diseases like preeclampsia or infectious diseases. The sampling method relied on the use of a simple random sampling technique.

The collection of data was carried out using direct interviews and reviewing medical record during maternal and neonatal discharge. Immediately after delivery, the infants were weighed on a calibrated electronic weighing scale when they were not dressed and neonatal birth weight was recorded.

The history taking of all the participants was done in detail including demographic, obstetric, medical, and surgical history, and general physical examination and calculation of body mass index. Laboratory tests were complete blood count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), liver and kidney testing, coagulation, interleukin-6 (IL-6), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). Sample blood taken was the maternal venous blood pre-delivery. The hs-CRP, ESR, IL-6, and TNF- $\alpha$  levels of serum were examined. ESR was assessed by using the method by Westergren whereas IL-6 and TNF- $\alpha$  were evaluated by using enzyme linked immunosorbent assay (ELISA) per manufacturer instructions. Fetus development was measured by use of third trimester ultrasonography; which began at 32 weeks of gestation

and carried out every two weeks. An incidence of standard fetal biometric measurements was taken, which comprise of biparietal diameter, head circumference, abdominal circumference, and femur length, to determine the weight of the fetus and gestational age. After delivery, neonatal outcomes of birth weight and Apgar scores at 1 and 5 minutes were measured.

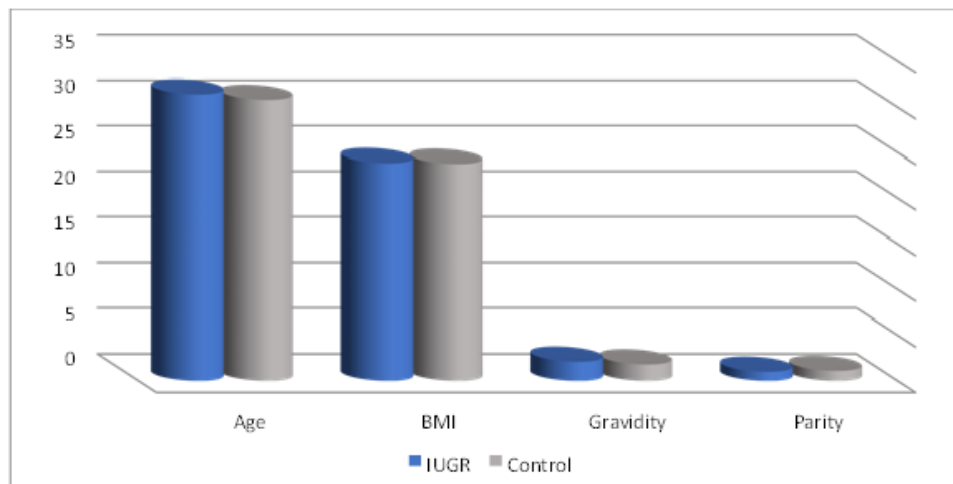
Ethical approval was obtained from the relevant ethical committee, and written informed consent was secured from all participants. Data were analyzed using SPSS version 26. Quantitative variables were expressed as mean  $\pm$  standard deviation and compared using Student's t-test, while categorical variables were analyzed using the chi-square test. A p-value  $\leq 0.05$  was considered statistically significant.

### 3. Results

**Table (1): Distribution of maternal characteristic between studied groups.**

|  | IUGR (N=54)      | Control (N=54)   | P-value |
|--|------------------|------------------|---------|
| <b>Age (year)</b><br>Mean $\pm$ SD             | 31.4 $\pm$ 3.7   | 30.8 $\pm$ 4.1   | 0.426   |
| <b>BMI (kg/m<sup>2</sup>)</b><br>Mean $\pm$ SD | 23.84 $\pm$ 1.47 | 23.75 $\pm$ 1.92 | 0.785   |
| <b>Gravidity</b><br>Mean $\pm$ SD              | 2.1 $\pm$ 0.5    | 2.2 $\pm$ 0.7    | 0.394   |
| <b>Parity</b><br>Mean $\pm$ SD                 | 1 $\pm$ 0.5      | 1.1 $\pm$ 0.4    | 0.253   |

According to maternal characteristic, there was no statistically significant difference regarding to age, BMI, gravidity & parity between studied groups.



**Figure (1):** Distribution of maternal characteristic between studied groups.

**Table (2): Distribution of inflammatory markers between studied groups.**

|                                       | IUGR (N=54)     | Control (N=54)  | P-value |
|---------------------------------------|-----------------|-----------------|---------|
| <b>ESR (mm/hr)</b><br>Mean $\pm$ SD   | 6 $\pm$ 1.7     | 2.7 $\pm$ 0.99  | <0.001  |
| <b>Hs CRP (mg/L)</b><br>Mean $\pm$ SD | 1.65 $\pm$ 0.67 | 0.35 $\pm$ 0.02 | <0.001  |
| <b>IL-6 (pg/mL)</b> Mean $\pm$        | 8.6 $\pm$ 1.5   | 1.6 $\pm$ 0.4   | <0.001  |

|   |                 |                |        |
|---|-----------------|----------------|--------|
| SD  |                 |                |        |
| <b>TNF-<math>\alpha</math> (pg/mL)</b><br>Mean $\pm$ SD | 1.65 $\pm$ 0.42 | 0.9 $\pm$ 0.21 | <0.001 |

IUGR: Intra-uterine growth restriction, ESR: Erythrocyte sedimentation rate.

According to inflammatory markers, there was highly statistically significant difference regarding to ESR, Hs CRP, IL-6 & TNF- $\alpha$  between studied groups.

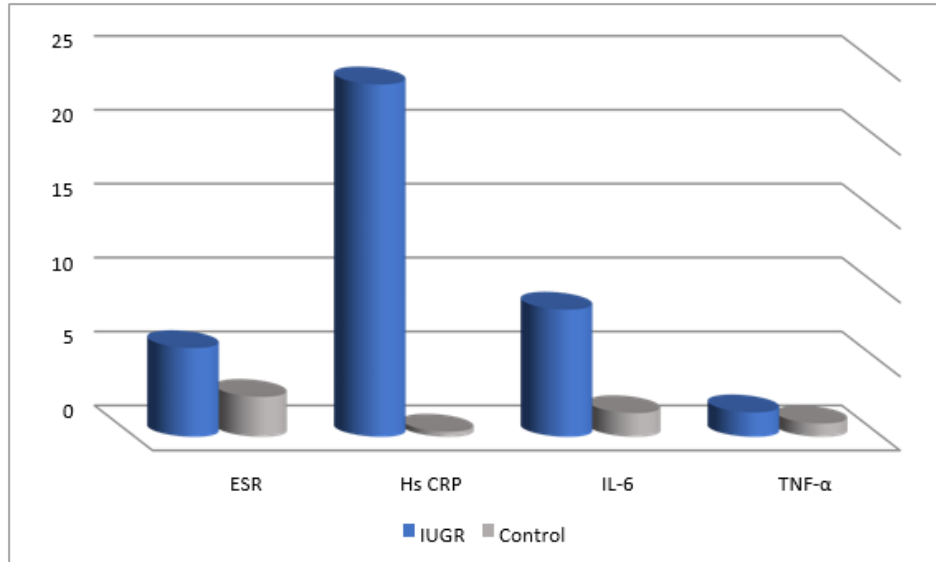


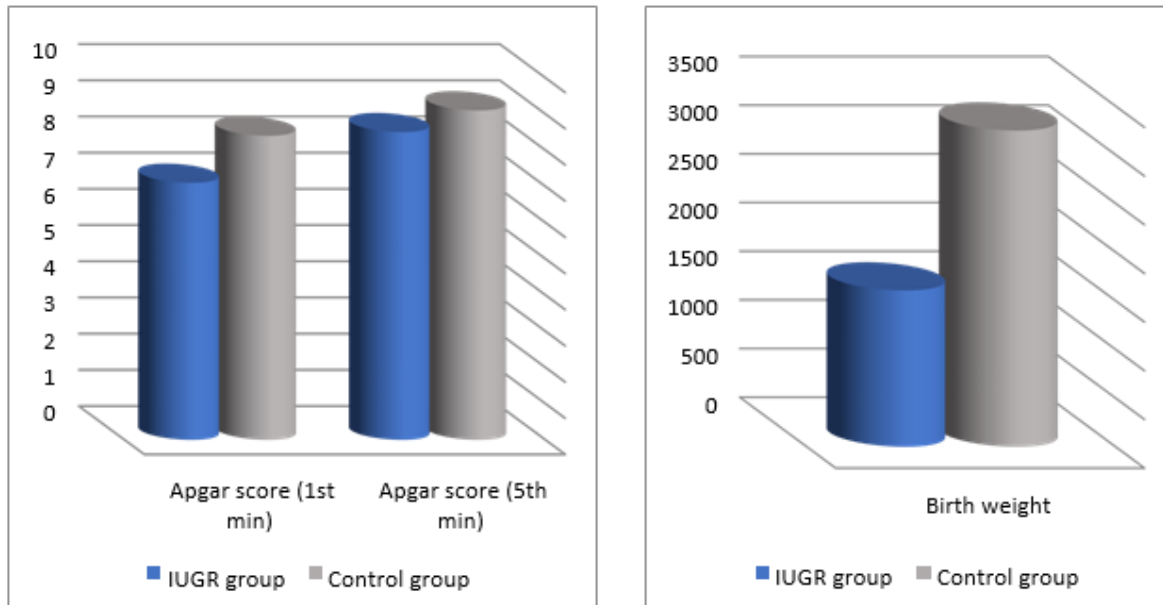
Figure (2): Distribution of inflammatory markers between studied groups.

Table (3): Distribution of neonatal outcome between studied groups.

|  | IUGR group<br>(N=54) | Control group<br>(N=54) | P-value |
|--|----------------------|-------------------------|---------|
| <b>Apgar score (1<sup>st</sup> min)</b><br>Mean $\pm$ SD | 7.1 $\pm$ 0.85       | 8.4 $\pm$ 0.82          | <0.001  |
| <b>Apgar score (5<sup>th</sup> min)</b><br>Mean $\pm$ SD | 8.5 $\pm$ 0.72       | 9.1 $\pm$ 0.68          | <0.001  |
| <b>Birth weight (g)</b><br>Mean $\pm$ SD                 | 1605.4 $\pm$ 285.2   | 3248.5 $\pm$ 62.7       | <0.001  |

### IUGR: Intra-uterine growth restriction

According to neonatal outcome, there was highly statistically significant difference regarding to Apgar score (1st min), Apgar score (5th min) & birth weight between studied groups.



**Figure (3): Distribution of neonatal outcome between studied groups.**

#### 4. Discussion

IUGR denotes a reduction in growth rate that may transpire in fetuses. precise etiology of IUGR remains unidentified as it impacts over thirty million neonates annually.

It was thought that genetic factors play a role in approximately one-third cases, and maternal, foetal, or placental factors account for the remaining two-thirds. (16)

IUGR foetuses are more vulnerable to perinatal morbidity, such as sepsis, asphyxia and perinatal death. In addition, IUGR was a significant risk factor for development of DM & cardiovascular diseases in childhood & adulthood. For these reasons, it is important to understand the pathogenesis of IUGR and take the necessary precautions. (17, 18)

Immunological balance is of great importance during pregnancy. It is known that some biomarkers and cytokines are responsible for endothelial damage and placental dysfunction in pregnancy. Furthermore, the alteration of the immune balance mediates perinatal complications, such as preterm delivery or abortion. (19,20)

There are few studies in literature investigating relationship between inflammation & IUGR. a study comparing CRP levels in maternal serum & umbilical cord blood during delivery, CRP levels were higher in IUGR group (21,22). There were studies showing that high CRP levels detected in the early weeks of pregnancy can predict which infants will be small for their gestational age at birth. (23)

Recent investigations have examined influence of pro-inflammatory cytokines, including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-6, IL-8, IL-12, IL-18, & IL-23, as well as anti-inflammatory cytokines such as IL-4, IL-10, & IL-13, in aetiology of intrauterine growth restriction (IUGR). these investigations were unable to ascertain which pro- or anti-inflammatory cytokines were significant in development of IUGR, perhaps due to complex & heterogeneous character of condition. (24)

Current study indicated that, based on maternal characteristics, there were no statistically significant differences in age, BMI, gravidity, & parity among analysed groups.

Our findings corroborate those of Kirici et al. (2023) (25), who sought to assess alterations in maternal serum inflammatory markers in pregnancies affected by IUGR. They reported no statistically significant differences between IUGR group control group concerning age, BMI, gravidity, and parity.

Nnamani et al. (2021) (26) aimed to determine whether there was a relationship between prenatal Doppler indices & perinatal outcomes, as well as to assess predictive ability of UA Doppler ultrasonography in distinguishing between normal & growth-restricted pregnancies (27). With control group averaging 1.33 (SD = 1.36) & case group averaging 1.42 (SD = 1.31), they found no discernible difference in mean parity between two groups. Procalcitonin & CRP levels in maternal serum & fetal cord blood samples from cases of idiopathic IUGR were compared to a control group of newborns that were appropriate for gestational age (AGA) by Karlı et al. (2019) (28). Regarding age, BMI, & gestational period, they found no statistically significant differences between IUGR group & control group.

Our data indicated a very significant difference in ESR, Hs CRP, IL-6, & TNF- $\alpha$  across tested groups.

This was consistent with results of Kirici et al. (2023) (29), that IUGR group & control group differed statistically significantly in terms of ESR, Hs CRP, IL-6, & TNF- $\alpha$ . By comparing groups with & without placental insufficiency, Bartha et al. (2003)(30), aimed to evaluate maternal serum concentrations of two inflammatory cytokines in females with IUGR. They found that TNF- $\alpha$  levels in groups under study differed statistically significantly. (31)

A statistically significant difference in CRP levels between IUGR group & control group was found by Karlı et al. (2019). (28)

In their 2019 study, Kara et al. sought to compare serum levels of interleukin-6 (IL-6), sialic acid (SA), & hs-CRP in pregnancies complicated by IUGR & preeclampsia (PE) to those in healthy pregnancies.

The goal of Al-Azemi et al. (2017) (29) was to quantify the production of cytokines by maternal peripheral blood lymphocytes from pregnant female with IUGR foetus & other female with healthy fetus, as well as to look into connection between cytokine profiles & IUGR. When comparing IUGR group as a whole to normal pregnancy control group, they found no Discernible differences in IL-6 levels. (30)

Our results showed that there was a highly statistically significant difference between study groups in terms of birth weight & Apgar scores at 1<sup>st</sup> & fifth minutes.

In a similar vein, Kirici et al. (2023) (29) statistically significant difference in birth weight & Apgar scores at 1 & 5 minutes between IUGR group & control group. Karlı et al. (2019) (29) demonstrated that IUGR group & control group differed statistically significantly in terms of birth weight & Apgar score at one-minute mark.

In their 2015 study, Erkenekli et al.(32) aimed to determine if pregnant female with FGR have higher levels of plasma neopterin & CRP than women with normal pregnancies. They found that birth weights of groups under study differed statistically significantly.

## 5. Conclusion

pro-inflammatory cytokines, ESR, hsCRP, IL-6, & TNF- $\alpha$  levels were elevated in IUGR cases. Consequently, it was evident that a proinflammatory condition was implicated in pathophysiology of IUGR.

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