Study of Role of Maternal Serum Ferritin in the Prediction of Asymmetric Intrauterine Growth Restriction

Amr Kamel El-Fayomy, Walid Abdallah Abdelsalam, Ahmed Ismail Mohamed, Mostafa Ahmed Mostafa*

Department of Obstetrics and Gynecology, Faculty of Medicine, Zagazig University, Egypt

***Corresponding author:** Mostafa Ahmed Mostafa, **Mobile:** (+20) 01093016946, **E-mail**: m.nagar9294@gmail.com

ABSTRACT

Background: Late-onset asymmetric Intrauterine Growth Restriction (IUGR) is associated with an increase in the maternal serum ferritin level, which is thought to be caused by a decrease in placental extraction ferritin from the systemic circulation. **Objective:** The aim of the current study was to predict asymmetric IUGR by measuring maternal serum ferritin at 30-32 weeks of gestation. **Patients and methods:** A cross sectional study was conducted on a total of 550 women. At 30-32 weeks of gestation, maternal serum ferritins were assessed. Of the studied women, 84 women were selected and divided into two groups; 42 cases of elevated serum ferritin level taken as a case group and the first included 42 cases with normal serum ferritin level served as a control group. At 30-32 weeks of gestation, we assessed maternal serum ferritin. **Results:** Serum ferritin is good marker for detecting cases of IUGR at cut off ≥12.45 with a sensitivity of 85.7%, a specificity of 35.7%, a positive predictive value of 57.1% and a negative predictive value of 71.4%., while serum ferritin is good marker for excluding cases of IUGR at cut off ≥20.5 with a sensitivity of 33.3%, a specificity of 85.7%, a positive predictive value of 70% and a negative predictive value of 56.3%.

Conclusion: Serum ferritin level with a cut off level ≥12.45 ng/dl in addition to ultrasonography has as a predictive value of asymmetric IUGR

Keywords: Serum Ferritin, Ultrasonography, Asymmetric Intrauterine Growth Restriction.

INTRODUCTION

Asymmetric, late-onset (type II), intrauterine growth restriction (IUGR) is characterized by pathologically delayed fetal growth beginning in late pregnancy due to uteroplacental insufficiency. For comparison, the phrase "Small for Gestational Age" (SGA) refers to a newborn whose birthweight is below the 10th centile for its gestational age, even if the infant is otherwise healthy but just small [1]. Fetal growth restriction has multiple root reasons that can be broken down into these three groups: complications can arise on all levels, from the mother (maternal diabetes, hypertension, connective tissue illnesses or heart diseases) to the developing baby (fetal exposure to teratogens, fetal virus infection, fetal abnormalities) to the placenta) [2]. Maternal serum ferritin has been shown to distinguish between normal small group and small fetuses with pathological growth limitation in a few studies [3]. Fetuses with IUGR have a higher risk of experiencing fetal distress, dying in utero, developing neurological abnormalities, and having meconium aspiration after birth [4].

Seventy-eight percent of IUGR cases are asymmetric, and this is associated with factors such as decreased umbilical blood flow, a brain-sparing impact, oligohydramnios, and a low ponderal index [5].

The identification of suitable prediction diagnostics for IUGR remains one of the top priorities in obstetrics despite the lack of a direct causal therapy and the fact that the majority of IUGR patients are idiopathic [5].

Many biomarkers, including, adiponectin, lactate dehydrogenase, metastin s-endoglin, pregnancy associated plasma protein, endothelin-1, as well as leptin, have been proposed as potential early indicators of IUGR. Most of these tests either have low sensitivity, are difficult to come by, require invasive procedures like amniocentesis, or all three [5].

Due to its low cost and widespread availability, the primary iron storage protein ferritin has been proposed as a suitable replacement for the current screening test. Its concentration increases in response to low oxygen levels or during the acute phase of an infection [6].

Maternal serum ferritin levels are elevated because placental extraction of ferritin from the systemic circulation is diminished in late-onset asymmetric IUGR pregnancies. Its predictive value has been looked into before, but only in a few short studies with a limited sample size [5]. In the current study, we aimed to predict asymmetric IUGR by measuring maternal serum ferritin at 30-32 weeks of gestation.

PATIENTS AND METHODS

A cross sectional study was conducted on women attended at the Zagazig University Department of Obstetrics and Gynecology's Obstetric Outpatient Clinic and Maternity Hospital.

Assuming the mean ferritin level was 19.3 (SD 6.83) vs 14 (SD 5.18) in IUGR versus normal growth, about 550 pregnant women at 30-32 weeks were recruited for our study. Cases were chosen based on either a known first day of the last menstrual cycle or a first trimester ultrasound report.

Fasting blood samples were taken to detect serum ferritin level to obtain 42 women with elevated serum ferritin forming the case group. Another 42 women with normal serum ferritin level and firstly recruited delivering adequate for gestational age neonates at term were selected to form the control group.

Inclusion criteria: All included women had singleton at 30-32 weeks of pregnancy with reliable date.

Exclusion criteria: Women with many pregnancies or those under the age of 20 Pregnancy-related diabetes, maternal smoking, low body mass index, and anemia all increase the risk of needing a blood transfusion. Disease

of the liver or kidneys heart disease and hypertension, acute infections during pregnancy characterized by a high leukocytic count or C-reactive protein are associated with preterm birth. Idiopathic uterine pregnancy with twins was excluded from the study.

All patients were subjected to:
a) **Complete clinical history taking**: The current scenario (main complaint) both mild and severe preeclampsia, hyperglycemia in pregnancy, premature membrane rupture, and time since last period and pattern of menstruation. The patient's obstetric history was recorded, including her age, parity, number of pregnancies, if any had miscarriages or complications, and the outcomes of those pregnancies, as well as her gestational age as determined by the date of her last menstrual period, or an ultrasound performed during the first trimester.
b) **Complete clinical examination.**
c) **Investigation:** Complete blood count (CBC) and serum ferritin assessment: According to **Salem *et al.*** [6], all pregnant women were represented by a single sample obtained between 30 and 32 weeks. Ferritin levels were measured using the Enzyme-Linked Fluorescent Assay (ELFA) method. Selection of cases depended on sure date of first day of the last menstrual period or having ultrasound report in the first trimester.

d) **Ultrasound imaging:** Both the case and control groups were subjected to repeated obstetric ultrasound scans. At 37 weeks, IUGR cases also underwent an umbilical artery Doppler ultrasound [6]. According to the World Health Organization's Fetal Growth Charts, IUGR was diagnosed if the fetal weight was below the 10th percentile [7].

APGAR score: The APGAR score was then used to determine the health of newborns (0-10). We also recorded the newborn's birth weight, length, and length to head circumference ratio, as well as any other clinical signs of IUGR [6].

Ethical Consideration:
This study was ethically approved by the Institutional Review Board of the Faculty of Medicine, Zagazig University [ZU-IRB #(9045)]. Written informed consent was obtained from all participants. This study was executed according to the code of ethics of the World Medical Association (Declaration of Helsinki) for studies on humans.

Statistical Analysis:
The collected data were introduced and statistically analyzed by utilizing the Statistical Package for Social Sciences (SPSS) version 20 for windows. Qualitative data were defined as numbers and percentages. Chi-Square test and Fisher's exact test were used for comparison between categorical variables as appropriate. Quantitative data were tested for normality by Kolmogorov-Smirnov test. Normal distribution of variables was described as mean and standard deviation (SD), and independent sample t-test/ Mann-Whitney U test was used for comparison between groups. To evaluate the relationship between 2 normally distributed variables, Pearson correlation coefficients had been used. P value ≤0.05 was considered to be statistically significant.

RESULTS
Table 1 showed that there was no statistically significant difference between case and control groups.

Table (1): The demographic and clinical characteristics of the studied groups.

Variable		Studied groups		X²	P-value
		Case group (N=42)	Control group (N=42)		
Age	Mean ± SD	27.4 ± 5	27.7 ± 3.7	0.397	0.693
	Range	20 – 34	21 – 35		
Parity	Nulliparous	23 (54.8%)	21 (50%)	0.19	0.66
	Multiparous	19 (45.2%)	21 (50%)		
History of previous preterm labour	Positive	2 (4.8%%)	6 (14.3%)	3.455	0.27
	Negative	40 (95.8%)	36 (85.7%)		

Table 2 showed that there was statistically significant level of serum ferritin in case group compared to control group, otherwise there is no statistically significant difference between case group and control group regarding to other laboratory parameters.

Table (2): Comparison between case group and control group regarding laboratory findings.

Laboratory Findings		Studied groups		t-test	P-value
		Case group (N=42)	Control group (N=42)		
Erythrocytes (10⁶cells /µl)	Mean ± SD	4.05 ± 0.5	3.9 ± 0.69	1.033	0.304
Hemoglobin (gm/dl)	Mean ± SD	11.5 ± 0.36	11.4 ± 0.39	1.675	0.098
Leucocytes (10³/cc)	Mean ± SD	8.4 ± 2.1	8.7 ± 1.8	0.716	0.476
Hemtocrit (%)	Mean ± SD	37.2 ± 2.6	38 ± 2.1	0.716	0.476
CRP (mg/l)	Mean ± SD	3.66 ± 0.88	4.19 ± 1.02	1.261	0.212
Ferritin level (ng/ml)	Mean ± SD	19.29 ±4.80	14 ± 3.40	2.03	0.001

Table 3 showed that there was statistically higher value of resistance index and pulse index of umbilical artery in case group compared to control group, while there was statistically lower value of resistance index and pulse index of cerebral artery in case group compared to control group.

Table (3): Comparison between case group and control group regarding Doppler indices.

Doppler indices		Studied groups		t-test	P-value
		Case group (N=42)	Control group (n=42)		
Resistance index umbilical artery	Mean ±SD	0.71 ± 0.05	0.6 ± 0.06	8.48	0.001
	Range	0.62-0.83	0.43-0.72		
Pulse index umbilical artery	Mean ±SD	1.25 ± 0.14	0.88 ± 0.1	13.31	0.001
	Range	0.93-1.54	0.64-1.08		
Resistance index middle cerebral artery	Mean ±SD	0.74 ± 0.09	0.81 ± 0.05	4.82	0.001
	Range	0.52-0.94	0.68-0.9		
Pulse index middle cerebral artery	Mean ±SD	1.63 ± 0.06	1.88 ± 0.14	10.94	0.001
	Range	1.52-1.75	1.53-2.09		

Table 4 showed that there was statistically significant difference between case group compared to control regarding asymmetric IUGR and Apgar score at (1st minute), also there is statistically higher percent of neonatal intensive care admission in case group compared to control group.

Table (4): Comparing case group and control groups regarding pregnancy outcome.

Pregnancy outcome		Studied groups		t/ χ 2	P-value
		Case group (N=42)	Control group (N=42)		
Gestational age at delivery (days)	Mean ± SD	272.4 ± 6	274.1 ± 6.9	1.153	0.252
	Range	258-281	257-286		
Mode of delivery	Vaginal	15 (35.7%)	21 (50%)	1.75	
	CS	27 (64.3%)	21 (50%)		0.19
Birth Weight (gm)	Mean ±SD	2167.7 ± 144.5	3412.4 ± 388	19.48	0.001
	Range	1854-2418	2772-4198		
Asymmetric IUGR	Yes	31 (74%)	5(11.9%)	6.65	0.01
	No	11 (26%)	37(88.1%)		
Apgar score (1st minute)	Mean ±SD	6.7 ± 2.6	8.1 ± 0.93	*2.3	0.021
	Range	1-10	6-10		
Apgar score (5th minute)	Mean ±SD	8.7 ± 0.77	9 ± 0.58	*1.76	0.082
	Range	7-10	8-10		
Admission to NICU	Yes	15 (35.7%)	5 (11.9%)	6.65	0.01
	No	27 (64%)	37 (88.1%)		

t: t test; χ²: Chi square test; *: Mann-Whitney U test; NICU: Neonatal intensive care unit.

Table 5 showed that there was significant direct relation between Serum ferritin level (ng/ml) in asymmetric IUGR and erythrocytes count , while there is significant inverse relation between serum ferritin level (ng/ml) in asymmetric IUGR and Apgar score (1st minute).

Table (5): Correlation between serum ferritin level and the characteristics, doppler indices and APGAR score of IUGR cases (N=36).

Variables	Serum ferritin level (ng/ml) in IUGR cases	
	r	P-value
Age (years)	0.073	0.647
BMI	-0.108	0.495
Gestational age at delivery (days)	-0.054	0.733
Birth Weight (gm)	-0.247	0.115
Systolic Blood pressure	-0.260	0.097
Diastolic Blood pressure	-0.052	0.745
Erythrocytes (10^6 /µl)	0.403**	0.008
Hemoglobin (gm/dl)	-0.201	0.201
Hematocrit (%)	0.001	0.996
Leucocytes (10^3/cc)	0.001	0.996
Resistance index umbilical	-0.078	0.622
Pluse index umbilical	0.125	0.429
Resistance index middle cerebral	-0.250	0.111
Pluse index middle cerebral	0.087	0.583
Apgar score (1st minute)	-0.377 *	0.015
Apgar score (5th minute)	-0.162	0.305

r: correlation coefficient, **: direct relation, *: inverse relation.

Figure 1 and **table 6** showed that serum ferritin is good marker for detecting cases of intrauterine growth retardation at cut off ≥12.45, while serum ferritin is good marker for excluding cases of intrauterine growth retardation at cut off ≥20.5.
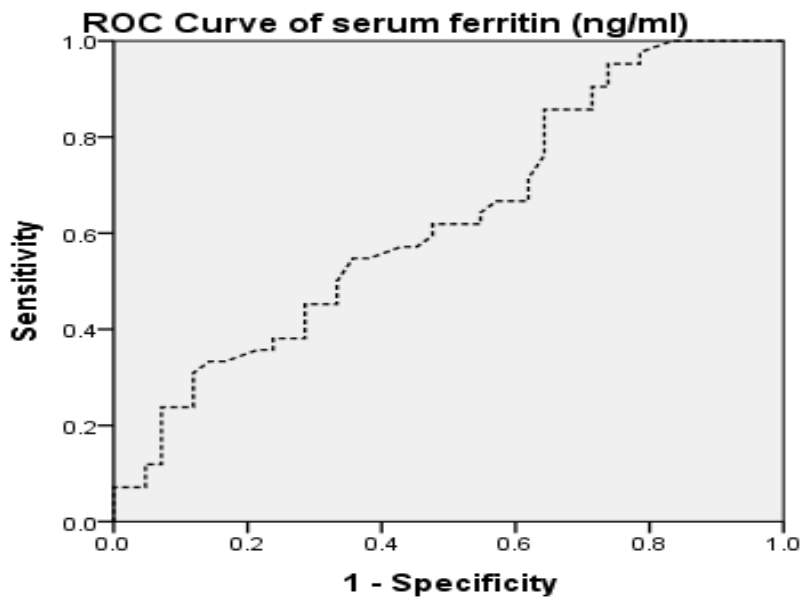

Figure (1): ROC curve of serum ferritin as predictor of asymmetric IUGR. Area under curve (AUC) was 0.628 with (95% CI 0.51-0.747, P=0.043).

Table (6): Performance of serum ferritin as a predictor of asymmetric IUGR.

Cut off level	Sensitivity	Specificity	PPV	NPV	Accuracy
≥12.45	85.7%	35.7%	57.1%	71.4%	60.7%
≥14.05	71.4%	38.1%	53.6%	57.1%	54.7%
≥15.95	61.9%	52.4%	56.5%	57.9%	57.1%
≥20.5	33.3%	85.7%	70.0%	56.3%	59.6%

DISCUSSION

Predictive markers of increased risk of IUGR have also been developed using measurements of maternal serum ferritin. The principal intracellular iron storage protein, ferritin is a globular protein complex made up of 24 protein subunits. Because of its role as an acute phase protein, its serum concentration rises in response to challenges such as hypoxia and infection. With each passing week of pregnancy, ferritin levels drop. Concentration reaches a plateau after the first 30–32 weeks of pregnancy, when it is at its lowest [8].

Regarding demographic data we found mean ages of 27.4 (SD 5) and 27.7 (SD 3.7) in 2 studied groups case and control group and for mean gestational ages (days) 272.4 (SD 6) and 274.1 (SD 6.9).

The same age group was also found in the study of **Fakher et al.** [9], which intended to examine the relationship between maternal serum ferritin fluctuations throughout pregnancy and fetal birth weight classifications such as "normal," "small for gestational age," and "intrauterine growth restriction". Women who became pregnant were anywhere between the ages of 17 and 40, with the mean age being 27.52 (SD 4.997) years old. The gestational ages varied between 38 and 40 weeks, with a mean value of 39.068 (SD 0.753) weeks.

As regard fetal birth weight we found that the mean weight was 2167.7 (SD 144.5) and 3412.4 (SD 388) in the two studied groups (case and control groups).

We found a highly significant relationship between pregnant women with low birth weight and pregnant women with normal weighted neonates above and below cutoff value of ferritin (≥12.45ng/dl). Our results indicate that maternal serum ferritin is associated with lower birthweight infants (P<0.001).

Regarding mode of delivery, we found that number of cases of vaginal delivery was 15 (35.7%) and 21(50%) in two studied groups and number of cases with CS were 21 (50%) and 27 (64.3%), we found no correlation between maternal serum ferritin and the mode of delivery (P=0.19).

In agreement with our study, **Salem et al.** [6] showed that the mean birth weight was 2134 (SD 143) and 3419 (SD 352) and maternal serum ferritin was found to have a stronger association with birth weight (P<0.001).

In contrast with our study, **Bindal et al.** [8], a long-term, prospective study performed by Indian researchers at New Delhi's Deen Dayal Upadhaya Hospital. There were 326 pregnant women that participated in the study. Serum samples from all pregnant women were collected in trace-mineral-free evacuated tubes at 25 weeks and 30 to 32 weeks for chemiluminescence analysis of serum ferritin. Mean birth weights (gm) were 2674.9 and 2199.8 in the two groups, showing a negative connection between serum ferritin value and neonatal birth weight (correlation coefficient = -0.36).

Rahman et al. [10] reported that birth weight is negatively correlated with mother plasma ferritin at GW30 in their prospective cohort research. Babies born to mothers in the highest plasma ferritin tertile weighed, on average, 93 gm less than those born to mothers in the lowest ferritin tertile.

Our study and the study of **Abdel-Malek et al.** [11] showed that high maternal ferritin, as measured between gestational weeks 26 and 36, has been linked to an increased risk of premature birth, fetal growth restriction, and low birth weight. These findings corroborate those of prior investigations.

Ferritin, hemoglobin, hematocrit, and erythrocyte levels were all considerably higher in women who gave birth to babies with low birth weights. Mothers whose babies were born with low birth weights had a considerably greater mean blood ferritin concentration than those whose babies were born at a normal weight (P>0.005) [12]. Women with a ferritin level above 13.6 ng/dl, an erythrocyte count above 3.76x10(12)/l, hemoglobin above 117 g/dl, and a hematocrit above 32.9% between weeks 30 and 32 of pregnancy all had a significantly higher probability of having a low birth weight newborn for gestational age, as determined by ROC curve analysis (P<0.05) [12].

In the other hand in In our study we found a non-significant differences between pregnant mothers with IUGR and pregnant mothers with normal growth fetuses level as regards blood cell count, hematocrit value, hemoglobin level, total leucocytic count and C-reactive protein (P>0.05).

As regarding Doppler indices in our study, there were highly significant differences between pregnant women with elevated serum ferritin level and pregnant women with normal serum ferritin. The mean RI of umbilical artery was 0.71 (SD 0.05) and 0.6 (SD 0.06) in case and control group, respectively, and the mean of PI of umbilical artery was 1.25 (SD 0.14) and 0.88 (SD 0.1) in case and control group respectively (P<0.001).

The mean of RI of middle cerebral artery was 0.74 (SD 0.09) and 0.81 (SD 0.05) in case and control group, respectively and the mean of PI of middle cerebral artery was 1.63 (SD 0.06) and 1.88 (SD 0.14) in case and control group respectively (P<0.001).

In agree with our study in **Salem et al.** [6], in addition to Doppler indices at 37 weeks of pregnancy, serum ferritin levels at 30-32 weeks were found to be substantially correlated with a previous history of asymmetric IUGR.

As regarding APGAR score, In our study, we found an inverse relation between maternal serum ferritin level and APGAR score (at 1st minute) as in cases with increased serum ferritin there is decreased in APGAR score (at 1st minute) and as regarding neonatal admission to NICU, our study found that there high percent of neonates admitted to NICU in case group (35.7%) compared to control group (11.9%). This

agreed with **Salem *et al.* [6]** in which significant differences were seen between IUGR and AGA newborns in birthweight, admission to neonatal intensive care units as well as APGAR scores.

Our study showed that AUC was 0.628 and a cutoff level was 12.45 ng/dl. Sensitivity of serum ferritin level was 85.7%, specificity was 35.7%, positive predictive value was 57.1%, negative predictive value was 71.4%. To our knowledge very few studies used maternal serum ferritin to predict asymmetric IUGR.

CONCLUSION
Serum ferritin level with a cut off level ≥12.45 ng/dl in addition to ultrasonography has as a predictive value of asymmetric IUGR. More research into the utility of maternal serum ferritin levels as a biomarker for IUGR differentiation is warranted.

Supporting and sponsoring financially: Nil.
Competing interests: Nil.

REFERENCES
1. **Lee P, Chernausek S, Hokken-Koelega A *et al.* (2003):** International Small for Gestational Age Advisory Board. International Small for Gestational Age Advisory Board consensus development conference statement: management of short children born small for gestational age, April 24-October 1, 2001. Pediatrics, 111(6 Pt 1):1253-61.
2. **Zur R, Parks W, Hobson S (2020):** The placental basis of fetal growth restriction. Obstetrics and Gynecology Clinics, 47(1): 81-98.
3. **Akkurt M, Akkurt I, Altay M *et al.* (2017):** Maternal serum ferritin as a clinical tool at 34-36 weeks' gestation for distinguishing subgroups of fetal growth restriction. J Matern Fetal Neonatal Med., 30(4):452-6.
4. **Sharma D, Shastri S, Sharma P (2016):** Intrauterine Growth Restriction: Antenatal and Postnatal Aspects. Clin Med Insights Pediatr., 10:67-83.
5. **Ozgu-Erdinc A, Cavkaytar S, Aktulay A *et al.* (2014):** Mid-trimester maternal serum and amniotic fluid biomarkers for the prediction of preterm delivery and intrauterine growth retardation. J Obstet Gynaecol Res., 40(6):1540-6.
6. **Salem S, Ashoush S, Bayoumi H *et al.* (2019):** Role of Maternal Serum Ferritin in the Prediction of Asymmetric Intrauterine Growth Restriction. Evidence Based Women's Health Journal, 9(3):494-500.
7. **Kiserud T, Piaggio G, Carroli G *et al.* (2017):** The World Health Organization Fetal Growth Charts: A Multinational Longitudinal Study of Ultrasound Biometric Measurements and Estimated Fetal Weight. PLoS Med., 14(1):e1002220. doi: 10.1371/journal.pmed.1002220.
8. **Bindal N, Godha Z, Kohli R *et al.* (2015):** Role of maternal serum ferritin as a predictive marker in intrauterine growth restriction. Int J Reprod Contracept Obstet Gynecol., 4(3):804-8.
9. **Fakher D, Abd Alhakam F, Fouad W (2015):** Maternal serum ferritin in relation to birth weight. Al-Azhar Assiut Medical Journal, 13:174-9.
10. **Rahman S, Siraj M, Islam M *et al.* (2021):** Association between maternal plasma ferritin level and infants' size at birth: a prospective cohort study in rural Bangladesh. Global health Action, 14(1):1870421. doi: 10.1080/16549716.2020.1870421.
11. **Abdel-Malek K, El-Halwagi M, Hammad B *et al.* (2018):** Role of maternal serum ferritin in prediction of preterm labour. J Obstet Gynaecol., 38:222-5.
12. **Milasinović L, Visnjevac N, Bogavac M *et al.* (2013):** Significance of serum ferritin level in the prediction of delivery of low birth weight newborns for gestational age. Srp Arh Celok Lek., 141(5-6):337-43.