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Original Research Article

To Analyze the Incidence and Role of HLA Alloantibodies & T Lymphocytes and the Ratio of T -Helper & T- Suppressor (Th: Ts) in Pre-Eclampsia and Eclampsia Compared to Normal Pregnancy

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Conflict of interest: Nil

Abstract:

Background: A thorough knowledge of the immunological processes underlying these conditions is essential to develop diagnostic and therapeutic strategies appropriate for preeclampsia and eclampsia.

Aim: This study aims to understand the immune processes behind preeclampsia and eclampsia and identify possible therapy targets.

Hypothesis: Compared to a normal pregnancy, eclampsia is thought to have significantly greater T cells and HLA alloantibodies. Moreover, we speculate that eclampsia and preeclampsia markedly change the T lymphocyte ratio to T cells.

Materials & Methods: The current study was started once the institutional human ethics committee approved the study protocol, which was submitted for approval. The study was conducted in the RDJM medical college and hospital, Turki, Muzaffarpur. A total of 183 samples will be tested in each of the following three categories: 100 normotensive samples, 83 pre-eclamptic and eclamptic samples, and so on. a) Normal Pregnancy: This describes a pregnancy that is singleton and devoid of any evident problems. If blood pressure is 140/90 mm Hg or above, or if it has increased by 15 mm Hg diastolic or 20 mm Hg systolic over the previous blood pressure that was detected on at least two occasions six hours apart, then hypertension is considered to be present in the presence of b) preeclampsia and c) eclampsia.

Results & Conclusion: The research provides crucial information for comprehending the relationship among HLA alloantibodies, T lymphocytes, and eclampsia. The results show that these immunological components are significantly different in preeclampsia and eclampsia compared to a normal pregnancy and that the ratio of Th to Ts is also considerably changed. These results have significant implications for diagnosing and managing eclampsia and preeclampsia; more research is needed to realize these implications fully. Future studies should identify specific HLA alleles and T cell subsets associated with preeclampsia and eclampsia and explore the potential benefits of altering the immune response to provide novel therapies for the illness.

Keywords: Preeclampsia: Eclampsia; Intrauterine growth retardation; T lymphocytes, Human leukocyte antigen; T helper cells.

Introduction

Worldwide, preeclampsia is a pregnancy complication that affects 5-8 percent of pregnancies. Its defining features are high blood pressure and harm to vital organs including the liver and kidneys [1-3]. Preeclampsia is a severe pregnancy complication that results in about 70,000 maternal fatalities and 500,000 fetal and neonatal deaths annually; most of these deaths take place in nations that are categorized as developing [4-6]. Preeclampsia affects between 5–15% of pregnancies in India [4–5], compared to 5-8% of pregnancies globally [5–6]. Apart from its significant role in maternal mortality, it also plays a noteworthy role in preterm delivery and fetal development limitations resulting from medical intervention. A significant amount of blood research is being done to investigate the kind of rejection and the survival of fetal allografts after response [5]. Many immunological traits can significantly influence the graft's success [1, 3].

These include the lack of alloantibodies, the presence of spouse-shared HLA antigens, a weakened immune system, and tolerance to paternal antigens. Knowing exactly what lymphocytes do in the cell-mediated immune response is essential when dealing with pre-eclampsia-complicated pregnancy. Creating efficient treatments to stop and treat this condition requires this understanding [2.4]. Previous research has established a connection between HLA alloantibodies and preeclampsia; however, the exact function of these immunological components in developing the disease remains unclear [1–5]. Further investigation is required to close the knowledge gap on the exact function that T lymphocytes and HLA alloantibodies play in developing preeclampsia. This is true even when there is already much knowledge on the topic. This study aims to assess the role that T lymphocytes and HLA alloantibodies play in the development of preeclampsia to identify potential therapeutic targets. What part do T cells and HLA alloantibodies play in the development of preeclampsia, and how may this information be used to create new treatment options for the illness? This study will look into this particular research question. Understanding the immune systems behind preeclampsia and eclampsia, as well as identifying possible targets for therapy, are the goals of this study. Preeclampsia and eclampsia are expected to have much greater levels of T lymphocytes and HLA alloantibodies than in normal pregnancy. Additionally, we think that there is a considerable change in the ratio of T lymphocytes to T lymphocytes in preeclampsia and eclampsia.

Materials & Methods

The current inquiry was conducted once the study's protocol was approved. The institutional human ethics committee was asked to approve the protocol. The study was conducted in the RDJM medical college and hospital, Turki, Muzaffarpur.

A total of 183 samples will be tested, including 100 normotensive samples and 83 pre- and eclamptic samples. We shall categorize these samples into four different groups. a) Normal Pregnancy: This describes a pregnancy that is singleton and devoid of any evident problems. If the blood pressure is 140/90 mm Hg or higher, or if it has increased by 15 mm Hg diastolic or 20 mm Hg systolic over the previous blood pressure that was observed on at least two occasions that were six hours apart, hypertension is considered to be present in cases b) preeclampsia and c) eclampsia.

Antibodies that, according to screening, are lymphocytotoxic: Five milliliters of serum were drawn from women at the time of delivery and during the latter portion of their pregnancy. When the diagnosis was made, these samples were obtained. These were examined using a two-stage micro lymphocytotoxicity assay (a well-characterized HLA-typed panel) with a minimum of fifty cells.

a) The nylon wool column technique may isolate T and B lymphocytes and CD4+ and CD8+ T cells from peripheral blood. Heparinized peripheral venous blood was drawn from pre-eclamptic patients, healthy control subjects, and typical pregnant women. It was shown that the best method for obtaining peripheral blood mononuclear cells was the Ficoll-opaque technique. 0.5 milliliters of cell suspension were mixed with 0.02 milliliters of Dynabeads M-450 CD4 (Dynal UK) and TM M-450 CD8 in the appropriate amounts to separate CD4 and CD8 cells. After that, the mixture was incubated on a medium-tilting, moderately rotating apparatus for sixty minutes at a temperature of four degrees Celsius. Five milliliters of random venous blood were drawn into simple vials with each research group member's informed written permission obtained beforehand. The blood was centrifuged for twenty minutes at 3000 revolutions per minute to extract the serum. After that, the serum was kept in aliquots at -20 degrees Celsius until it was used to measure immunoglobulins (IgG, IgM, and IgA) using an ELISA kit and the immune turbidimetry technique.

Inclusion Criteria: Pregnant patients with a diagnosis of preeclampsia based on accepted clinical criteria (e.g., elevated blood pressure, proteinuria, and end-organ dysfunction). For comparison purposes, pregnant women who did not have preeclampsia (for instance, pregnancies with normal blood pressure or pregnancies with other difficulties).

Exclusion Criteria: Unless subgroup analyses were performed, pregnant patients with pre-existing autoimmune disorders (e.g., systemic lupus erythematosus, rheumatoid arthritis) or chronic medical conditions (e.g., diabetes, chronic hypertension) unrelated to preeclampsia were excluded from the study to minimize the number of confounding variables.

Statistical Analysis: The statistical analysis was performed using IBM SPSS version 20. The means of the variables in the two groups were compared using an Unpaired 't' test.

The authors employed a method known as one-way analysis of variance (ANOVA) when they wished to compare the means of two or more groups. Multiple regression analyses were performed to ascertain the averages of the various groups. It was demonstrated that a significance level of $P < 0.05$ worked well."

Results

Table 1: Clinical Characteristics of the Study Population

Characteristics	Normal Pregnant Women (N=100)	Preeclampsia (N=31)	Eclampsia (N = 42)
Age (Years)	32.4 (30 – 35)	33 (29 – 35)	32.5 (28 – 34)
No. of Primiparous women	12	8	16
Systolic blood pressure (mm of Hg)	109 (105 – 116)	163 (140 – 170)	170 (148 – 180)
Diastolic Pressure (mm of Hg)	66 (60 – 70)	94 (90 – 110)	100 (94 – 116)
Gestational age at blood collection (weeks)	36 (34 – 37)	36 (30 – 38)	36 (30 – 38)
Gestational age at delivery (weeks)	39 (38 – 40)	38 (30 – 38)	37 (31 – 39)
Fetal birth weight (g)	3250 (3000 – 3650)	3010 (2800 – 3200)	2780 (1425 – 3450)
No. of IUGR	0 (0%)	1 (0%)	8 (0%)
Proteinuria (g/dl)	0.9 (0.4 – 1.2)	4.1 (3.8 – 4.8)	3.9 (2.8 – 4.1)

The current investigation was conducted with 183 prenatal women to compare the clinical characteristics of preeclampsia patients, eclampsia patients, and normotensive pregnant women (Table 1). Pregnant women without high blood pressure made up the control group. In addition, none of these people had proteinuria. On the other hand, the normotension group's mean age was 32.4 years, with a 3.05-year standard deviation. The mean age of the pre-eclamptic group was 33 years, while the mean age of the eclamptic group was 32.5 years, with a standard deviation of 2.82 years."

Table 2: The number of study subjects showed positive HLA alloantibodies in preeclampsia and eclampsia compared to normal pregnancy

Human leukocyte Antigens (HLA)	Normal Pregnant Women (N=100)	Preeclampsia (N=31)	Eclampsia (N = 42)
HLA-C	2	8	32
HLA-E	3	11	16
HLA-F	1	4	17
HLA-G	1	8	12

Table 2 presents the study's findings by contrasting the proportion of individuals with positive HLA alloantibodies in preeclampsia with those in a typical pregnancy. Two patients with normal pregnancy showed HLA class 1a (HLA-C). In contrast, eight participants had preeclampsia, and the count increased to 32 in eclamptic subjects. It was discovered that ordinary pregnant women belonged to HLA class 1a.

Compared to the preeclampsia and standard pregnant women groups, a higher percentage of individuals in the eclampsia group had positive HLA class 1b, which includes subtypes like E, F, and G.

Table 4: Analysis of T lymphocytes and the ratio of T -helper & T- Suppressor (Th: Ts) towards the tolerance mechanism responsible for the fetus's survival.

Subset	Average Pregnant Women (N=100)	Preeclampsia (N=31)	Eclampsia (N=42)
CD4 cells			
CXCR3	7.98 (4.60 – 9.81)	10.56 (5.92 – 11.89)	12.99 (10.12 – 14.18)
CCR4	12.56 (9.33 – 17.35)	14.78 (10.65 – 18.56)	17.10 (15.26 – 18.29)
CXCR3/CCR4	0.63 (0.35 – 0.70)	0.71 (0.56 – 0.98)	0.75 (0.62 – 0.90)
CD25	5.77 (4.2 – 6.83)	3.67 (2.2 – 4.61)	3.17 (2.88 – 3.45)
CD8 cells			
IL – 17A	6.15 (4.89 – 7.22)	9.17 (6.12 – 12.65)	10.22 (8.12 – 12.34)
NK cells	1.07 (0.88 – 1.59)	2.99 (1.8 – 3.5)	3.24 (2.14 – 4.2)

"Moreover, compared to the prevalence of CXCR CD4 cells, which was demonstrated to be much higher in preeclampsia participants than in ordinary pregnant people, the prevalence of CCR4 CD4 cells was also dramatically altered in preeclampsia patients. Furthermore, compared to controls and pre-eclamptic individuals, the ratio of CXCR3 to CCR4 CD4 cells was significantly higher in preeclampsia. The CD4 CD25 regulatory T cell proportion was lower in eclamptic pregnant women. However, Th17 cells were more prevalent in them than healthy pregnant women. As a result, eclampsia was shown to have a more significant proportion of Th17 /Treg cells than the control

group. Additionally, it was demonstrated that the subsets of CD8 and NK cells present in eclamptitis had an increased prevalence of IL-17 cells. We also looked for evidence of a potential relationship between the expression of CXCR3 or CCR4 in the CD4 subset in preeclampsia and controls and the preponderance of IL-17-producing cells, but we could not uncover any. The proportion of IL-17-producing lymphocytes varied statistically significantly between patients with preeclampsia and eclampsia in the pre-eclamptic patient group: 3.47 (2.41–4.40)% versus 4.15 (3.16–4.73)% for IL-17+ CD4 cells, 9.17 (6.12 – 12.65)% versus 10.22 (8.12 – 12.34)% for NK cells, and 2.99 (1.8 – 3.5) % versus 3.24 (2.14 – 4.2) % for NK cells."

Discussion

In the current study, a methodology was applied to examine the following: the function of T lymphocytes, the ratio of T-helper cells to T-suppressor cells (Th: Ts) concerning the tolerance mechanism that allows the fetus to survive, and the prevalence of HLA alloantibodies and immunoglobulins in preeclampsia and eclampsia in comparison.

"It was discovered that 76% of the women with preeclampsia had HLA positivity and reactivities towards HLA classes I and II. Early in the pregnancy, there were a lot of positive beads. As the due date drew nearer, the HLA antibody kinetics consistently declined. Preeclampsia's increased allo-response may play a role in endothelial dysfunction and inadequate placental perfusion, leading to complement system activation. HLA-C antibodies were detected in the bodies of every woman who underwent eclampsia testing. Human trophoblast cells generate HLA class Ia (HLA-C) and class Ib molecules (HLA-E, -F, and -G) as part of the immune regulatory process at the fetomaternal interface. These substances have a part in suppressing immune reactions."

Th17 cells have the opposite impact on the inflammatory balance as immunosuppressive Tregs because they produce IL-17 and activate other pro-inflammatory cytokines. This is due to IL-17 production by Th17 cells. Interestingly, the embryonic route from which Th17 cells and Tregs originate is distinct from the lineage that gives rise to Th1 and Th2 cells. An exclusive dichotomy was found in their development: based on whether the ancestral cells are activated in the presence of TGF-beta or TGF-beta plus inflammatory cytokines, Treg cells, or Th17 cells originate from them [8]. Cell formation provided the standpoint from which this dichotomy was identified. Thus, the increased ratio of Th17 to Tregs seen in preeclampsia may be partially explained by changes in TGF-beta signalling.

Moreover, it is also conceivable that the decreased frequency of Tregs in preeclampsia results from the phenomenon that some members of this subgroup have differentiated into Th17 cells. The fact that it is seen as a possibility lends credence to this conclusion. This theory may account for the increase in Th17 cell predominance observed in preeclampsia [9]. Apart from the observations in preeclampsia, other pregnancy-related disorders have also been linked to alterations in Th17 cells. This implies that the inflammatory status during human pregnancy is significantly influenced by the balance of Th17 cells and Tregs and the balance of Th1 and Th2 cells. The importance of IL-17-producing cells in the patho-mechanism of preterm labour was demonstrated by a recent experiment [32]. The incidence of decidual IL-17-producing cells is much higher in instances of inevitable abortion than it is in normal pregnancy, but not in cases of missed abortion, according to the results of another research [10].

This shows that these cells could not be involved in the early phases of abortion but rather in the production of inflammation later on. Another research found that individuals with unexplained recurrent spontaneous miscarriages had an increased prevalence of Th17 cells in the decidua and peripheral blood [11]. They noted that in these people's decidua and peripheral blood cells, there is a comparable upregulation of the Th17-associated component known as RAR-related orphan receptor gamma, or ROR-c [12]. This component is expected to downregulate the production of IL-2 and Fas ligands required to form Th17 cells [13]. Th17 cell differentiation depends on this component. According to a recent study [36], eclampsia patients' peripheral blood mononuclear cells (and decidua) have more significant levels of this factor's mRNA than pregnant women in good health. When the two groups were compared, this was validated.

"Consequently, the increased prevalence of Th17 cells in the peripheral blood of patients with preeclampsia may be partially explained by the increased production of this transcription factor. It has also been demonstrated that CD4 lymphocytes and CD8 and NK cells can produce IL-17 [14,15]. For the first time in this part of India, our results show that preeclampsia is linked to a higher frequency of CD8 and NK cells that express IL-17 in addition to CD4 cells. These lymphocyte subsets may produce IL-17, which might lead to the development of a pro-inflammatory milieu throughout the body."

Global research [16–18] has revealed that natural killer (NK) cells are essential to controlling the immune system during human pregnancy, indicating that the innate immune system, not the adaptive immune system, is responsible for this

function. The aberrant activation of natural killer (NK) cells in the placenta, which can happen locally and systemically, is the etiology of eclampsia and preeclampsia. This activation has many applications. We found that NK cells expressing IL-17 are more common in eclampsia than in healthy pregnancies. This discovery raises the possibility that the presence of NK cells that express IL-17 causes the aberrant activation of NK cells in this scenario.

"Based on these results, we calculated the percentage of Th1 and Th2 cells in healthy pregnancy and eclampsia using cell surface chemokine receptor markers. It was also shown that Th2 cells were identified using CCR4, whereas the Th1 fraction was identified using CXCR3. Based on these markers, we determined that a change in the Th1 direction characterizes eclampsia.

The Th1-biased shift of T lymphocytes in eclampsia may be partially explained by the altered ratio of Th17 ⁄ Treg cells. This is because IL-17 promotes the synthesis of other pro-inflammatory cytokines, including IFN-c [19,20]. However, there was no indication of any correlation between the preponderance of CXCR3 cells and IL-17-producing cells in the CD4 subset. Consequently, rather than altering the balance between Th1 and Th2 in eclampsia, it is more likely that Th17 cells and IL-17 directly impact the inflammatory state. This is caused by IL-17 and Th17 cells.

Our study's conclusions show that IL-17, a pro-inflammatory cytokine, is essential for the pathophysiology of eclampsia. In this context, the altered ratio of Th17 /Treg cells is especially noteworthy. More than likely, this action is done directly instead of adjusting the ratio of Th1 to Th2.

Based on the information we know, it is challenging to say if this discovery is a cause or an effect in the development of eclampsia. However, given the complexity of the etiology, this particular change could be both a significant signal and a contributing factor to immune dysfunction in eclampsia. One of its drawbacks is that our study only has a small number of participants. Further investigations, including the measurement of circulating IL-17 levels, are required to determine the biological significance of the pathophysiology of eclampsia alterations that have been found.

Conclusion

The research provides crucial information for comprehending the relationship among HLA alloantibodies, T lymphocytes, and eclampsia. The results show that these immunological components are significantly different in preeclampsia and eclampsia compared to a normal pregnancy and that the ratio of Th to Ts is also considerably changed. These results significantly affect the diagnosis and management of preeclampsia and eclampsia, and more research is needed to explore these implications fully. Future studies may identify specific HLA alleles and T cell subsets associated with preeclampsia and eclampsia and explore the potential benefits of altering the immune response to develop novel treatments for the condition."

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