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Neutrophil Activation Through the Expression of CD11a, CD11b and CD11c and Its Role With Complement C3 And C4 Levels in Patients With Pre-eclampcia

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Abstract

Background The leukocyte integrin that plays a major role in neutrophil activities is CD11b. In addition to mediating neutrophil adherence to endothelial cells, CD11b binds to the complement component iC3b and directs phagocytosis and intracellular lysis of microorganisms.

Objective To determine whether neutrophil activation, through the increased surface expression of the cell surface markers CD11a, CD11b, CD11c have a correlation with the values of complement components C3 and C4 in pregnant women with pre-eclampsia.

Methods This study was conducted at the AlKadhemiya Teaching Hospital. Patients were 60 pregnant women in labour subdivided into three groups:
Group A: 20 pregnant women with severe pre-eclampsia.
Group B: 20 pregnant women with mild-moderate pre-eclampsia.
Group C: 20 normotensive pregnant women (control group).
We performed the following laboratory measurements for all groups: total white blood cell WBC count, cell surface expression of CD11a, CD11b, CD11c by direct immunofluorescent technique, and serum complement C3 and C4 levels by radioimmunodiffusion method (RID).

Results There was a significant difference in neutrophil activation as detected by the cell surface expression of CD11a, CD11b, CD11c being higher in the pre-eclamptic group than the control group. The incidence of neutrophil activation was significantly higher among patients in group A compared to the other groups. There was a significant difference in the serum level of C3 and C4 in the pre-eclamptic group being higher in group A and B than group C.

Conclusions No correlation was found between the markers of neutrophil activation (CD11a, CD11b, CD11c) and the serum levels of (C3, C4) in the pre-eclamptic group. The incidence of adverse maternal and neonatal outcome is significantly higher among patients in group A compared to the other two groups.

Key words Preeclampsia, complement serum level, CD11a, CD11b, CD11C, neutrophil.

Introduction

Women with chronic hypertension and pregnancy-induced hypertension are at substantial risk for developing pre-eclampsia/eclampsia, a disease with high fetomaternal morbidity and mortality. However, the etiology of this disease is still unknown [(1)]. Preeclampsia complicates 3-5% of first pregnancies and 1% of subsequent pregnancies with around 5-10% of cases being severe [(2)].

Hypertension with proteinuria or oedema or both induced by pregnancy after the 20th week of gestation [3].

The presentation is very variable and although hypertension and proteinuria are the two signs most easily detected they are not consistently present, 20% of women with preeclampsia are normotensive and 30% have no premonitory proteinuria [3].

The increased incidence of pregnancy induced hypertension noted among patients over the age of 35 years probably reflects undiagnosed chronic hypertension with superimposed pregnancy induced hypertension [4]. During an inflammatory response, neutrophil activation leads to neutrophil binding and transmigration through endothelial cells. This occurs through an interaction between endothelial cell adhesion molecules and surface receptors on polymorphonuclear neutrophils. Activation of leukocyte integrins mediates firm adherence of activated neutrophils to the endothelium, with subsequent release of substances mediating vascular damage [5].

Surface expression of L-selectin (CD62L), which is involved in the initial adhesion process, is downregulated as adherent neutrophils emigrate through vascular endothelium [6].

Several lines of evidence have documented neutrophil activation in pre-eclampsia as follows:

1. Neutrophil degranulation products, including elastase and lactoferrin, are present in higher concentrations in the serum of women with pregnancy-induced hypertension [7].
2. The production of leukotrienes and superoxides by leucocytes are also increased [8].
3. Neutrophil integrin expression is altered [9].

The leucocyte integrins represent a subclass of a large family of proteins that mediate interactions between cells and their extracellular environment [6].

The leucocyte integrins-known as CD11a/CD18, CD11b/CD18 and CD11c/CD18 (CD=cluster of differentiation)-mediate the interactions of leucocytes with each other, with extracellular particles and with endothelial cells [5]. Integrins have found to play a role in platelet aggregation, immune functions, and tissue repair and tumor invasion [10].

The leucocyte integrin that plays a major role in neutrophil activities is CD11b. On stimulation, CD11b is translocated to the neutrophil cell surface. In addition to mediating neutrophil adherence to endothelial cells, CD11b binds to the complement component iC3b and directs phagocytosis and intracellular lysis of microorganisms. CD11b also recognizes fibrin and fibrinogen and can mediate neutrophil adherence to the extracellular matrix [11].

CD11a, which is crucial to lymphocyte adherence, is also present on the neutrophil surface and plays a role in antibody-dependent killing.

CD11c is also found on neutrophils, also binds to complement component iC_3b and appears to play a role in neutrophil-endothelial interactions [12]. So we intended in this study to focus on the role of these molecules in patients with pre-eclampsia.

Methods

This study was conducted on 60 pregnant women presented with labor pain attending Alkadhemiya Teaching Hospital starting from the 1st of October, 2001 to the end of April, 2002. Forty pre-eclamptic women were studied and 20 apparently healthy pregnant women as a control. The following inclusion criteria were followed; Pre-eclampsia was diagnosed by the occurrence of hypertension (upper limit of normal blood pressure value was 139/89 mmHg) in combination with proteinuria and/or edema, developing after 20 weeks gestation in a previously normotensive nonproteinuric patient. Age range from 18-40 years, less than 6 weeks

gestational, singleton pregnancy. The exclusion criteria were:

Multiple pregnancy, history of essential hypertension, diabetes mellitus, renal disease, hepatic disease, blood disorders, epilepsy or other medical diseases, history of drug intake other than supplements.

Women included in this study were subdivided into three groups:

Group A: Twenty pregnant women in labor with severe pre-eclampsia which was evidenced by a systolic blood pressure of >160 mmHg and diastolic blood pressure of >110 mmHg with proteinuria >5 gm/24 hours and/or edema.

Group B: Twenty pregnant women in labor with mild-moderate pre-eclampsia which was evidenced by a systolic blood pressure of 140-159 mmHg and a diastolic blood pressure of 90-109 mmHg with proteinuria and/or edema.

Group C: Twenty apparently healthy pregnant women in labor of comparable age, parity and stage of pregnancy as a control group.

Blood pressure was recorded in the sitting position with a cuff that is large enough for the subjects arm on at least two occasions 6 hours apart.

Korotkoff phase 5 (K5) disappearance of the sound was used to detect the diastolic blood pressure.

Proteinuria was diagnosed by collecting clean catch midstream urine sample in a clean dry container, and then urine protein was determined using the reagent strips (Albustix).

A reading of 2+ (1 gm/L) or more or equal to 1+ (0.3 gm/L) if the specific gravity is less than 1030 was considered to be positive result for proteinuria (significant proteinuria).

After medical, surgical and obstetric history was taken for all women who were subjected to full physical and obstetric examination and they were followed during their labor, delivery, postnatal period and peurperium with follow up of their newborn during their postnatal period.

In addition to the routine laboratory tests done, we performed the following laboratory measurements for all groups:

- Total white blood cell WBC count.
- Determination of cell surface adhesion molecule (CD11a, CD11b, CD11c) by direct immunofluorescent technique.The technique described by Eggleton et al (1989) [13] was used for neutrophil separation.

Preparation of slides:

10 μl of cell suspension per well on teflon coated slides, left to dry, then fixed by fixator (prepared by mixing 90 parts of 95% ethanol, 5 parts methanol, 5 parts of 100% isopropanol).

Slides dipped in fixator for 30 minutes and allowed to dry at room temperature, then slides checked with microscope for even spread of cells, then stored at -20 C till assayed.

Direct immunofluorescent test (DIFT):

The teflon slides precoated with neutrophil suspension were removed from freezer and allowed to reach room temperature, then washed with PBS, then slides were layed flat in humidity chamber then 10 μl of fluorochrome conjugated monoclonal antibodies at 1/10 dilution were added to each well. Cover chamber and slides were left undisturbed in incubator at 37 C. Slides then transferred to staining jar filled with PBS at room temperature, then we placed a drop of mounting media on each well of slides (9 parts glycerol to 1 part carbonate buffer PH=9) to enhance fluorescence and retard fading on exposure to UV light, then cover slides were lowered into place slowly to avoid bubbles.

Examination of the slides: slides were examined with fluorescence microscope at 490nm. In each spot 200 neutrophils were counted, the positive labeled cells were identified by their bright green color. The percentages of positive cells were found using the following formula: (labeled cells/200)×100

Measurement of serum complements C3 and C4 levels:

The serum level of C3 and C4 for each patient was estimated using the Radioimmunodiffusion method (RID), whichwas done according to manufactuers instruction.

Statistical analysis:

The statistical analysis of the data was performed using the following tests: Chi square test, Student t-test and Correlation coefficient test.

Results

Figure 1 shows the mean WBC count for all groups. There was no significant difference in the mean total WBC count for all groups (p > 0.05).

Figure 2 shows the mean percentage positive cells for CD11a for all groups. We found a higher percentage among patients of group A (49.4% ± 2.63) compared to those in group B (30.75% ± 1.67) and group C (16.65% ± 1.30) which was statistically significant (p < 0.0001).

Figure 3 shows the mean percentage of positive cells for CD11b. We found a statistically significant higher percentage among patients of group A (41.9% ± 1.57) compared to group B (24.15 % ± 1.11) and group C (16.95% ± 1.33) (p < 0.001).

Figure 4 shows the mean percentage of positive cells for CD11c. We found a higher percentage among patients of group A and B (33.1% ± 1.29, 18.05% ± 1.58 respectively) compared to group C (15.15 % ± 1.13) being statistically significant for A and C, B and C (p < 0.0001) but statistically not significant for A and B (p > 0.05).

Figure 5 shows the mean serum concentration of C3 and C4 for all groups. We found a statistically higher concentration among the pre-eclamptic group as compared to the control group (p < 0.0001) but there was no correlation with disease severity with p value for A and B (p > 0.05).

To evaluate the relationship between the percentage of positive cells for CD11a, CD11b, CD11c and the serum complement C3 and C4 levels in the pre-eclamptic group, the correlation coefficient was calculated and no correlation was found between the two parameters.

Table 1 shows the relation between the mean percentage positive cells for CD11a, CD11b, CD11c and the mean serum complement C3 and C4 levels for the pre-eclamptic group.

Table 1: The relationship between CD11a, CD11b, CD11c and C3, C4 among the pre-eclamptic group

Group A		C3 (mg/dl)	C4 (mg/dl)
CD11a (% positive cells)	Correlation Coefficient	-0.129	-0.077
	Significance	0.589	0.748
CD11b (% positive cells)	Correlation Coefficient	-0.50	0.378
	Significance	0.835	0.10
CD11c (% positive cells)	Correlation Coefficient	0.121	-0.34
	Significance	0.587	0.887
Group B		**C3 (mg/dl)**	**C4 (mg/dl)**
CD11a (% positive cells)	Correlation Coefficient	-0.254	-0.005
	Significance	0.280	0.985
CD11b (% positive cells)	Correlation Coefficient	0.078	0.118
	Significance	0.747	0.621
CD11c (% positive cells)	Correlation Coefficient	-0.320	0.04
	Significance	0.169	0.868

Discussion

Pre-eclampsia is characterized by platelet activation, vasoconstriction and vascular damage [14], all changes which strongly imply disordered endothelial cell function. Neutrophils have been implicated in the pathogenesis of atherosclerosis [15] and through their ability to produce reactive oxygen species, have been presumed to play a role in the vascular damage of pre-eclampsia.

The expression of neutrophil cell adhesion molecules have been described as a useful marker of in vivo activation in various pathologic situations, including chronic renal failure [16], endotoxemia [17], trauma and sepsis [18].

Neutrophil priming and activation can best be identified by a transformation in cellular adhesion molecule expression — most notably by a rise in the integrins, predominantly CD11b and to a lesser degree CD11a, CD11c and a fall in the level of the selectin CD62L. These are constitutively expressed on human neutrophils and are upregulated on the cell surface [19].

The total WBC count does not change during pregnancy [20]. In our study we found that the total WBC count remained within the normal range in the three groups with mean value of 9202.5 cell/mm^3 $\pm$ 577.63 in group A, 8059 cell/mm^3 $\pm$ 619.72 in group B, 7650 cell/mm^3 $\pm$ 482.5 in group C.

The present study showed that polymorphonuclear neutrophils are activated in pre-eclamptic women presented with labour pain through the changes of several markers. The neutrophil response was evaluated by quantitating the cell surface expression of the integrins CD11a, CD11b, CD11c.

The present study showed a significant increase in cell surface expression of CD11a, CD11b, CD11c in group A (49.4%, 41.9%, 33.1% respectively) and group B (30.75%, 24.15%, and 18.05% respectively) as compared to group C (16.65%, 16.95%, and 15.15% respectively).

This result is consistent with that of Sabatier et al. 2000 who found that neutrophils were activated in pre-eclamptic women and in patients with isolated intrauterine growth restriction through the increased cell surface expression of Cd11b and CD62L [21].

In our study we measured the serum complement levels, C3 and C4 and it was found that a significant increase in the levels of C3 in group A and B (210.4 mg/dl, 192.7 mg/dl respectively) as compared to group C (135.6 mg/dl) and increased levels of C4 in group A and B (52.42 mg/dl, 48.75 mg/dl respectively) as compared to group C (37.2 mg/dl) but with no correlation with disease severity as there was no statistical difference between group A and B (p > 0.05).

While Mellenbakken et al, 2001 [22] found only a decrease in the level of C4 but no changes in the level of C3 between normotensive and pre-eclamptic subjects.

de-Messias et al, 2000 [23] in their study on Brazilian pre-eclamptic women showed a significantly higher level of C3 and C4 in the pre-eclamptic group when compared to the normal pregnancies, but none of the studied variables showed statistically significant differences regarding the severity of pre-eclampsia.

These results confirm the activation of complement in pre-eclampsia, suggesting that this activation is related to the disease manifestations.

In our study we could not find a positive correlation between the markers of neutrophil activation and the markers of complement activation in the pre-eclamptic women and it may be that other factors of the complement cascade operate on the neutrophil or that the effect of complement on neutrophil is local and cannot be detected systemically, this is in agreement with the study of Mellenbakken et al, who found no correlation between neutrophil activation and systemic complement activation in patients with pre-eclampsia[22].

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