Measurement of the Maternal Serum 25 - Hydroxyvitamin D, Neutrophil - to - Lymphocyte Ratio and Paraoxonase 1 Levels in the Prediction of Spontaneous Preterm Birth

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Abstract

Background: The main factor contributing to perinatal morbidity and mortality worldwide is preterm birth. Studies have linked a number of etiological factors, including vitamin D, oxidative stress (paraoxonase 1), and inflammatory markers, to the development of spontaneous preterm labour. O**bjective**: To investigate the value of maternal serum25hydroxy vitamin D, paraoxonase 1, and neutrophil to lymphocyte ratio in spontaneous preterm birth as markers of different pathogenesis pathways and to determine the correlation among them. **Patients and Methods:** Prospective Case-control study in AL- Yarmouk Teaching Hospital/ Baghdad during the period (January- October) 2021, was performed on pregnant women referred to the hospital, 45 pregnant women who delivered spontaneous preterm labor were regarded as a case group and another 45 pregnant women without signs and symptoms of preterm labour with comparable gestational age who continued pregnancy till term regarded as a control group, maternal serum level of 25-hydroxyvitamin D, serum paraoxonase1 and neutrophil to lymphocyte ratio was measured in both groups. **Result:** The level of 25 –hydroxyvitamin D and paraoxonase 1 level were significantly lower in case group women. p-value <0.05. The result showed that more preterm women had severe vitamin D deficiency <10 ng/ml. In the case group, a non-significant negative moderate correlation was found between vitamin D and paraoxonase1, and a non-significant negative very weak correlation was found between neutrophil to lymphocyte and paraoxonase1, regarding the control group there was a non-significant positive weak correlation was found between vitamin D and paraoxonase 1, and non-significant negative very weak correlation was found between neutrophil to lymphocyte and paraoxonase 1. **Conclusion:** The three parameters of three pathways act independently in the pathogenesis of preterm labour. Paraoxonase 1has the highest sensitivity among three variables and can be recommended for screening of preterm labour. The specificity of the three parameters is low suggesting a poor diagnostic performance.

Keywords: 25 -Hydroxyvitamin D, Paraoxonase 1, Neutrophil- to - Lymphocyte Ratio, Prediction, Spontaneous Preterm Birth.

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INTRODUCTION

Preterm birth (PTB) is a significant contributor to neonatal mortality rates worldwide and to long-term health issues for infants who survive. The W.H.O. and the UN both view PTB prevention as essential to enhancing the care provided to expectant mothers and newborn children [(1).] Preterm labour (PTL) is the term used to describe deliveries that occur before 37 full weeks of pregnancy[(2),]less than 259 days have passed since the first day of the last menstrual cycle. [(3)].

The phrase "spontaneous preterm labour" (SPTL) refers to labour that begins without warning, has intact membranes, and results in delivery before 37 full weeks of pregnancy[(4)]. Worldwide, preterm birth is linked to 50% of newborn morbidity and 50% to 75% of neonatal mortality. [(5),]Preterm labour occurs in 5–10% of cases, but a further 5% of labours are complicated by 'threatened' preterm labours that continue to deliver at term[(6),] Although preterm labour accounts for the main cause of perinatal mortality and morbidity, survival rates are improving as neonatal intensive care advances[(7)], However, long-term morbidities, like cerebral palsy, blindness and lung disease, are common in this group of infants[(7)]. Preterm birth rates are increasing in almost all countries with reliable data[(2)], This is associated to assisted reproduction, rising incidence of multiple pregnancies, and a greater propensity for obstetric intervention, especially in the industrialised world [(2).]

Preterm births have surpassed all other causes of death in the globe for children under the age of five [(8).] Of the 6.3

million kids who passed away before the age of five in 2013, 52% perished from an infection, and 44% did so while still in the womb [(2).]

The immune system and inflammation are linked to vitamin D, and many different cell types, including immune cells like antigen-presenting cells, T cells, B cells, and monocytes, have vitamin D receptors [(9).] Preterm birth and vitamin D may be related in this way. Many complications, including premature birth, have reportedly been linked to low maternal serum vitamin D levels. A high neutrophil-to-lymphocyte ratio, which is a reliable indicator of inflammation, is produced when systemic inflammation occurs as a result of an increase in neutrophil count and a decrease in the circulation of lymphocytes. Recent studies found an association between PON1 activity and pro-inflammatory. Due to an imbalance between the generation of reactive oxygen species and the body's ability to detoxify them, pregnancy is a physiological situation that is highly susceptible to oxidative stress. Preterm birth, intrauterine foetal development retardation, and preeclampsia are just a several of the complications that a high level of oxidative stress during pregnancy may cause[(10)].

Aim of the Study: To investigate the value of maternal serum 25 hydroxyvitamin D, Neutrophil to Lymphocyte ratio and paraoxonase 1, in spontaneous preterm birth as markers of different pathogenetic pathways and to determine the correlation among them.

Patients and Methods: This is a prospective case-control study carried out at the Department of Obstetrics and Gynecology of Al-Yarmouk Teaching Hospital. From (January2021till October 2021, the protocol of the study was approved by the Scientific Council of the Obstetrics and Gynecology Specialization / Iraqi Board for Medical Specializations.

The study included 90 pregnant women who have collected from the inpatient obstetric world and the consultation clinic of AL-Yarmouk Teaching Hospital. Informed verbal consent was obtained from all pregnant women before enrollment in the study.

The participating pregnant women were divided into two groups:

Group A: Includes 45 pregnant women presented with spontaneous preterm labor at gestational age from 24 to 36 weeks plus 6 days, whether delivered by cesarean section or by vaginal delivery, this group was considered as the case group, **Group B:** Includes 45 pregnant women of comparable gestational age without signs and symptoms of labour, who completed their pregnancy till term. This group was considered a control group.

Gestational age was calculated based on reliable last menstrual period (LMP) and confirmed by ultrasonography done early in pregnancy.

Inclusion Criteria: Maternal age 15-44 years, Single viable pregnancy, Gestational age 24-36^{+6}.

Clinical assessment

1. A detailed history was taken from all participants including name, age, and history of present illness, obstetrical, gynecological, medical, surgical, and social history.
2. General and systemic examination including vital signs.
3. Abdominal examination for symphysis fundal high, fetal lie, presentation, fetal heart rate assessment, uterine contractions, and estimation of fetal weight.
4. Speculum and vaginal examinations were done looking for cervical dilatation and amniotic fluid membrane (intact or ruptured).
5. Investigations: all women involved in the study had been sent for the following investigations: Complete blood count, blood sugar level, midstream urine serum vitamin D, and serum paraoxonase 1.

Sample collection and preparation: Five milliliters of venous blood were collected by venipuncture from each woman who participated in this study placed into two tubes, the first one is ethylenediaminetetraacetic-acid (EDTA) for assay of complete blood count (CBC) and blood sugar level, the second is a gel tube for serum vitamin D and serum paraoxonase 1 level assessment. The gel tube was centrifuged for 15 minutes at 1000, upper serum was collected carefully then divided into two Eppendorf tubes, Each tube was labeled with the patient's name and then stored at _20°C. Avoided repeated free thaw cycles until sent to a laboratory.

Maternal Neutrophil to Lymphocyte Ratio (NLR): Determined with the same hematology analyzer (Norma icon 5, Hungary) that was used for the calculation of the neutrophil-to-lymphocyte ratio (NLR).

Measurement of Vitamin D: by ELISA kits according to the manufacturer's instruction using Cobas e 411 analyzers devise from HITACHI (Roche diagnostic GmbH) Which is a fully automatic analyzer that used a patent electrochemiluminescence technology for immunoassay.

Vitamin D deficiency was defined as less than 30 ng/ml. Obtained results of serum vitamin D levels were divided into 4 categories. Severe vitamin D deficiency (< 10 ng/ml), moderate deficiency (10–20 ng/ml), suboptimal vitamin D level (20–30 ng/ml), and the level over 30 ng/ml was considered normal[(10).]

Measurement of Serum paraoxonase 1 levels: by Sandwich ELISA Kit (Shanghai Yehua), optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the

concentration of Human PON1. Can calculate the concentration of Human PON1 in the samples by comparing the OD of the samples to the standard curve.

Statistical Analysis: The data were analyzed with the aid of SPSS (Statistical Package for Social Sciences) version 24.

An AUC of 0.5 suggests no discrimination (ability to diagnose patients with and without the disease), 0.7-0.8considered acceptable, 0.8-0.9 is considered excellent, and more than 0.9 is considered outstanding, the p-value of less than 0.05 was considered to be significant. Correlation coefficient r measures the strength and the direction of a linear relationship between two variables on scatterplots. The value of r is between +1 and -1. There are three types of value: Positive correlation: when two variables change in the same direction, Negative correlation: when two variables change in opposite directions, and Zero correlation: no relationship between the variable.

RESULTS

Table (1) describes the demographic characteristics of the study groups; the control group ladies were older than the case group. Gestational age (G.A.) of cases and controls represented the G.A. at the time of taking samples; those participants of the control group had non-significantly higher gravidity & parity.

Table (1): Demographic characteristics of the study groups

Variables	study group (No.=45)	Control group (No.=45)	p-value
	Mean± S.D		
Maternal age (yrs.)	23.12± 5.19	29.08± 2.28	0.001
G.A. (wks.)	32.77± 2.29	33.6± 1.13	0.87
Gravidity	2.88± 1.88	3.29± 1.9	0.449
Parity	1.81± 1.65	2± 1.59	0.677

`Table 2 and illustrate that the control group of women had a significantly higher level of Vitamin-D (ng/ml) & Paraoxonase-1(ng/ml), while the case group had a non-significantly higher level of NLR.

Table (2): Comparison between the study groups in different variables

Variables	Case group N=45 Mean± S.D.	Control group N=45 Mean± S.D.	p-value
Vitamin-D (ng/ml)	10.86± 4.75	17.61± 9.88	0.033
NLR	5.07± 3.39	4.06± 2.31	0.23
Paraoxonase-1(ng/ml)	8.58± 5.21	13.26± 9.27	0.011

Figure 3 shows that more of the case group ladies had significantly severe vitamin D deficiency (<10 ng/ml) & moderate deficiency (10-20ng/ml) than the control group ladies and more control group ladies had significantly higher vitamin D levels including (21-30 ng/ml) & >30ng/ml.

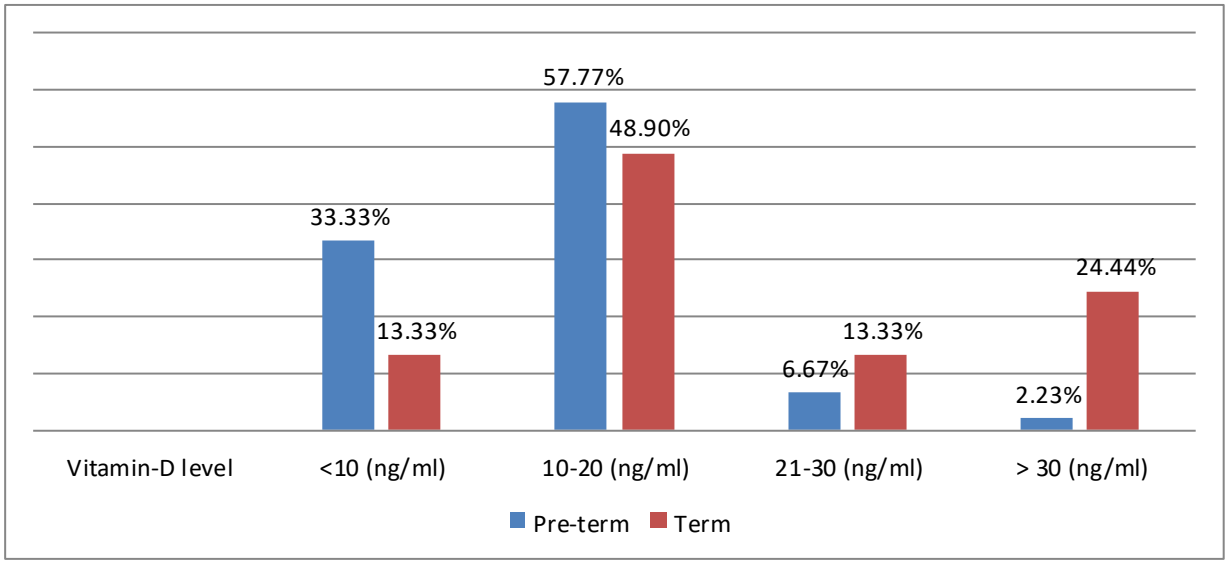

Fig. 3: Comparison between the study groups in vitamin D levels

Table (3): shows the correlation among different biochemical and Hematological markers of the case group; where a non-significant negative moderate correlation was found between Vitamin D &NLR, a significant positive weak correlation was found between Vitamin D & Paraoxonase-1and a non-significant negative weak correlation was found between NLR & Paraoxonase-1.

Table (3): Correlation among different biochemical and Hematological markers of the case group

Variables	Pearson (*r*) correlation	P-value
Vitamin D &NLR	-0.421	0.245
Vitamin D & Paraoxonase-1	0.193	0.034
NLR & Paraoxonase-1	-0.112	0.581

Table (4): shows the correlation among different biochemical and Haemotological markers of the control group; where a non-significant negative weak correlation was found between Vitamin D &NLR, a non-significant positive weak correlation was found between Vitamin D & Paraoxonase-1 and a non-significant negative weak correlation was found between NLR & Paraoxonase-1.

Table (4): Correlation among different biochemical and Haemotological markers of the control group

Variables	Pearson (*r*) correlation	P-value
Vitamin D &NLR	-0.21	0.674
Vitamin D & Paraoxonase-1	0.20	0.428
NLR & Paraoxonase-1	-0.138	0.520

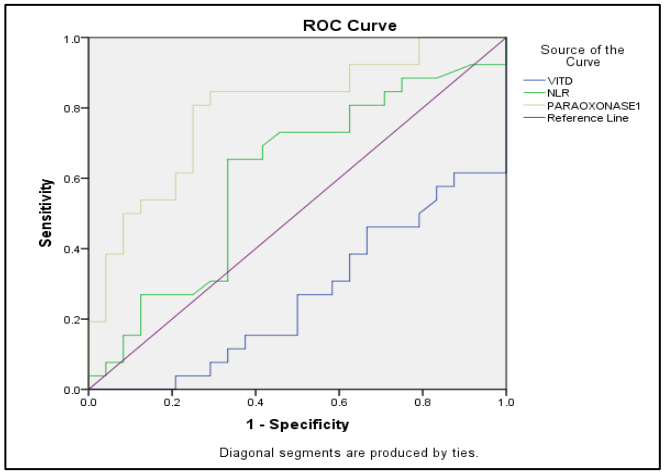

Figure (4): ROC curves showing the diagnostic criteria of the three biochemical and Hematological markers

Figure. (4) shows the ROC curves for the Diagnostic criteria of the three biochemical and Hematological markers as the paraoxonase-1 was with the greatest AUC (Area Under Curve), followed by NLR, and then Vitamin D.

Table (5) displays the diagnostic criteria of these biochemical and hematological and Hematological markers, paraoxonase-1 was with the highest sensitivity to predict significantly pre-term delivery than the other two markers.

Table (5): Diagnostic criteria of the biochemical and Hematological markers

Variabl	Cut	AU	Sensit	Specif	P	N	P-

es	-off poi nts	C%	ivity	icity	P V	P V	val ue
Vitamin D	8.3 (ng/ ml)	26%	61%	52%	58 %	25 %	0.0 04
NLR	3.95	60%	63%	67%	65 %	64 %	0.2 07
Paraox onase-1	7.18 (ng/ ml)	79%	84%	55%	65 %	77 %	<0. 001

DISCUSSION

Preterm birth is the most important problem in modern obstetrics PTB risk factors are complex, and its prevention is a worldwide health problem [(10).] Prematurity prevalence varies from 5% in certain high-income nations to more than 20% in some low-income countries, despite current attempts by primary, secondary, and tertiary treatments to reduce PTB rates. **(11)**.

Regarding the level of 25 hydroxyvitamin D, the current study revealed that ladies in the case group had a lower concentration of vitamin D compared to the control group with a p-value of 0.033, Many studies have shown the association between vitamin D levels and adverse pregnancy outcomes counting to spontaneous preterm birth**(12)**.

These results agreed by several studies, like one done by Milene Saori Kassai et al, at the Municipal Hospital in Brazil revealed that women who delivered at preterm gestation and their preterm newborns had lower serum 25(OH)D concentrations in comparison to women who delivered at the full term **(14)**.

In a study carried out by Wagner CL et al, they measured the strength of the relationship between serum 25(OH) D concentrations and preterm birth at 3-time points during pregnancy: baseline below16 weeks, from 16 to 26, and at more than 27 weeks. It was hypothesized that the 25(OH) D value near delivery was significantly correlated with preterm birth, Women who had serum concentrations below 20ng/mL had 3.3 times of odds of preterm birth compared to those with serum concentrations equal or above 40ng/mL. Also, serum vitamin D level of 40ng/mL in the third trimester was correlated with a 47% reduction in preterm births**(9)**.

While in a prospective cohort study done by Lixia Yang, et al China, in 2016 which involved two hundred participated pregnant women were divided into preterm and term groups regardless of age difference, smoking, drinking, education level, body mass index, serum vitamin D level calculated in both groups reveal that no significant difference of vitamin D levels across different stages of pregnancy**(14)**.

About paraoxonase -1 the present study found a significantly low level with a p-value of 0.011 among case group ladies when compared to the term group which is similar to a study done by Ozlem Bozoklu Akkar et al, in Sivas state hospital / Turkey (2016) which investigate the association of maternal serum 25-hydroxyvitamin D, paraoxonase 1 and neutrophil-to-lymphocyte ratio in women having early spontaneous preterm birth the study found decreased maternal serum 25-hydroxyvitamin D and paraoxonase 1 level in preterm group women **(12)**.

In the current study, the level of NLR was non significantly higher in a preterm group with a p-value of 0.23, While in research done by Yoksel Korban et al, in Turkey which included 138 pregnant women were divided into 3 groups: early preterm, late preterm, and control, NLR found to be significantly higher in a preterm group compared to control **(15)**.

The present study showed that paraoxonase-1 was with the highest sensitivity to predict significantly pre-term delivery than the other two markers. The sensitivity of the maternal serum vitamin D to predict case birth was 61%, of PON-1 was 84%, while NLR was 63%, along with the specificity of 52% for vitamin D, and 55% for PONS-1 & 67% for NLR, in comparison to the present findings; a retrospective cohort study was done by Liong S, et al in Australia for the prediction of preterm delivery using low maternal serum levels of vitamin D as a predictor; which provided a 66.7% sensitivity, 100% specificity, 100% positive predictive value, and 96.7% negative predictive value**(16)**.

CONCLUSIONS

- The three parameters of three pathways act independently in the pathogenesis of preterm labour.
- Paraoxonase 1has the highest sensitivity among three variables and can be recommended for screening of preterm labour.
- The specificity of the three parameters is low suggesting poor diagnostic performance.

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