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Serum relaxin in preeclamptic and normotensive pregnant women at the Lagos University Teaching Hospital

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Abstract

Background: Preeclampsia is a pregnancy-specific multi-systemic progressive disorder that is unique to human pregnancy occurring in the second half of pregnancy. Reliable biochemical markers for prediction, diagnosis and management of preeclampsia would have a great impact on maternal health and several of these markers have been suggested. Relaxin is a peptide that has shown promising effect in the treatment of cardiovascular diseases and it is believed to increase vascular endothelial growth factor and placenta growth factor both of which promote angiogenesis and placentation in pregnancy. Thus, relaxin may play an important regulatory role in maternal vascular adaptations during pregnancy.

Aim: This study therefore aimed to find out the difference, if any, in serum relaxin levels in preeclamptic and normotensive pregnant women at Lagos University Teaching Hospital.

Methods: This was an analytical comparative cross-sectional study involving pregnant women with preeclampsia (study group- 48 had mild preeclampsia while 42 had severe preeclampsia) and age-, parity- and gestational age-matched women without preeclampsia (comparative group). Venous samples were collected from eligible participants after counselling and obtaining informed consent. Serum relaxin was determined using the Quantikine Human Relaxin-2 Immunoassay according to manufacturer's instructions. A structured questionnaire was also administered to collect relevant sociodemographic and clinical information. Data were entered and analyzed using the IBM Statistical Package for Social Sciences (SPSS Statistics) Version 23. Armonk, NY: IBM Corp. Categorical variables were summarized and presented as frequency and percentages. The test of normality of continuous variables was performed using the Kolmogorov-Smirnov test. The continuous variables such as age that were normally distributed were presented as mean (± standard deviation). All tests were two- tailed and statistical significances was set at $p < 0.05$.

Results: The mean level of serum relaxin in women with preeclampsia was significantly lower than that of the normotensive women (0.24+0.03ng/ml vs. 0.42±0.05ng/ml; $p < 0.001$). However, there was

no significant difference in the mean serum relaxin levels in participants with mild preeclampsia when compared to those with severe preeclampsia (0.24 + 0.02ng/ml vs. 0.23ng/ml + 0.01; p = 0.271).

Conclusion: Women with pre-eclampsia have significantly lower levels of serum relaxin than normotensive pregnant women. However, there is no significant difference in mean serum relaxin levels in women with mild when compared to those with severe preeclampsia. Therefore, development of pre-eclampsia is associated with lower serum levels of relaxin, though the relaxin levels may not indicate the severity of disease.

Key words: ELISA, Lagos, normotensive, preeclampsia, serum relaxin

Introduction

Preeclampsia has been previously defined as the onset of hypertension accompanied by significant proteinuria after 20 weeks of gestation. However, it is currently defined as a multisystem progressive disorder characterized by the new onset of elevated blood pressure and proteinuria or the new onset of hypertension and end-organ dysfunction with or without proteinuria in the last half of pregnancy or postpartum.[1-3] Severe cases of preeclampsia and eclampsia are very common in Nigeria with a high case fatality and a prevalence ranging between 2% and 16.7%.[2]

Worldwide about 76,000 pregnant women die from preeclampsia (PE) and eclampsia each year and it also results in 500,000 infant deaths globally each year, with a higher incidence noted in developing countries as opposed to developed countries.[2] The prevalence of preeclampsia reported in different parts of Nigeria ranges from 2-16%.[2]

The pathophysiology of preeclampsia involves both maternal, fetal, and placental factors.[4] Although there are many theories to explain the onset of this pregnancy- specific disorder, the most plausible theory is that of defective trophoblastic invasion, the result of which is inappropriate remodeling of the uterine spiral arteries and the second step of which is the maternal response to endothelial dysfunction and imbalance between angiogenic and antiangiogenic factors, leading to the manifestation of preeclampsia.[3,5-8]

The role of relaxin, a peptide hormone in the aetiopathogenesis of preeclampsia is currently being unveiled. Some authors infer that it plays a regulatory function in maternal cardiovascular and renal physiologic changes in pregnancy.[9,10] It is also implicated in reducing vascular stiffness during uterine artery remodelling in early pregnancy as it is believed to increase local production of vascular endothelial growth factor and placenta growth factor both of which promote angiogenesis and placentation in pregnancy. In addition, it has been shown to increase endometrial vascularization in the first trimester.[9-12] Hence, women who are deficient in serum relaxin levels may be at increased risk of developing preeclampsia. Relaxin on the other hand is a potent vasodilator of maternal blood vessels in pregnancy.[9-12]

Major Relaxin receptors are found in cardiovascular and trophoblastic tissues. These receptors mediate many signaling pathways and biological activities.[13] Recently, studies have shown that relaxin was associated with neovascularization of the endometrial lining of the uterus, potentially via specific induction of vascular endothelial growth factor, insulin-like growth factor binding protein-1, basic fibroblast growth factor, several cytokines and cyclooxygenases.[12,13] In other studies, it is also shown to have a different protective effect in the cardiovascular system against

major cardiac diseases including heart failure, oxidative stress, inflammatory changes in the myocardium, vasoconstriction, and fibrosis.[13,14] Relaxin also has a good efficacy and safety margin when administered to women with cardiac diseases.[13,15]

Thus, the functions of the endothelium in modulating the cardiovascular and renal adaptations associated with pregnancy may be altered in response to increased relaxin; Therefore, relaxin may have a possible role in the aetiopathogenesis and treatment of preeclampsia and its sequelae. This study, therefore, was aimed to determine the difference, if any, in serum relaxin levels between women with preeclampsia and normotensive pregnant women at the Lagos University Teaching Hospital, Lagos, Nigeria.

Materials and Methods

This was an analytical comparative cross-sectional study carried out in the delivery ward (Were all the participants in labour at the time of recruitment into the study?) of the Lagos University Teaching Hospital (LUTH). The Lagos University Teaching Hospital (LUTH) is the teaching hospital of the College of Medicine, University of Lagos. It acts mainly as a referral center for other government-owned and private hospitals in Lagos state. The study was carried out from August 10 2018 to June 20th 2019. The participants were pregnant women with preeclampsia (study group) and their age-, parity- and gestational age-matched normotensive pregnant women (comparative group).

Inclusion and Exclusion Criteria:

Pregnant women who presented to the labour ward during the study period with or without preeclampsia who gave informed consent and did not have underlying renal or cardiac disease, chronic hypertension and did not conceive via in-vitro fertilization with donor eggs were included in the study

Sample size determination

The sample size for the study was determined using the formula for comparing means of two independent variables;[16]

$$N_{1} = \frac{2\,(Z_{\alpha/2} + Z_{\beta})^2 * \delta^2}{d^2}$$

Where;

n = Minimum sample size

$Z_{\alpha/2}$ = Standard normal deviate corresponding to the probability of type 1 error α at 5% = 1.96

Z_{β} = Standard normal deviate corresponding to the probability of making type II error β of 10%. Power at 80% = 0.842

δ = standard deviation (standard deviation between the two groups)

δ_1 = standard deviation of preeclampsia cases[16]

δ_2 = standard deviation of comparison group[16]

d = Detectable difference in mean between the two groups corresponding to the margin of error[16] at 5% = 12

Where;

$\delta = (\delta_1 - \delta_2)$

$\delta = (268-241)$

$\delta = 27$

d= Detectable difference in mean[16] = 376-364= 12

Therefore, the sample size n

$$= \frac{2(1.96+0.842)^2 \times (27)^2}{(12)^2}$$

$$= \frac{2(2.802)^2 \times 729}{144}$$

$$= \frac{15.702 \times 729}{144}$$

= 79.49 approximately 80

Therefore, calculated sample size (n) = 80

Attrition rate of 10% was added to make up for non-response using the formula;

ns = n/1 – nrr

Where;

ns = new minimum sample size

nrr = non-response rate = 10% = 0.1

Therefore, ns = 80/1 – 0.1 = 88. Which is approximately 90

Total number of participants (n1+n2) therefore will be 180.

Participant Recruitment: Participants were consecutively consenting pregnant women who met the inclusion criteria. For each study group participant recruited a similar comparative group participant was recruited matched for age (±3 years), parity (±2) and gestational age (±5 weeks)

Definition of terms

Preeclampsia

The criteria for diagnosis for preeclampsia were blood pressure of at least 140/90 mmHg recorded on two separate occasions at least 4 hours apart and in the presence of at 2++ proteinuria in a clean catch urine sample detected after 20 weeks' gestation, in a previously normotensive woman after[2]

Mild preeclampsia was defined as systolic blood pressure measurements ≥ 140mm Hg- < 159 mmHg and or diastolic BP of 90-109mm Hg) taken on 2 occasions at least 4 hours apart, with significant proteinuria in a previously normotensive woman after 20 weeks of gestation.

Severe preeclampsia was defined as markedly elevated blood pressure measurements (systolic ≥ 160 mm Hg and or diastolic ≥ 110 mm Hg) plus significant proteinuria ≥ 2+.[2]

Outcome measures: The primary outcome measure was the mean serum relaxin levels in preeclamptic and normotensive women while the secondary outcome was to compare the mean serum level of relaxin in women with mild and severe preeclampsia.

Laboratory Method: Serum relaxin was determined using the Quantikine Human Relaxin-2 Immunoassay '(DRL200, R&D Systems Inc., Minneapolis, MN, USA 2019) according to the manufacturer's protocol to determine the serum relaxin concentration of the samples. This assay is based on the sandwich ELISA principle. The assay range was 0.007ng/ml-0.5ng/ml (7.8 - 500 pg/mL), with sensitivity of 0.00457ng/ml.

Ethical Approval

This study was approved by the Health Research and Ethical Committee of the Lagos University Teaching Hospital: ADM/DCST/HREC/APP/2400. All the ethical principles were adhered to.

Data Analysis: Data were entered and analyzed using the IBM Statistical Package for Social Sciences (SPSS Statistics) Version 23. Armonk, NY: IBM Corp. Categorical variables were summarized and presented as frequencies and percentages. The test of normality of continuous variables was performed using the Kolmogorov-Smirnov test. Continuous variables that were normally distributed were presented as mean (± standard deviation). The non-normally distributed continuous variables were presented as medians and interquartile ranges. The Student's independent sample t-test was used to compare the mean of normally distributed continuous variables among the preeclamptic and normotensive participants while Mann-Whitney U test was used to compare the median of the non-normally distributed variables. Analysis of variance was used to assess the differences in mean relaxin levels across the study groups of normotensive, mild and severe preeclampsia participants Post hoc Bonferroni test was then conducted to determine the pairwise differences within the groups.

RESULTS

Two hundred and twenty-two participants were initially approached and screened for inclusion in

the study, following which forty-two (18.9%) of the participants were excluded while one hundred eighty participants were recruited into the study.

There were no statistically significant differences in the mean ages, median parities and the mean gestational ages at recruitment between the participants in the two groups. There were no significant differences in the levels of education between the two groups; however, women in the comparison group were significantly more likely to have been professionals with regards to occupation. Women in the study group were significantly more likely to be unbooked patients and to have been delivered by Caesarean section. Fifty-seven (63.3%) of the study group had proteinuria of 2+, 26 (28.9%) had 3+ and 7 (7.8%) had 4+ proteinuria. Nineteen (21.1%) of the comparison group had trace proteinuria (Table 1).

In Table 2, the mean level of serum relaxin in women with preeclampsia was 0.24±0.03ng/ml which was lower than the mean level of 0.42±0.05ng/ml in the normotensive comparison group (P<0.001).

As shown in Table 3, after conducting one-way analysis of variance, there was a statistically significant difference in the serum relaxin levels across the participant groups (normotensive vs mild preeclampsia vs severe preeclampsia, 0.42±0.05ng/ml vs 0.24±0.02ng/ml vs 0.23±0.01ng/ml, P<0.001).

The post hoc Bonferroni test showed that there was a statistically significant difference in the mean serum relaxin levels between women with mild preeclampsia and normotensive women (0.24±0.02ng/ml vs 0.42±0.05ng/ml, P<0.001). Also, there was a statistically significant difference in the mean serum relaxin levels between women with severe preeclampsia and normotensive women (0.23±0.01ng/ml vs 0.42±0.05ng/ml, P<0.001). In contrast, there was no statistically significant difference in the mean serum relaxin levels between women with severe preeclampsia and those with mild preeclampsia (0.23±0.01ng/ml vs 0.24±0.02ng/ml, P<0.001) (Table 4).

Discussion

This study aimed to determine and compare the levels of serum relaxin in pregnant women with or without preeclampsia between 20-41weeks gestation, to document the effect of relaxin on women with preeclampsia and its severity **(**This study was about the levels of relaxin in women with preeclampsia compared to normotensive pregnant women. It was not about the effect of relaxin on women with preeclampsia and its severity.).

The mean serum relaxin level among preeclamptic participants was significantly lower than that in the normotensive participants. This is in agreement with the finding of Emiel et al,[9] who observed that the serum relaxin level in women with preeclampsia was lower than in the normotensive pregnant women The finding of this study is different from that of Lafayette et al who found no significant difference in mean serum relaxin levels between preeclamptic and normotensive participants.[11] The difference in this finding may be that their study used small sample size comprising mainly of Caucasians compared to this current study with a relatively larger sample size conducted in a black population. However, both studies share a similarity of recruiting their study participants in the third trimester.

Bramham et al in their study also tried to find out if relaxin will have an important physiological role in renal adaptation in pregnant women with chronic kidney disease or chronic hypertension or (both).[15] It was found that women without superimposed pre-eclampsia had higher relaxin concentrations than did healthy controls, but there were no differences between women with chronic kidney disease or chronic hypertension (or both) with and without superimposed pre-eclampsia[15]

Further subgroup analysis revealed that there

Table 1. Socio-demographic and clinical characteristics of preeclamptics and normotensive pregnant women.

CHARACTERISTICS	PREECLAMPSIA N=90	NORMOTENSIVE N=90	TOTAL (%)	STATISTICS
Maternal Age (years)	**Frequency(%)**	**Frequency(%)**		**P-value**
20-24	11(12.2)	13(14.4)	24(13.3)	**0.987**[*]
25-29	31(34.4)	29(32.2)	60(33.3)	
30-34	29(32.2)	30(33.3)	59(32.8)	
≥35	19(21.1)	18(20.0)	37(20.6)	
Mean Age ±SD	37±2.32	37±2.87		0.86[**]
Parity (median, IQR)	0(0-1)	1(0-2)		0.369****
0-2	33(36.7)	41(45.6)	74(41.1)	0.394[***]
≥ 2	57(63.3)	49(54.4)	106(58.9)	
Mean Gestational age ± SD	36±2.32	37±2.87		0.78[**]
Educational Qualification				
No formal education	17(18.9)	10(11.1)	27(15.0)	0.053*
Primary	6(6.7)	4(4.4)	10(5.6)	
Secondary	26(28.9)	16(17.8)	42(23.3)	
Tertiary	41(45.6)	60(66.7)	101(56.1)	
Occupational status				
Professionals	43(47.8)	62(68.9)	105(58.3)	0.016[*]
Skilled non-professional	27(30.0)	17(18.9)	44(24.4)	
Unskilled	20(22.2)	11(12.2)	31(17.3)	
Systolic Blood pressure(mmHg) (mean ± SD)	168.2 ± 20.1	120.5 ± 12.2		< 0.001*
Diastolic Blood pressure(mmHg) (mean ± SD)	107 ± 15.5	73.56± 8.3		< 0.001[**]
Mode of Delivery				
Caesarean section	58(64.4)	23(25.6)	81(45.0)	0.001
Spontaneous vaginal delivery	32(35.6)	67(74.4)	99(55.0)	
Booking status				
Booked	31(34.4)	82(91.1)	113(62.8)	0.001[*]
Unbooked	59(65.6)	8(8.9)	67(37.2)	

**Student's t-test; ***Fischer's test; *Pearson's Chi-square, ****Mann Whitney U

was a statistically significant association between normotensive participants and participants with mild as well as severe preeclampsia, as serum relaxin level decreased across each group. However, no significant difference between participants with mild and those with severe preeclampsia was found, indicating that serum relaxin is not affected by the severity of preeclampsia. This is similar to the previous study by Emiel et al which also showed no significant difference in serum relaxin

TABLE 2. Comparison of serum relaxin levels in participants with preeclampsia and normotensive pregnant women.

ANALYTE	NORMOTENSIVE PARTICIPANTS N=90	PREECLAMPTIC PARTICIPANTS N=90	T- STATISTICS	P-VALUE
Serum relaxin (mean ± SD) ng/ ml	0.42±0.05ng/ml	0.24±0.03ng/ml	0.376	<0.001#*

Student T-test,

Table 3. One-way analysis of variance in mean serum relaxin across the study groups.

	NORMOTENSIVE N=(90)	MILD PREECLAMPSIA N= (48)	SEVERE PREECLAMPSIA N= (42)	P-VALUE
Serum Relaxin (mean ± SD) ng/ml	0.42 ± 0.05	0.24 ± 0.02	0.23 ± 0.01	0.001£*

ANOVA£*

Table 4. Post-hoc Bonferroni pair-wise comparison of serum relaxin levels among the three groups

PREECLAMPSIA STATUS	NORMOTENSIVE (N=90)	MILD PREECLAMPSIA (N=48)
Mild (N=48)	< 0.001*$	
Severe (N=42)	< 0.001*$	0.271$

levels between the participants who had mild preeclampsia and those with severe preeclampsia in their study, with serum relaxin level of 715 pmol/L (IQR 414–715) in those with severe preeclampsia and 1176 pmol/L (IQR 766-1552) in those with mild preeclampsia.[9] Our study also differs from the case control study involving 51 women with preeclampsia by Rehfeldt et al, which found no significant difference in the serum relaxin levels in women with preeclampsia. The finding of a low serum relaxin levels in preeclamptic women suggests that serum relaxin level may play a role in the identification, but, not in the categorization of preeclampsia; it may not be a marker of severity in preeclampsia.[10]

The clinical implication of this finding may be that relaxin if done routinely in high risk pregnant women possibly across trimesters may be used to identify women at risk of developing the disease hence the need to institute appropriate measures in its prevention.

Study limitations

This is a hospital-based study, therefore, the finding may not be a true representation of the general obstetric population in Lagos

We were limited also by the fact that only a single measurement of serum relaxin was available for each woman rendering us unable to examine inter-individual differences in the analytes variation during each trimester of pregnancy.

Strength of study

This is an analytical cross sectional study that involved a relatively large sample size when compared with other similar studies.

All samples were analysed at the same time by a senior laboratory scientist at the same laboratory. The quality control was ensured throughout this study.

Recommendation

The finding of low level of mean serum relaxin in preeclamptic participants suggests that serum relaxin may have a role in aetiopathogenesis of preeclampsia, this will require further evaluation for possible therapeutic role of relaxin in preeclampsia.

There is also need for further robust multicenter longitudinal study involving larger population of preeclamptic women to explore further on the cause-effect relationship of relaxin and preeclampsia, following which possible interventional study may be done using relaxin to evaluate its therapeutic effect in preeclampsia.

Conclusion

Women with pre-eclampsia have significantly lower levels of serum relaxin than normotensive pregnant women. However, there is no significant difference in mean serum relaxin levels in women with mild when compared to those with severe pre-eclampsia. Therefore, development of pre-eclampsia is associated with lower serum levels of relaxin, though the relaxin levels may not be indicative of the severity of the disease.

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Disclosure

None declared.

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