Research Article

Assessment of the Relationship Between Luekotriene B4 and Luekotriene D4 Levels in Prediction of Preeclampsia.

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ABSTRACT

The objective of this study was to assess the relationship of levels of luekotriene D4 and luekotriene B4 in prediction of preeclampsia. Two hundred and ten blood samples were collected from patients. Twenty seven patients were developed PE. These patients were followed for up to five months (first reading at 16-20 week, second reading at 21-28 week and third reading at 29-40 week). Patients suffered from any other disease were not included in the current study. The control group consisted of one hundred and eighty subjects. They were pregnant women without preeclampsia and other complications. These patients also were followed for up to five months (first reading at 16-20 week, second reading at 21-28 week and third reading at 29-40 week). Three patients were escaped. Compared with the control, levels of serum LTD4 and LTB4 was increased when compared preeclamptic patients with the non-preeclamptic group (P < 0.001). There was a significant differences in third reading (29-40 week) of serum LTD4 and LTB4 levels (p ≤ 0.001) and second reading (21-28 week) of serum LTD4 and LTB4 levels (p ≤ 0.001) when compared to first reading (16-20 week) of serum levels of serum LTD4 and LTB4 in the preeclamptic patients. No Significant correlations were observed for the levels of serum LTD4 and LTB4 with ages, BMI and parity while Significant correlations were observed for the levels of serum LTD4 and LTB4 with MAP of the preeclamptic group (p < 0.001).

Keywords: luekotriene D4, luekotriene B4, preeclampsia.

INTRODUCTION

Preeclampsia(PE), a human-pregnancy-specific disease defined as the occurrence of hypertension and significant proteinuria in a previously healthy woman on or after the 20th week of gestation, occurs in about 8-12% of pregnancies[1,2]. It is the most common medical complication of pregnancy whose incidence has continued to increase worldwide, and it is associated with significant maternal morbidity and mortality, accounting for about 50,000 deaths worldwide[3].

Risk factors for preeclampsia include nulliparity, multifetal gestations, previous history of preeclampsia, obesity, diabetes mellitus, vascular and connective tissue disorders like systemic lupus erythematosus and antiphospholipid antibodies, age > 35 years at first pregnancy, smoking and African American race. Among primiparous women, there is a disparity among ethnic groups as the risk in African American women is twice that of Caucasian women[4].

The connection between these risk factors and preeclampsia is poorly understood. The differences in risk among ethnic groups suggest a strong role for genetic factors in the pathogenesis of preeclampsia. Most theories on the etiology of preeclampsia suggest that the disease is a cascade triggered by combination of abnormal maternal inflammatory response, endothelial cell activation/damage with deranged hemodynamic milieu, and deranged immunity[5,6]. The precise trigger that unifies the deranged vascular, immune and inflammatory responses remains to be elucidated[7]. PE is considered a multisystem disorder, affecting several organs and maternal systems, including the vascular system, liver, kidney and brain. Despite the intensive research in this area, the etiology of PE remains unknown. PE seems to have a multifactorial cause and is also known as the "disease of theories". In fact, there are several hypotheses raised to explain its etiology. Some those theories propose modifications in the trophoblastic invasion, immunologic intolerance between maternal and feto placental tissue, inflammatory changes in pregnancy and genetic modifications, underlying PE development[7].

Although its unknown cause, it is consensual that there are modifications occurring at different levels, like changes in placental perfusion, increased inflammatory response with changes in leukocyte activation, activation of the coagulation system, endothelial dysfunction and changes in lipid metabolism. The most accepted theory describes two stages for PE, stage1 - reduced placental perfusion; stage 2 - multisystem maternal syndrome[8]. The complications of preeclampsia are include eclampsia, HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome, liver rupture, pulmonary edema, renal failure, disseminated intravascular coagulopathy (DIC), hypertensive emergency, hypertensive encephalopathy and cortical blindness[9]. The role of leukotrienes in hypoxia and PE appears to be linked to the transcription of nitric-oxide synthase (iNOS)[10,11]. Chemical or physical hypoxia has been shown to increase gene transcription of iNOS and subsequent NO production. This increase has

also been shown to perpetuate lipid peroxidation and leukotriene B4 (LTB4) and leukotriene D4 (LTD4) generation. LTB4 is suppressed in pregnancy to inhibit the activation of neutrophils and other leukocytes. Conversely, this inhibition is not observed in PE and the failure of this mechanism may contribute to the maternal inflammatory syndrome[12].

LTB4 changes may initiate a dysregulation of the inflammatory response, thus contributing to the maternal syndrome of PE[13].

METHODS

Two hundred and ten blood samples were collected from patients admitted to AL- Zahraa Teaching Hospital during the period from June 2013 to January 2014. Twenty seven patients were developed PE later on after follow up. Their ages range from 21-38 years. These patients were followed for up to five months (first reading at 16-20 week, second reading at 21-28 week and third reading at 29-40 week) for serial assessment for estimation of serum levels of LTD4 and LTB4 in the PE patients. Patients suffered from any other disease were not included in the current study. This group consisted of one hundred and eighty subjects. They were pregnant women without preeclampsia and other complications.

The control group samples were collected from AL-Zahraa Teaching Hospital in Al najaf City. These patients also were followed for up to five months (first reading at 16-20 week, second reading at 21-28 week and third reading at 29-40 week). Their ages range from 21-36 years. Blood samples were obtained from pregnant women with preeclampsia and Non-PE groups by vein puncture. Samples were allowed to clot at room temperature for 30 minutes, and then centrifuged at 3000 Xg for 20 minutes. Sera were removed and stored at -18 °C until analysis in disposable serum tubes.

RESULTS

The evaluation of the data indicated that the enrolled patients were distributed according to different trends. They were distributed according to the age, Parity, BMI and MAP. The characteristics of the enrolled preeclamptic patients are mentioned in Table (1, 2 and 3) by mean and the standard deviation (Mean ± SD).

There was significant (p < 0.001) elevation of the concentrations of LTD4 and LTB4 in preeclamptic patients when compared with those of the non-preeclamptic group (Table 4) (A,B,C). 27 patients were followed up (the first reading from 16-20 week, the second reading from 21-28 week and the third reading from 29–40 week) for serial assessment of serum levels estimation of LTD4 and LTB4 in preeclamptic patients (Table 4).

There was a significant differences in third reading (29-40 week) of LTD4 and LTB4 levels (p≤0.001) and second reading (21-28 week) of LTD4 and LTB4 levels (p≤0.001) when compared to first reading (16-20 week) of serum

levels of LTD4 and LTB4 in the preeclamptic patients (Table 4).

The linear regression analysis was used to examine the relationship of the levels of LTD4 and LTB4 with ages of the preeclamptic and Non-preeclamptic group.

No Significant correlations were observed for the levels of LTD4 and LTB4 with ages of the preeclamptic and Non-preeclamptic group.

The linear regression analysis was used to examine the relationship of the levels of LTD4 and LTB4 with MAP of the preeclamptic and Non-preeclamptic group. Significant positive correlations (Table 5, Figure 1 and 2) were observed for the levels of LTD4 and LTB4 with MAP of the preeclamptic.

The linear regression analysis pointed out significant positive correlation between LTB4 with LTD4 in preeclamptic patients (Figure 3).

Table 1: Characteristics of preeclamptic patients (16-20 week) included in the study.

Variable	Mean ± SD	Range
Age (years)	28.93 ± 5.45	21-38
Body mass index (kg/m^2)	24.26 ± 2.61	20-29
Systolic pressure (mmHg)	116.44 ± 6.57	97-124
Diastolic pressure (mmHg)	75.26 ± 5.76	65-86
Mean arterial pressure (mmHg)	88.99 ± 5.04	78.3-98
Parity	2.26 ± 1.23	0-6

Table 2: Characteristics of preeclamptic patients (21-28 week) included in the study.

Variable	Mean ± SD	Range
Age (years)	28.93 ± 5.45	21-38
Body mass index (kg/m2)	25.16 ± 2.15	20-30.16
Systolic pressure (mmHg)	151.16±7.45	140-170
Diastolic pressure (mmHg)	97.56 ± 6.33	90-114
Mean arterial pressure(mmHg)	108.05 ± 4.11	106-118.6
Parity	2.26 ± 1.23	0-6

Table 3: Characteristics of preeclamptic patients (21-40 week) included in the study.

Variable	Mean ± SD	Range
Age (years)	28.93 ± 5.45	21-38
Body mass index (kg/m^2)	27.31 ± 2.06	22.2-33
Systolic pressure (mmHg)	152.24 ± 6.07	145-172
Diastolic pressure (mmHg)	98.04 ± 4.41	89-115
Mean arterial pressure (mmHg)	118.45 ± 4.13	106.7-126.6
Parity	2.26 ± 1.23	0-6

Table 4: Serum LTB4 and LTD4 levels in preeclamptic patients and the non-preeclamptic group (16-20 week).

Parameter	Subject	NO.	Mean ± SD	Range	P-value
LTB4 ng/ml	PE	27	5.10 ± 0.54	3.6-5.7	< 0.001
	NON-PE	180	3.41 ± 0.65	2.3-4.5	
LTD4 pg/ml	PE	27	114.63 ± 2.47	110-119	< 0.001
	NON-PE	180	86.67 ± 6.75	75-98	

Note: P-value < 0.001 mean significant

Table 5: Serum LTB4 and LTD4 levels in preeclamptic patients and the non-preeclamptic group (21-28 week).

Parameter	Subject	NO.	Mean ± SD	Range	P-value
LTB4 ng/ml	PE	27	6.93 ± 0.77	5.6-8.9	< 0.001
	NON-PE	180	3.45 ± 0.73	2.1-4.5	
LTD4 pg/ml	PE	27	122.22± 4.64	114-129	< 0.001
	NON-PE	180	87.70 ± 5.81	77-100	

Table 6: Serum LTB4 and LTD4 levels in preeclamptic patients and the non-preeclamptic group(29-40 week).

Parameter	Subject	NO.	Mean ± SD	Range	P-value
LTB4 ng/ml	PE	27	9.11± 1.04	6.5-10.9	< 0.001
	NON-PE	180	3.57± 0.70	2.1-4.5	
LTD4 pg/ml	PE	27	133.04 ± 5.58	122-140	< 0.001
	NON-PE	180	88.19 ± 5.80	78-109	

Table 7: Serial assessment of serum levels of LTD4 and LTB4 in 27 preeclamptic patients

Parameter	First Reading 16-20 week	Second Reading 21-28 week	Third Reading 29-40 week
LTB4 ng/ml	5.10 ± 0.54 B	6.93 ± 0.77 B*	9.11± 1.04 B*
LTD4 pg/ml	114.63 ± 2.47 C	122.22 ± 4.64 C*	133.04 ± 5.58 C*

Table 8: Univariate analysis of LTB4 and LTD4 with MAP of preeclamptic patients and Non-preeclamptic group.

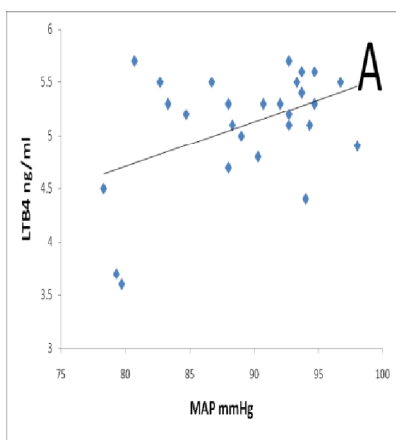|	Preeclamptic		Non-Preeclamptic	
Parameter	r	P	r	P
LTB4 (ng/ml)	0.43	< 0.001	0.11	N.S
LTD4 (pg/ml)	0.34	< 0.005	0.07	N.S

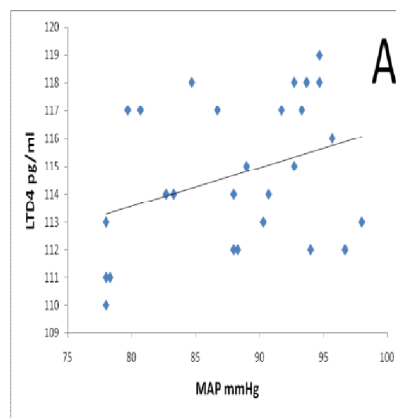**Figure 1:** The correlation of MAP with LTB4 in: A) preeclamptic patients

Figure 2: The correlation of MAP with LTD4 in: A) preeclamptic patients
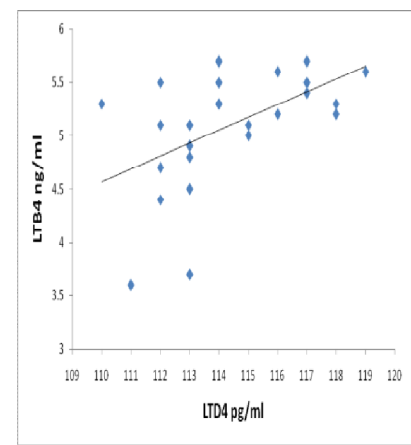

Figure 3: The correlation of LTB4 with LTD4 in preeclamptic patients

DISCUSSION

When the results of LTD4 and LTB4 concentrations of patients were compared with the control group, the highest levels of the control group were 119 pg/ml and 5.7 ng/ml respectively, the lowest values of LTD4 and LTB4 concentrations in the group of patients were 110 pg/ml and 3.6ng/ml respectively. Thus it is clear that only the value LTD4 of the patients and the control group did not overlap, in contrast LTB4 do not do so. These evidences suggest the use of measurements of LTD4 concentration as additional parameters in the management and prediction of PE. We can suggest a cut off value for LTD4 after more investigations. No Significant correlations were observed for the sera level of LTD4 and LTB4 with ages of patients and the control group. These results are in agreement with Schwartz LB[14] who found that there were no significant changes in serum leukotrienes with respect to maternal age. The current results indicated that alterations of inflammatory markers in PE are independent on ages of patients. The reasons seem to be unclear and further investigations may be required to explain such reasons. The linear regression analysis was used to examine the relationship of the levels of LTD4 and LTB4 and with mean arterial

pressure (MAP) of the patients and the control group. Significant positive correlations were observed for the level of LTD4 and LTB4 with the mean arterial pressure (MAP) of the patients, significant correlations could not be obtained in the group of healthy pregnant women. These results are in agreement with those reported by Shakow S.[15] who found that in hypertensive pregnant women there is a release of placental factors that initiate a cascade of cellular and molecular events leading to endothelial and vascular smooth muscle cell dysfunction and thereby increased vascular resistance and arterial pressure. It was found a positive correlation between serum levels of LTD4 and diastolic and systolic blood pressure in PE. Endothelial dysfunction of PE has been associated with an exaggerated maternal inflammatory response to pregnancy. It has been hypothesized that placental hypoxia resulting from uteroplacental arterial insufficiency, amplifies the release of inflammatory stimuli into the maternal circulation[16].

The elevation of serum LTB4 and LTD4 levels in preeclamptic female may explain the increased blood pressure as has an inhibiting effect on nitric oxide and stimulatory action on endothelin-1 and prostaglandins. Carreras L. (2008) showed that MPO can induce

endothelial dysfunction and injury to ultra structure of placenta and umbilical vascular endothelium. This injury may play a role in the pathogenesis of pregnancy induced hypertension[16]. In the current study, LTB4 and LTD4 were showed increase in preeclamptic patients when compared with non-preeclamptic group. This agreement with[17] which show elevated serum concentration of LTB4 and LTD4 in PE. PE is known to be associated with alterations of the arachidonate metabolism (I-10). In this condition, arachidonic acid availability is in fact increased in the maternal district and reduced in the fetal one in contrast with normal pregnancy.

The cyclooxygenase pathway was repeatedly investigated in pre-eclampsia, because of the suggested pivotal role for the so called "PGI/TXA balance" in the regulation of vascular homeostasis. Concerning cyclooxygenase metabolites, regarding high thromboxane A and lower prostaglandin E2 levels both in fetal and maternal district of hypertensive pregnant women. For what concerns prostacyclin, conflicting results have previously been reported in the literature: while numerous studies demonstrated reduced prostacyclin production in serum from cases of preeclampsia[16]. In other investigations this finding was not confirmed in the present study we could not demonstrate a general lowering of prostacyclin production in preeclamptic pregnant women. Lipoxygenase derivatives of arachidonate may be involved in the pathogenesis of gestational hypertension and especially in the increase of endothelial cell membrane permeability observed in preeclampsia. Besides circulating blood cells, also vascular tissue has been found to produce leukotrienes, especially in the adventitia. Our data confirm that PE–patients can produce LTB4 and LTD4 at detectable levels and show that such production is significantly increased in serum of preeclamptic pregnant even in the less severely affected patients. It is known fact-that LTB4 is a potent chemotactic and vasopermeabilizing agent and a mediator of ischemic tissue damage. Our results suggest therefore a possible involvement of the lipoxygenase products of arachidonate in the natural history of PE. It seems interesting that the observed increase in LTB4 occurs also in mild gestational hypertension and in absence of any change in placental prostacyclin.

However, it should be stressed that the relative role of tissue and platelet-derived arachidonate metabolites in the pathogenesis of this condition needs to be further investigated[18,16].

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