Correlation of Interleukin-6 in Vaginal Secretions with Neonatal Infection, Histologic Chorioamnionitis, and Umbilical Cord pH in Patients with Premature Prelabor Rupture of Membranes

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Abstract:
The objective of this study was to evaluate the diagnostic value of interleukin-6 bedside test in vaginal secretions for neonatal infections, histologic chorioamnionitis and neonatal acidemia in pregnant ladies with prelabor premature rupture of membranes above 34 weeks. The study was a prospective clinical study performed on 50 patients with prelabor premature rupture of membranes above 34 weeks. Interleukin-6 in vaginal secretions was determined in less than 20 minutes by an immunochromatographic bedside test.

Results: A positive strong correlation was present between vaginal interleukin-6 and each of the three parameters (p=0.000): histologic chorioamnionitis(r=0.561), early onset neonatal infection(r=0.836), and umbilical pH(r=0.723). The test showed sensitivity (87.5%, 72.7%, 95%), specificity (88.4%, 84.6%, 80%), positive predictive value (87.5%, 80%, 90.4%) and negative predictive value (88.4%, 78.5%, 96.4%) respectively.

Conclusion: Vaginal interleukin-6 represents an effective bedside test for the screening and prediction of early onset neonatal infection, histologic chorioamnionitis and neonatal acidemia in premature prelabor rupture of membranes.

Introduction:
Preterm premature rupture of membranes (PROM) has been attributed to ascending infection and a choriodecidual inflammatory response (ie; on the maternal side). However, on the fetal side, those most at risk of morbidity have a systemic proinflammatory cytokines response.1 Microbial invasion of the amniotic cavity is frequently observed in patients with preterm premature rupture of membranes (PROM). It is a major risk factor for neonatal infection and adverse neonatal outcomes.2,3

In both term and preterm infants, early warning signs and symptoms of neonatal infection are often minimal, subtle, and non-specific and can easily be misinterpreted as being due to non-specific causes.4 The clinical course may be alarmingly fulminant leading to septicemic shock, disseminated intravascular coagulopathy, and death within hours of the onset of the clinical manifestations.5

Infected infants must therefore be promptly identified and differentiated from non-infected infants, and antibiotics started without delay. However, as microbiological culture results and antimicrobial susceptibility data are not usually available until at least 48 hours after the specimen reaches the laboratory, early identification of genuine sepsis is a major diagnostic problem. A wide variety of hematological and biochemical markers have been investigated for the evaluation of clinical sepsis.6 Chemokines and cytokines are endogenous mediators that orchestrate the inflammatory cascade of the human body.7

A high Interleukin-6 was the most sensitive test (sensitivity 82%) in detecting amniotic fluids containing bacteria, in many studies.8 They found good correlation between amniotic fluid IL-6, chorioamnionitic microbial colonization and preterm delivery. IL-6 had better diagnostic value than Gram staining, glucose levels, white blood cell count and leukocyte esterase of AF. However, transabdominal amniocentesis is by no means non invasive and is not applied to all patients with PROM. Hence, noninvasive method to screen for maternal-fetal infection, by detection of proinflammatory cytokines, using vaginal fluid would be useful; as their presence in cervical or vaginal secretions is associated with microbial invasion of AF, as suggested in many researches.5

The purpose of this study was to determine whether detection of IL-6 by immunochromatographic bedside test in vaginal fluid among women with PPROM, might predict neonatal infection, histologic choriarnionitis or neonatal acidemia. We also evaluated the correlations, if any, between all parameters studied.
Subjects and Methods:
From the period of 1/1/2005 to 1/7/2005, fifty patients admitted in Obstetrics and Gynecology department at Alsalama hospital, Abu Dhabi, UAE, were recruited in this study. Evaluation of Interleukin-6 in cervicovaginal secretions was performed after patient admission to the in-patient ward.

Inclusion criteria included:
1. Pregnancy ≥ 34 weeks.
2. Prelabor rupture of membranes: as documented by sterile speculum examination confirming the pooling of fluid in the posterior vaginal fornix, an alkaline pH, ferning and direct visualization of fluid leakage from the cervical canal.9

Exclusion criteria:
1. Patients clinically suffering from chorioamnionitis at presentation, such as fever, uterine tenderness, or foul vaginal discharge.
2. Fetal distress as detected by fetal heart rate.
3. Fetal malformations.
4. Diabetes mellitus, pregnancy – induced hypertension

Interleukin-6 evaluation:
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 strip (Pall Germain science, Paris, France) was applied to the sample, and the results were interpreted as follows; a positive test shows 2 bands after 15 minutes, denoting the presence of interleukin-6. A negative test shows only the control band. While, the absence of two bands denotes failure of the test. The detection threshold of IL-6 was 100 pg/ml in vaginal secretions. The result was available in < 20 minutes.5

Histologic chorioamnionitis:
Histologic evaluation of the placenta 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 a.10 Four grades of inflammation were used to assess the amnion, chorion-decidua, umbilical cord and chorionic plate. Grade 2 inflammation, characterized by multiple foci of 5 or more polymorphonuclear leukocytes or a larger focus in the subchorionic 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.

Neonatal infection:
Proven neonatal infection was diagnosed by clinical signs of infection plus positive blood culture or elevated neonatal CRP (normal value, <5 mg/l) or chest radiography showing pulmonary infection. Probable neonatal infection was suggested by clinical, radiographic, and laboratory findings (mainly elevated neonatal CRP) in cases with negative blood cultures.6 We included all neonates whether with proved or probable infection, in our results. Cord artery pH was also evaluated to detect acidemia by preheparinized syringes. Cut-off for normal arterial pH was 7.10.11

Statistical Analysis:
Statistical analysis was done using the SPSS program. Chi-square was used to compare between variables. Pearson correlation was used to test association among different parameters. Accuracy (sensitivity, specificity, positive and negative predictive values) was done to test the validity of interleukin-6 test in predicting different outcomes.

Results:
A total of 50 cases were investigated. The mean gestational age at delivery was 35± .792 weeks, and mean birth weight was 2300± 264.717 grams (table I).

Table I: Characteristics of the study group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean maternal age (years)</td>
<td>28±9.829</td>
</tr>
<tr>
<td>Mean gestational age at delivery (wk)</td>
<td>35± .792</td>
</tr>
<tr>
<td>Ethnic origin (Arabs)</td>
<td>80%</td>
</tr>
<tr>
<td>(Non-Arabs)</td>
<td>20%</td>
</tr>
<tr>
<td>Mean birth weight (gms)</td>
<td>2300± 264.717</td>
</tr>
</tbody>
</table>

Neonatal infections were noted in a total of 24 cases including bacteria detection (Escherichia coli 10 cases, GBS in 6) and abnormal laboratory findings in 8 cases.

Table II: Associations between neonatal infection and different parameters

<table>
<thead>
<tr>
<th>Neonatal Infection</th>
<th>Present (n=24)</th>
<th>Absent (n=26)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal IL-6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>17 (70.8%)</td>
<td>4 (15.4%)</td>
<td>0.000*</td>
</tr>
<tr>
<td>Negative</td>
<td>7 (29.2%)</td>
<td>22 (84.6%)</td>
<td></td>
</tr>
<tr>
<td>Umbilical artery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH &lt; 7.1</td>
<td>18 (75%)</td>
<td>2 (7.7%)</td>
<td>0.000*</td>
</tr>
<tr>
<td>&gt; 7.1</td>
<td>6 (25%)</td>
<td>24 (92.3%)</td>
<td></td>
</tr>
<tr>
<td>Histologic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chorioamnionitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>21 (87.5%)</td>
<td>3 (11.5%)</td>
<td>0.000*</td>
</tr>
<tr>
<td>Absent</td>
<td>3 (12.5%)</td>
<td>23 (88.5%)</td>
<td></td>
</tr>
</tbody>
</table>

*P <0.05 is significant

Table II shows a highly significant association between neonatal infection and other parameters:
positive vaginal interleukin 6, low umbilical artery pH and histologic amnionitis (p=0.000).

Table III: Accuracy of vaginal Interleukin-6 test in prediction of different outcomes

<table>
<thead>
<tr>
<th>Vaginal Interleukin-6</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal infection</td>
<td>72.7 %</td>
<td>84.6 %</td>
<td>80%</td>
<td>78.5 %</td>
</tr>
<tr>
<td>Chorioamnionitis</td>
<td>87.5 %</td>
<td>88.4 %</td>
<td>87.5%</td>
<td>88.4%</td>
</tr>
<tr>
<td>Neonatal acidosis</td>
<td>95%</td>
<td>93.1%</td>
<td>90.4%</td>
<td>96.4%</td>
</tr>
</tbody>
</table>

Table III represents the validity of vaginal IL-6 test in predicting different outcomes. Correlations among different parameters by Pearson correlation revealed: A positive significant (p<0.0001) correlation between IL-6 and histologic amnionitis, neonatal infection and acidosis (r=0.723, 0.561, 0.836 respectively). When correlating other parameters together, the results showed a positive strong correlation between neonatal infection and neonatal acidosis (r=0.723), neonatal infection and histologic chorioamnionitis (r=0.760) and lastly choriomnionitis and neonatal acidosis (r=0.886).

**Discussion:**

An intense inflammatory reaction at the site of prematurely ruptured membranes was noted as early as 1950, and this suggested infection. McGregor and colleagues,12 in 1987, demonstrated that in vitro exposure to bacterial proteases reduced the bursting load of fetal membranes.

No available, non-invasive tests currently provide early screening of fetal infection to help obstetricians determine the need for antibiotic treatment, cessation of tocolysis, or even early delivery. Antenatal detection of neonatal infection would also make it possible to target more accurately the population of newborn infants who require antibiotic treatment.5

Several studies were done correlating IL-6 with either chorioamnionitis, neonatal sepsis or both. Goffinet et al.,13 in 2005, demonstrated in their study that interleukin-6 detection in vaginal secretions allows the identification of a small group of women at high risk of neonatal infection, independently of other markers. Jun et al.,14 in 2000, demonstrated that cervical fluid Interleukin-6 determinations are of value in the assessment 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. Rizzo et al.,15 in 1998, evaluated the value of IL-6 in cervical secretions to diagnose microbial invasion of the amniotic cavity in patients with premature rupture of membranes. They concluded that intra-amniotic infection was associated with increased levels of IL-6, in cervical secretions related to amniotic levels.

On the other hand, Hitti et al.,16 in 2001, while evaluating the role of the IL-6 and other cytokines as predictors of amniotic fluid infection, found that IL-6 was not associated with amniotic fluid infection.

In our study, 50 patients attending the antenatal unit, complaining with premature prelabor rupture of membranes after 34 weeks of age were recruited, and levels of vaginal interleukin-6 were evaluated. Interleukin-6 showed 72.7% sensitivity, 84.6% specificity, 80% PPV and 78% NPV for prediction of neonatal infection and 87.5% sensitivity, 88.4% specificity, 87.5% PPV, and 88.4% NPV for histologic chorioamnionitis, while highest test accuracy was for neonatal acidosis where sensitivity was 95%, specificity, 93%, PPV 90%, and NPV was 96%. Among group of infected newborns (24 neonates), 70.8% had positive IL-6 versus only 15.4% among the non infected group (26 neonates) (p=0.000). A similar study by Kayem et al.,5 in 2005, evaluated IL-6 levels in cervicovaginal secretions using an immunochromatographic test, it showed 79% sensitivity to detect clinical neonatal infection, 56% specificity 30% PPV, and 92% NPV.

Various mechanisms may explain the association between IL-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. It may also be a sign of a primary inflammation process at the amnio-chorionic-decidual surface. Another possibility is that IL-6 levels in vaginal secretions may mirror the IL-6 in amniotic fluid after membrane rupture.17

When other parameters were correlated together, a positive strong association was noticed between neonatal infection and neonatal acidosis (p=0.000). However, no relationship between sepsis and any umbilical artery blood gas parameter was found in a study done in 1999 on 93 neonates with umbilical artery pH <7.0.18 Also in our work, we observed a positive strong correlation between histologic chorioamnionitis and neonatal acidosis (r=0.886)(p=0.000). Recently, as reported in a retrospective cohort study no relationship was found between the degree of fetal inflammation and umbilical arterial pH.19 The difference between their results and ours might be explained by the fact that their study population was between 23 to 34 weeks gestation, while our sample was >=34 weeks. Accordingly the mechanism of fetal acidosis might be different.
Conclusion:
Determination of interleukin-6 by immunochromatographic bedside test of vaginal secretions is a sensitive, non-invasive prenatal vaginal marker of neonatal infection, histologic chorioamnionitis, and neonatal acidosis in the setting of premature rupture of membranes, even in small units with limited equipments. We recommend multicentric studies to evaluate its clinical applications in larger populations.

References:


