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Relation between interleukin-6 in the cervicovaginal fluid and subclinical chorioamnionitis in patients with preterm premature rupture of membranes

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ABSTRACT

Objective: To evaluate the accuracy of the interleukin-6 (IL-6) in the cervicovaginal fluid to diagnose subclinical chorioamnionitis and neonatal infection in patients with preterm premature rupture of membranes (PPROM). **Methods:** One hundred and twenty (120) pregnant women > 34 weeks, and < 37 weeks gestation with PPROM were included in this study. Patients included in this study were subjected to standard examination, trans-abdominal ultrasound, sterile speculum examination to detect amniotic fluid pooling through the cervical canal and for assessment of the IL-6 in the cervicovaginal secretions. After delivery, all neonates were examined for detection of neonatal infection and all placentae and membranes were histological examined for detection of chorioamnionitis. **Results:** The sensitivity & specificity of Interleukin-6 test to diagnose neonatal infection were 82.8% & 89.3%; respectively, while the Interleukin-6 test sensitivity & specificity to diagnose chorioamnionitis were 90.7% & 91.0%; respectively. The positive predictive value (PPV) & negative predictive value (NPV) of Interleukin-6 test to diagnose neonatal infection were 86.9% & 85.9%; respectively, while the Interleukin-6 test PPV & NPV to diagnose chorioamnionitis were 87.5% & 93.4%; respectively. The accuracy of Interleukin-6 test to diagnose neonatal infection was 86.3%, while the accuracy of Interleukin-6 test to diagnose chorioamnionitis was 90.9%. **Conclusions:** Detection of Interleukin-6 in the cervicovaginal secretion is a sensitive, non-invasive prenatal marker for neonatal infection and subclinical chorioamnionitis in patients with PPROM.

1. Introduction

Premature rupture of membranes (PROM) is rupture of the fetal membranes before the onset of labor, while rupture of fetal membranes before 37 weeks gestation, is defined as preterm premature rupture of membranes (PPROM)[1]. PPROM is usually associated with significant perinatal and maternal infectious morbidities[2,3]. PPROM occurs approximately in 2%-8% of all pregnancies and it is associated with 20% of perinatal deaths[4]. Chorioamnionitis is the most important complication of PPROM[5]. The ability to diagnose subclinical chorioamnionitis could lead to subsequent decrease in perinatal morbidity & mortality[6,7]. No available, non-invasive tests currently provide early screening of fetal infection to determine the need for antibiotic treatment, cessation of tocolysis or even early delivery. Antenatal detection of neonatal infection would make it possible to target more accurately the population of newborn infants who require antibiotic treatment[8]. Interleukin-6 (IL-6) is one of the inflammatory cytokines produced by macrophages, amnion, chorion and decidual cells[9]. Interleukin-6 is elevated in maternal serum & amniotic fluid in patients with PPROM with microbial invasion before development of symptoms and signs of chorioamnionitis[10,11]. So this study was designed to evaluate the accuracy of the interleukin-6 in the cervicovaginal fluid to diagnose subclinical chorioamnionitis and neonatal infection in patients with preterm premature rupture of membranes.

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2. Materials and methods

One hundred and twenty (120) pregnant women >

34 weeks, and < 37 weeks gestation with PPROM were included in this study after informed consent and approval of the study protocol by the institute ethical committee. Patients with multiple pregnancies or > 37 weeks gestation or not sure of dates or on antibiotic therapy before admission were excluded from this study. Patients with fetal distress or vaginal bleeding or preterm labor or clinical chorioamnionitis (maternal fever, maternal tackycardia, fetal tackycardia, uterine tenderness, maternal leucocytosis and positive C reactive proteins) were also excluded from this study. The diagnosis of PPROM was based on patient's history of sudden gush of water, pooling of amniotic fluid, positive ferning pattern, positive nitrazine test, confirmed by visualization of fluid passing from the cervical canal during sterile speculum examination and trans-abdominal ultrasound to measure the amniotic fluid index (AFI $\leqslant$ 5 cm in PROM)[12, 13]. The gestational age was calculate from the first day of LMP and confirmed by early ultrasound scan (done before 20 weeks gestation).

2.1. Methods

Patients included in this study were subjected to standard examination, trans-abdominal ultrasound, sterile speculum examination to detect amniotic fluid pooling through the cervical canal and for assessment of the interleukin-6 in the cervicovaginal secretions.

Interleukin-6 evaluation: the cervico-vaginal secretions were collected from the posterior vaginal fornix, after insertion of a sterile vaginal speculum[15-20]. Micro liter of secretion were collected by aspiration with sterile plastic pipette. The test strip (Pall Germain science, Paris, France) was applied to the sample and the results were interpreted as follows; a positive test shows 2 bands or lines after 15 minutes, denoting the presence of interleukin-6 in the cervicovaginal secretions. A negative test shows only the control band or line. While, the absence of two bands or lines denotes invalid test. The detection threshold of Interleukin-6 was 100 pg/mL in vaginal secretions. The result was available in less than 20 minutes[8]. After delivery, all neonates were examined for detection of neonatal infection and all placentae and membranes were histological examined for detection of chorioamnionitis.

Histological chorioamnionitis: histological evaluation of the placentae and membranes was done within 48 hours. Infection was defined in the presence of acute inflammatory cells in any of the tissue samples, as described by Salafia *et al*[14]. Four grades of inflammation were used to assess the amnion, choriondecidua, umbilical cord and chorionic plate. Grade 2 inflammations, characterized by multiple foci of 5 or more polymorph nuclear leukocytes or a larger focus in the sub-chorionic fibrin, was used as the cutoff for clinically important placental inflammation because this grade has been shown to be a sensitive indicator of culture-proven amniotic infection[14].

2.2. Neonatal examination

All neonates were examined after delivery to diagnose early-onset GBS sepsis. The diagnosis of early-onset GBS sepsis in new-borns was diagnosed by clinical signs of infection (fever or hypothermia, tachycardia, tachypnea or hyperventilation, abnormal white blood cells or increase in immature forms) plus positive blood culture or elevated neonatal CRP value >10 mg/L (normal value < 5 mg/L) or chest radiography showing pulmonary infection[15].

2.3. Statistical analysis

Data were collected, and statistically analyzed using Statistical Package for Social Sciences (SPSS); computer software version [15]. Numerical variables were presented as mean and standard deviation (±SD), while categorical variables were presented as number and percentage. Sensitivity: is the proportional detection of individuals with the disease of interest in the population. Specificity: is the proportional detection of individuals without the disease of interest in the population. PPV: is the proportion of all individuals with positive tests, who have the disease. NPV: is the proportion of all individuals with negative tests, who are non-diseased.

3. Results

The mean age of the studied population was (28.7 ± 7.34) years and the mean gestational age was (35.5 ± 6.28) weeks. One hundred and twenty (120) pregnant women > 34 weeks and < 37 weeks gestation with PPROM were included in this study and the neonatal examination after delivery revealed: neonatal infection in 53 neonates (true positive), the Interleukin-6 test was positive in 42 (79.2%) of them, while it was false negative in 11 (20.8%) of them and the neonatal examination after delivery revealed: no neonatal infection in 67 neonates (true negative), the Interleukin-6 test was negative in 59 (88.1%) of them, while it was false positive in 8 (11.9%) of them.

The histological examination of the placentae and membranes of the studied population after delivery revealed; chorioamnionitis in 49 cases (true positive), the Interleukin-6 test was positive in 44 (89.8%) of them, while it was false negative in 5 (10.2%) of them and the histological examination of placentae and membranes revealed; no chorioamnionitis in 71 cases (true negative), the Interleukin-6 test was negative in 64 (90.1%) of them, while it was false positive in 7 (9.9%) of them, (Table 1). In this study; the sensitivity & the specificity of Interleukin-test to diagnose neonatal infection were 82.8% & 89.3%; respectively, while the Interleukin-6 test sensitivity & the specificity to diagnose chorioamnionitis were 90.7% & 91.0%; respectively. The positive predictive value (PPV) & negative predictive value (NPV) of Interleukin-6 test to diagnose neonatal infection were 86.9% & 85.9%; respectively, while the Interleukin-6 test PPV & NPV to diagnose chorioamnionitis were 87.5% & 93.4%; respectively. The accuracy of Interleukin-6 test to diagnose neonatal infection was 86.3%, while the accuracy of Interleukin-6 test to diagnose chorioamnionitis was 90.9%, (Table 2).

Table 1

Relation between Interleukin-6 test and histological chorioamnionitis

Variables	Histological chorioamnionitis (Total number = 120 patients)	
	Positive histological chorioamnionitis (49 cases = True positive)	Negative histological chorioamnionitis (71 cases = True negative)
Positive Interleukin-6 (IL-6) test	44 (89.8%)	7 (9.9%) False positive
Negative Interleukin-6 (IL-6) test	5 (10.2%) False negative	64 (90.1%)

Table 2

Accuracy of Interleukin-6 test in prediction of neonatal infection and histological chorioamnionitis

Variables	Interleukin-6 test				
	Sensitivity	Specificity	PPV	NPV	Accuracy
Neonatal infection	53/(53+11)× 100 = 82.8%	67/(67+8)×100 = 89.3%	53/(53+8)×100=86.9%	67/(67+11)×100 = 85.9%	53+67/(53+67+8+11)×100 = 86.3%
Histological chorioamnionitis	49/(49+5)× 100 = 90.7%	71/(71+7)×100 = 91.0%	49/(49+7)×100 =87.5%	71/(71+ 5)×100 = 93.4%	49+71/(49+71+7+5)×100 = 90.9%

Sensitivity = True positive /(True positive + false negative) × 100; Specificity = True negative /(True negative + false positive) × 100; PPV = True positive /(True positive + false positive) × 100; NPV = True negative /(True negative + false negative) × 100; Accuracy = True positive + true negative /(True positive + true negative + false positive + false negative) × 100.

4. Discussion

When PPROM occurs, there are significant risks of fetal morbidity & mortality[16-18]. Amniotic fluid markers have been developed to detect subclinical chorioamnionitis[19]. Interleukin-6 is elevated in maternal serum & amniotic fluid in patients with PPROM with microbial invasion before development of symptoms and signs of chorioamnionitis[10,11]. The value of interleukin-6 in cervical secretion to diagnose microbial invasion of the amniotic cavity in patients with premature rupture of the membranes was evaluated by Rizzo and colleagues and they concluded that intra-amniotic infection is associated with increased levels of Interleukin-6 and measurement of Interleukin-6 in cervical secretions may help to identify intra-amniotic infection among pregnancies with preterm premature rupture of membranes. On the other hand, Hitti *et al*, while evaluating the role of the Interleukin-6 and other cytokines as predictors of amniotic fluid infection, they found that Interleukin-6 was not associated with amniotic fluid infection, but, Popowski and colleagues concluded that the predictive value of Interleukin-6 in detection of chorioamnionitis or neonatal infection is higher but its clinical usefulness is limited by the various threshold used in the studies and the lack of routine measure[20-22].

In this study, the sensitivity & specificity of Interleukin-test to diagnose neonatal infection were 82.8% & 89.3%; respectively, while the Interleukin-6 test sensitivity & specificity to diagnose chorioamnionitis were 90.7% & 91.0%; respectively. The PPV & NPV of Interleukin-6 test to diagnose neonatal infection were 86.9% & 85.9%; respectively, while the Interleukin-6 test PPV & NPV to diagnose chorioamnionitis were 87.5% & 93.4%; respectively.

Also, the diagnostic value of an interleukin-6 test for neonatal infection in cases of preterm premature rupture of membranes was evaluated by Kayem *et al*, and they concluded that, Interleukin-6 protein determination by this new immunochromatographic test is a non-invasive prenatal vaginal marker of neonatal infection with 79% sensitivity, 56% specificity, 30% PPV and 92% NPV[8].

Various mechanisms may explain the association between Interleukin-6 in vaginal secretions and neonatal infection. High cytokine levels in cervical secretions may reflect overall increased production throughout the maternal genital tract in response to intra-amniotic infection or it may also be a sign of a primary inflammation process at the amnio-chorionicdecidual surface[23].

Cervical secretions were collected from 286 women hospitalized for preterm labour with intact membranes at 24-34 weeks' gestation by Goffinet & colleagues and they concluded that the detection of Interleukin-6 mRNA by RT-PCR in vaginal secretions allows identification of a small group of women at high risk of neonatal infection, independently of other markers of infection. The value of interleukin-6 concentrations in cervical fluid samples to detect microbial invasion of the amniotic cavity, duration of the latency period and risk of neonatal complications in PPROM was studied by Jun & colleagues and they concluded that the cervical fluid interleukin-6 is an indicator of the likelihood of microbial invasion of the amniotic cavity, impending preterm delivery and the occurrence of significant neonatal complications in the setting of preterm premature rupture of membranes, also, Yoshio Matsuda et al, found that the measurement of Interleukin-6 in cervicovaginal fluid is a useful marker to detect patients who are more likely to develop neonatal infection after PPROM[24-26].

In this study; the accuracy of Interleukin-6 test to diagnose neonatal infection was 86.3%, while the Interleukin-6 accuracy to of diagnose chorioamnionitis was 90.9%. The detection of interleukin-6 the cervicovaginal secretion of patients with preterm premature rupture of membranes was a sensitive and non-invasive prenatal marker of neonatal infection and subclinical chorioamnionitis.

Conflict of interest statement

No actual or potential conflict of interest in relation to this article exists.

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