RESEARCH ARTICLE

Cord Blood Interleukin-6 as a Predictor of Early Onset Sepsis in High Risk Neonates

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Abstract

IntroductionThe purpose of this study was to evaluate accuracy of cord blood interleukin-6 (IL-6) as an early marker of early onset neonatal sepsis (EOS) in neonates with maternal risk factors..
Objectives: To evaluate accuracy of cord blood IL-6 as a predictor of early onset neonatal sepsis (EONS) in neonates with prenatal risk factors for sepsis.
Patients and methods:
The study included 80 neonates divided into two groups. Group(I) included 25 full-term and 25 preterm neonates. Group (II) included thirty healthy age and sex matched controls with mean gestational age 35.2±3.8. All patients were subjected to adequate history taking, full clinical examination, complete blood count, blood culture, cord blood interleukin-6 and serum high sensitive CRP for elevated IL-6 level.
Results:IL-6 level was significantly higher in the group (I) compared with group(II). No significant correlation between gestational age and level of IL-6. The best cutoff value for serum interleukin-6 as a marker for early detection of neonatal sepsis is ≥50 pg/ml with sensitivity 96 % and specificity 100%.
Conclusion: Cord blood IL-6 can be a sensitive predictor of early onset sepsis for neonates with high risk of sepsis.

Key words: cord blood, interleukin-6, maternal risk factors, neonatal sepsis.

Background:

Early-onset neonatal sepsis (EONS) is a serious complication with a mortality rate ranging from 1.5% in term to almost 40% in very-low-birth weight infants.[1]Neonatal sepsis may be categorized as early-onset or late-onset. Of newborns with early-onset sepsis, 85% present within 24 hours, 5% present at 24-48 hours, and a smaller percentage present within 48-72 hours.[2] EONS is more rapid in premature neonates and it is associated with acquisition of microorganisms from the mother. Trans placental infection or an ascending infection from the cervix may be caused by organisms that colonize the mother's genitourinary (GU) tract and pass through the colonized birth canal at delivery. The microorganisms most commonly associated with early-onset infection includeGroupB Streptococcus (GBS), Escherich iacoli,Coagulasenegative Staphylococcus, Haem ophilus influenzae and Listeria monocytogenes . Different biomarkers have been used to identify patients early in the course of neonatal sepsis. Fetal inflammatory response syndrome (FIRS) has been defined by high levels of pro-inflammatory cytokines in fetal blood.[3]

Interleukin-6(IL-6)(an early marker of infection) is a rapid response inflammatory protein with a short plasma half life, in EOS,

infection is commonly acquired in utero resulting in the development of chorioamnionitis. [4] Chorioamnionitis activates the fetal immune system leading to fetal inflammatory response syndrome (FIRS). In some neonates, FIRS is amplified and these neonates become symptomatic after birth with clinical manifestations suggestive of EONS. FIRS can be diagnosed by an increased number of pro-inflammatory cytokines in cord blood, of which interleukin-6 (IL-6) is one of the early mediators released in the circulation.[4,5]

Patients and Methods

This study was carried out from December, 2012 and August, 2013. Eighty patients were selected at the delivery time from obstetric emergency department in Minia University hospital for children .Neonates involved in this study were classified into two groups:

Group I: Fifty newborn infants with prenatal risk factor for EONS. Twenty five were full term while 25 were preterm. Twenty nine (58%) were males while 21(42%) were females. The prenatal risk factors include any of the following: Premature rupture of membrane more than 18h, unclean foul smelly turbid vaginal secretion, foul smelling liquor, maternal pyrexia (temp.>38C), maternal leukocytosis (leukocytes>14000), dysuria and

prolonged labor more than24h.

Group II: Thirty newborn infants without prenatal risk factor for EONS included as control. Fifteen were full term while 15 were preterm; 12(40%) were males and 18(60%) were females.

Both groups (I&II) were subjected to thorough history taking including: gestational age, sex, obstetric history (maternal temperature, duration of labor, amount and color of vaginal secretions).General examination including anthropometric measures, vital signs, chest, heart, abdominal, neurological examination. Umbilical stump status (infected or not)and neonatal activity (doing or not doing well).Criteria of neonatal sepsis included alteration in at least 2 of the following clinical signs:

 1-Respiratory distress: apnea, tachypnea, or hypoxemia.

2-Cardiological: tachycardia or bradycardia.

3-Hemodynamics: bad color, poor peripheral hypo perfusion, hypotension.

4-Neurological: irritability, lethargy, hypotonia, hypo activity, seizures.

5-Gastrointestinal: poor feeding, abdominal distension, feeding intolerance.

6-Temprature: fever $>38^C$ or hypothermia C.

7-Metabolic: metabolic acidosis or hyperglycemia. [6]

Plus elevated immature/ total neutrophils ratio (I/T ratio)>0.2 or presence of toxic granules or elevated white blood cell count > 25000 mm3 at the time of evaluation.

Early onset neonatal sepsis is defined as: Sepsis occurs at

Laboratory investigations carried out to neonates were included complete blood count (CBC), blood culture, cord blood interleukin-6 level and cord blood high sensitive C reactive protein(HsCRP) were assayed immediately after collection at labor by ELISA technique using (Ani Biotech Oy, Orgenium laboratories, Finland). Cases with elevated cord IL-6levels or CRP were followed up by serum HsCRP using (ACCUBIND ELISA Microwells (Monobind inc.100 North Pointe Drive Lakeforest, CA 92630 USA).

The study was approved by the local research ethics committee of the faculty of medicine in Minia University and a written informed consent was obtained from the parents of all neonates to share in the study.

Statistical analysis:

Data were statistically analyzed using the SPSS software package, version 16 (SPSS Inc., Chicago, IL, USA) on a personal computer. Numerical data were expressed as range, mean± SD, median, and percentiles.

Non numerical data were expressed as frequencies. Comparative studies were done using Student t test and chi square test. (p valu < 0.05 was considered significant). Pearson correlation test was used to detect correlation between different parameters. Receiver operating characteristic (ROC)Curve analyses with measurement of area under the curve (AUC) were performed to identify the appropriate cut-off values. The study approved by ethics committee of pediatric department minia university & informed parents' consents

Results

Our study included 80 neonates who further subdivided into 2 groups. Group I included 50neonates with prenatal risk factors for infection and group II included 30 healthy neonates as a control. There were significant differences between both groups regarding length, Apgar score, history of prolonged labor, hospital admission (p<0.001); birth weight(p<0.01)and H.C (p=0.004). While, there was no significant difference between both groups regarding gestational age, sex, mode of delivery.(table 1)

Regarding to clinical data, 33(66%) of group(I) neonates had respiratory distress, chest retraction (68%), cyanosis (26%), grunting (58.1%),while all neonates

included were normal in group(II).There were significant differences between both groups regarding chest retraction, respiratory distress , cyanosis and grunting (p<0.001) while no significant difference between both groups regarding convulsion, skin mottling, pallor, jaundice, abdominal distention and organomegaly (fig.1).

Regarding obstetric and maternal signs of groups I, (100%) of mothers of the neonates had PROM. Fever was found in30 mothers (60%), and lower abdominal tenderness were detected in 17 mothers (34.7%).Twenty four (48%) of the mothers had history of ante partum antibiotic, while in 48 of them (96%)there was history of intra partum antibiotics.(Figure 2).Table 2shows some laboratory data of group I neonates. Significant difference between group I and II regarding Cord blood IL-6and HsCRP (p wereStreptococci positive, (15%) were E. coli and klebsella detected in (20%)of these septicemia neonates. Table 4 shows the difference between cord IL6 and HsCRP and some laboratory data in the infected and non infected group I neonates.in the infected group I patients, mean cord IL-6was118± 32 , cord HsCRP was30.1± 10, WBC was 16.8± 8.3 and serum HsCRP after 72h was54.9± 22.5 . while in non infected group I patients, Cord

IL-6was56.5± 24, cord HsCRP was25.4 ± 19.5, WBC was 12± 4.3 and serum HsCRP after 72h was 37.2± 23.5. There were significant difference between infected and non infected group I patients regarding cord IL-6 (P=0.001), WBC (P=0.001) and HsCRP after 72h (P=0.01) while no significant difference between them as regard cord blood HsCRP (p=0.33).(table 4)

Studying the correlation between cord IL-6 level and some lab data among group I neonates, There was significant positive correlation between IL-6 and WBCS (p =0.01, r= 0.33), toxic granules(P=0.001. r= 0.46) and serum HsCRP after 72h (p=0.002, r= 0.44)while there was no correlation between cord IL-6 and Hb (p = 0.8, r =0.03), platelets (p = 0.9, r= -0.01), neutrophils (p=0.5, r=0.1) and cord blood HsCRP(p =0.54, r=0.09).(table 5, figure 3,4)

Cut off point of cord IL-6 was ≥ 84 while of cord CRP was ≥ 21 sensitivity of cord IL-6 was 90% and specificity was 96.7% while for cord CRP sensitivity was 85% and specificity was 56.7% and for serum HsCRP after 72h 65% and specificity was 90%.(complete term before abbreviation) NPV of I L-6 was 93.5% and (complete term before abbreviation) PPV was 94.7 % while NPV of CRP was 85 % and PPV 56.7%. . For serum HsCRP after 72h,

PPV was 81.2 and NPV 79.4(Fig 5).

Discussion

IL-6 is an early and sensitive marker for neonatal sepsis.[8] In the present study, cord IL-6 was significantly higher in the case group compared with the control group. Same results were found byCernada et alwho found that serum interleukin-6 was higher in the case group than the control group.[9]Emphasizing the role of this useful inflammatory marker in detection of neonatal sepsis.[10]The inflammatory response is mediated by cytokines that are used as neonatal infection markers, especially interleukin-6 (IL-6). IL-6 is an inducer of hepatic protein synthesis, promotes production and liberation of C-reactive protein, and can be detected early when there is bacterial bloodstream invasion. It acts as a signal for T-cell activation, promotes antibody secretion by B cells and differentiation of cytotoxic T cells, and stimulates liberation of other cytokines, particularly TNF-a.[11]

IL-6 is an important neonatal inflammatory mediator. There is several studies demonstrating an elevation of plasma IL-6 levels in septic newborn infants. Balance between pro and anti-inflammatory cytokines is determinant for their blood levels. Low IL-10 plasma level in infected newborn infants

contribute to an increase of IL-6.[12]

Significant positive correlation were found between interleukin-6 and gestational age. Greater IL6 elevations in term infants compared to preterm infants until the inflammation becomes sever.[13] significant differences in IL6 levels were seen in term versus preterm infants when the acute chorioamnionitis was mild or moderate may be related to the relative immaturity of the preterm immune system, as has been demonstrated in vivo and in vitro. While others [3,5] reported that immature infants seems to be capable of synthesizing this cytokine.

HsCRP was high in the studied group compared with control group and no significant correlation between IL-6 and cord HsCRP was found while a significant positive correlation was obtained between cord IL-6 and CRP after 72hours.After a trauma or the invasion of microorganisms an acute localized inflammatory reaction is initiated by activation of local resident cells. The contact with bacterial endo- or exotoxins initiates the release of prostaglandins, leukotriene, and histamine, which results in vasodilatation, elevated vascular permeability, and attraction and activation of further inflammatory cells.[14,15]Activated fibroblasts, leukocytes,

and endothelial cells produce pro-inflammatory cytokines including IL-1, TNF-α, and IL-6 are responsible for the development of fever, lethargy, arthralgia, and headache, they activate the vascular endothelial cells, regulate proliferation of T- and B-lymphocytes, activate macrophages, have pro-coagulatory effects on endothelial cells, and they induce the production of acute-phase-proteins in the hepatocytes of the liver.

Acute-phase-proteins form a heterogeneous group and include components of the complement system, coagulation factors, protease inhibitors, metal binding proteins, HsCRP, and other proteins that increase or decrease by more than 25% during an inflammatory reaction.[14,16]

HsCRP, the major acute-phase reactant in humans, derives mainly from hepatocytes in response to interleukin-6 (IL-6) and is then secreted into the systemic circulation.[17]

HsCRP level is not recommended as a sole indicator of neonatal sepsis but may be used as part of a sepsis workup or as a serial study during infection to determine response to antibiotics, duration of therapy, and/or relapse of infection. [18]

Serum concentrations of HsCRP increase several hundredfold in response to bacterial infection, making it an attractive diagnostic

test for neonatal sepsis. Several hours are needed for HsCRP levels to increase in serum (including activation of neutrophils, elaboration of interleukin-6, and induction of hepatic synthesis

of HsCRP) therefore limiting the sensitivity of this test in diagnosing EONS. HsCRP levels are consistently 48 hours after the onset of infection.[19]Various factors apart from sepsis can cause a rise in CRP levels especially Meconium aspiration syndrome, perinatal asphyxia, surgery, etc.. but a negative CRP rules out infection with high certainty i.e. it has a high Negative Predictive Value.[20]Based on sensitivity and specificity of the results, cord blood interleukin-6 at cut off point of >84pg\ml or more, can be used as a marker for the diagnosis of EOS in neonates with suspicion of infection with.[21]IL-6 can be a valid marker for predicting NS. It may be considered for early diagnosis and control of sepsis in neonatal care units.[22]Also, IL-6 had high sensitivity and specificity for early detection of neonatal sepsis comparing with the sensitivity of HsCRP.[21]

Conclusions

Cord blood interleukin-6 increases significantly in neonates with risk factors for early onset neonatal sepsis can be used as an early marker to diagnose early onset sepsis.

The combination of IL-6 (early and sensitive marker) with serum HsCRP (late and specific marker) can elicit better sensitivity than either marker alone.

Conflict of interest

Acknowledgment

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Table (1): Comparison between group (I) and (II) regarding some demographic data:

Data		Group I N=50	Group II N=30	P-Value
Gestational age (Wk.)	Range	26-38	28-42	0.5
	Mean ±SD	34.8±3.4	35.2±3.8	
Maturity	Full term	25 (50%)	15 (50%)	0.99
	Preterm	25 (50%)	15 (50%)	
Sex	Male	29(58%)	12(40%)	0.1
	Female	21(42%)	18(60%)	
Maternal age (Year)	Range	19-35	19-33	0.3
	Mean ±SD	25.3±4.1	24.5±3.4	
Birth weight (Kg)	Range	1.2-4	1.6-3.8	0.01*
	Mean ±SD	2.4±0.6	2.8±0.6	
Length (Cm)	Range	40-56	43-60	0.001*
	Mean ±SD	47.2±3.8	53.2±4.6	
HC (Cm)	Range	28-37	29-37	0.0-4*
	Mean ±SD	32.9±2.1	34.4±2.3	
Apgar score at 1 min	Range	3-8	8-10	0.001*
	Mean ±SD	5.9±1.8	8.1±0.4	
Apgar score at5 min	Range	5-10	10-12	0.001*
	Mean ±SD	7.8±1.5	10.1±0.4	
Mode of delivery	NVD	30(60%)	17(56.7%)	0.7
	C.S	20(40%)	13(43.3%)	
Prolonged labor	YES	27(54%)	0	
	NO	23(46%%)	30(100%)	-------
Signs of vaginal Infection	YES	5(10.2%)	30(100%)	
	NO	44(89.8%)	0	--------
Admission	Yes	35(70%)	0	
	No	15(30%)	30(100%)	---------

Significant(p<0.05)   NVD= normal vaginal delivery

Table (2): Some laboratory data for group (I) patients

Data		Total N=50
WBCs	Range	5.8-42.3
	Mean ±SD	15.5±6.8
Hb gm/dl	Range	11-21.4
	Mean ±SD	16.02±2.6
Platelets x10^3/cmm	Range	79-560
	Mean ±SD	272.5±124.3
Neutrophil x10^3/cmm	Range	1.9-24.3
	Mean ±SD	5.2±3.8
Toxic granules	Range	1.2-18.6
	Mean ±SD	7.4±5.1
Blood culture	Positive	13(26%)
	Negative	1(2%)
Serum HsCRP (72h after labor)	Positive	4-41
	Negative	44.28+24.5

Table (3): Comparison between group (I), (II) regarding Cord IL-6 and Cord CRP.

Data		Group (I) N=50	Group (II) N=30	p-value
Cord IL-6 pg/ml	Range	.8-9.5	20-178	0.001*
	Mean ±SD	5.3±5.6	80.1±42.1	
Cord CRP: mg/l	Range	0-7	0-64	0.001*
	Mean ±SD	2.7±2.1	27.1±16.3	

*= Significant(p<0.05).

Table (4) : Comparison between infected and uninfected neonates of group I some laboratory data.

| Variable	Group (I) Infected patients (N=20)	Group (II) Non infected patients (N=30)	*P* -value
Cord blood IL-6 (pg/ml)	118± 32	56.5± 24	0.001*
Cord blood CRP (mg/l)	30.1± 10	25.4 ± 19.5	0.33
WBC	16.8± 8.3	12± 4.3	0.01*
Serum HsCRP after 72h (mg/l)	54.9± 22.5	37.2± 23.5	0.01*

*= Significant(p<0.05)

Table (5): Correlation between laboratory data and cord IL-6 in group I

Data	r	p-value
WBCs	0.33	0.01*
Hb level	0.03	0.8
Platelets count	-0.01	0.9
Neutrophil count	0.1	0.5
Toxic granules	0.46	0.001*
Cord CRP level	0.09	0.54
Serum HsCRP level after 72h	0.44	0.002*

*= Significant(p<0.05).
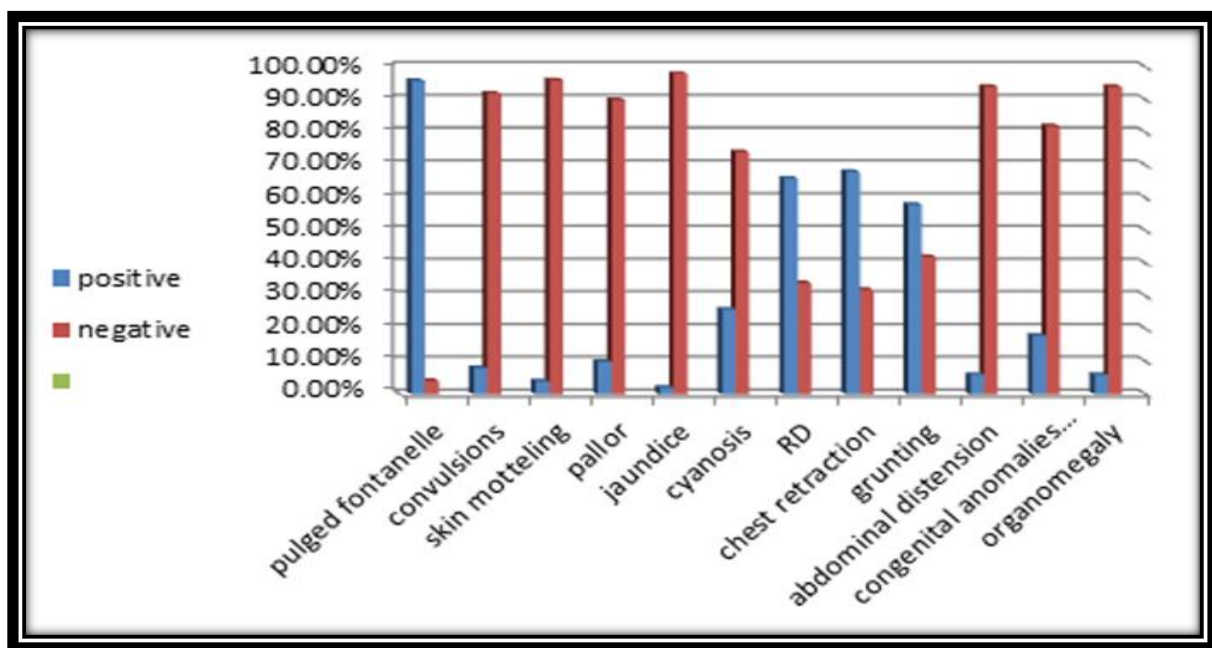
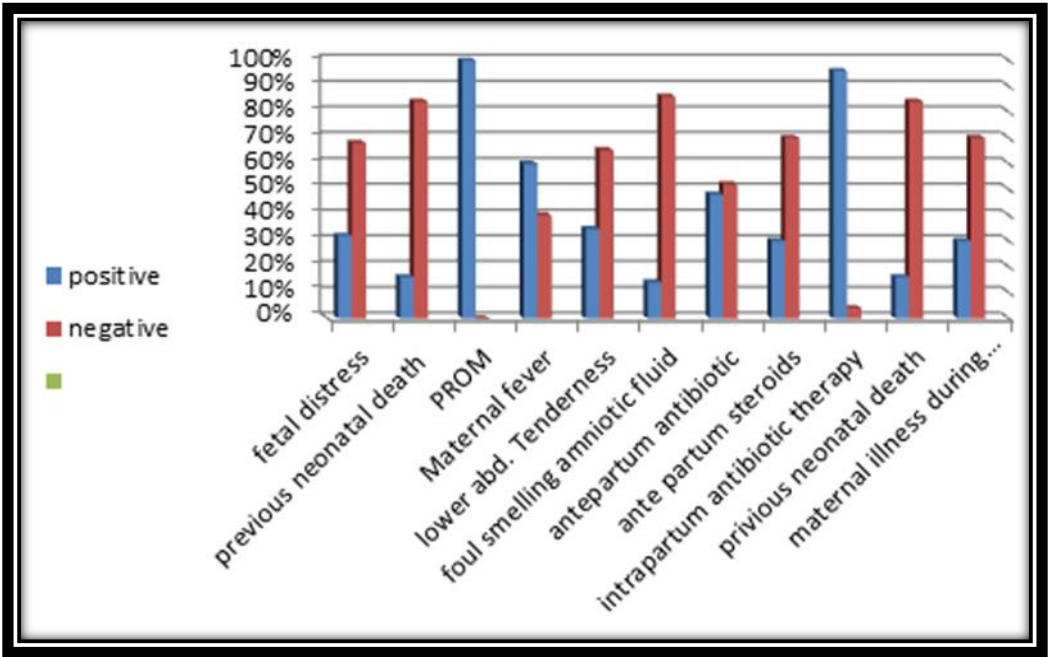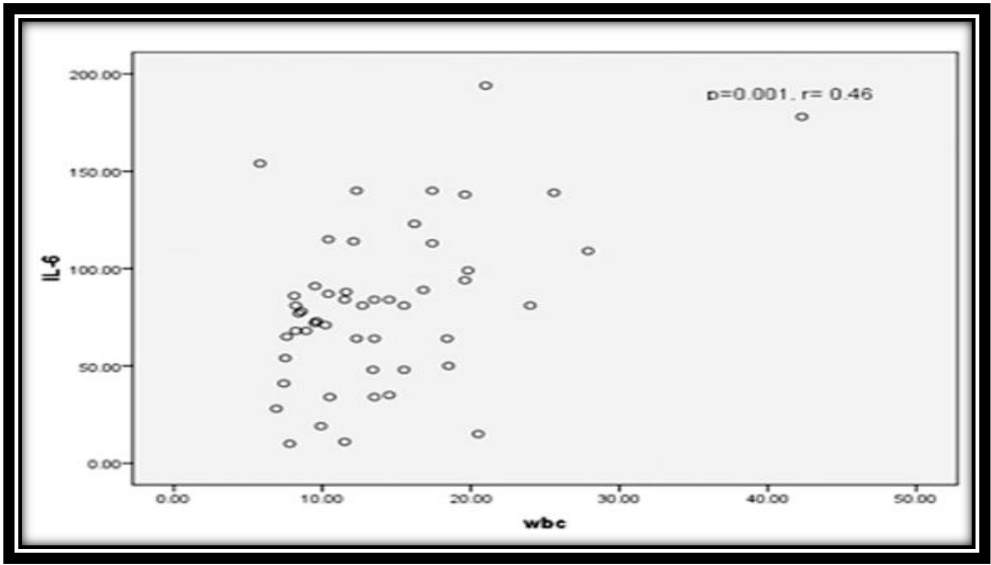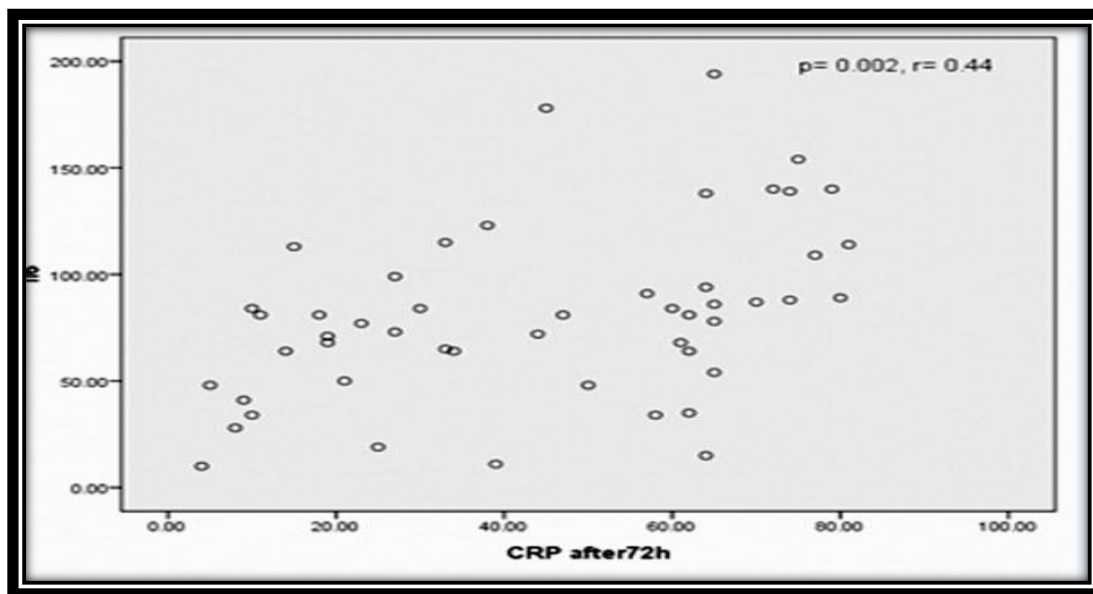**Figure (1): Some clinical signs in group I patients.**

Figure (2): Frequency of some prenatal risk factor for group I patients.

Figure(3): Correlation between WBC and IL-6.

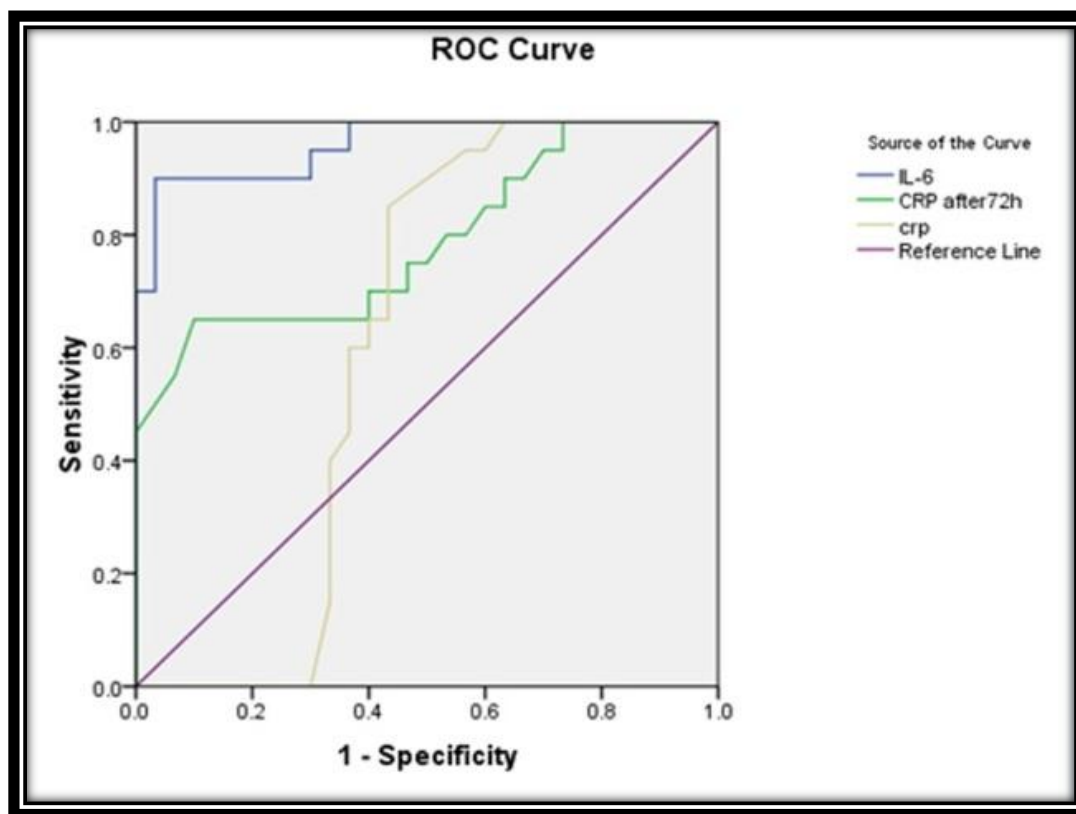**Figure(4): Correlation between CRP after 72h and IL-6.**

Figure (5): ROC (Receiver Operating Characteristic Curve) of IL-6 and CRP validity in group I

cases.

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