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Evaluation of C-Reactive Protein, Some Interleukins, Trace Elements and Immunoglobulin Level in Preeclamptic Women Attending Federal Teaching Hospital, Owerri

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ABSTRACT: Along with bleeding and infection, preeclampsia is one of the fatal trifecta of pregnancy complications that significantly increases maternal morbidity and mortality. The purpose of the study was to measure the levels of immunoglobulin, trace elements, C-reactive protein, and certain interleukins in preeclamptic women undergoing treatment at the Federal Teaching Hospital in Owerri, Imo state. About 50 preeclamptic individuals, 50 hypertensive pregnant women, and 50 seemingly healthy pregnant women made up the test group, which also included 50 control subjects. Standard techniques were used to determine the individuals' serum levels of immunoglobulins (IgA and IgM), interleukin, zinc, magnesium, iron, and C-reactive protein. SPSS version 27 was used to analyse the test outcomes. Preeclampsia women had significantly higher mean values of CRP (50.04±1.77) mg/L, TNF-alpha (29.84±2.25)pg/mL, IL-6 (40.08±2.44)pg/mL, Zn (22.14±1.95) µmol/L, and Fe (185.56±9.47) µg/dL than control (p=0.000, p=0.000, p=0.000, and p=0.000). In preeclamptic women, the mean IgA level (293.78±13.00) mg/dl was considerably lower (p=0.000) than in the control group. When comparing preeclamptic women to control, there was no significant difference (p=0.316 and p=0.517) in the mean values of Mg (0.87±0.15)mmol/l and IgM (112.66±9.32) mg/dl. Preeclamptic women had significantly higher mean values of CRP (50.04±1.77) mg/L, TNF-alpha (29.84±2.25) pg/mL, and Zn (22.14±1.95) µmol/L than control (p=0.000, p=0.000, p=0.000, and p=0.000). When comparing hypertensive pregnant women to the control group, the mean values of Fe (185.56±9.47)µg/dL and IgM (112.66±9.32) mg/dl were considerably lower (p=0.000 and p=0.000), respectively.When comparing preeclamptic women to the control group, there was no statistically significant difference (p=0.290 and p=0.286) in the mean values of IgA (293.78±13.00) mmol/l, mg (0.87±0.15) mmol/l, and IL-6 (40.08±2.44) pg/ml.In the preeclamptic group, there was a substantial positive connection (p<0.05) between magnesium and TNF-alpha.Iron and zinc, however, did not significantly correlate with a few immunological markers in the preeclamptic group (p>0.05). Elevations of C-reactive protein, TNF-alpha, IL-6, zinc, and iron have been linked to preeclampsia.IgA levels are lowered in patients with the condition, but there is no discernible correlation with IgM levels.In preeclamptic women, there was a noteworthy positive correlation observed between magnesium and TNF-alpha.

KEY WORDS: C-Reactive Protein, Interleukins, Trace Elements, Immunoglobulin, Preeclamptic Women

INTRODUCTION

Preeclampsia (PE) is a pregnancy condition marked by the start of elevated blood pressure and frequently a noticeable protein build-up in the urine.Preeclampsia is defined as follows: onset at 20 weeks gestational age; 24-hour proteinuria of 30 mg/day or, if unavailable, a protein concentration of 30 mg in a minimum of two random urine samples collected at least 4-6 hours but no more than 7 days apart; a systolic blood pressure of 140 mmHg or a diastolic blood pressure of 90 mmHg as measured twice, using an appropriate cuff, 4-6 hours and less than 7 days apart; and the disappearance of all these abnormalities before the end of the sixth week postpartum.However, some cases of pregnancy-related hypertension in conjunction with anomalies in the laboratory or clinical setting, or with intrauterine growth restriction, should also be regarded as possible preeclampsia[1].

Seizures that occur in preeclamptic women are known as eclampsia, and hypertensive pregnancy is mainly associated with preeclampsia that is superimposed [2].

Due to prematurity and the disease's impact on the foetus, there is a significant rate of foetal mortality and morbidity. Fifteen percent of premature deliveries are the result of women being delivered earlier than recommended in order to stop preeclampsia from progressing. Maternal mortality is widespread in developing nations when prenatal care is inadequate and limits preeclampsia surveillance, accounting for 50,000 fatalities annually.

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Heterodimeric proteins called immunoglobulins are made up of one or more units, each of which has four polypeptide chains: two light chains (L) and two heavy chains (H) that are exactly the same [3]

Serum contains antibodies belonging to the following five immunoglobulin classes, or isotypes: IgG, IgM, IgA, IgE, and IgD. The kind of thick chain they include sets them apart. IgMs have μ-chains, IgAs have α-chains, IgEs have ε-chains, and IgDs have δ-chains. IgG molecules have heavy chains known as γ-chains ([4]). Each immunoglobulin class can operate in a different kind of immunological response or at a different stage of the body's defense due to variations in heavy chain polypeptides. The Fc domain contains the majority of the amino acid sequences that convey these functional distinctions.

The quantities of trace elements, such as copper, iron, manganese, selenium, and zinc, that regulate the antioxidant defense system vary during a typical pregnancy, and they are essential for the growth and development of the fetus [5]. Zinc, manganese, and copper are necessary trace elements for superoxide dismutase (SOD), an antioxidant enzyme, to function at its best [6]. It has been discovered that some trace elements lower the chance of developing pre-eclampsia.

The liver is the only organ that produces the class of acute phase reactants, which includes C-reactive protein (CRP). Its levels have been demonstrated to increase dramatically after acute inflammation [7].

As a result, it has been proposed that CRP serves as a marker of inflammation in a number of illnesses, such as myocardial infarction, rheumatoid arthritis, psoriasis, and tuberculosis [4]. Within hours of acute inflammation, the rate of CRP synthesis and secretion rises, reaching a peak level in 24–48 hours. It has been demonstrated that the primary function of CRP is to identify potentially hazardous autogenous chemicals that are produced from injured tissue and to bind and eliminate them from blood. Additionally, there is a correlation between its levels and the disease's severity [8].

These employees also noted that although CRP levels decrease following a 12-week course of treatment, values do not return to normal. Pre-eclampsia is characterized by the persistence of pro-inflammatory macrophages during pregnancy, as well as increased levels of interleukin (IL)-6, tumor necrosis factor-α, and interferon-γ. In contrast, anti-inflammatory cytokines, such as IL-4 and IL-10, are reduced. These results imply that favorable perinatal outcomes in a typical pregnancy depend on a proper balance between pro- and anti-inflammatory macrophage subsets in the placenta. Owing to a lack of data, the goal of this study is to identify the biomarkers that are most useful in the diagnosis of preeclampsia.

MATERIALS AND METHOD

Study Area

The study was conducted among patients with preeclampsia attending antenatal at the Federal Teaching Hospital, Owerri, Imo State, Nigeria. Owerri is the capital of Imo State in Nigeria, set in the heart of Igboland. It is also the state's largest city, followed by Orlu, Okigwe and Ohaji/Egbema. Owerri consists of three Local Government Areas including Owerri Municipal, Owerri North and Owerri West, it has an estimated population of about 1,401,873 as of 2016 and is approximately 100 square kilometres (40 sq mi) in area. Owerri is bordered by the Otamiri River to the east and the Nworie River to the south.Rain falls for most months of the year with a brief dry season. The Harmattan affects the city in the early periods of the dry season and it is noticeably less pronounced than in other cities in Nigeria. The average temperature is 26.4 °C.

Ethics, Advocacy and Pre-survey Contact

The ethical clearance was obtained from the health institution where the research was conducted; that is Federal medical Centre Owerri.

Sample size determination

Sample size was calculated using the formula below determined.The lowest prevalence rate of preeclampsia is in southern Nigeria is 2.8 %

$n=z^2p(q)/d2$

d^2

where

n=sample size

q=1.p

p= the prevalence of preeclampsia in Nigeria using a confidence interval set at 95% is 1.96 %

q=1-p

d= degree of accuracy set at 0.05

$n= \frac{1.96^2 x 0.028 (1-0.028/ 0.05^2)}{} = \frac{3.8416 x 0.272/0.0025}{} = 41.797$

The minimum sample size is 50 . From the sample size, approximately patients with preeclampsia was selected from the subject population.

Selection Criteria

Inclusion criteria: Key Diagnostic Criteria (Based on WHO Guidelines)

People which have:

1. New-onset hypertension: Systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, measured on two occasions at least 4 hours apart after 20 weeks of gestation.
2. Proteinuria:
   ≥ 300 mg /24 hours of protein in urine collection
   ≥ 1+ protein on dipstick testing (if dipstick is used)

Presence of other complications, including:

3. Elevated liver enzymes: AST (aspartate aminotransferase) or ALT (alanine aminotransferase) levels at least twice the upper limit of normal also where considered .
4. Renal dysfunction: Serum creatinine > 1.1 mg/dL
5. Pulmonary edema: Fluid accumulation in the lungs
6. Additional Considerations:

Gestational Age: where considered atleast 20 weeks and above of gestation.

Exclusion Criteria:

Certain conditions can mimic preeclampsia, so it's important to rule out other possibilities, so people with:

* Chronic hypertension
* Chronic kidney disease
* Gestational hypertension (high blood pressure without proteinuria) where Excluded.

Study Design

This is a case control study that was carried out among patients with preeclampsia attending the antenatal clinic of federal teaching hospital Owerri. The test group comprises of about 50 patients with preeclamptic, 50 hypertensive pregnant women and 50 healthy women has also serve as control , whose age matched with the test population. A structured questionnaire was issued to them for the purpose of obtaining some information regarding their medical and demographic characteristics, in addition to their hospital records. Those who qualify to participate in the research were made to sign a written letter of consent.

Sample Collection

Five milliliters (5 ml) of venous blood was collected and dispensed into a plain container. The tube was properly labeled with the subject's name, sample number and date of collection. The blood was allowed to clot at room temperature, and serum separated and harvested into a clean dry well labeled sample bottles following centrifugation at 3000 rpm for 5 minutes. The sample was stored in a freezer at -20^{oc}, prior to use.

Laboratory Procedures

The reagents employed were all commercially purchased and the manufacturer's standard operating procedure was strictly followed.

The determination of Serum Immunoglobulin IgM, Serum Immunoglobulin IgA and C-reactive protein **were by** Using ELISA Method while the **determination of** Selenium, copper and zinc level was by using the Atomic Absorption spectrophotometers (AAS) assay.

Serum concentrations of Interleukins, TNF-α was determined with the Human Cytokine/Chemokine Panel I Merck Millipore (Cat. No. MPXHCYTO-60K, Millipore Corporation, Billerica), according to the manufacturer's instructions on the Luminex fully automated analyzer.

Statistical Analysis

Results were presented in mean ± standard deviation (SD). All data obtained in the study were analyzed using student t-test (spss.21) and Pearson correlation coefficient. The level of significance was set at $p < 0.05$.

RESULT

Table 1: Mean =+STD of CRP, TNF-alpha, IL-6, Mg, Zn, Fe, IgA and IgM in Preeclamptic Women versus Control

Parameter	Preeclamptic Women	Control	t-value	p-value
CRP (mg/L)	50.04±1.77	12.00±2.33	64.95	0.000
TNF-alpha (pg/mL)	29.84±2.25	12.25±2.00	29.15	0.000
IL-6 (pg/mL)	40.08±2.44	22.11±2.31	26.69	0.000
Mg (mmol/l)	0.87±0.15	0.82±0.16	1.01	0.316
Zn (µmol/L)	22.14±1.95	16.93±1.31	11.09	0.000
Fe (µg/dL)	185.56±9.47	65.52±4.19	57.92	0.000
IgA (mg/dl)	293.78±13.00	342.73±39.79	5.85	0.000
IgM (mg/dl)	112.66±9.32	110.98±8.89	0.65	0.517

KEY:
CRP: C-reactive protein
TNF-alpha: Tissue Necrosis Factor
IL-6: Interleukin-6
Mg: Magnesium
Zn: Zinc
Fe: Iron
IgA: Immunoglobulin A
IgM: Immunoglobulin M
*: Significant

The mean value of CRP (50.04±1.77)mg/L, TNF-alpha (29.84±2.25)pg/mL, IL-6 (40.08±2.44)pg/mL, Zn (22.14±1.95) µmol/L and Fe (185.56±9.47) µg/dL was significantly increased (p=0.000, p=0.000, p=0.000 and p=0.000) in preeclamptic women when compared to control.

The mean value of IgA (293.78±13.00)mg/dl was significantly decreased (p=0.000) in preeclamptic women when compared to control.

There was no significant difference (p=0.316 and p=0.517) in the mean value of Mg (0.87±0.15)mmol/l and IgM (112.66±9.32)mg/dl in preeclamptic women when compared to control.

DISCUSSION

Along with bleeding and infection, preeclampsia is one of the fatal trifecta of pregnancy complications that significantly increases maternal morbidity and mortality [10].

According to the current study's findings, preeclamptic pregnant women had a considerably higher mean value of C-reactive protein than hypertensive and control pregnant women. An objective and sensitive measure of the body's total inflammatory activity is C-reactive protein (CRP) [11]. The increased maternal inflammatory response during pregnancy is the cause of the elevated C-reactive protein level. The outcome is consistent with a study by [12] that discovered preeclamptic pregnant women had greater serum CRP concentrations than hypertensive pregnant women.

In addition, women with severe preeclampsia had significantly higher CRP levels than patients with mild preeclampsia. ($p<0.001$) Numerous observations are similar to this one [13].

According to the current study, preeclamptic women had a considerably higher mean value of TNF-alpha than control women. Preeclampsia during pregnancy is associated with an elevated level of TNF-α because endothelial cells, the target cell type, are injured [14]. The results of this investigation are in line with a publication by [15], which stated that endothelial cell injury and induced inflammation cause TNF-α to be produced in preeclampsia [16].

This study showed that preeclamptic women had a considerably higher mean value of IL-6 than control women. An inflammatory reaction is what causes an increase in interleukin-6. Also, histopathological chorioamnionitis is the cause of increased IL-6 in preeclamptic women [17]. A well-known association exists between preterm labor and newborn neurological damage and elevated IL6. The outcome is consistent with the research from [18], who looked at prospective changes in inflammatory markers during pregnancy and the inflammatory status 20 years later in women who experienced a pre-eclamptic pregnancy complicated by compared to matched controls. They discovered that pre-eclampsia was linked to both short- and long-term changes in inflammatory status. The hallmark of pre-eclampsia is impaired vascular remodeling, which lowers placental perfusion and produces a hypoxic environment for fetal and placental tissues. As a result, pro-inflammatory cytokines like IL-8 and IL-6 are also markedly elevated in the bloodstream of preeclamptic women [19].

Compared to controls, preeclamptic women's magnesium levels did not significantly rise. [20] corroborated this finding, finding no discernible variation in the plasma magnesium levels of the patients and controls. However, a number of additional investigations revealed that preeclampsia was associated with much lower serum magnesium levels than normal pregnancy. The current study's findings show that preeclamptic women had significantly higher serum zinc concentrations than control groups. The outcome deviates from other authors' findings, which show a notable drop in zinc content. As to [21], the mechanisms that underlie the correlation between the level of serum zinc and PE remain incompletely comprehended. The fact that zinc can boost antioxidant levels or act as necessary substrates or cofactors for the proper activation of antioxidant enzymes, such superoxide dismutase (SOD), is one fundamental theory explaining how zinc might reduce oxidative stress [22].Since zinc is a cofactor of the antioxidant enzyme SOD, a zinc shortage may result in a drop in SOD, which has been linked to a reduction in the antioxidant capacity of cells and an imbalance between oxidants and antioxidants [23].

By the methods described above, zinc deficiency may lead to an imbalance between antioxidants and lipid peroxides (LPO), which may encourage the onset and progression of PE. Given that the patients in this study were hospitalized, the elevated level observed may have resulted from the preeclamptic women receiving zinc treatment.

Compared to controls, preeclamptic women had significantly higher iron levels. Despite the fact that it was much lower in pregnant hypertensive women. The production of hydroxyl and methoxyl radicals in the luminal and mucosal contents of the gastrointestinal tract confirms the role of iron in free radical damage. Local iron excess and iron-mediated oxidative stress have been demonstrated in the intestinal mucosa, liver, spleen, bone marrow, and placenta. [24]

The findings are consistent with the report of [25], which suggests that released iron free radicals from an ischemic placenta may play a role in the etiology of preeclampsia.

According to the current study report, preeclamptic women's mean IgA values were substantially lower than those of the control group. While the mean value of IgA did not differ considerably from that of normotensive pregnant women, it was significantly lower in preeclamptic women. This demonstrates unequivocally that the large drop in IgA may be a marker of a successful, typical pregnancy.

To provide safety for the developing placenta and fetus, there may be a slight reduction of the mother's immune system response. It is possible to propose that in order for a pregnancy to be successful, the mother's immune is less sensitive to the allograft that is forming.The outcome is consistent with the research conducted (12), which published a related conclusion. Furthermore, there was no discernible variation in the average IgM level between preeclamptic and control women.Even yet, there was a drop in comparison to pregnant women with hypertension.Preeclampsia-related immunosuppression, increased urine losses of immunoglobulins—particularly the intermediate group of macroglobulins—or a decline in their synthesis could all be contributing factors to the drop in IgM.

The outcome is consistent with research conducted by [26,27], which found that preeclampsia with magnesium considerably elevated TNF-α levels in comparison to normal and preeclampsia.

CONCLUSION

Elevations of C-reactive protein, TNF-alpha, IL-6, zinc, and iron have been linked to preeclampsia. IgA levels are lowered in patients with the condition, but there is no discernible correlation with IgM levels. In preeclamptic women, there was a noteworthy positive correlation observed between magnesium and TNF-alpha.

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