Comparison of Lymphocyte Transformation in Normotensive and Preeclamptic Women

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Abstract

Background: *Preeclampsia is a severe form of hypertensive disorders in pregnancy with multisystemic effects. Although the exact aetiology is not known, lymphocyte response during preeclampsia may be a cause or effect.*

Objectives: *To determine if or not lymphocyte transformation occurs in normal pregnancy and in preeclampsia and compare results between the two.*

Method: *This was a cross-sectional comparative study. Two millilitres of venous blood was obtained from 37 preeclamptic women and 38 normal pregnant women. Phytohaemagglutinin was used to induce lymphocyte transformation after determining the baseline count. Data analysis was with Graphpad prism 7.1. Level of significance was set at $p<0.05$.*

Results: *Forty women were recruited in each group of normotensive and preeclamptic patients from which 38 and 37 samples respectively were eligible for analysis. The mean baseline lymphocyte count was not statistically different between the two groups ($p = 0.5731$). There was no significant increase in lymphocyte transformation with phytohaemagglutinin in and between the groups ($p = 0.335$).*

Conclusion and Recommendation: *During normal pregnancy and in preeclampsia, there was no increase in lymphocyte activity noted. Further research on lymphocyte subpopulation and uterine natural killer cells activity in pregnancy is recommended using modern technology.*

Keywords: *Lymphocytes, transformation, phytohaemagglutinin, preeclampsia*

Introduction

Preeclampsia is a syndrome which affects virtually all maternal organ systems. Despite extensive researches, the aetiopathogenesis of pre-eclampsia is not yet fully understood. Preeclampsia is comprehended as an abnormality of the maternal immune system that prevents it from recognizing the fetoplacental unit[1]. Excessive production of immune cells like lymphocytes causes secretion of tumor necrosis factor alpha which induces apoptosis of the extravillous cytotrophoblasts[1, 2]. The major pathological changes are in the placental bed[3]. Altered immune cells are believed to play a role in the pathogenesis of preeclampsia[4]. During pregnancy there is gradual increase in lymphocytes due to increased spontaneous Deoxyribonucleic acid (DNA) synthesis[5, 6]. The proinflammatory $CD4^+$ and cytokines increase during preeclampsia while the regulatory T cells and anti-inflammatory cytokines decrease[7,8]. All these are products of lymphocytes. It is not clear whether the altered immunity is a cause of preeclampsia or a consequence of the disease.

Lymphocyte transformation is applied in immune function testing, bacterial and viral testing, testing for metal poison and environmental pollutant. Lymphocyte transformation may play a significant role in finding the aetiopathogenesis of preeclampsia. This study, set to identify the role of immune system through lymphocytes transformation in preeclampsia using mitogens

Materials and Methods

It was a cross sectional comparative study of preeclamptic women as cases and normal pregnant women as controls. The study was conducted from September, 2017 to April, 2018 in the department of Obstetrics and gynaecology of Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto. Inclusion criteria were women with preeclampsia and normal pregnancy beyond 20 weeks of gestational age, while exclusion criteria were medical disorders that were not preeclampsia, clinical evidence of infection and use of antiviral drugs. The sample size for the study was determined using the standard formula for calculation of minimum sample size for group comparison.

$$n = \frac{Z^2_{1-\alpha/2}(2\sigma^2)^9}{d^2}$$

Where: n = Minimum Sample size

$Z^2_{1-\alpha/2}$ = Standard error associated with confidence interval.

σ = estimated standard deviation (assumed to be equal for each group)

d= desired precision.

The calculated sample size was approximately 31 women and 20% attrition was added to get 37 women for each group. For convenience, 40 women for each group were enrolled into the study. The cases were recruited using the convenient sampling method while the women in both the cases and controls were matched for gestational age only.

All working reagents were obtained from Sigma–Aldrich (St. Louis, Missouri, USA). The lymphocytes transformation assay was done as described by Bayun[10]. Two millilitres of blood were aseptically collected from each study participant and dispensed into a K_2EDTA container. The blood sample and balanced salt solution were dispensed into a 10ml test tube and the contents of the tube were carefully mixed; using a Pasteur pipette. The diluted blood sample was layered onto 3ml of Ficoll-paque solution in a centrifuge tube and centrifuged at 400rpm for 30mins at room temperature. The upper layer of the segments formed was removed, while the lymphocyte layer at the interface was pipetted into another centrifuge tube. The isolated lymphocyte was washed in 6ml of the balanced salt solution and finally suspended in 0.5ml of the balanced salt solution. Lymphocyte viability test was done by the trypan blue exclusion test.

The lymphocytes were prepared into concentrations of 1.0×10^6 cells/ ml of blood and cultured in the wells of microtitre culture plates, using TC 199 as culture medium which had been enriched with AB blood group serum. Exactly 0.1ml of phytohaemagglutinin was then added to each well of the microtitre plate. The culture mixtures were then incubated at 37°C for 72 hours. At the end of the incubation period, cultures were centrifuged at 1,500 rpm for 7 minutes and the isolated cells were fixed in 1:10 glacial acetic acid/ alcohol mixture and re-centrifuged. The cell pellets were finally resuspended in 0.5ml of culture medium. Thin blood film was prepared from the isolated cells in microscope slides. The films were allowed to dry, stained with Leishmans stain. The percentage of transformed T cells was determined for each sample. The lymphoblasts (as the transformed cells), were counted in a light microscope using x100 objective and the count expressed in percentage. Stimulation index (SI) was calculated as the mean ratio of the stimulated cells divided by the unstimulated cells[11].

Ethical approval for this study was obtained from UDUTH Health Research and Ethics Committee. The data was recorded in Microsoft excel and analysed using GraphPad prism 7.1 software. A *p* - value of < 0.05 was considered statistically significant.

Results

Five samples were found to be haemolysed and were excluded from analysis. Two of the samples were from the normotensive pregnant women (control) group and three samples were from the preeclamptic women (cases) group.

A Shapiro Wilk test ($p < 0.05$) and a visual inspection of the histograms, normal Q-Q plots and box plots of the lymphoblast and stimulation index showed that the data was not normally distributed as it was positively skewed.

Table 1. *Baseline characteristics of the study participants*

Characteristics	Preeclampsia	Normal pregnancy	*p*-value
Mean age (years)	28.9±6.8	24.0±4.9	*0.001*
Parity	1	0	*0.006*
Mean gestational age(weeks)	36.0±3.8	36.2±3.7	*0.832*

The mean ages of the preeclamptic and normal pregnant women were 28.9 (SD 6.8) and 24.0 (SD 4.9) years respectively. The mean ages (p =0.003) and modal parity (p=0.006) were statistically different between the two groups while the gestational age shows no statistically significant difference (p = 0.832). Table 1

Table 2. *Baseline lymphocyte count, lymphoblast and stimulation index*

	Preeclampsia	Control	*p* – value
Baseline lymphocyte count (x 10^9)	2.1 ± 1.2	1.96 ± 0.8	*0.5731†*
Lymphoblast (%)	46.5	57.9	*0.335ū*
Stimulation index	2.1	1.7	*0.335ū*

† - t-test, ū - Mann Whitney U test

Though the mean unstimulated cell count was higher with the Preeclamptic group, this however did not attain statistical significance (p= 0.573). While the control group recorded a median increase in lymphoblast transformation (p = 0.335), the Preeclamptics had a higher stimulation index (p=0.335) as depicted in Table 2.

Discussion

Preeclampsia known as disease of theories has multi-systemic effects. The immune system is one system that has a very significant role either as a cause or effect[1,4]. From this study the preeclamptic women were found to be older than the normal pregnant women which was statistically different. This was similar to findings by Kumari *et al*[12]. The observed difference may be because the two groups of the participants were not matched for age.

The unstimulated lymphocytes count was slightly lower in the normal pregnancy group compared to the preeclamptic women. Though the difference was not statistically significant this may indicate an ongoing inflammatory process in preeclampsia. This is similar to findings by Yuvuscan *et al* and *Brien et al*, who demonstrated slightly higher lymphocyte count in preeclamptic women compared to the normal pregnant women[13,14].

Also Ceyhan *et al* and Mukayana *et al* found no significant difference in lymphocyte count between the preeclampsia and normotensive pregnant women[15, 16]. Clinically, total lymphocytes count may not be of any clinical significance in diagnosing or assessing the severity of pre-eclampsia in our environment. However assessing CD4+ CD8+ lymphocytes and T regulatory cells may help in assessing the severity of the disease[7, 8]. An imposing number of mechanisms have been proposed to explain the aetiopathogenesis of preeclampsia. One of these mechanisms is immunological maladaptive tolerance among maternal, placental and fetal tissues. This may explain the higher lymphocytes counts observed in this study.

Lymphoblast that results from the incubation of lymphocytes with phytohemagglutinin (PHA) stimulates the transformation of autologous lymphocytes but in preeclampsia the lymphocytes response is poor[17]. In this study lymphocytes transformation with PHA was slightly higher in the normal pregnant women compared to the preeclamptic women however not statistically significant. This may indicate that lymphocytes during normal pregnancy and preeclampsia are not significantly stimulated by phytohaemagglutinin or it may be the failure of the lymphocytes to initiate adequate inflammatory response during pregnancy and preeclampsia. However the lymphocytes in normal pregnancy and preeclampsia may not be responsive to phytohaemagglutinin but may be stimulated by other mitogens. This was similar to the work of Petrucco *et al* on phytohemagglutinin[18]. Curzen and co-workers demonstrated that there was no statistically significant difference in Mytomycin-induced lymphocytes transformation between normal pregnant and preeclamptic women[19]. Although Metthiesen *et al* found a higher lymphocytes transformation with phytohemagglutinin in preeclampsia patients compared to normotensive women, there was no statistically significance found[20]. Lymphocytes have been demonstrated to be responsible for their spontaneous DNA activity[18]. During pregnancy human placental lactogen, human chorionic gonadotrophic hormone, cortisol and progesterone may be responsible for the inhibition of lymphocyte DNA activity[18, 21]. It has also been demonstrated that mesenchymal stem cells can inhibit the proliferation of lymphocytes in respond to phytohaemagglutinin[22]. However only cortisol was shown *in vitro* to have a regular inhibition at physiological concentration[18]. These may explain the failure of significant lymphocytes activation during pregnancy and preeclampsia.

The stimulation index with PHA between the normal pregnant and preeclamptic women was not significantly different since the transformation was not statistically significant. There was no significant lymphocyte transformation in preeclampsia. This had been demonstrated by Petrucco *et al*,[18] Curzen *et al*[19] and Metthiesen *et al*[20]. Lymphocytes transformation neither occurred significantly in normotensive pregnancy nor in preeclampsia in this study as in another report by Comings[23]. It can be deduced from this research that the mitogen used did not induce the lymphocytes in both normal pregnant and preeclamptic women. The failure of the lymphocytes to be initiated by this mitogen may disprove the immune theory proposed for preeclampsia. This may not hold until other available mitogens are used for lymphocytes transformation in normal pregnancy and preeclampsia. It may also be that lymphocytes are not involved significantly in the aetiopathogenesis of preeclampsia.

If the immune theory holds to be true in the aetiopathogenesis of preeclampsia in humans, immune modulators may play a role in the prevention or treatment of the disease. This study could downplay the role of immune modulation in the prevention or treatment of pre-eclampsia. However the limitation of this study is the non-matching of the participants for age and parity.

Conclusion

There was no involvement of lymphocytes transformation in preeclampsia from this study. This may be due to failure of the lymphocytes to respond to the mitogen used, counter effect of hormones of pregnancy or perhaps that the immune system plays little or no role in the pathogenesis of preeclampsia

Recommendation

Further researches that will focus on the functions and subpopulations of lymphocytes including the uterine natural killer cells using modern techniques of assessing immune responses are recommended.

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