WEST AFRICAN JOURNAL OF MEDICINE

ORIGINAL ARTICLE

Adenosine Deaminase Activity in Subjects with Normal Pregnancy, Pregnancy Induced Hypertension and Pre-eclampsia

L'Adénosine l'Activité de Deaminase dans les Sujets avec la Grossesse Normale, la Grossesse l'Hypertension Incitée et Pre-eclampsia

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ABSTRACT

BACKGROUND: **Both pregnancy and adenosine deaminase (ADA) are associated with depressed cellular mediated immunity. There is little information on ADA activity in pregant Africans.**

OBJECTIVE: **To determine the serum levels of adenosine deaminase (ADA) in normal pregnancy and pregnancy complicated by hypertension in Nigerian women.**

METHODS: **One hundred and twenty-five pregnant women comprising 35 normal non-pregnant women, 35 normal pregnant women, 35 pregnant women with pregnancy induced hypertension and 20 patients with pre-eclampsia were recruited for the study. Serum adenosine deaminase enzyme (ADA) activity was measured by the Giusti and Galanti spectrophotometric method in all study subjects.**

RESULTS: **The mean serum ADA level in the non-pregnant women was higher than that in the normal pregnant women (23.21 ± 6.3 v 14.69 ± 3.2, $p<0.001$). Amongst the pregnant women, mean serum ADA in the hypertensive and pre-eclamptic women was significantly higher than that in the normal pregnant group ($p<0.001$).**

Conclusion: **These findings indicate a probable decrease in cellular immunity in normal pregnancy and an enhanced cell mediated immunity in pre-eclampsia. WAJM 2009; 28(3): 161–164.**

Keywords**: Adenosine deaminase; pregnancy; pregnancy-induced hypertension; pre-eclampsia.**

RÉSUMÉ

CONTEXTE: *Tant la grossesse que l'adénosine deaminase (ADA) sont associées à l'immunité négociée cellulaire déprimée. Il y a peu de renseignements sur l'activité d'ADA dans les Africains pregant.*

OBJECTIF: *déterminer les niveaux de sérum d'adénosine deaminase (ADA) dans la grossesse normale et la grossesse compliquée par l'hypertension dans les femmes nigérianes.*

MÉTHODES: *Cent vingt-cinq femmes enceintes comprenant 35 femmes non-enceintes normales, 35 femmes enceintes normales, 35 femmes enceintes avec la grossesse ont incité l'hypertension et 20 patients avec pre-eclampsia ont été recrutés pour l'étude. L'adénosine de sérum deaminase l'enzyme (ADA) l'activité a été mesurée par le Giusti et Galanti spectrophotometric la méthode dans tous les sujets d'étude.*

RÉSULTATS: *le sérum moyen le niveau d'ADA dans les femmes non-enceintes était plus haut que cela dans les femmes enceintes normales ($23.21 + 6.3$ v $14.69 + 3.2$, $p <0.001$). Parmi les femmes enceintes, le sérum moyen ADA dans le hypertensive et femmes pre-eclamptic étaient de façon significative plus hautes que cela dans le groupe enceinte normal ($p <0.001$).*

CONCLUSION: *Ces conclusions indiquent une diminution probable dans l'immunité cellulaire dans la grossesse normale et une cellule améliorée a négocié l'immunité dans pre-eclampsia. WAJM 2009; 28 (3) : 161–164.*

Mots clé: *l'Adénosine de-aminase, la grossesse, l'hypertension, pre-eclampsia*

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Abbreviation: ADA, Adenosine Deaminase.

INTRODUCTION

Adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4. ADA) is a key purine enzyme essential for proliferation, maturation and function of lymphoid cells.[1] It irreversibly catalyzes the deamination of adenosine and 2'-deoxyadenosine to inosine and 2'deoxyinosine respectively.[2] ADA originates from the monocyte/ macrophage lineage and therefore reflects the involvement of the cellular Immune system.[3] Congenital deficiency has been associated with severe combined immunodeficiency disease.[1,4] ADA is considered as a non specific marker of cell mediated immunity.[1,3] Changes in serum ADA have been associated with diseases in which there is involvement of cellular immunity[5,6] Although Pregnancy is a physiological state, it has been associated with depressed cellular mediated immunity.[7,8]

Hypertension in pregnancy and pre-eclampsia are frequent complications of pregnancy.[9]

Hypertension complicates 6–20% of pregnancies all over the world and is a common cause of maternal and perinatal mortality.[10] Pre-eclampsia is one of the leading causes of maternal and fetal morbidity and mortality and it is associated with a probable enhancement of cell mediated immunity.[11] Endothelial cell dysfunction and immunological disorders are thought to be the major effector mechanism of pre-elampsia.[12] Knowledge of the ADA activity may give some understanding of the role of cell mediated immunity in pre-eclampsia. This is so because ADA is thought to be produced from lymphoctes and monocyte-macrophage cell system. The aetiology and pathogenesis of pre-eclampsia is not fully understood yet although it is reported that T cell activation may be responsible for the altered immune response in pre-eclampsia.[12]

There is paucity of information on ADA activity in pregnancy (normal and complicated) in Africans.

In the present study, maternal serum ADA levels were measured in normal uncomplicated pregnancies and in pregnancies complicated with hypertension and pre-eclampsia; these were compared with normal non-pregnant women.

SUBJECTS, MATERIALS AND METHODS

A total of 125 pregnant women comprising 35 normal non-pregnant women, 35 normal pregnant women, 35 pregnant women with pregnancy-induced hypertension and 20 women with pre-eclampsia were recruited into the study after an informed consent was sought. The patients were recruited from the Obstetrics and Gynaecology out-patient Clinics of the Lagos University Teaching Hospital, Lagos, Nigeria. The laboratory analysis was carried out at the Clinical Pathology Department of the College of Medicine of the University of Lagos. Ethical clearance was obtained from the ethics committee of the hospital. The age range of the patients was 20-40years and the pregnant patients were all in the third trimester of pregnancy carrying singleton pregnancies.

The normal non-pregnant females used as control group were recruited from amongst the medical students and hospital workers. The normal pregnant patients consisted of women with uncomplicated pregnancies who had normal blood pressure readings throughout pregnancy with no glycosuria or proteinuria. There was no history of any significant illness during pregnancy.

Criteria was defined as a blood pressure of 140/90 mm Hg or greater, found on 2 consecutive occasions at least 4 hours apart.

Patients with pregnancy induced hypertension were those with hypertension but without proteinuria or other symptoms of pre-eclampsia e.g. severe headaches, visual symptoms or epigastric pain. Significant proteinuria was defined as 2+ proteinuria or more on two consecutive occasions. Pre-eclampsia was defined as hypertension in combination with significant proteinuria developing after 20 weeks of gestation in a group of previously normotensive women.[13] Systolic amd diastolic blood pressure were determined using the first and fifth Korotkoff sounds.

Patients with a history of hypertension before 20 weeks of gestation were excluded. Patients with renal disease, diabetes mellitus, cardiac disease or sickle cell disease were also excluded from the study.

A volume of 5ml of fasting venous blood was collected from each subject into serum vacutainer tubes with clot activator. The serum was separated as soon as clot retraction had taken place and frozen at -20^0C until analysis.

Adenosine deaminase enzyme activity was determined by the Giusti and Galanti method[14] which is based on the formation of ammonia produced when adenosine deaminase acts in the presence of excess adenosine. The substrate used was adenosine; this was incubated with the specimen for one hour at 37^0C to give inosine and ammonia. Ammonia was then determined by the Charney and Marbach modification of Berthelot's reaction. Ammonia formed a blue indophenol with sodium hypochlorite and phenol in alkaline solution with sodium nitroprusside as the catalyst.

Each sample had its own blank, the ADA activity was calculated thus:

$$A_{blank} - A_{sample} / A_{blank} - A_{standard} \times 50u/L$$

All reagents were prepared with double distilled water with sulphuric acid and potassium permanganate to render the water ammonia free.

Statistical Analysis

This was performed by the SPSS version 11.0 statistical package. The statistical significance of differences between means of normal pregnancy, pregnancy induced hypertension and pre-eclampsia was estimated using the student "t" test. Differences were considered significant at <0.05.

RESULTS

There was no significant difference in the mean age values for the non-pregnant and pregnant women. The mean systolic and diastolic blood pressure in the non-pregnant and the normal pregnant women was not significantly different ($p>0.05$). The mean systolic blood pressure in the hypertensive women (141±11mmHg) was significantly lower than that found in the pre-eclamptic women (151±11mmHg), $p<0.01$; however, there was no significant difference in the

diastolic blood pressure. Five pre-eclamptics had 3+ proteinuria while eight and seven had 2+ and 1+ proteinuria respectively. (Table 1).

Table 2: Characteristics of Study Population

	Normal Non-pregnant	Normal Pregnant	Hypertensive (Pregnancy-induced)	Pre-eclamptic
Number	35	35	35	20
Maternal Age	30 ± 2.6	32 ± 4.6	33 ± 3.6	35 ± 4.2
Mean Blood				
Systolic	110 ± 8.8	110 ± 6.8	141 ± 11*	151 ± 11
Diastolic	70 ± 5.6	72 ± 8.4	96 ± 8	99 ± 8.5
Proteinuria	Negative	Negative	Negative	1+, 7; 2+, 8; 3+, 5

*Compared to pre-eclamptic group, p<0.01. Blood pressure is in mmHg; average values are mean±SD.

The mean total ADA activity of the pregnancy-induced hypertensive and pre-eclamptic group was significantly higher than the ADA activity in the normal pregnant group (p<0.01). There was no significant difference between the hypertensive and pre-eclamptic groups (p>0.05).

The ADA activity in each group of pregnancy (normal, hypertensive and pre-eclamptic women) was significantly lower than that found in the non-pregnant group (p<0.01) (Table 2).

Table 2: Adenosine Deaminase Activity in various Study Groups

Group	Number	Mean ± SD ADA Activity
Non-Pregnant	35	23.21 ± 6.3
Normal Pregnant	35	14.69 ± 3.2*
Pregnancy Induced Hypertension	35	17.72 ± 4.9*†
Pre-eclampsia	20	17.92 ± 5.5*†

*Compared to non-pregnant group, p<0.01; †Compared to normal pregnancy, p<0.01

DISCUSSION

In the present study, the ADA activity in the pregnant groups was significantly lower than the non-pregnant group in the third trimester. However the ADA activity in the hypertensive and pre-eclamptic groups were significantly higher than that in the normal un-complicated pregnancy. These findings agree with those reported by Yoneyama *et al*[1]. In our present study however, the absolute figures are higher compared to their figures of 14IU/L in the normal non pregnant and 10.5IU/L in the normal pregnant group. In a another study in which ADA was assessed in the three trimesters of pregnancy, Jaqueti *et al.*[7] reported a significant decrease in ADA activity in pregnancy compared to the non-pregnant controls but no significant difference in ADA activity between the three trimesters.

These results illustrate a reduced ADA activity in pregnancy, which may be associated with depressed cell mediated immune status in pregnancy[1] since the highest concentrations of ADA are found in the lymphocytes and monocytes.[3] A recent study by Kafkasli *et al*[15] also reported a lower maternal plasma ADA in normal pregnancy compared with non-pregnant women. However there is no other African data to compare our results with. A previous study on a white population reported an increase in normal pregnancy but that study did not include a control group[16] therefore the results were inconclusive.

Oestradiol and cortisol which are in high concentrations in pregnancy have been implicated as inhibitors of ADA activity; this may also offer an explanation for the significant decrease in ADA activity in pregnancy;[17] the clinical significance of the lower ADA in normal pregnancy compared to non-pregnant women remains uncertain.[15]

The regulatory mechanism for ADA activity in pregnancy is not very well understood yet. A decrease in the conversion of adenosine to inosine by ADA has been reported[18] and a consequent increased level of adenosine has been reported in normal pregnancy.[18] The decrease in ADA in pregnancy may have a positive effect on pregnancy by modulating immunity through the increase in adenosine which has been thought to influence cytokine production[18].

Our present study found an increase in ADA activity in the pregnancy induced hypertension and the pre-eclamptic groups. An increase in the ADA levels in pre-eclampsia as seen in this study was also reported by Yoneyama[12] and Karabulut[19] in separate studies. This may be related to enhanced cell mediated immunity in pre-eclampsia since the enzyme increases in situations where cellular immunity is stimulated. Pre-eclampsia is characterized by endothelial cell dysfunction, lipid peroxidation and alterations of immune responses and lymphocyte function may be involved in it's pathogenesis.[12] ADA activity increases during mitogenic and antigenic responses of lymphocytes and conversely, lymphocyte blastogenesis is inhibited by ADA inhibition. Activation of cell mediated immunity may activate leukocytes and activated leukocytes produce cytokines[20], adenosine is said to modulate immune triggered cytokine production.[21]

In a study on pre-eclamptic women, Yoneyama *et al.*[22], reported to have an increase in interferon gamma cells and Th1 cytokines, both of which activate the monocyte/macrophage cell system. This is a likely contributor to the elevated ADA in pre-eclampsia.

In conclusion, the findings in this study show a reduction in ADA activity in pregnancy with an increase in ADA in patients with pre-eclampsia. The clinical significance of the high ADA levels in pre-eclampsia is yet to be ascertained, but it is a pointer to the role of cell mediated immunity in pre-eclampsia.

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