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Serum endocan level as a predictor of chorioamnionitis among pregnant women with preterm premature rupture of fetal membranes at Aminu Kano Teaching Hospital, Nigeria

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Abstract

Chorioamnionitis is associated with maternal and perinatal morbidity and mortality. Inflammatory biomarkers such as endocan are said to be increased in chorioamnionitis. This study aimed to determine the predictive value of serum endocan as a biomarker for chorioamnionitis among pregnant women. A prospective longitudinal study conducted in Aminu Kano Teaching Hospital. Ninety-five pregnant women with Preterm Premature Rupture of Fetal Membranes (PPROM) were recruited for the study. The serum endocan level of each participant was assessed on admission and analysed. The women were followed up to delivery and monitored for clinical signs of chorioamnionitis. At delivery, another sample was taken to assess the serum endocan level. Data was collected and analysed using the Statistical Package for Social Sciences (SPSS) version 23. Data were expressed as frequencies and percentages for qualitative variables. The quantitative variables underwent normality testing using the Kolmogorov-Smirnov test. Student T test was used to compare continuous variables, and Chi-square test/Fisher's exact test was used to compare categorical variables, and $p<0.05$ was considered statistically significant. The incidence of histological chorioamnionitis in pregnant women with PPROM in AKTH was found to be 38%. The median serum endocan levels were found to be higher in pregnant women with PPROM and histological chorioamnionitis than those with PPROM only, but there was no statistically significant difference ($p=0.582$). The findings have shown that serum endocan is not a good biomarker for detecting chorioamnionitis in pregnant women with PPROM.

Key words: PPROM, chorioamnionitis, inflammatory biomarkers, serum endocan.

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Introduction

Preterm Premature Rupture of Membranes (PPROM) is associated with significant perinatal morbidity and mortality.[1-4] It contributes remarkably to maternal, perinatal and neonatal morbidities and mortalities in both high and low-income countries.[4,5]

The exact etiology of PPROM is poorly understood, but there are several risk factors, such as uterine overdistention, prenatal invasive procedures, among others.[5,6]

Chorioamnionitis, the commonest complication of PPROM, is defined as an acute inflammation of amniotic membranes, most often of bacterial origin.[7,8] Studies done in Abuja and Enugu, Nigeria, reported chorioamnionitis occurring in 12.5% and 8.3% of pregnant women with PPROM, respectively.[2,5] Chorioamnionitis can be suspected clinically based on premature rupture of membranes occurring 12 hours prior to delivery, fever, uterine tenderness, and fetal tachycardia.[8] However, it may not manifest early clinically as the clinical signs have low predictive probability.[9] Definitive diagnosis of chorioamnionitis can only be made by amniocentesis, culturing the amniotic fluid and histological diagnosis of the placenta following delivery.[10,11]

Several inflammatory markers and acute phase reactants have been studied in PPROM; however, there is no ideal marker that has been established clinically to predict or detect chorioamnionitis before delivery.[2,6,12] Traditionally, inflammatory biomarkers have been measured in amniotic fluid from amniocentesis, which is invasive and not always available in non-specialist centres.[3] Maternal serum also offers a sample for measuring inflammatory markers, which is easier, more accessible and non-invasive to the fetus.[13,14]

Endocan, also called Endothelial Specific Molecule-1 (ESM-1), is a proteoglycan in the circulation that has been considered to be a new endothelial biomarker.[15] It is secreted by the vascular endothelium into the bloodstream in healthy individuals, and its amount is said to be increased in inflammation.[16] Hence, studies have shown endocan to be a novel marker for inflammation as well as a prognostic factor in some disease conditions associated with endothelial dysfunction.[6,12,15-25]

Two studies done in Turkey reported elevated serum levels of endocan in PPROM, however, a USA study reported no change in levels of endocan compared to normal healthy pregnancy [6,12,15]

This study aims to determine the predictive value of serum

endocan as a biomarker for chorioamnionitis among pregnant women with PPROM at Aminu Kano Teaching Hospital (AKTH).

Materials and Methods

This is a prospective longitudinal study conducted at the Aminu Kanu Teaching Hospital (AKTH), a tertiary hospital situated in Kano state, Northern Nigeria. The study participants were recruited among pregnant women with PPROM who fulfilled the inclusion criteria. Pregnant women who presented at a gestational age between 28-36 weeks with PPROM confirmed by leakage of liquor from the cervix during sterile speculum examination were selected for the study. The detail of the study was explained to the participants, and written informed consent was obtained.

Each participant was admitted into the antenatal ward, and blood samples for serum endocan were taken. Conservative management was instituted, and the patients were commenced on erythromycin; those with gestational age less than 34 weeks received two doses of 12 mg dexamethasone 12 hours apart. They were all monitored for clinical signs of infection, fever, uterine tenderness and change in colour from perineal pad or purulent vaginal discharge and fetal tachycardia. This was done at least dwith the help of the nurses on duty. The conservative management was stopped if any clinical evidence of infection was observed or the appropriate gestational age was reached (37 weeks). Another blood sample for serum endocan level was taken at delivery and analysed. Immediately following delivery, the placenta of the participants was thoroughly examined by the researcher and research assistants, and samples were taken from suspicious sites for histology within 30 minutes of delivery. The samples were immersed in 10% formalin and taken to the histopathological laboratory for standard examination. The neonatal parameters, such as APGAR score, temperature, development of jaundice, SCBU admission, number of days spent were all recorded.

Sample size determination and sampling approach

The minimum sample size (n) was calculated using the formula for calculating sample size for sensitivity and specificity;

$$n = \frac{P(1-P)Z^2_{1-\alpha/2}}{d^2}$$ [35]

n = minimum sample size required in each group

P = Prevalence of chorioamnionitis from a previous study =12.5% =0.125^2

$Z^2_{1-\alpha/2}$ = Standard normal deviation which corresponds to 5% level of significance =1.96% (obtained from normal distribution table)

d = margin of error =0.05

$$n = \frac{0.125\,(1-0.125) \times 1.96}{0.05^2}$$

$$n = \frac{0.125 \times 0.875 \times 1.96}{0.0025}$$

$$n = \frac{0.214}{0.0025}$$

$$n = 85.75$$

Sample size calculated =86

Using a 10% attrition rate calculated sample size is 94.5=95

Data collection

Data was collected using a well-structured proforma from the participants following obtaining written informed consent.

Sampling technique

Convenience sampling technique was used to recruit each consecutive patient who met the inclusion criteria until the sample size is attained.

Ethical consideration

Ethical approval was obtained from the ethical committee of AKTH according to the latest principles of the Helsinki declaration. Informed written consent was obtained from the participants after details of the study have been explained to them.

Statistical analysis

The data collected was analysed using Statistical Package for Social Sciences (SPSS) software version 23. Data was expressed as frequencies and percentages for qualitative variables, while the quantitative variables underwent normality testing using the Kolmogorov-Smirnov test. Student T test was used to compare continuous variables, and Chi square test/Fisher's exact test was used to compare categorical variables, and $p<0.05$ was considered statistically significant. A receiver operator curve was carried out to determine a cut-off value for maternal serum endocan levels predicting Chorioamnionitis. Sensitivity, specificity, positive predictive value, and negative predictive value were also determined.

Results

A total of 95 pregnant women with PPROM who met the inclusion criteria were recruited for the study over a period of 9 months, from November 2022 to July 2023. However, 9 pregnant women were lost to follow up, giving a follow up rate of 90.53%. Only the 87 participants were included in the analysis.

The mean age of the participants who had PPROM only was 30.4 years (±6.4), and the mean age for those with PPROM complicated by chorioamnionitis was 26.2 years (±6.1). The mean age of the participants with PPROM only was significantly higher than the mean age of the participants with PPROM complicated by chorioamnionitis (p=0.03).

The PPROM only group had the majority of its participants to be multigravidae (75.9%), while less than half of the participants with chorioanmionitis were multigravidae (45.5%). Most of the participants in both groups had gestational age ranging from 33 to less than 37 weeks, with 70.4% in participants with PPROM only while 84.4% in those with PPROM with histological chorioamnionitis (Table 1).

The incidence of histological chorioamnionitis was found to be 38%. Table 2 shows that a total of 8 patients had clinical chorioamnionitis (9.30%) and only 4 of them had histological chorioamnionitis (12.1%). The participants with PPROM with histological chorioamnionitis had significantly longer duration from rupture of

membranes to admission than those with PPROM only (p=0.018). The latency period in the participants with PPROM only was found to be significantly longer than those with PPROM complicated by chorioamnionitis (p=0.026).

The modes of delivery in both groups were mostly vaginal delivery (68.9% and 75.8% in PPROM only and PPROM complicated by chorioamnionitis, respectively).

Table 3 revealed the neonatal outcome of the participants, and it shows no statistically significant differences in the outcome between the two groups. Table 4 shows the median serum endocan level at presentation in the participants with PPROM only to be 61.9pg/ml, with a first quartile value of 39.3pg/ml, and a third quartile value of 99.1pg/ml while the median serum endocan level was found to be 72.2pg/ml in the participants with PPROM complicated by chorioamnionitis with the first quartile value of 48.7pg/ml and a third quartile value of 107.4pg/ml. Prior to delivery, the median serum endocan level in the participants with PPROM only to be 62.6pg/ml, with a first quartile value of 49.6pg/ml, and a third quartile value of 91.7pg/ml while the median serum endocan level was found to be 75.1pg/ml in the participants with PPROM complicated by chorioamnionitis with the first quartile value of 49.7pg/ml and a third quartile value of 106.4pg/ml. The median serum endocan level was found to be higher in the participants with PPROM complicated by chorioam-

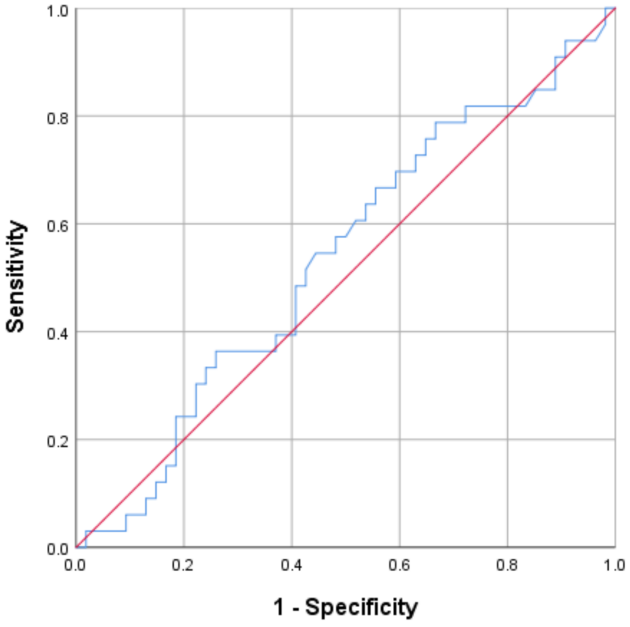

Figure 1. Rotator Operator Curve (ROC).

Table 1. Sociodemographic and reproductive characteristics of the study participants.

Maternal characteristics	Without chorioamnionitis (n=54) n(%)	With chorioamnionitis (n=33) n(%)	Test statistic	p
Age (yrs)			T test	0.003#
≤20	1 (1.9)	6 (18.2)		
21-30	26 (48.1)	21 (63.6)		
>30	27 (50.0)	6 (18.2)		
Mean (SD)	30.4±6.4	26.2±6.1		
Tribe			χ^2=3.204*	0.557
Hausa	42 (77.8)	29 (87.8)		
Yoruba	6 (11.1)	1 (3.0)		
Igbo	3 (5.6)	1 (3.0)		
Others	3 (5.6)	2 (6.1)		
Religion			Fisher's exact	0.144
Islam	46 (85.2)	32 (97.0)		
Christianity	8 (14.8)	1 (3.0)		
BMI			T test	0.907
≤18.5	4 (7.4)	3 (9.1)		
18.5-24.9	28 (51.9)	18 (54.5)		
>25.0	22 (40.7)	12 (36.4)		
Mean ± SD	24.0±4.7	24.2±5.5		
Educational level			χ^2=10.065*	0.006
Primary	0 (0.0)	1 (3.0)		
Secondary	0 (0.0)	1 (3.0)		
Tertiary	19 (35.2)	20 (60.6)		
Others	28 (64.8)	11 (33.3)		
Parity			χ^2=8.292	0.006
Primigravidae	13 (24.1)	18 (54.5)		
Multigravidae	41 (75.9)	15 (45.5)		
Gestational age (weeks)			T test	0.202
28-30	5 (9.3)	2 (6.1)		
31-32	11 (20.4)	3 (9.1)		
33-less than 37	38 (70.4)	28 (84.8)		
Mean ± SD	34.0±2.1	34.5±1.8		

*Chi square not valid; #p value significant at <0.05. Others: Nupe, Igala. SD, Standard Deviation; BMI, Body Mass Index

nionitis but it is not statistically significant (p=0.582).

The Area Under the Rotator Operator Curve (ROC) (AUC) was 0.54 at a cut off value of 71.08pg/ml with a sensitivity of 45.5% and a specificity of 44.4% for the serum endocan level taken at presentation (Figures 1 and 2). The threshold for detecting histological chorioamnionitis in pregnant women with PPROM is 71.08 pg/ml.

Discussion

The incidence of histological chorioamnionitis in pregnant women with PPROM in AKTH was found to be 38%. The median serum endocan levels were found to be higher in pregnant women with PPROM and histological chorioamnionitis than those with PPROM only, but there was no statistically significant difference (p=0.582). The threshold for detecting histological chorioamnionitis was found to be 71.08pg/ml at a sensitivity of 45.45% and specificity of 44.44%.

The mean age of the participants with PPROM only was found to be significantly higher than the mean age of the participants with histological chorioamnionitis (p=0.01). This finding is different

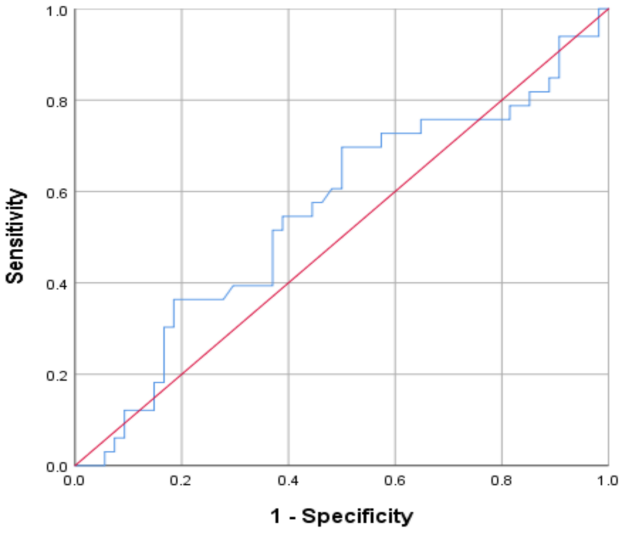

Figure 2. Showing Rotator Operator Curve (ROC) curve of serum endocan level in latent phases of labour.

Table 2. Participants' characteristics since admission.

Characteristics	PPROM without chorioamnionitis (n=54) n (%)	PPROM with chorioamnionitis (n=33) n (%)	Test statistic	p
Clinical chorioamnionitis	4 (7.4)	4 (12.1)	Fisher's exact	0.705
Rupture to admission interval in hours Median (Q1,Q3)	4.0 (2.0,8.0)	6.0 (4.0, 8.0)	U=623.5	0.018*
Latency period in days Median (Q1,Q3)	3.0 (2.0,5.25)	2.0 (2.0, 3.0)	U=642.5	0.026*
Mode of delivery			χ^2=0.444	0.626
SVD	37 (68.5)	25 (75.8)		
CS	17 (31.5)	8 (24.2)		

*p value significant at <0.05. PPROM, Preterm Premature Rupture of Fetal Membranes; SVD, Spontaneous Vaginal Delivery; CS, Caesarean Section.

Table 3. Table showing the neonatal outcome.

Characteristics	PPROM without chorioamnionitis (n=54) n (%)	PPROM with chorioamnionitis (n=33) n (%)	Test statistic	p
APGAR score 1st minute			χ^2=0.099*	1.000
0-3	1 (1.9)	1 (3.0)		
4-7	41 (75.9)	24 (72.7)		
8-10	12 (22.2)	8 (24.2)		
Median (Q1,Q3)	7 (6.8,7.0)	7 (6.0,7.5)	U=884.0	0.947
Temperature			χ^2=0.002	1.000
<35.6	10 (18.5)	6 (18.2)		
35.6- <37.2	44 (81.5)	27 (81.8)		
≥37.2	0 (0.0)	0 (0.0)		
Mean ± SD	35.9±0.5	36.0±0.6	0.654	
Jaundice	10 (18.5)	9 (27.3)	0.920	0.424
SCBU admission	23 (42.6)	13 (39.4)	0.086	0.825
Number of days in SCBU				
Median (Range)	5 (1 to 16)	7.0 (1 to 13)	U=109.5	0.186

PPROM, Preterm Premature Rupture of Fetal Membranes; SD, Standard Deviation; SCBU, Special Care Baby Unit.

from other studies, which also observed higher ages among pregnant women with PPROM only, yet without any statistically significant differences between the two groups.[21,23,26] These differences may be due to differences in methodological approach; most of the studies mentioned were retrospective studies. There was no statistically significant association in the other maternal characteristics such as tribe, religion, educational status, BMI, parity and gestational ages. This is similar to the findings in other studies.[23,26]

The study found that the incidence of histological chorioamnionitis in pregnant women with PPROM to be 37.9% which is similar to 35.6% obtained from a study done in Pennsylvania, USA[21] but lower than a median prevalence of 50% reported in a systemic review and meta-analysis done on maternal inflammatory markers for chorioamnionitis in PPROM.[13] These differences maybe as a result of differences in methodological approaches and sample population. The incidence of clinical chorioamnionitis was found to be 9.3%. This finding is lower than 14.1% obtained in a study done in Calabar.[5] The reduced occurrence of clinical chorioamnionitis could be attributed to the exclusion of patients with prolonged PPROM of more than 24 hours from the study. This stands in contrast to the aforementioned study where a significant number of their participants had prolonged PPROM. However, only 12.1% of all the participants had both clinical and histological diagnosis of chorioamnionitis. A study done in Morocco reported 22% of normal placental tissue to have histological chorioamnionitis and inferred that histological chorioamnionitis may not necessarily be associated with infection, it may be from sterile inflammation from extra placental causes.[8] Another study done in Jos reported histological chorioamnionitis in 60% of participants who did not have any clinical evidence of chorioamnionitis.[23] It has also been argued that non-specific reactive changes from labour may lead to chorioamnionitis.[23,27] Nonetheless, some authors have suggested that histological diagnosis of chorioamnionitis confirms clinically suspicious chorioamnionitis as well as infra clinical chorioamnionitis.[8]

The median duration of rupture of fetal membranes from onset to presentation was found to be higher in pregnant women with PPROM and histological chorioamnionitis than in those with PPROM only, and it was found to be statistically significant (p=0.018). This may be as a result of the pregnant women with longer duration are exposed more to having infections as against those that present early and placed earlier on antibiotics. Hence reduction or prevention of chorioamnionitis. The median latency period was found to be higher in pregnant women with PPROM only and lower in pregnant women with PPROM and histological chorioamnionitis and it was found to be statistically significant (p=0.026). This finding is different from the results obtained in the USA which showed no statistically significant association between latency period in the two groups.[21] This reduction in latency period in the chorioamnionitis group may be as a result of early intervention in the patient with the slightest suspicion of chorioamnionitis.

There was no statistically significant association between mode of delivery and presence of histological chorioamnionitis (p=0.626). This is similar to the findings in Jos, Plateau.[23]

The median serum endocan level at presentation was found to be higher in participants with PPROM complicated by histological chorioamnionitis than in participants with PPROM only, but it was not statistically significant. This is in contrast to several studies done on biomarkers in chorioamnionitis, where the biomarkers were found to be significantly higher in patients with chorioamnionitis.[9] Serum endocan level has been shown by 2 studies done in Turkey to be significantly higher in pregnant women with PPROM than in normal pregnancy.[6,12] Some studies have also been done on serum endocan level as a marker for severity of infection.[23,25] This finding may mean that serum endocan level may be high in patients with PROM and also in patients with infection as a biomarker for inflammation, but may not be used in chorioamnionitis in pregnant women, probably as a result of all the physiological changes of pregnancy. It may also be as a result of differences in race and geographical location, as the 2 studies done on serum endocan level in PPROM and those on serum endocan level as a maker of severity of infection were not conducted in Africa. Similarly, same finding was obtained concerning the median serum endocan level in latent phase of labour, which was also higher in pregnant women with histological diagnosis of chorioamnionitis than in those with PPROM only but not statistically significant.

There was no statistically significant association between the neonatal outcomes in the two groups. This is similar to the study done in Jos, Plateau, which reported no statistically significant association in the neonatal outcome in the pregnant women with PPROM only and those with chorioamnionitis.[23]

The sensitivity and specificity of serum endocan level in detecting chorioamnionitis was found to be both low at 45% and 44.4% respectively. This finding is slightly comparable to the result reported from by Ovayolu *et al.* on serum endocan level in detecting PPROM where they obtained a sensitivity and specificity of 64.5% and 34.1% respectively.[6] The other study done by Iflazoglu also reported similar finding with sensitivity and specificity of 48% and 64% respectively.[12] These findings from the two studies done in Turkey also obtained low sensitivity and specificity of serum endocan level in detecting PPROM. This is in contrast to several studies done on biomarkers detecting chorioamnionitis in pregnant women with PPROM. A systemic review and meta-analysis of diagnostic test accuracy studies on detecting chorioamnionitis in pregnant women with PPROM also reported low sensitivity and specificity of the biomarkers.[13] In contrast to these findings, a study done in USA reported C-reactive protein to be 76.9% sensitive and 31.9% specific for detecting chorioamnionitis.[21] Another study done in China on predicting chorioamnionitis in patients with PPROM using 5 inflammatory biomarkers (WBC, CRP, IL-6, PCT and neutrophil count) reported that a combination of two biomarkers (C reactive protein and WBC count) had the best predictive value with a sensitivity and specificity of 60.22% and 76.11% respectively.[26] A study done in Nigeria reported chorioquick which

Table 4. Table showing serum endocan level in both groups.

Characteristics	PPROM without chorioamnionitis (n=54)	PPROM with chorioamnionitis (n=33)	p
At presentation Median (Q1,Q3)	61.9 (39.3, 99.1)	72.2 (48.7, 107.4)	0.582
Prior to delivery Median (Q1,Q3)	62.6 (49.6, 91.7)	75.1 (49.7, 106.4)	0.391

PPROM, Preterm Premature Rupture of Fetal Membranes.

contained IL-6 to have 7.5% sensitivity and 87.9% specificity in detecting chorioamnionitis in pregnant women with PROM.[9] These dissimilarities may be as a result of differences in the type of inflammatory biomarkers used in different studies.

Conclusions

The findings from this study have shown that serum endocan levels in pregnant women were not significantly higher in pregnant women with PPROM with histological chorioamnionitis therefore, it is not suitable as an effective biomarker for detecting chorioamnionitis in pregnant women with PPROM.

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Informed consent: informed written consent was obtained from the participants after details of the study had been explained to them.

Patient's consent for publication: the patients gave their written consent to use their personal data for the publication of this case report and any accompanying images.

Availability of data and materials: all data generated or analyzed during this study are included in this published article.