Evaluation of serum Predictive Markers for Placental Inflammatory Response in Preterm delivery

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ABSTRACT:

BACKGROUND:

Placental inflammation represents a crucial pathogenic process responsible for preterm labor& neonatal complications for example low birth weight, premature delivery, cerebral palsy ,sepsis , and neonatal death. Being able to predict preterm labor with histological chorioamnionitis is important. However, there may be an urgent requirement for sensitive and noninvasive markers to predict inflammatory response of Placenta

OBJECTIVE:

To assess the usefulness of inflammatory markers in maternal serum to predict placental inflammation in patients with threatening premature Labor.

PATIENTS AND METHODS:

A prospective study conducted in the department of Obstetrics and Gynecology of Al-Yermook Teaching Hospital for a period of one year from 1st of May 2015 to the 30th of April 2016 The study included 74 pregnant women presented with preterm labour or preterm prelabour rupture of membrane. Measurement of differential counts of leukocyte, C-reactive protein in maternal serum and histological examination of placenta post delivery done to identify placental inflammatory status. Comparison of neutrophil to lymphocyte ratio is done in patients with no inflammation of placenta and those with placental inflammatory response

RESULTS:

The level of neutrophil to lymphocyte ratio in patients with inflammatory response in the mother and/ or the fetus (8.3±5.4), (11.8±9.2) respectively were significantly higher than those with no placental inflammation (5.2±3.1). It showed higher Predictive accuracy; with 77.9% specificity, 71.4% sensitivity 81.5% positive predictive value, and 65.8% negative predictive value for prediction of PIR. Regarding CRP the results show significant relationships among women with no PIR and those with MIR alone or MIR with FIR (P values = 0.011, 0.005, 0.003) respectively. Also revealed that women with inflammatory response in the mother or both mother and fetus who had high levels of neutrophil to lymphocyte ratio had a shorter admission to delivery interval (mean=2.6 days, 3.2±7.5) than those with no placental inflammation (mean=4.1days).

CONCLUSION:

A placental inflammatory change may be simply and quickly verified at low expense by measuring the NLR .The Maternal blood NLR can be considered a useful, quick, noninvasive prenatal method to predict placental inflammatory response and for diagnosis of HCA in pregnant women presented with preterm labor.

KEYWORDS: preterm delivery, serum inflammatory markers, placental inflammatory response

INTRODUCTION:

Preterm delivery, described as childbirth going on at gestation of less than 37 completed weeks[1] , is a main responsible of mortality and morbidity in neonate.

Kids who are born prematurely have higher rates of respiratory illnesses ,sensory deficits, cerebral palsy and learning disabilities. The morbidity connected with prematurity often extends to a later life, resulting in many physical, psychological and economic costs. Causal factors linked with preterm birth incorporate maternal or fetal medical disease,

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environmental factors, socioeconomic factors, infertility medications, iatrogenic prematurity and roughly 45–50% of cases are idiopathic[2]. Despite the etiology of preterm birth is thought to be multifactorial , the main contributor to the premature delivery is Intrauterine inflammation [3] . Intrauterine infection is the only pathological process for which both a firm causal join with preterm delivery and a direct molecular path physiology characterized the Preterm birth has been found .Intrauterine infection will initiate and activate those biochemical pathways that involves the generation of prostaglandins, cytokines, chemokines, and matrix-degrading proteins. Eventually prompting cervical ripening and uterine contractions [1 ,4,6] .The response of the umbilical cord and placenta to the inflammation, as markers of intrauterine inflammation, is ordered as fetal inflammatory response (FIR)(funisitis) and maternal inflammatory response (MIR). Histological chorioamnionitis (HCA) is viewed as similarly as a marker of inflammation of the placenta in mother [3].Most intrauterine infections are subclinical in nature and ultimately difficult to detect prior labor or membranes rupture[4]. Intrauterine infection is accurately detected by histological examination of the placenta. Histological chorioamnionitis may be frequently asymptomatic, Further more clinical signs and symptoms consisting of tachycardia of mother or fetus, uterine tenderness, fever, and foul amniotic fluid, lack both specificity and sensitivity; and more than one third of patients with these symptoms do no longer have histological prove of chorioamnionitis, as other different reasons may generate comparative clinical signs.

Moreover in over half of cases, intrauterine infections might be happen without microbiological-prove of amniotic fluid infection because of sampling limitations, fastidious organisms and prior antibiotics exposure. Molecular methods for fastidious microorganisms enhance the diagnostic yield, but their sensitivity and specificity are unknown and are not widely available. Histological examination of the placenta and amniotic fluid evaluation are essential tools of detecting intrauterine infection and inflammation. but, an essential obstacle of those strategies is that detection of HCA via histological examination of the placenta may be workable just after delivery similarly; amniocentesis, an invasive method, is unavoidable. Hence, those strategies not appropriate for fast analysis of intrauterine inflammation prenatally, Therefore, many researchers have tried to create a noninvasive, prenatal, and furthermore fast system for detecting intrauterine infection and inflammation. Noninvasive diagnostic methods to evaluate the chance of HCA encompass (estimation of maternal serum CRP levels and leukocyte counts, vaginal or cervical secretions) appear to be more pertinent to clinical practice because of the feasibility and accessibility[3] [4]. Absolute neutrophil count (ANC), C-reactive protein (CRP) and procalcitonin (PCT) are biomarkers that can be used as adjunctive tests in the detection of infection, inflammation, sepsis.[5]

AIM OF STUDY:

To evaluate the usefulness of inflammatory markers in maternal serum as predicter of placental inflammation in women with impending premature delivery

PATIENTS AND METHODS:

A prospective study conducted at AL-Yarmouk Teaching hospital/ Baghdad/ Iraq in the Department of Obstetrics & Gynecology in cooperation with laboratory department (hematology unit, biochemistry unit and histopathology unit) for a period between 1st of May 2015 to 30th of April 2016.

Ethical approval was obtained from the scientific council of Obstetrics & Gynecology Specialization/ Arab Board of Health Specializations.

A study included 74 pregnant women with singleton gestation, premature delivery between 28 and 37 weeks of gestation

Exclusion criteria including :no cervical cerclage , no fetal congenital anomalies, Intrauterine growth restriction nor intrauterine fetal death, no preexisting illness in mother, no history of administration of antenatal Antibiotics, corticosteroid & tocolytics. All examined contributors signed a written knowledgeable consent.

A pre tested, self-administered questionnaire were used to collect the socio demographic data including maternal age, gravidity, parity, past obstetrical, gynecological, medical, surgical history, and administration of antenatal Antibiotics, corticosteroid & tocolytics

At the time of admission, maternal blood was sampled, before use of corticosteroids, antibiotic, or tocolytics, and the samples sent to the laboratory for estimation of concentration of C-reactive protein (CRP)and leukocyte differential counts.

Leukocyte estimation was done by taking 1-2ml of venous blood samples collected into Ethylene Diamine Tetra Acetic Acid (EDTA), shaking the test tubes and placing tubes in automated cell counter The procedure give WBCs count and its differentiation; with the percentage of each type. Neutrophil to Lymphocyte ratio (NLR) was described as the absolute Neutrophil count to absolute Lymphocyte count.

CRP measured by *taking*2ml of venous blood collected in a tube without anticoagulant. Centrifuged clotted blood sample and collected clear serum .If the test could not be performed within 24 hours after the preparation of test samples, they should be immediately frozen below-10degrees, Frozen specimens must be completely thawed, thoroughly mixed, and brought to room temperature prior to testing.

To assess the clinical significance of maternal inflammatory markers as a prognostic marker, we assess the outcome of pregnancy for all the included participants. We described outcome of pregnancy as the admission to delivery time interval.

After delivery pieces of Placenta, membranes and umbilical cord were examined by histopathologist. Placenta from included women was tested fresh. As minimum two segments from the placental discs has been obtained by a non-toothed forceps and scalpel for microscopic examination. One of the disc segments has been obtained halfway between placenta margin and cord insertion. The other was obtained near to the insertion of umbilical cord. Every segment of placenta was measuring 1.5cm in length, 0.5cm in depth and 1cm in breadth including decidual floor, chorionic plate and the center of a placental lobule.

As a minimum one segment of umbilical cord 2 cm from the disc insertion site and a rolled strip of extra-placental membranes has been additionally tested histologically. The biopsy placed in 4% buffered neutral formaldehyde-saline solution. After 12 to 24 hours of primary fixation, blocks have been sectioned and stained with hematoxylin and eosin (H&E). Slides reviewed of the placenta, umbilical cords, and membranes for histological examination to identify Placental inflammatory status and was categorized as:

Fetal inflammatory responses (**FIR**) described as funisitis or chorionic plate vasculitis.

Maternal inflammatory responses **(MIR)** described as deciduitis ,chorioamnionitis, or free membranitis without funisitis or chorionic plate vasculitis

The study subjects were categorized into 3 groups in keeping with histopathological outcomes

Group I: Patients without inflammation of placenta, no MIR and no FIR

Group II: Patients with MIR

Group III: Patients with combined MIR and FIR

Statistical analysis

The data analyzed using Statistical Package for Social Sciences (SPSS) version 21.

Categorical data was represented by percentage tables and frequency.

Testing association between categorical variables was done by Pearson's Chi-square test.

The normality of data distribution was tested using Shapiro-Walk test.

The data presented as mean and standard deviation or median and inter- quartile range, according to their distribution.

Analysis of Variances (ANOVA) or Kruskal-Wallis test was used to compare the continuous variables among study groups, according to their distribution.

Receiver Operator (ROC) curve was used to assess the sensitivity and specificity of laboratory parameters and markers for diagnosis of premature labor caused by placental infection.

Multivariate regression model was used to assess risk factors for placental inflammatory response in patients with preterm delivery. P – Value less than 0.05 was considered significant.

RESULTS:

A total **74** pregnant women have been included in this study , and subdivided into three groups based on the presence and absence of Placental inflammatory response, MIR and FIR according to histological examination*:*

Group **I**: patients with no placental inflammation, no **MIR** and no **FIR**.

Group **II***:* patients with **MIR**

Group **III**: patients with combined **MIR** and **FIR**

Fig.1 shows the distribution of Study groups according to the presence and absence of MIR and FIR.

On histological testing of placenta of the study groups, 66.2 %(49/74) had evidence of placental inflammatory response. Among the 49 patients with placental inflammatory response PIR; MIR alone was showed in 43.2% (32/49) and combined MIR and FIR was identified in 23.0% (17/49).

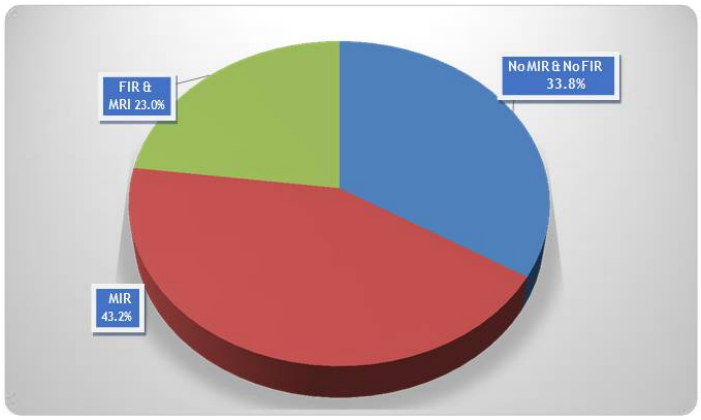

Figure -1 The distribution of Study groups according to the presence or absence of MIR and FIR.

Table 1 : Neutrophil, Lymphocyte, NLR and CRP were shown in table 1..

There were significant differences in the mean lymphocyte counts and mean neutrophil counts among patients with no placental inflammation, MIR alone, and those with MIR and FIR. The mean neutrophil counts in both patients with MIR alone and those with MIR and FIR were significantly higher than those in patients with no any placental inflammation (P value < 0.001). The lymphocyte counts in both patients with MIR alone and those with MIR and FIR were significantly lower than those in patients with no any placental inflammation (P value < 0.001. Because the neutrophil and lymphocyte counts in all PIR groups were significantly different from those of women without PIR, we investigated the diagnostic and prognostic significance of NLR.The NLR was significantly higher in both women with MIR alone and in those with MIR and FIR than in women without any placental inflammation, and differences could be distinguished among all three groups; the NLR in women with FIR was significantly higher than that in women with MIR alone. Regarding to CRP the results show significant relationships among women with no PIR, MIR or FIR and those with MIR alone or MIR with FIR (P values = 0.011, 0.005, 0.003) respectively, while non-significant differences appeared when comparison is done between those with no PIR and women with FIR (P value =0.211).

Table 1: Mean values of Neutrophil, Lymphocyte, NLR and CRP of the study groups

Serum inflammatory markers	Group I (n = 25) Mean± SD	p–value a	Group II (n = 32) Mean± SD	p-value b	Group III (n = 17) Mean± SD	p-value c	p-value d
Neutrophil (cells/ml)	7593.0±3122.0	**<0.001**	10511.5±4399.5	0.298	111523.1±3819.0	**<0.001**	**<0.001**
Lymphocyte (cells/ml)	1632.5±809.1	**<0.001**	1357.5±557.7	0.787	1311.3±610.0	**0.01**	**<0.001**
NLR	5.2±3.1	**<0.001**	8.3±5.4	**0.007**	11.8±9.2	**<0.001**	**<0.001**
CRP	7.2±13.2	**0.011**	16.5±21.5	0.211	22.5±35.7	**0.005**	**0.003**

a: a: Comparison among groups I, II & III.
b: b: comparison between groups I and III;
:c c :Comparison between groups II and III;
Dd : Comparison between groups I and II.

Table 2 : the Usage of ROC curve analysis, show the comparison of the diagnostic indices and the predictive values of leukocyte differential counts, CRP ranges, and the NLR in predicting PIR. The NLR had the highest AUC with a cut-off value of 6.51, and it had a sensitivity of 71.4%, specificity of 77.9%, positive predictive value (PPV) of 81.5%, and negative Predictive value (NPV) of 65.8% as a predictor for PIR. For CRP levels, had small AUC with a cut-off value of 7.38, along with 56.8% sensitivity, 82.9% specificity, 81.7% PPV, and 59.8% NPV.

Table 2: Diagnostic significance of leukocyte differential counts, CRP and NLR in study groups.

	S sensitivity (%)	S specificity (%)	P PPV (%)	NNPV (%)	C cutoff value al
Neutrophil	49.8	84.9	81.6	57.3	11362
Lymphocyte	50.3	33.6	46.8	31.9	1 287
Monocyte	72.2	37.7	69.3	52.1	387
Basophil	14.5	95.5	66.7	44.3	69
Eosinophil	8.4	99.4	88.9	44.3	350
CRP	56.8	82.9	81.7	59.8	7.38
NLR	71.4	77.9	81.5	65.8	6.51

Table 3 Show comparisons among study groups regarding gestational age at time of admission to hospital and sampling (weeks) and gestational age at delivery. Early and moderately preterm births were more common in group II & III while late preterm birth was more common in group I. Comparison among groups regarding time of admission to delivery interval (days revealed that women with PIR had shorter admission to delivery interval than those without PIR.

Table 3: Times of delivery interval

Pregnancy outcome	Group I (n=25)	p -value a	Group II (n=32)	p-value b	Group III (n=17)	p-value c	p-value d
Gestational age at hospitalization(wk) Median (IQR)	32.9 (24.5–36.6)	**<0.001**	30.3 (24.7–36.5)	0.313	29.8 (24.1–36.4)	**<0.001**	**<0.001**
Gestational age at delivery (wk) Median (IQR)	34.0 (25.0–37.2)	**<0.001**	30.5 (25.2–37.0)	0.453	30.3 (24.5–36.9)	**<0.001**	**<0.001**
Early preterm birth (24-27^{+6}wk) No.%	3 (12)	**0.030**	9 (28.1)	0.465	7 (41.2)	**0.014**	**<0.001**
Moderate preterm birth (28-33^{+6}wk) No.%	9 (36)		15 (46.9)		8 (47.1)		
Late preterm birth (34–36^{+6}wk) No.%	13 (52)		8 (25)		2 (11.8)		
Admission to delivery interval (days) Mean ± SD	4.1±9.5	0.195	2.6±6.8	0.791	3.2±7.5	0.561	0.528

S significant values are bold, IQR=inter quartile range,

a: a: Comparison among groups I, II & III.

b: b: comparison between groups I and III.

c: c:comparison between groups II and III;

d: d:Comparison between groups I and II.

DISCUSSION:

Placental inflammation represents a crucial pathogenic process responsible for preterm labor& neonatal complications for example low birth weight, premature delivery, cerebral palsy, sepsis, and neonatal death. Being able to predict preterm labor with histological chorioamnionitis is important. Noninvasive diagnostic technique used to evaluate the hazard and chance of histological chorioaminnitis incorporate estimation of leukocyte counts and c reactive protein levels in maternal serum[3]. HCA has been demonstrated to be linked with elevated blood concentration of inflammatory markers, that is the neutrophil to lymphocyte ratio must be influenced throughout the inflammatory processes of histological chorioaminnitis.3.

The current study revealed that the NLR is elevated on the time of admission in patient with HCA (NLR 8.3±5.4 for women with MIR alone) and (NLR 11.8±9.2 for those MIR with FIR) with (P-value<0.007 and p-value<0.001) respectively; than those with no PIR (low NLR 5.2±3.1).

This result came in agreement with the results of **OzlemBozokluAkkar et al;** which showed that the NLR value(r=0.281) of the preterm group was significantly higher than that of the term group(r=0.27), (p<0.05). [7]. In another cross sectional comparative study done by **UdinSabarudin et al;** they concluded that an average NLR was greater in preterm labor (12.62 ± 6.44) than term labor (5.17 ± 1.64) with significant differences (p<0.001).[(8)] **H.K. DAGLAR et al;** in another prospective controlled study they concluded that the NLR values were significantly higher in those with preterm labor (5.29±2.98) compared to those with term pregnancy (4.77±3.18) ($p < 0.01$).[(9)]

Regarding HCA the current study showed that maternal NLR at the time of admission has extra

diagnostic usefulness with sensitivity (71.4%) &specificity (77.9%) than CRP level with sensitivity & specificity (56.8%), (82.9) respectively as shown in table 2 , this came in agreement with **Min-A Kim et al;** in retrospective study they found that; neutrophil to lymphocyte ratio in ladies with funisitis was significantly higher than in ladies with HCA alone and revealed better predictive accuracy than CRP.[3]. CRP in maternal serum may not be taken into consideration a dependable indicator of either clinical or histological chorioamnionitis according to 55%**Smith EJ et al**; in retrospective study, they found no difference between both groups in CRP levels, so CRP were found to be not effective predictors of clinical or HCA.[10] In review was conducted by **RF Lamont et al;** Three studies (**Fisk et al., Hawrylyshyn et al, and Nowak et al)** concluded that CRP was a useful diagnostic tool for chorioamnionitis so these studies disagree with the current study. The other five studies **(Bankowska et al, Kurki et al, Sereepapong et al,Yoon et al, and Farb et al)** concluded that the overall performance of CRP in the diagnosis of chorioamnionitis was poor, which came in agreement with current study.[11]

This study (table 3) showed significantly shorter delivery time interval from admission among group II &/or group III who had high NLR level (2.6±6.8days, 3.2±7.5days) respectively than group I with normal NLR (4.1days), This came in agreement with a study of **Min-A Kim et al;** on1 who compared the clinical effectiveness of NLR and cervical length to predict preterm delivery. They concluded that combination of markers have better sensitivity (64·2%) and specificity (88·3%) to predict preterm delivery. [12]

CONCLUSION:

A placental inflammatory change may be simply and quickly verified at low expense by measuring the NLR. Maternal blood NLR can be considered a useful, quick, noninvasive prenatal method for diagnosis of histological chorioamnitis in pregnant women presented with preterm labour.

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