Original Article

Plasma immunoglobulins (IgG, IgM, IgA) change in pregnancies of pre-eclampsia and eclampsia compared to normotensive pregnant and non-pregnant

DOI: https://doi.org/10.47648/zhswmcj.2024.v0601.01

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Abstract

Background: Pre-eclampsia is an unpredictable pregnancy complication; severe pre-eclampsia affects the brain, even seizures, and induces eclampsia. Materials and Methods: This study was conducted among a total of 155 subjects comprising pre-eclampsia (n=44), eclampsia (n=47), normotensive pregnant (n=35), and normotensive non-pregnant (n=29) women. Plasma immunoglobulin content was determined by indirect Enzyme-Linked Immunosorbent Assay (ELISA). Significant differences in immunoglobulin contents across the groups were assessed by one-way analysis of variance (ANOVA) and between two groups by pair-sample t-tests. Results: Results showed significant ($p<0.05$) differences in IgG and IgM levels, while no significant changes were observed in the IgA level across groups. The IgG level was found to be increasing from non-pregnant (6.86±0.6 g/L) to the eclampsia (7.42±0.9 g/L) to pre-eclampsia (7.53±0.7 g/L); there had also significant ($p<0.05$) changes among eclampsia (7.42±0.9 g/L), normotensive pregnant (6.96±0.5 g/L), and non-pregnant (6.86±0.6 g/L). Furthermore, immunoglobulin M (IgM) had significant ($p<0.05$) variations among the complicated pregnancies (pre-eclampsia and eclampsia), normotensive pregnant (2.09±0.4), and non-pregnant (2.64±0.2) women. At the same time, the highest amount of IgM was found in eclampsia (2.74±0.3 g/L) and the lowest in pre-eclamptic subjects (1.92±0.1 g/L). Conclusion: Significant alterations were observed in IgG and IgM levels across the groups, while changes ($P>0.05$) in the IgA level among the pre-eclamptic, eclamptic, pregnant, and non-pregnant women were found to be independent.

Key words: Plasma immunoglobulins; Pre-eclampsia; Eclampsia; Normotensive pregnant; Non-pregnant

Received on: 05.08.2023; Accepted on: 15.10.2023

Introduction

Pre-eclampsia and eclampsia (PE/E) are associated with unknown etiology, less understood disease pathways, and unexplained proper cellular and molecular mechanisms.[1,2] Occurrence of pre-eclampsia and eclampsia is much higher in underdeveloped countries than that in developed countries.[3] In Bangladesh, the prevalence of pre-eclampsia is 14.4%, 10% of pre-eclamptic patients have no previous history of hypertension, and 5.4% of pregnancies are superimposed on chronic hypertension.[4] Study reported that 20% of maternal deaths in Bangladesh are attributable to pre-eclampsia and eclampsia, the second leading direct cause of maternal mortality.[3] Moreover, knowledge regarding PE/E is not up to the mark among the general population,3 and no antenatal care (ANC) visits are more likely to be associated with pre-eclampsia.[4]

Pregnancy is a well-tolerated homograft where blastocyst affixes to the mother's uterus as a homograft, induces immune suppression by inhibiting T-cell activity.[5, 6] The genetic composition of the fetus differs from that of the mother; even then, it is not rejected. [6,7] Thus, the maintenance procedure of pregnancy is fulfilled.

Several studies revealed that pre-eclampsia is a two-stage process linked to higher vascular resistance of uterine arteries and lower uteroplacental blood flow, prevents

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adequate blood and oxygen flow to the developing fetus, damages the maternal liver, kidney, and brain, and progresses to eclampsia- a severe condition involving seizures. No known cure for eclampsia is reported except for the baby's delivery or the placenta.[2, 4, 8-10]

Recently, genetic factors linking immunological pathways to predisposition to pre-eclampsia have been identified.[8] However, the etiology of pre-eclampsia is multiple. In addition to immune suppression in pregnancy[5], alters the immune system, including changes in cytokines and immunoglobulin contents.[9, 11, 12] Persistent placental hypoxia causes inadequate placentation. The change in cytokine profiles may be due to an alteration in immune regulation, inadequate fetal allo recognition, or inflammatory triggers present during implantation.[9] Moreover, a particular type of pre-eclampsia could be auto-immune and represent immune dystrophy.[12]

Previous studies reported IgG and IgM levels were increased[13], IgA levels were decreased, or IgM remained unchanged in pregnancy and pre-eclampsia.[5, 11] As per the continuation of the research from the previous study for new findings on a broader scale[13], the present study reports immunoglobulin content in a large population of pre-eclamptic, eclamptic, normotensive pregnant, and non-pregnant women.

Materials and methods

Reagents: Ninety-six well micro-titer plates, anti-human IgG, IgA and IgM, peroxidase-conjugated anti-human IgG, IgA and IgM, and Tetra-methyl benzidine (TMB) were purchased from Sigma Chemicals Co, USA. Standard immunoglobulin (IgG, IgA, and IgM) were obtained from Sera-Pak®, Immuno, Bayer, USA.

Study design and population: This cross-sectional study was conducted with a sample size of 155 comprising pre-eclampsia (n=44), eclampsia (n=47), normotensive pregnant (n=35), and normotensive non-pregnant (n=29) women of 25 to 40 gestational weeks who had antenatal care during the pregnancy from January 2020 to June 2020. The first three groups of pregnant women were recruited from Dhaka Medical College Hospital, Sir Salimullah Medical College Mitford Hospital, and other clinics in Dhaka City. Age-matched non-pregnant (n=29) healthy women were collected from the community clinics.

Selection of patients and ethical approval: Exclusion criteria were lower than <25 gestational weeks of pregnancy and having other severe illnesses or co-morbidities (liver diseases, cardiovascular diseases). At the beginning of the study, verbal and written consents were taken from each patient according to the 'Helsinki Declaration', and patients' confidentiality was also maintained. Ethical permissions were taken from the heads of the Department of Obstetrics and Gynecology, Dhaka Medical College Hospital, and Sir Salimullah Medical College Mitford Hospital. Ethical permissions were also obtained from other Community Clinics and Hospitals.

Collection of the blood Samples: Two milliliters (2 ml) of venous blood sample were collected aseptically in a heparinized specimen tube from each study subject (n=155).

Analysis of immunoglobulins: The plasma immunoglobulin content was determined by indirect Enzyme-Linked Immunosorbent Assay (ELISA) described by Islam et al. (2004).14 One hundred microliter (100 µl) of anti-human IgG, IgA, and IgM (Sigma Chemicals Company, USA; diluted 1:1000 with Phosphate buffered saline or PBS) were pipetted to coat the wells of the microtiter plate (Nunc Immuno plate, Denmark), incubated overnight at 4ºC, washed more than three times with PBS (containing 0.5% Tween 20), and dried by wads of paper towels. The wells were blocked with 100 µl of sheep serum solution, incubated at 37ºC for 60 minutes, and washed and dried as indicated above. A 100 µl of diluted test sera and serially diluted standard immunoglobulins (IgG, IgA, and IgM) were pipetted into the pre-marked wells, incubated, and treated similarly. Then, 100 µl of diluted peroxidase-conjugated anti-human IgG, IgA, or IgM (diluted 1:500 with 0.1% BSA) were added into the well, incubated, and treated as indicated above. Finally, 100 µl of substrate solution (0.001% Tetra-methylbenzidine/TMB in 0.1M sodium acetate buffer containing H2O2) was added to each well of the plates and incubated in the dark at room temperature for 50 minutes. The peroxidase reaction was stopped by adding 50 µl of 10% sulfuric acid to each well. The plate was then read at 450 nm by an ELISA reader (Labsystems, MultiskanEX, Finland).

Statistical analysis: Data were analyzed using the SPSS software package (version 23.0 SPPS Inc. Chicago, IL, USA) and Microsoft Excel (2019). Significant differences in immunoglobulin contents across the groups were assessed by one-way analysis of variance (ANOVA). Moreover, paired-sample t-tests were employed to compare or differentiate the two (pair) immunoglobulin contents between the two groups, and the chi-square test was employed to observe differences among categorical variables, especially socio-demographic variables.

Results

Socio-economic and clinical parameters of the pre-eclampsia, eclampsia, normotensive pregnant, and non-pregnant women are shown in Table 1. No significant differences (P>0.05) were observed among mean ages (years) of the four groups of women [pre-eclampsia (25.4±4.38), eclampsia (24.6±4.4), normotensive pregnant (24.8±4.9), and non-pregnant (25.5±4.5)]. Similarly, all four groups were independent (P>0.05) for gestational weeks and monthly family incomes. Mean gestational ages were 35.8 (±3.8) weeks for pre-eclampsia, 35.9 (± 2.38) weeks for eclampsia, and 36.2 (± 2.85) weeks for normotensive pregnant mothers.

Most women across groups were multiparous (51.1%-52.3%), and the rest were primiparous (47.7%-48.9%). No significant difference in parity was observed among the four groups of women. Moreover, monthly family incomes were 20000 (±6000), 22000 (±4000), 24000 (±5000), and 21000 (±4500) BDTs for pre-eclampsia, eclampsia, normotensive pregnant, and non-pregnant, respectively.

Table 2 and Figure 1 describe the plasma immunoglobulin contents in different groups of complicated pregnancies (Pre-eclampsia and Eclampsia), normotensive pregnant and non-pregnant women. One-way analysis of variance (ANOVA) showed Significant ($p<0.05$) differences in IgG and IgM levels, while no significant variations were observed in the IgA level across groups. The IgG level was found to be increasing from non-pregnant (6.86± 0.6 g/L) to the eclampsia (7.42 ± 0.9 g/L) to pre-eclampsia (7.53± 0.7 g/L); there had also had significant ($p<0.05$) changes among eclampsia (7.42± 0.9 g/L), normotensive pregnant (6.96 ± 0.5 g/L), and non-pregnant (6.86 ± 0.6 g/L). Furthermore, immunoglobulin M (IgM) had significant ($p<0.05$) variations among the complicated pregnancies (pre-eclampsia and eclampsia), normotensive pregnant (2.09 ± 0.4), and non-pregnant (2.64 ± 0.2) women. At the same time, the highest amount of IgM was found in eclampsia (2.74 ± 0.3 g/L) and the lowest in pre-eclamptic subjects (1.92±0.1g/L).

Table No. 1: Socio-economic Profile and maternal characteristics of the study subjects

Socio-economic variables (n=155)	**Pre-eclampsia (n=44)**	**Eclampsia (n=47)**	**Normotensive Pregnant (n=35)**	**Non-Pregnant (n=29)**	**statistics**
Age (years) (Mean± SD)	25.4± 4.38	24.6± 4.4	24.8± 4.9	25.5± 4.5	P>0.05*
Gestational ages (weeks) (Mean± SD)	35.0± 3.8	34.9± 2.38	36.0± 2.85	-	P>0.05*
Parity (n %)					
Primi-parity	21 (47.7)	23 (48.9)	17 (48.6)	14 (48.3)	P>0.05**
Multi-parity	23 (52.3)	24 (51.1)	18 (51.4)	15 (51.7)	
Monthly Family Income (BDT) (Mean± SD)	20000±6000	22000±4000	24000±5000	21000±4500	P>0.05*

*Analysis of Variance (ANOVA); **Chi-square test

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Table No. 2: Serum immunoglobulin levels of Pre-eclampsia, Eclampsia, Normotensive pregnant mothers, and non-pregnant subjects

Immunoglobulin (g/L)	[x]Pre-eclampsia (n=44)			[y]Eclampsia (n=47)			[z]Normotensive pregnant (n=35)			[w]Non-pregnant (n= 29)		
	% n	Mean ±SD	95% CI (lower-upper	% n	Mean ±SD	95% CI (lower-upper	% n	Mean ±SD	95% CI (lower-upper	% n	Mean ±SD	95% CI (lower-upper
IgG[a]												
5.96-6.99	20.5 (9)	7.53±0.7	7.33-7.74	29.8 (14)	7.42±0.9	7.16-7.69	42.9 (15)	6.96±0.5	6.78-7.15	55.2 (16)	6.86±0.6	6.62-7.07
7.01-7.99	54.5 (24)			55.3 (26)			57.1 (20)			45.8 (13)		
8.01-12.0	25.0 (11)			14.9 (07)			00.0 (00)			00.0 (00)		
IgM[b]												
1.63-2.73	100 (44)	1.92±0.1	1.88-1.96	44.2 (19)	2.74±0.3	2.68-2.86	94.3 (33)	2.09±0.4	1.94-2.24	62.1 (18) .64±0.2 2.56-2.72	2.64±0.2	2.56-2.72
2.74-4.58	00.0 (00)			55.8 (24)			5 . 7 (02)			37.9 (11)		
IgA[c]												
2.80-3.70	43.2 (19)	3.73±0.4	3.61-3.85	40.4 (19)	3.75±0.5	3.61-3.89	37.1 (13)	3.70±0.3	3.61-3.79	37.9 (11) .64±0.6 3.40-3.88	3.64±0.6	3.40-3.88
3.71-4.58	56.8 (25)			59.6 (28)			62.9 (22)			62.1 (18)		

Human normal serum IgG=5.0-12.0 g/L, IgM=0.3-2.3 g/L, and IgA=0.5-3.5 g/L (Young, 1990)

ANOVA-test

[a]xy: t=0.094, P=0.926	[a]xz: t= 5.766, P=0.000	[a]yz: t= 2.899, P=0.007*	[a]xw: t=4.381, P=0.000**	[a]yw: t=3.604, P=0.001**	[a]zw t=.814, P=0.419
[b]xy: t=-16.28, P=0.000*	[b]xz: t= -2.192, P=0.035*	[b]yz: t= 7.272, P=0.000*	[b]xw: t=-17.280, P=0.000**	[b]yw: t=1.738, P=0.087	[b]zw: t= -6.174, P=0.000**
[c]xy: t= -0.171, P=0.865	[c]xz: t= 0.179, P=0.859	[c]yz: t= 0.400, P=0.692	[c]xw: t=.732, P=0.466	[c]yw: t=.557, P=0.579	zw: t= .490, P=0.626
a=IgG, b=IgM, c=IgA	x=Pre-eclamptic, y=Eclamptic, z=normotensive pregnant, and		w=Non-pregnant		

Pair-sampled t- Test (between the two groups of subjects)

[a]xyzw: F=8.194 (3,151), P=0.000** (a=IgG differs among 4 groups)	[a]xyz: F=6.410 (2,123), P=0.002** (a=IgG differs among 4 groups)
[b]xyzw:F=71.611 (3,151), P=0.000** (b=IgM differs among 4 groups)	[b]xyz:F=81.272 (2,123), P=0.000** (b=IgM differs among 4 groups)
[c]xyzw:F=0.350 (3,151), P=0.789 (no differences among groups)	[c]xyz:F=0.136 (2,123), P=0.873 (no differences among groups)

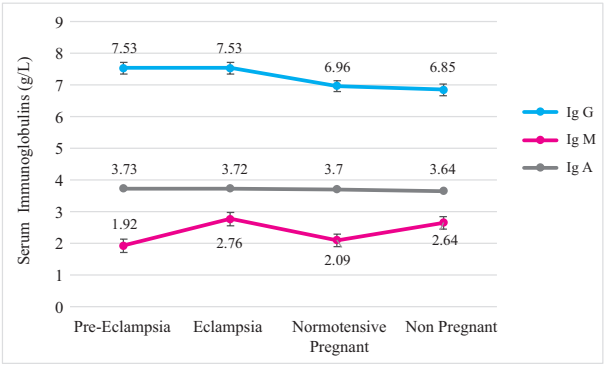

Figure No. 1: Plasma Immunoglobulin levels among Pre-Eclampsia, Eclamptic, Normotensive, and Non-pregnant mothers

Discussion

In the present study, a significant alteration was observed in the immunoglobulin G (IgG) and M (IgM) levels, but likely an insignificant change in the IgA level among the pre-eclamptic, eclamptic, pregnant, and non-pregnant women. Previously, similar outcomes were observed by Ahsan et al. (2008)[13], where increment of both IgG and IgM were noticed. However, studies (2016)[5,15] showed alteration among all immunoglobulin levels but decrement in IgG and IgM. Although the possible reason is still unknown and remains not fully understood; however, undiagnosed infections, co-existing other medical disorders, and influential factors can play roles in the increment/decrement of both immunoglobulin G (IgG) and M (IgM) levels.

A non-significant difference in immunoglobulin A levels between pre-eclamptic and normotensive pregnant women is reported in a recent study[15]. At the same time, earlier studies showed no changes (P>0.05) in both serum IgA and lgM levels.[16,17] However, both elevation[13,18] and decrement[5,15,19] of immunoglobulin A levels in eclamptic or pre-eclamptic women than the normotensive or non-pregnant women are available in the works of literature.

In this study, IgG levels increased from non-pregnant (6.86± 0.6 g/L) to eclampsia (7.42 ± 0.9 g/L) to pre-eclampsia (7.53± 0.7 g/L); also, significant changes were observed across eclampsia, pregnant and non-pregnant subjects. However, higher IgG, IgM, and IgA immunoglobulins levels in hypertensive mothers than in non-hypertensive were also reported.[18] Increased level of IgG in both pre-eclampsia and eclampsia and the only increment of IgM in eclampsia may be due to the release of cytokine, induced by stress or inflammation in them, which stimulate humoral 'immune system' to increase or regulate the immunoglobulin secretion.[20-22] Moreover, the enhanced level of IgG in pre-eclampsia and eclampsia may also be caused by an infection in the complicated pregnancy.[23-26] Furthermore, studies reported that pregnancy modifies the immune response[27,28] to develop immune tolerance to sustain and maintain blastocyst implantation in the mother's uterus.[25] The regulatory T cells (Tregs) have been reported to inhibit the immune response to self-antigens and suppress excessive immune responses deleterious to the host.[29] Pre-eclampsia may be due to poor placentation followed by oxidative stress or Inflammation[24], which initiates cytokine release[20,21], which regulates humoral immunity.[22] Implantation, placentation, and the first and early second trimester of pregnancy resemble "an open wound" that requires a robust inflammatory response.[24] Furthermore, semen may cause infection[8,25], and infection in pre-eclampsia caused by Chlamydia pneumonia has been reported to increase immunoglobulin levels.[26]

In this study, IgG was not influenced by age, parity, and socio-economic condition across the four groups despite elevating serum immunoglobulin G levels in pre-eclamptic and eclamptic subjects than those of normotensive pregnant and non-pregnant, which agrees with previous studies.[13,16] Contrary to the present study, recent[15] and earlier study[19] reported a significantly decreased level of IgG in pregnant women. Moreover, in a previous study Jha and Pandey reported (1983) significantly decreased level of IgG in pregnant women with hypertension and pre-eclamptic toxemia than in normotensive pregnant women, while serum IgA and lgM showed no significant changes.[19] Reduction in the level of serum IgG decreased selective trans-placental passage of IgG from mother to fetus. This reduction is pronounced when pregnancy involves toxemia, anemia, essential hypertension, or nephritis. These conditions add pathological stress to the mother's physiological stress (i.e., pregnancy). A depression of IgG synthesis or the formation of immune complexes can possibly lower the IgG level. The profound endocrinological changes of pregnancy and pre-eclampsia could be responsible for the suppressed immunological status of the mother.[2, 8, 21] A more convincing explanation for lowering the level of IgG in toxemia is the loss of IgG in the urine. Thus, retention of macroglobulin, too large to be filtered, and molecular sieving of intermediate and low molecular weight proteins in the urine through the defective glomerular basement membrane.[19,21]

Conclusion

Significant alterations were observed in IgG and IgM levels across the groups, while changes (P>0.05) in the IgA level among the pre-eclamptic, eclamptic, pregnant, and non-pregnant women were found to be independent. Possible reasons for the alteration of immunoglobulin levels remain not fully understood. However, undiagnosed infections, co-existing other medical disorders can play important roles. Longitudinal study is therefore warranted to obtain conclusive results for the increment of IgG and IgM levels in pre-eclamptic and eclamptic subjects than in normotensive pregnant and non-pregnant subjects.

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