Evaluation of Levels of Some Inflammatory Cytokines in Preeclamptic Women in Owerri

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Authors’ contributions
This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information
DOI: 10.9734/JPRI/2021/v33i42A32384
Editor(s): (1). Dr. Rafik Karaman, Al-Quds University, Palestine.
Reviewers: (1) Musa Ibrahim Kurawa, Bayero University, Nigeria.
(2) Kaustav Chakraborty, S.B.S. Government College, University of Gour Banga, India.
Complete Peer review History: https://www.sdiarticle4.com/review-history/72825

Received 15 June 2021
Accepted 19 August 2021
Published 25 August 2021

ABSTRACT

Preeclampsia is a serious and life-threatening pregnancy complication. In this study, the levels of inflammatory cytokines were measured in preeclamptic women in Owerri, Imo State. A total of 120 pregnant women aged 18-45 years at 20-40 weeks of pregnancy were recruited; 60 were preeclamptic women (test group) while 60 were normotensive pregnant women (control group). Preeclampsia was determined by the presence of ≥2+ protein in the urine using combi 2 dipstick for urinalysis and sphygmomanometer blood pressure reading of ≥ 140/90 mmHg. From the demographic data obtained in the studied subject through questionnaire, it showed that nulliparity and family history of high blood pressure were the most dominant risk factor of preeclampsia. The mean of the pro-inflammatory cytokines (IL-6 and TNF-α) in the test group were 4.33±1.42 pg/ml and 8.23±2.73 pg/ml while that of the control group were 3.38±1.22 pg/ml and 6.89±1.88 pg/ml. There was a significant increase in the pro-inflammatory cytokines of the preeclamptic women when compared to the control group. The mean of the anti-inflammatory cytokines (IL-10 & IL-4) of the group were 78.88±14.28 pg/ml and 27.92±7.22pg/ml while the control group was 91.39± 18.46

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1. INTRODUCTION

Preeclampsia is universally defined as hypertension and significant proteinuria developed at or after 20 weeks of pregnancy in an otherwise normotensive woman [1-4]. Gestational hypertension is the presence of new hypertension (usually systolic BP > 140 mmHg and/or diastolic BP > 90 mmHg) occurring in the second half of pregnancy, while preeclampsia is the combination of gestational hypertension with proteinuria [5,6]. In the absence of proteinuria, hypertension together with evidence of systemic disease such as thrombocytopenia or elevated levels of liver transaminase is required for diagnosis [7].

Preeclampsia is a multisystem disorder unique to human pregnancy. It is a common complication of pregnancy associated with high maternal morbidity, mortality and uterine fetal growth restriction [8].

Worldwide, preeclampsia affects estimated 2-10% pregnant women [9,10]. More than 4 million women across the world develop this disorder every year and an estimated 50,000 - 76,000 women and 500,000 infants die of this condition every year [10]. In Nigeria, it is estimated that 3-10% are complicated by Hypertensive Disorder in Pregnancy (HDP) [11-16].

Risk factors for preeclampsia include nulliparity, multifetal gestations, previous history of preeclampsia, obesity, diabetes mellitus, vascular and connective tissue disorders like systemic lupus erythematosus and antiphospholipid antibodies, age > 35 years at first pregnancy, smoking, and African American race [8]. The symptoms of preeclampsia may include edema of the hands and face/eyes and weight gain. Severe preeclampsia may present headache, abdominal pain, agitation, decreased urine output, nausea, vomiting and vision changes.

Pregnancy has been described as a state of ‘mild’ controlled inflammation [17]. However when this inflammatory response becomes exaggerated it results in the development of preeclampsia. In pregnancy, inflammation is tightly controlled to prevent excessive inflammation, and is probably achieved by reducing the production of proinflammatory cytokines and/or increasing the production of anti-inflammatory cytokines [18,19]. However, the cytokine profile in preeclampsia shows that the production of type 1 cytokines, which induce inflammation, is dominant while the production of type 2 cytokines, which regulates inflammation, is suppressed [20].

Cytokines are low molecular weight regulatory proteins that are secreted by many cells of the immune system in response to a number of stimuli. The proinflammatory cytokines tumor necrosis factor α (TNF-α), and interleukin (IL) 6 are typically secreted by activated Th1 and Th17 cells to instigate a cytotoxic and inflammatory immune response to foreign pathogens or injury. According to Gadonski et al., during preeclampsia, these cytokines are significantly increased in the maternal circulation and the placenta, resulting in chronic systemic and local placental inflammation, which contributes to the pathophysiologic complications that manifest during preeclampsia. Supporting a role for these cytokines are findings of elevated tumor necrosis factor (TNF)-alpha, and interleukin (IL)-6 plasma levels in preeclamptic women [22-24,18].

In addition to an increase in proinflammatory T cells and inflammatory cytokines, it has been suggested that preeclampsia is characterized by a decrease in Regulatory T cell (Tregs), IL-10 and IL-4, the anti-inflammatory cytokine produced by Tregs [25]. Heo et al., [26] stated that interleukin 10 and 4 are important during pregnancy because of their ability to inhibit secretion of Th1 inflammatory cytokines and thus provide an important counterbalance for controlled inflammation at the fetal-maternal interface.

<table>
<thead>
<tr>
<th>Keywords:</th>
<th>Preeclampsia; pregnant women; IL-6; TNF-α; IL-10; IL-4.</th>
</tr>
</thead>
</table>

| pg/ml and 31.25±6.90 pg/ml. There was a significant decrease in the result obtained from the test group when compared to the control group. The levels of the pro-inflammatory cytokines were higher than the levels of the anti-inflammatory cytokines of the test group when compared with the control group. Hence there is need to manage the disorder with an anti inflammatory drugs in order to enhance obstetric care, thereby reducing maternal mortality and morbidity that may occur from haemostatic abnormalities. |

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However, according to studies by Gu et al.[27] and Lochner et al.,(2008), pro-inflammatory cytokines such as interferon γ (IFN-γ), IL-2, and TNF-α are downregulated by IL-10. Several studies have observed lower levels of IL-10 and IL-4 in circulation and in the placenta of preeclamptic patients [28,29,30].

However, most of these studies were done in countries outside of Africa and the generalizability of these findings to women in Nigerian settings may be limited. Therefore, this study will be done to provide data on the level of inflammatory cytokines in pregnant women with preeclampsia in Owerri, South-East of Nigeria, Africa. I believe these findings will contribute to the body of knowledge on the effects of this disorder on pregnant women residing in this area.

2. MATERIALS AND METHODS

The Study Area

This study was carried out in Federal Medical Centre Owerri in Imo State, Nigeria.

Study population and sample size

A total of 120 subjects all women aged from 18-45 years were recruited for the study. Sixty pregnant women clinically diagnosed as preeclampsia and 60 normotensive pregnant women, all in their third trimester (28-40 weeks) between the ages of 18-45 years attending maternity clinic of Federal medical Centre Owerri, Vaden Specialist Hospital Owerri, Life-Spring Specialist Hospital Owerri and Emekuku Specialist Hospital Owerri were recruited for the study. The sample size was obtained using the formula by Naing et al. (2006). Prevalence rate of preeclampsia is 3.4% [16].

\[ n = z^2 \times P \times (1-P)/d^2 \]

Where

- \( n \) = Sample size
- \( P \) = prevalence rate 3.4%
- \( z \) = confidence interval 95% - 1.96
- \( d \) = Degree of accuracy - 0.05

\[ N = 1.96^2 \times 0.034(1-0.034)/0.05^2 = 50 \]

Therefore, the minimum sample size was 50. Considering 10% attrition, a sample size of 60 was used for the study. The control subjects for this study were randomly selected and were appropriately matched for maternal and gestational age with those of the test group.

Study Design

A case-control study was carried out on 2 groups.

Group 1 = 60 Preeclamptic Pregnant Subjects, Group 2 =60 Normotensive Pregnant Subjects,

Inclusion Criteria

- Pregnant women who were diagnosed of preeclampsia in their third trimester between the ages of 18-45 years. Preeclampsia is defined as hypertension with significant proteinuria after 20 weeks of gestation. Hypertension: Blood pressure of >140/90 mmHg on at least two occasions 6 hours apart; Significant Proteinuria: urinary protein excretion of >300 mg/day quantitatively or ≥2+ on dipstick examination

- Normotensive pregnant women in their third trimester between the ages of 18-45 years.

Exclusion Criteria

Those that were excluded from the study were:

- Pregnant women with evidence of chronic infection like HIV, chronic renal disease, tuberculosis and other inflammatory disease.
- Pregnant women who have past history of diabetes, systemic or endocrine disorder.
- Pregnant women with previous history of hypertension.
- Pregnant women who were in active labour.
- Pregnant women using any kind of anticoagulant drugs.
- Pregnant women who smokes and drink alcohols.
- Pregnant women who did not give their informed consent.
- Pregnant women in need of emergency care or having an at-risk pregnancy such as gestational diabetes; gestational hypertension.
Sample Collection

About 8mls of participants venous blood was drawn from the ante cubital vein of the lower arm by the researcher for haematological and biochemical analysis. About 2.5mls was dispensed into a vacutainer, containing EDTA for determination of haematological parameter, 2.0mL into 0.25mL of 3.2% trisodium citrate anticoagulated container for haemostatic test and 3.5mls into a plain container to obtain serum. The sample in the citrate anticoagulated test tube was centrifuged for 5 minutes at 3000 rpm to separate the plasma. The sample in the plain test tube was allowed to clot at room temperature and centrifuged to separate the serum. The collected samples were analysed immediately for haematological and coagulation tests while the sera for biochemical tests were stored at -20°C prior to analysis.

Laboratory Procedures

All reagents were commercially purchased and the manufacturer’s Standard Operating Procedures (SOP) will be strictly followed. The analysis of the parameter was done at the Medical Laboratory Unit of Federal Medical Centre, Owerri by three medical laboratory Scientists in the laboratory with the researcher observing and assisting in the analysis.

Dipstick Urinalysis [31]

Medi-Test Coombi 2 urine test stripe was used.

Procedure

The test stripe was completely immersed in a well mixed sample of urine for a short period of time, and was then extracted from the container. Supporting the edge of the stripe over the mouth of the container, the excess urine was removed. The stripe was then left to stand for the time necessary for the reactions to occur (1 to 2 minutes), and finally the colours that appear was compared against the chromatic scale provided by the manufacturer.

Alpha Tumour Necrosis Factor (TNF- α)

Human Alpha Tumour Necrosis Factor Commercial ELISA Kit by MELSIN Medical Co Limited was used.

Procedure

Dilutions of standard were prepared to get a concentration of 80 pg/mL, 40pg/mL, 20pg/mL, 10pg/mL, 5pg/mL and 0pg/mL. Fifty (50μl) of standard was added to the well, 10μl of testing sample was added into another well. Then sample diluent 40μl was added to testing sample well. 100μl of HRP-conjugate reagent was added to each well, and cover with an adhesive strip and incubate for 60 minutes at 37°C. 5. Each well was aspirated and washed, repeating the process four times for a total of five washes. Each well was wash by filling with Wash Solution (400μl) using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, the remaining Wash Solution was removed by aspirating or decanting. The plate was inverted and blotted it against clean paper towels. 50μl of chromogen solution A and 50μl chromogen solution B was added to each well and gently mixed and incubated for 15 minutes at 37°C. It was protected from light. Then 50μl Stop Solution was added to each well. The color in the wells should changes from blue to yellow. Optical density of the samples was read in a microtiter plate reader at 450nm wavelength within 15 minute taking the blank well as zero concentration.

Calculation

A standard curve of optical density against concentration of standard was plotted and the concentration of the tests determine from there.

Interleukin-6 (IL-6) Assay

Human Interleukin 6 Commercial ELISA Kit by MELSIN Medical Co Limited was used.

Procedure

Dilutions of standard were prepared to get a concentration of 48 ng/mL, 24 ng/mL, 12 ng/mL, 6 ng/mL, and 3 ng/mL. Fifty (50μl) of standard was added to the well, 10μl of testing sample was added into another well. Then sample diluent 40μl was added to testing sample well.
100μl of HRP-conjugate reagent was added to each well, and cover with an adhesive strip and incubate for 60 minutes at 37°C. Each well was aspirated and wash, repeating the process four times for a total of five washes. Each well was wash by filling with Wash Solution (400μl) using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, the remaining Wash Solution was removed by aspirating or decanting. The plate was inverted and blotted it against clean paper towels. 50μl of chromogen solution A and 50μl chromogen solution B was added to each well and gently mixed and incubated for 15 minutes at 37°C. Then 50μl Stop Solution was added to each well. The color in the wells should changes from blue to yellow. Optical density of the samples was read in a microtiter plate reader at 450nm wavelength within 15 minute.

Calculation
A standard curve of optical density against concentration of standard was plotted and the concentration of the tests determined from there.

Interleukin-10 (1L-10) assay
Human Interleukin 10 Commercial ELISA Kit by MELSIN Medical Co Limited was used.

Procedure
Dilutions of standard were prepared to get a concentration of 240 ng/mL, 160 ng/mL, 80 ng/mL, 40 ng/mL, and 20 ng/mL. Fifty (50μl) of standard was added to the well, while 10μl of testing sample was added into another well.

Then sample 57luents 40μl was added to testing sample well. 100μl of HRP-conjugate reagent was added to each well, and cover with an adhesive strip and incubate for 60 minutes at 37°C. Each well was aspirated and wash, repeating the process four times for a total of five washes. Each well was wash by filling with Wash Solution (400μl) using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, the remaining Wash Solution was removed by aspirating or decanting. The plate was inverted and blotted it against clean paper towels. 50μl of chromogen solution A and 50μl chromogen solution B was added to each well and gently mixed and incubated for 15 minutes at 37°C. It was protecting from light. Then 50μl Stop Solution was added to each well. The color in the wells should changes from blue to yellow. Optical density of the samples was read in a microtiter plate reader at 450nm wavelength within 15 minute taking the blank well as zero concentration.

Statistical Analysis
All statistical analysis was performed using Statistical Package SAS (ver. 13.0). The results were expressed as mean plus or minus standard deviation (X ± S.D) in tabular form. Chi-square and student’s t-test were used for comparison of differences in the two groups. Level of significance was set at p<0.05. The tests of association were performed using Pearson’s correlation. Results were represented graphically to show nature of association.

3. RESULTS
Table 1 shows the demographic data of study and control subjects. The subjects ranged from 18 to 45years with the mean ± SD lower in test group (30.19±4.76years) than control (30.33±5.79) and the difference was not statistically significant (P>0.05). In both the study and control group, the age group 36–years had the highest number of subjects recruited for the
study 24 (40.0%) and 20 (33.3%) respectively. The least number was seen in the age group 18-24 years, preeclampsia 8 (13.3%) while normotensive had 10 (16.7%).

Gestational age was found to be lower in test group (32.90±3.46 weeks) compared with the control (34.68±3.32 weeks) and the difference was not statistically significant (p>0.05). Those in the gestational age 32 to 36 weeks were highest in both groups, preeclampsia had 28 (46.7%) while 30 (50.0%) were recruited in the control group. With respect to parity, most of the subjects in the test group were nulliparious 28 (46.7%), followed by multiparious 23 (38.3%) while 9 (15.0%) were secondigravidae. When compared with the control group, it was found to be statistically significant ($\chi^2=7.948$, P=0.018).

According to marital status, most of the study participants in both test and control group were married (90.0% and 95.0%) respectively. There was no statistical significant difference among the groups (p=0.901). Majority of the subjects were Christians, accounting for 96.7% and 98.3% of study test and control group respectively. There was no significant association between them ($\chi^2=0.087$, P=0.768).

Table 1. Demographic data of test and control subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Preeclamptic Women N(%)</th>
<th>Normotensive Pregnant Women N (%)</th>
<th>Chi Square ($X^2$)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age Group (years) 18-24</td>
<td>8 (13.3)</td>
<td>10 (16.7)</td>
<td>0.872**</td>
<td></td>
</tr>
<tr>
<td>25-30</td>
<td>16 (26.7)</td>
<td>18 (30.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31-35</td>
<td>12 (20.0)</td>
<td>12 (20.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36*</td>
<td>24 (40.0)</td>
<td>20 (33.3)</td>
<td>0.704</td>
<td></td>
</tr>
<tr>
<td>Mean±SD (years)</td>
<td>30.19±4.76</td>
<td>30.33±5.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational Age (weeks) 28-31</td>
<td>15 (25.0)</td>
<td>12 (20.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32-36</td>
<td>28 (46.7)</td>
<td>30 (50.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>37-40</td>
<td>17 (28.3)</td>
<td>18 (30.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SD (years)</td>
<td>32.90±3.46</td>
<td>34.68±3.32</td>
<td>0.431</td>
<td>0.807**</td>
</tr>
<tr>
<td>Parity Nulli Second Multi</td>
<td>28 (46.7)</td>
<td>24 (40.0)</td>
<td>7.948</td>
<td>0.018*</td>
</tr>
<tr>
<td>Multi</td>
<td>9 (15.0)</td>
<td>22 (36.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SD (years)</td>
<td>23 (38.3)</td>
<td>14 (23.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Religion Christianity</td>
<td>58 (96.7)</td>
<td>59 (98.3)</td>
<td>0.559**</td>
<td></td>
</tr>
<tr>
<td>Muslims</td>
<td>2 (3.3)</td>
<td>1 (1.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family History of HBP</td>
<td>39 (65.0)</td>
<td>22 (36.7)</td>
<td>9.636</td>
<td>0.002*</td>
</tr>
<tr>
<td>Yes</td>
<td>21 (35.0)</td>
<td>38 (63.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>58 (96.2)</td>
<td>57 (95.0)</td>
<td>0.647**</td>
<td></td>
</tr>
<tr>
<td>Antenatal Visit</td>
<td>2 (3.8)</td>
<td>3 (5.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8 (13.3)</td>
<td>6 (10.0)</td>
<td>0.2087</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>17 (28.3)</td>
<td>15 (25.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occupation Student Civil Servant</td>
<td>8 (13.3)</td>
<td>6 (10.0)</td>
<td>0.627</td>
<td>0.731**</td>
</tr>
<tr>
<td>Self Employed</td>
<td>17 (28.3)</td>
<td>15 (25.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marital Status Single Married</td>
<td>6 (10.0)</td>
<td>3 (5.0)</td>
<td>0.901**</td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>54 (90.0)</td>
<td>57 (95.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Most of the subjects recruited for the study were self-employed, accounting for 58.3% and 65.0% of the test and control subject respectively. The least among them were students (unemployed) accounting for 8.0% and 6.0% of test and control subject respectively. There was no significant association between the study group in respect to occupation (χ²=0.53%, P=0.765). More of the test subject had family history of HBP, (65.0%) while less of the control subjects had family history of HBP (36.7%). There was a significant association between both group (χ²=9.190, P=0.002). Most of the tests and control subjects attended care (96.2% and 95.0% respectively).

Table 2 shows the mean ± SD of IL-6, TNF-α, IL-10 and IL-4 of preeclampsia and normotensive pregnant women. The mean ± SD of IL-6 of test subjects (4.33±1.42 pg/ml) was higher than that of control group (3.38±1.22 pg/ml) and when compared statistically, there was a significant difference (P=0.0001). TNF-α of test group (8.23±2.73 pg/ml) was higher than control (6.89±1.88 pg/ml) and the difference was significant (p=0.002). Mean ± SD of IL-10 value of test group (78.88±14.28 pg/ml) was lower than control (91.39±18.46 pg/ml). The P value shows a significant difference with the control group. Interleukin-4 of test group (27.92±7.22 pg/ml) was significantly (p=0.011) lower when compared to that of control (31.25±6.90 pg/ml).

4. DISCUSSION

The socio-demographic data obtained from the studied subject showed that majority of the preeclamptic women recruited for the study were above 35 years of age (40%). Preeclampsia has been linked to multiple risk factors, one of the suggested risk factors is advanced maternal age. The result from this study agrees with the findings of previous researchers (Osungbade et al., 2011) [32-35], who stated that maternal age is a risk factor in preeclampsia. Increased age of women is an important risk factor due to increased villous reaction leading to preeclampsia in a woman greater than 30years [36].

In relation to parity, the prevalence of preeclampsia was higher in nulliparious women (46.7%) when compared to multiparous women. Several studies have shown preeclampsia to be commonly encountered in nulliparous women. This is because nulliparity is due to initial trophoblastic invasion and how the mother reacts to it. The failure of the normal invasion of trophoblastic cells leads to mal adaptation of the spiral arterioles, which are related to the causation of preeclampsia [3]. The result in this study is in accordance with the findings from similar studies in Nigeria and other countries [37-39]. However, in a study by Ayogu et al., [40], on risk factor of hypertensive disorder of pregnancy (HDP), he noted that nulliparity is not a risk factor which is not in accordance with this study. This may be due to the method adopted for recruiting the subjects, as all the subjects were recruited consecutively on antenatal visit from 20 weeks prior to developing HDP and followed up 6 weeks post partum in his study. Moreso, other HDPs were considered in the study alongside preeclampsia and this maybe the reason for the disparity in the finding of this study.

It was observed in this study that family history of hypertension was a major risk factor for preeclampsia. A significant number of the preeclamptic women had family history of hypertension, (65.0%) while less of the normotensive pregnant women (36.7%) had family history of hypertension. It has been noted that family history of hypertension was the most dominant risk factor for preeclampsia in pregnant women. This finding was in keeping with the findings from other studies [41, 36, 40]. Moreso, from this study 8 (13%) of test subjects have had previous history of preeclampsia. History of preeclampsia has always been implicated as a risk factor in the development of preeclampsia in

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test (n=60)</th>
<th>Control (n=60)</th>
<th>T-Test</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/ml)</td>
<td>4.33±1.42</td>
<td>3.38±1.22</td>
<td>3.910</td>
<td>0.0001***</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>8.23±2.73</td>
<td>6.89±1.88</td>
<td>3.126</td>
<td>0.002**</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>78.88±14.28</td>
<td>91.39±18.46</td>
<td>4.154</td>
<td>0.001**</td>
</tr>
<tr>
<td>IL-4 (pg/ml)</td>
<td>27.92±7.22</td>
<td>31.25±6.90</td>
<td>2.582</td>
<td>0.011*</td>
</tr>
</tbody>
</table>

Significant level: *P<0.05, **P<0.001, ***P<0.0001 ns Not significant (P>0.05); KEY: IL-6 - Interleukin-6, TNF-α - Tumur Necrotic Factor, IL-10 - Interleukin-10, IL-4 - Interleukin 4
multiparous women. This is in accordance with the findings from other studies [42,43,44].

There was a difference observed in prevalence of preeclampsia among the different occupational groups surveyed in this study. Most of the pregnant women recruited for this study were self employed, accounting for 58.3% and 65.0% of the preeclamptic women and normotensive pregnant women respectively; others were civil servants and students. It has been observed in previous studies that low socio economic factors act as multiple risk factors for preeclampsia. Low socio economic factors are associated with Nutritional issues, reduced ante-natal care and unsanitary hygienic conditions. In Egypt, low socio-economic status of women doubled the risk of preeclampsia and eclampsia [45]. A study in Australia found working women compared to non working ones had a higher risk of developing preeclampsia and eclampsia [46]. This may be related to the stress that women get during work.

The pro-inflammatory cytokines (IL-6, TNF-α) are important mediators of maternal immune system and are secreted in excess by maternal immune cells in preeclampsia [47]. These cytokines are found to be responsible for the pathophysiological characteristics of preeclampsia [48]. TNF-α activates the endothelin system in placental, vascular, and renal tissues, while IL-6 activates the renin-angiotensin system [18]. Pro-inflammatory cytokines produced in preeclampsia may contribute to hypertension by inducing the production of vascular mediators that result in vasoconstriction and consequently hypertension [49]. Cytokines are suggested to be linked to hypertension by provoking inflammation, which would result in vascular injury and may also contribute to elevated blood pressure by causing kidney injury [47]. Chronic infusions of IL-6 or TNF-α into normal pregnant rats increase arterial pressure and affect renal hemodynamics [18]. That TNF-α may contribute directly to proteinuria is supported by a recent study on diabetic nephropathy in which a positive correlation was observed between plasma TNF-α levels and urinary protein concentrations [50]. TNF-α also has potent effects on endothelial and platelet function, it enhances coagulation, microvascular leakage, activation of vasoconstrictive endothelial cells, and production of antiangiogenesis factors like tissue factor [51,52]. The synthesis of these cytokines is increased in preeclampsia and amends the levels of anti-inflammatory cytokines [53, 54]. In the present study, the levels of IL-6 and TNF-α were found to be significantly increased in preeclamptic women when compare with normotensive pregnant women. Similar increases in the serum levels of these cytokines were also reported by Udenze et al. [24], Teran et al., [55], Aggarwal et al., [56] and Hentschke et al., [57], who reported increased IL-6 receptors in preeclampsia. Ozier et al., [58], found no difference in the levels of IL6 and TNF-α in preeclampsia when compared to normotensive pregnant women. Afshari et al., [59], found a significant increase in IL6 levels but no significant difference in TNF-α in preeclampsia. The disparity in the reports from these studies may be from differences in time of sampling, prospective or cross sectional as opposed to case control studies, amongst others. The Ozier et al., [58], study was a cross sectional study involving women at different gestational ages, the study design plus the relatively small sample size used may explain the difference in the findings of this group. In the Afshari et al., [59] study, some of the patients were on dexamethasome therapy and this may have affected the levels of the inflammatory cytokines. In this study, the preeclampsia and the controls were appropriately matched for maternal age and gestational age.

The resolution of inflammation plays an important role throughout pregnancy and is largely mediated by immune cells that produce interleukin (IL)-4 and IL-10 [60]. Interleukin-4 and IL-10 are pleiotropic anti-inflammatory cytokines that function mainly by suppressing the pro-inflammatory milieu. These anti-inflammatory cytokines are crucial for the functioning of T helper cell 2 (Th2) and regulatory T cells (Treg) for the successful progression of pregnancy [61]. Any modulation in their level may affect the functioning of several immunological and apoptotic pathways leading to pregnancy-associated syndromes like preeclampsia [62]. Several experiments on mice had reported that the scarcity of IL-10 leads to inflammation [63,64]. In a study by Chatterjee et al. [60], pregnant IL-4-deficient mice exhibited altered splenic immune cell subsets, increased levels of pro-inflammatory cytokines, placental inflammation, mild hypertension, endothelial dysfunction, and proteinuria compared to pregnant control mice.
In this study, the levels of IL-4 and IL-10 were found to be significantly decreased in preeclampsia when compared to normotensive pregnant women. The study done by Aggarwal et al., [56], Borceki et al., [29] and Pinheiro et al., [65], were in agreement with the finding in this study. From these observations, it may be presumed that in preeclampsia, IL-10 and IL-4 are not able to exert their regulatory effects on pro-inflammatory cytokines resulting in aggravated inflammatory responses [66-71].

5. CONCLUSION

Pregnancy has been described as a state of ‘mild’ controlled inflammation. However when this inflammatory response becomes exaggerated it results in the development of preeclampsia. A role has been suggested for inflammatory mediators in the pathogenesis and pathology of preeclampsia. The present study has demonstrated and confirmed the link between exaggerated inflammation and preeclampsia. The pro-inflammatory cytokines were elevated with a decreased in the levels of anti-inflammatory cytokines in preeclamptic women when compared with normotensive pregnant women in this study.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

An oral consent was gotten from the patients after; a structured questionnaire was administered to all respondents who were also part of clinical study. Participants were recruited among pregnant women who were booked for antenatal care. Thereafter demographic information which includes age, parity, place of residence, education, socio-economic status and medical and obstetrical history was collected using a questionnaire. The second group comprising of normotensive pregnant women as control (appropriately matched for maternal age and gestational age) was randomly selected from pregnant women attending antenatal clinic in the hospital.

ETHICAL APPROVAL

A letter of introduction was secured from the Head of Department, Medical Laboratory Science of Nnamdi Azikiwe University, Awka. The letter together with a written proposal on the study was submitted to the ethical committee of Federal Medical Centre Owerri to seek for ethical approval to carry out the study. After all considerations the Ethical committee approved my request.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here: https://www.sdiarticle4.com/review-history/72825