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Association of Serum Ferritin Level in Women with Preeclampsia

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Abstract
Pre-eclampsia, the most common hypertensive disorder of pregnancy, affects around 5% of all first-time mothers, and is an important cause of fetal and maternal morbidity and mortality worldwide. Increased serum ferritin level in pre-eclampsia, decreasing the antioxidant capacity of serum and exacerbating lipid peroxidation and endothelial-cell injury leads to vascular damage which further causes development of preeclampsia. The study was designed to evaluate the association of elevated serum ferritin level and preeclampsia. The knowledge of which expected to be used for prevention of preeclampsia and its complications. A case control study was conducted in the Department of Obstetrics and Gynecology, Institute of Child and Mother Health (ICMH), Matuail, Dhaka, among pregnant women at 32-38 weeks of gestation diagnosed as preeclampsia (cases) and without preeclampsia. Preeclampsia was diagnosed by measuring blood pressure, proteinuria or presence of severe symptoms. Serum ferritin level was measured. Data was collected and descriptive and inferential analysis was carried out using SPSS version 22.0. P-value less than 0.05 was considered as statistically significant. Mean iron (µg/dL) of case (135.76±13.53) and control (82.84±11.79). Mean serum ferritin level among case (127.75±11.77) and control (24.70±2.89). The difference was statistically significant (p<0.05) between two groups. Patients having serum ferritin level >120 ng/ml increased 4.83 times more chance to develop preeclampsia with 95% CI 2.92-8.00%. In multivariate regression analysis a subject with high ferritin level had 1.302 times significantly associated to developed preeclampsia with 95.0% C.I. 0.056 to 1.627%. Significant positive Pearson's correlation observed between serum ferritin level and systolic blood pressure (r=0.820, p=0.001). In this study, finding conveyed that patients with preeclampsia have higher serum ferritin levels in comparison with normal pregnant women. Elevated serum ferritin is found associated to develop preeclampsia.

Keywords: Serum ferritin level, preeclampsia, elevated serum ferritin

Introduction
Preeclamsia is a multisystem disorder that is primarily characterized by systolic blood pressure of 140 mm Hg or more or diastolic blood pressure of 90 mm Hg or more on two occasions at least 4 hours apart after 20 weeks of gestation in a woman with a previously normal blood pressure and proteinuria, 300 mg or more per 24 hours urine collection (Or this amount extrapolated from a timed collection) or protein/creatinine ratio of 0.3 mg/dl or more or dipstick reading of 2+ (Used only if other quantitative methods not available) or in the absence of proteinuria, new-onset hypertension with the new onset of any of the following: thrombocytopenia (Platelet <100,000x10^9/L), renal insufficiency (Serum creatinine concentrations>1.1 mg/dl or a doubling of the serum creatinine concentration in the absence of other renal disease), impaired liver function (Elevated blood concentrations of liver transaminases to twice normal concentration), pulmonary edema, new-onset headache unresponsive to medicine and not accounted by alternative diagnoses of visual symptom [1]. Preeclampsia is a multi-systemic disorder occurring after 20 weeks of gestation of unknown etiology. In severe cases, it increases the risk of organ damage threatening the lives of both mother and baby. Globally preeclampsia and other hypertensive disorders of pregnancy are a leading cause of maternal and infant illness and death [2]. Generally, 3%-5% of pregnancies are complicated with preeclampsia [3]. Incidence of preeclampsia worldwide is around 2-10% of all pregnancies according to the World Health Organization (WHO) its incidence is

seven times higher in developing countries (2.8% of live births) than in developed countries (0.4%) [4]. Some investigators Berhe *et al*. [5], Sebastian *et al*. [6] and Adane *et al*. [7] reported that in less-developed countries the incidence varied from 4.0% to 12.3%. Preeclampsia and eclampsia may increase the prevalence of various cardiovascular disease, including metabolic syndrome, impaired insulin metabolism, microalbuminuria, endothelial dysfunction [8], inflammatory factors and oxidative stress [9]. If preeclampsia is not diagnosed or treated, it is associated with abruptio-placenta, acute renal failure (ARF) disseminated intravascular coagulation (DIC), HELLP (H: haemolysis, EL: Elevated liver enzyme and LP: Low Platelet count) syndrome, cerebral haemorrhage and maternal death [10]; intrauterine growth retardation (IUGR), preterm delivery, low birth weight and neonatal death are common perienatal outcome associated with preeclampsia [11]. Effective management of preeclampsia may be divided into three categories including the prevention of preeclampsia, early detection and treatment [12]. Serum ferritin is a reliable indicator of total body iron status in non-diseased individuals, with low concentrations diagnostic of iron deficiency. However, high ferritin not always signifies iron excess. Ferritin is a major iron storage protein found in spleen, liver, bone marrow, mucosa of small intestine, placenta, kidney, testes, skeletal muscle and plasma. Several independent investigators have demonstrated through studies that vascular endothelium provides a single target organ system involved in PE. In Bangladesh iron supplementation in pregnant women is a common practice without measuring whether patient is iron deficient or not. If the women who are not anemic will take large doses of supplemental iron during pregnancy may build up the iron stores, ferritin. It reflects iron status of the patient and its high level may have role in the pathophysiology of pre-eclampsia. There are limited studies in the pathophysiology of preeclampsia. So this study is an attempt to determine the association of serum feritin with preeclampsia.

Materials and Methods

Study design: Case-control study.
Period of study: From September 2017-August 2019.

Place of study: This study was carried out in the Department of Obstetrics and Gynecology of Institute of Child and Mother Health (ICMH), Matuail, Dhaka, Bangladesh.

Study population: The study population was including the pregnant women between 32 to 38 weeks of gestation attending in the outdoor and indoor of the Department of Obstetrics and Gynecology of ICMH.

a. Case: Pregnant women with gestational age between 32 to 38 weeks with preeclampsia (age –18 to 35 years).

b. Control: Pregnant women with gestational age between 32 to 38 weeks without preeclampsia (age – 18 to 35 years). Controls were selected with a ratio of 1:1 matching with age and gestational age of cases that won't have preeclampsia.

Sample size determination: To calculate sample size for case control study the comparison between two means, the following formula was followed:

$$n = \frac{(Z_\alpha + Z_\beta)^2 \times (\sigma_1^{\ 2} + \sigma_2^{\ 2})}{(\mu_1 - \mu_2)^2}$$ [5].

Estimated sample size was = 43 (in each group).
Targeted sample size was 50 in each group
During the study period a total of 92 (46×2) were collected.
All values were taken from Fatima *et al*. [2].

Inclusion criteria

Case

Pregnant women with preeclampsia at 32-38 weeks (age – 18 to 35 years).

Control

Pregnant women without preeclampsia at 32-38 weeks (age - 18 to 35 years).

Exclusion criteria

- Known case of chronic hypertensive patient
- Pregnancy with GDM, type 2 diabetes
- Pregnancy with known case of Iron deficiency anaemia
- Pregnancy with cardiovascular disease
- Patient with thyroid diseases
- Pregnant women with any malignancy

Data collection

Case and control were selected purposively according to the availability of the respondent. Detailed Obstetric and medical history and clinical information were obtained by preformed structured questionnaire.

Measurement of Blood pressure

After 10 minutes of rest, BP was measured following standard procedure. Korotkoff phase – I (First beat heard) and phase – V (Disappearance of sound) was used to determine systolic (SBP) and diastolic blood pressure (DBP) [13].

Measurement of proteinuria

About 5 ml of midstream random urine sample was collected in a clean and dry test tube. The reagent strip was dipped into the urine for making sure that all the reagent areas have contacted the urine specimen. The excess urine was removed by running the edge of the strip against the rim of the test tube and was held in horizontal position to prevent mixing of the chemical from adjacent reagent areas and to prevent contamination of hand with urine. The strip was properly oriented near the appropriate color chart on the container label and read the results under good lighting. Urinary protein changes the color of the reagent strip from yellow to green. Urinary protein of 0.3 gm/l or more were considered as positive. The results are graded as nil (Less than 10 mg per dL), trace (10 to 20 mg per dL), 1+ (30 mg per dL), 2+ (100 mg per dL), 3+ (300 mg per dL) or 4+ (1,000 mg per dL) [14].

Study procedure

The study population was pregnant women attended in the department of Obstetrics and Gynecology, ICMH fulfilling the inclusion and exclusion criteria. All participants were in their 32-38 weeks of gestation, primi or multigravida. The purpose and procedure of the study were discussed with the patients. Informed written consent was taken from those

who agree to participate in the study. Ethical committee clearance was obtained from the institution. Thorough clinical examination was done in all the subjects. Blood were taken from the ante cubital vein using a sterile needle and syringe. The blood samples were centrifuged immediately in a cooling centrifuge. Serum ferritin was measured by ELISA method using two high-affinity monoclonal antibodies in an immune metric assay system (Delaware Biotech Kit, USA). The Intra-assay and inter-assay coefficient variation of ferritin were 5.7% and 6.6% respectively. For each and every subject separate data collection sheet were prepared. Data were collected from the patients on variables of interest using the structured questionnaire design by interview, observation, clinical examination, biochemical investigations of the patients.

Blood collection

Maternal blood samples were drawn from the antecubital vein (in an arm without intravenous infusion ongoing). 5 milliliters blood was drawn with proper aseptic precautions. The blood sample were transferred into a clean, dry test tube and taken to the laboratory.

Measurement of serum ferritin level

Abbassi-Ghanavati *et al*. [15]: After arrival in the laboratory, venous blood for the clients was withdrawn from cubital vein. Serum ferritin was measured by a sandwich ELISA technique using two high-affinity monoclonal antibodies in an immune metric assay system (Delaware Biotech Kit, USA) that was developed using polyclonal anti ferritin raised in rabbit against the human liver ferritin obtained from International Centre for Control of Nutritional Anemia (ICCNA, USA) and HRP anti ferritin conjugate. This method has been standardized against reagents obtained from ICCNA and International Committee for Standardization in Hematology (ICSH, UK).

Data analysis

Statistical analyses was carried out by using Windows based Microsoft XL and Statistical Package for Social Sciences (SPSS 22.0) where required. Quantitative variables were presented as means ± standard deviations and tested by the unpaired t-test. The qualitative observations were indicated by frequencies, percentages and Chi-Square test was used to analyze, shown with cross tabulation. Relative risk with 95% confidence interval was performed for serum ferritin level and Multivariate logistic regression analysis was done $p<0.05$ was consider as statistically significant difference.

Results

This study carried out to assess the association of serum ferritin level in women with preeclampsia. A total of 46 cases and 46 controls were selected for the study. Findings of the study are presented by graphs and tables.

Socio-demographic characteristics of the respondents

Socio-demographic characteristics of the respondents are shown in table 1. It was observed that more than two third (67.4%) patients belonged to age 20-30 years in case group and 29(63.0%) in control group. The mean age was 27.00±3.54 years in group I and 26.23±3.5 years in group II. Nearly half (43.5%) patients education level were SSC level in case group and 8(17.4%) in control group. Majority (82.6%) patient's occupations were house wife in case group and 42(91.3%) in control group. More than three fourth (80.4%) patients belonged to lower middle class in case group and 28(60.9%) in control group. The difference of educational status and monthly family income were statistically significant ($p<0.05$) between two groups.

Table 1: Socio-demographic characteristics of the respondents

Socio-demographic characteristics	Case (n=46)	Control (n=46)	p value
	N (%)	N (%)	
Age (in years)			
≤20	8(17.4)	11(23.0)	0.734^{ns}
21-30 years	31(67.4)	29(63.0)	
31 years & above	7(15.2)	6(13.0)	
Mean±SD	27.00±3.54	26.23±3.5	
Rang (min, max)	18,34	19,35	
Educational status			
Primary and below	25 (54.3)	24 (52.2)	0.001^{s}
SSC	20 (43.5)	8 (17.4)	
HSC and above	1 (2.2)	14 (30.4)	
Occupation			
Housewife	38 (82.6)	42 (91.3)	0.191^{ns}
Student	5 (10.9)	4 (8.7)	
Service	3 (6.5)	0 (0.0)	
Monthly family income (Taka)			
Lower middle class	37 (80.4)	28 (60.9)	0.039^{s}
Upper middle class	9 (19.6)	18 (39.1)	

S = Significant
NS = Not Significant
p value reached from Chi-Square test

Obstetrical characteristics of the respondents

The obstetrical conditions of the patients are shown in table 2. Almost two third (65.9%) patients were multi para in case group and 27(59.1%) in control group. Majority (87.0%) patients belonged to gestational age <37 weeks in case group and 42(91.3%) in control group. The mean gestational age was 34.73±5.11 weeks in case and 33.97 ± 5.77 weeks in control group. The difference was statistically not significant (p>0.05) between two groups.

Table 2: Obstetrical characteristics of the respondents

Obstetrical characteristics	Case (n=46)	Control (n=46)	p value
Number of pregnancies	N (%)	N (%)	
Primi para	16 (34.1)	19 (40.9)	^{a}0.519^{ns}
Multi para	30 (65.9)	27 (59.1)	
Gestational age			
<37	40 (87.0%)	42 (91.3%)	
≥37	6 (13.0%)	4 (8.7%)	
Mean ± SD	34.73 ± 5.11	33.97 ± 5.77	^{b}0.505^{ns}

NS = Not Significant
^{a}p value reached from Chi-Square test
^{b}p value reached from Unpaired t-test

Hematological status of the respondents

Hematological status are shown in table 3. Mean iron (µg/dL) of case (135.76±13.53) and control (82.84±11.79). Mean serum ferritin level among case (127.75±11.77) and control (24.70±2.89). The difference was statistically significant (p=0.05) between two groups.

Table 3: Hematological status of the respondents

Hematological status	Case (n=46) Mean ± SD	Control (n=46) Mean ± SD	p value
Iron (µg/dL)	135.76 ± 13.53	82.84 ± 11.79	0.001[s]
Serum ferritin (ng/ml) level	127.75 ± 11.77	24.70 ± 2.89	0.001[s]

S = Significant, p value reached from unpaired t test

Association of serum ferritin level and preeclampsia
High level of serum ferritin was more in case (73.9%) compared with control (0.0%) group which is statistically significant (P= 0.001). Respondents with high ferritin level have 4.83 times more chance to develop preeclampsia (RR=4.83; 95% CI = 2.92-8.00).

Table 4: Association of serum ferritin level and preeclampsia

Serum ferritin (ng/ml)	Case (n=46)	Control (n=46)	p value	RR (95% CI)
	N (%)	N (%)		
> 120	34 (73.9)	0 (0.0)	0.001	4.83 (2.92 – 8.00)
≤ 120	12 (26.1)	46 (100.0)		

Factors associated with preeclampsia in multivariate logistic regression analysis: In multivariate regression analysis a subject with high ferritin level had 1.302 times significantly associated to developed preeclampsia with (95.0% C.I. 0.056 to 1.627%). But age, education, monthly family income, parity and BMI were not significantly associated with preeclampsia.

Table 5: Factors associated with preeclampsia in multivariate logistic regression analysis

	B	S.E.	P value	OR	95% C.I. for	
					Lower limit	Upper limit
Ferritin (ng/ml)	-4.631	1.036	0.001	1.302	0.056	1.627
Age	-1.963	1.082	0.070	0.140	0.017	1.171
Education	-1.223	0.723	0.091	0.294	0.026	0.792
Monthly family income	-1.197	0.859	0.163	0.010	0.001	0.074
Parity	0.680	0.747	0.363	0.136	0.456	1.239
BMI	-1.155	0.355	0.282	0.305	0.035	2.653

Correlation between serum ferritin level and systolic blood pressure: Correlation between maternal serum ferritin level and systolic blood pressure is shown in figure 1 where positive correlation observed (r=0.820, p=0.001).

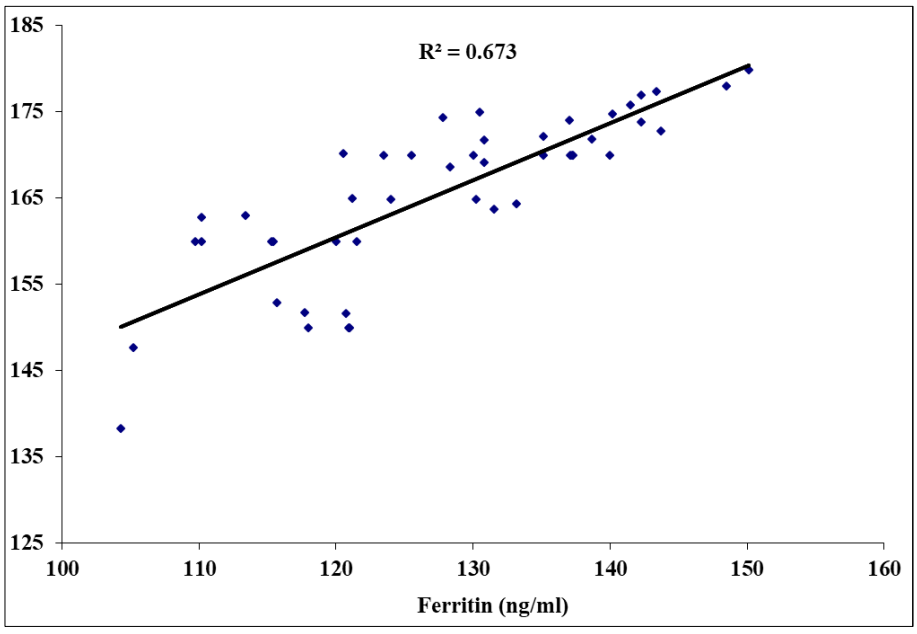

Fig 1: Scatter diagram showing positive significant Pearson's correlation (r=0.820, p=0.001) between serum ferritin level and systolic blood pressure.

Correlation between serum ferritin level and diastolic blood pressure: Correlation between maternal serum ferritin level and diastolic blood pressure is shown in figure 2 where positive correlation observed (r=-0.807, p=0.001).

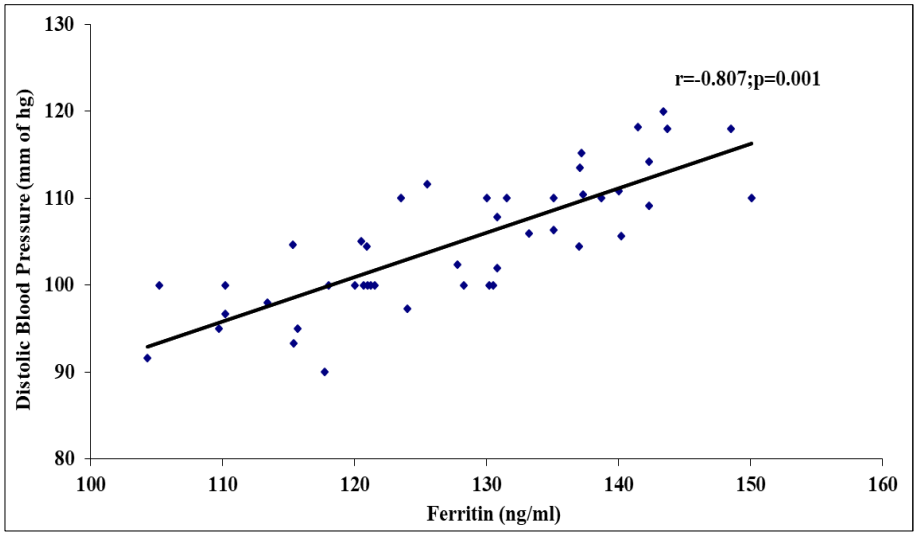

Fig 2: Scatter diagram showing significant positive Pearson's correlation (r=0.807, p=0.001) between serum ferritin level and diastolic blood pressure.

Discussion

Preeclampsia is still one of the leading causes of maternal and fetal morbidity and mortality. Despite active research for many decades, the etiology of this disorder remains exclusive to human pregnancy is an enigma. Recent evidence suggests there may be several underlying causes or predispositions of preeclampsia leading to endothelial dysfunction and causing hypertension, proteinuria and edema. Regarding the socio-demographic characteristics of the respondent's age and occupational status were almost alike between two groups, which is similar with Paul *et al.* [14], Aghade and Bavikar [16], Bakacak *et al.* [17] and Nugteren *et al.* [18] studies. However, poor educational level and poor socioeconomic status significantly more common in preeclampsia group in this study. Silva *et al.* [19] observed women with low educational level were more likely to develop preeclampsia OR=5.12; 95%CI: 2.20 - 11.93. Choe *et al.* [20] mentioned in their study that low maternal socioeconomic status is strongly associated with preeclampsia, which support with the present study. In this current study, it was observed that 65.9% patients had 2 or more pregnancy in preeclampsia group and 59.1% in control group. The difference was statistically not significant (p>0.05) between two groups. Grum *et al.* [21] study observed that primigravida to be associated to developing preeclampsia 2.68 times higher in primigravida comparing to the multigravida women having 95% CI: 1.38, 5.22. Many investigators Paul *et al.* [14], Sun *et al.* [22] and Yelikar *et al.* [23] and also reported that primigravida significantly associated to develop preeclampsia. In this current study, it was observed that 87.0% patients belonged to gestational age <37 weeks in preeclampsia group and 91.3% in control group, which is comparable with Aghade and Bavikar [16] and Dabbaghmanesh *et al.* [24] studies. In this present study, it was observed that the mean iron level was 135.76 ± 13.53 µg/dL and 82.84±11.79 µg/dL in preeclampsia and control group respectively, which is significantly higher in preeclampsia patients. Maitra *et al.* [25], Jana *et al.* [26] and Yesmin *et al.* [27] studies showed significantly higher level of serum iron in preeclamptic group as compared to the normotensive control group. In this present study, it was observed that the mean serum ferritin level was 127.75±11.77ng/ml in preeclamptia group and 24.70±2.89 ng/dl in control group. The mean serum ferritin level was significantly higher in pre-eclamptic patients than the control group. Similarly, Maitra *et al.* [25] study found that the mean serum ferritin concentration in cases and controls were 55.35µg/l and 17.19µg/l respectively. They showed significantly higher level of serum ferritin in preeclamptic group as compared to the normotensive control group. In our country Paul *et al.* [14] showed the mean serum Ferritin level of preeclamptic group was 167.11±10.43 ngm/ml and 17.0±3.03 ngm/ml in control. Serum Ferritin level is significantly higher in preeclamptic patients than the control group. In Bangladesh Yesmin *et al.* [27] study found the mean serum ferritin concentration of preeclamptic group was 99.91±7.84 ng/ml and control group was 18.47±2.03 ng/ml, which was also significantly higher in preeclamptic patients than the control group. Hubel *et al.* [28] reported that the total serum ferritin concentrations were approximately fivefold higher in preeclampsia than in women with healthy pregnancies, which are contributed to increased cellular damage in preeclampsia. It was also hypothesized that ferritin synthesis is increased in preeclampsia. This is attributed to hepatocellular, rather than placental damage. However, Brunacci *et al.* [29] study reported that increased serum iron and ferritin are rendered to decreased hepcidin levels, the peptide hormone that coordinates iron absorption and distribution, in women with pre-eclampsia. Although, it was reported by Muhsin *et al.* [30] that serum hepcidin levels were within the normal ranges in women with preeclampsia, yet significantly higher than controls. This striking increase in serum levels of iron and ferritin may even have the potential to be used diagnostically to warn of developing preeclampsia. Through effects on formation of oxygen free radicals and subsequent lipid peroxidation, ferritin might be a significant etiologic factor in the endothelial cell damage of preeclampsia (Kwon *et al.* 2007 and Hubel *et al.* 2004) [28, 31]. There was a significant difference in ferritin levels in women with preeclampsia compared with those of the women with normal pregnancies, 63.8 vs 22.47, respectively. Similar findings also observed by Jana *et al.* [26], Putra *et al.* [32], Fatima *et al.* [2], Hameed and Aboud [33], Siddique *et al.* [34] and Toldi *et al.* [35]. In this present study there was a significant positive Pearson's correlation was found between serum ferritin level and systolic blood pressure (r=0.820, p=0.001) and with diastolic blood pressure (r=0.807, p=0.001) in preeclampsia group. In this

present study, it was observed that 73.9% patients had high level of serum ferritin in preeclampsia group and not found in control group. Patients having serum ferritin level >120 ng/ml increased 4.83 times more chance to develop preeclampsia with 95% CI 2.92-8.00%. Paul *et al.* [14] study showed mean serum Ferritin level of preeclampsia group was almost 10 times higher than that of controls ngm/ml. Yesmin *et al.* [27] study reported that mean serum ferritin concentration of preeclamptic group was 5 times higher than that of control group. In multivariate regression analysis in this study it was observed a subject with high ferritin level had 1.302 times significantly associated to developed preeclampsia with (95.0% C.I. 0.056 to 1.627%). But age, education, monthly family income, parity and BMI were not significantly associated with preeclampsia in multivariate regression analysis.

Conclusion

The study finding suggest that patients with preeclampsia have higher serum ferritin level in comparison with normal pregnant women. So elevated maternal serum ferritin is associated with preeclampsia.

Limitations of the study

1. Follow up study is needed to observe whether the changes in serum ferritin that took place during preeclampsia sustain after pregnancy is over.
2. As estimation of total iron profile like total iron binding capacity was costly, so I investigated S. ferritin & S. iron level. If other parameters of iron profile been investigated, a better picture of iron status in preeclampsia women would have reflected.

Recommendations

Prospective studies are needed to see whether serum ferritin is casual to preeclampsia or a manifestation of preeclampsia.

Conflict of Interest

Not available

Financial Support

Not available

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