Evaluate the diagnosis of neonatal sepsis by measuring interleukins: A systematic review

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Neonatal sepsis is a dangerous and common disease among infants which is associated with high morbidity and mortality. Interleukins may be helpful for diagnosis of neonatal sepsis. Therefore, this study is conducted to investigate the role of interleukins in the diagnosis of neonatal sepsis. In this study, databases including PubMed, Cochrane Library, ISI and Google Scholar were searched up to 2016. Keywords were: Sepsis, neonatal, interleukins, prediction and diagnosis. Study inclusion criteria were: Articles about the relationship between the diagnosis of neonatal sepsis and interleukins; studies on babies; English and Persian articles and enough information from test results. Articles that had focused on adult sepsis or had used other markers except ILs or just their abstracts were available were excluded from the study. Of 100 searched studies, eventually, 16 articles were considered including 12 prospective studies, 3 cross-sectional studies and 1 retrospective study. IL6 has been studied more than other interleukins (50% of articles). ILs 6, 8 and 10 are among the initial markers of neonatal sepsis diagnosis. IL6 above 68 pg/ml had 85% sensitivity and 80% specificity, IL8 above 269.51 pg/ml had 80% sensitivity and 50% specificity, IL10 above 27 pg/ml had 60% sensitivity and 87% specificity and combined interleukins above 186.83 pg/ml had 75.63% sensitivity and 71.49% specificity in sepsis diagnosis. Interleukins can be helpful in the diagnosis of neonatal sepsis based on the results of this study. IL6 had the most sensitivity and IL10 had the most specificity for diagnosis of sepsis.

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1. Introduction

Neonatal sepsis is along with high neonatal morbidity (1–10 per 1000 live birth) and mortality (15–50%), especially in preterm babies.1,2 Neonatal infections are a major cause of infant mortality so that in our center, it was the third leading cause of neonatal death and had allocated about 25% of causes of neonatal death to itself.1 Neonatal sepsis is a major challenge for newborn specialists generally due to
non-specific symptoms as well as the absence of an early definitive diagnostic test\(^2\).

Since the amount of successful treatment depends on early initiation of appropriate antibiotic, empirical antimicrobial therapy usually starts very early in all infants with clinical signs of sepsis. On the other hand, this clinical practice exposes babies to adverse effects of antimicrobial agents as well. In addition, it increases the duration of hospitalization and costs and creates and expands the cases resistance to different types of bacteria.\(^3\) Early detection of neonatal sepsis is an essential prerequisite for improving survival and treatment outcomes.\(^4\)

It was found in a retrospective study on infants under investigation and treatment of neonatal sepsis with clinical symptoms suspected to primary infection during the first week of life that most of these infants did not require antibiotic. This means that only 10% of these infants had specified confirmed neonatal sepsis, while about 90% of babies were taking unnecessary antibiotics.\(^5\) Conventional hematological and microbiological methods which are routinely used for diagnosis of neonatal sepsis cannot reduce deaths and serious complications of neonatal sepsis. One of the wishes of babies’ specialists is to find an ideal biomarker or biomarkers to provide early, specific and valid detection of babies at risk of infection. Increasing understanding of the ability of the immune system of infants in response to infection will lead to a better identification of biomarkers for improving diagnosis, treatment and prognosis of neonatal sepsis in future.\(^6\)

Isolation of microorganisms from body fluids including blood, cerebrospinal fluid and urine are methods of gold standard for diagnosis of neonatal infection. But, microbiological culture is not available before at least 36–48 h.\(^7\) So accurate laboratory tests are required to rule out infection and reduce unnecessary antibiotic treatment.\(^8\) So hematological parameters and interleukins may be helpful for early diagnosis of neonatal sepsis.\(^9\) Many studies have tried to find valid initial reaction of cytokines for early diagnosis of neonatal sepsis.\(^10\) Inflammatory process in sepsis is very complex in terms of biochemical. Based on the results of laboratory and clinical studies, it has been clear that some pro-inflammatory cytokines reach their peak very quick within one to four hours after the onset sepsis.\(^11\) Analysis of immunological mediators may contribute to definitive and timely diagnosis of sepsis. Measuring cytokines as markers of sepsis has been taken into consideration in recent years\(^12\) and biochemical markers such as CRP, TNF-\(\alpha\) and ILs have been evaluated as the main indicators for early detection of neonatal sepsis.\(^13\) Cytokines are polypeptide messengers with low molecular weight which are created by macrophages and lymphocytes in response to antigenic stimulations or products of inflammation.\(^12\) One of identifying factors of neonatal sepsis is measuring interleukins. So that it is proposed to increase serum levels of interleukins 6, 8 and 10 as a valuable marker for early diagnosis and prediction of sepsis consequences. Levels of IL10 can predict the diagnosis of late neonatal sepsis before positive blood culture.\(^1\) IL10 is an anti-inflammatory cytokine that is often produced by T and B lymphocytes and macrophages and its’ level increases in the incidence of neonatal bacterial infections.\(^14\) Despite recognition of various biomarkers, no single biomarker could be used exclusively for the accurate diagnosis of neonatal sepsis. However, the combination of biomarkers may improve sensitivity of detection and treatment.\(^6\) The results of Boskabadi et al., (2013) showed that IL6 with boundary values of 10.85 pg/ml is distinctive to differentiate sepsis patients and healthy controls and values higher than 78.2 pg/ml from this factor can be used to predict neonatal mortality. IL8 in boundary values of 60.05 pg/ml is used for differentiation of specified infection from unspecified.\(^15\) Results of a study showed that the values of IL10 and CRP in specified infection (sepsis clinical trial) were higher than the control group. The values higher than 14 pg/ml for IL10 in diagnosis of neonatal sepsis had 77.7% sensitivity, 78.8% specificity, 73.6% positive predictive value and 90% negative predictive value.\(^16\)

As interleukins are known as one of the early inflammatory responses to infections, they are potentially
important in early diagnosis and hence proper management of infectious before the establishment of fulminant stage. Since neonatal sepsis is a major cause of mortality and morbidity in infants, and also early detection of neonatal sepsis leads to appropriate treatment and improve neonatal outcomes, this study has systematically reviewed the diagnosis of neonatal sepsis with interleukins.

2. Methods

2.1. Selecting ILs for diagnosis of neonatal sepsis

After initial review of searched articles, the list of interleukins were prepared to carry out a systematic review and only the articles were investigated that have worked on the role ILs in diagnosis of neonatal sepsis. In this regard, the articles containing interleukins such as IL6, IL8 and IL10 or their combination were enrolled for diagnosis of neonatal sepsis.

2.2. Search strategy

We used PubMed, EMBASE and Google Scholar databases in order to do a systematic review and find studies including measures for diagnosis of neonatal sepsis by interleukins. We used the keywords “neonatal sepsis”, “interleukin” and “diagnosis” to search the articles. 100 studies had inclusion criteria which were collected in a separate library file using EndNote software. Among these, 30 duplicated articles were removed. The searched articles were evaluated in terms of title and abstract and 40 articles were also excluded in this stage. From the 30 remaining studies, 15 articles were excluded due to incomplete data, lack of full text, and uncertainty of the type of the study and target group. Finally, 15 articles related to the subject of the study were selected.

2.3. Inclusion criteria

Articles were selected based on the following criteria: 1) Study population is the infants. 2) Neonatal sepsis is confirmed. 3) Interleukins are evaluated for detecting or predicting neonatal sepsis. 4) Articles are in Persian and English language. 5) There is sufficient data from test results.

2.4. Exclusion criteria

To prepare articles appropriate and relevant to the subject, the following articles were excluded: 1) Articles which had reviewed sepsis in adults or animals. 2) Articles which had used other markers than interleukins. 3) The articles that only their abstract was available.

2.5. Data extraction and quality assessment of articles

Articles with full text were received from mentioned databases. Extracted data from them was drawn in Excel software with following titles: name and family name of authors, year of study, study method, study area, subject group, control group, type of IL, IL measurement time, sensitivity, specificity, positive predictive value, negative predictive value and the results of the investigations.

We determined the methodological quality of papers using quality control tool for diagnostic accuracy of studies (QUADAS). This tool consists of 14 questions and “yes”, “no” and “uncertain” answers with scores of respectively 1, -1 and 0 and maximum score of 14.17

Of 100 found articles, finally, 16 articles were reviewed with a sample of 1650 infants (see Fig. 1). The searched articles were related to the years 1994—2016. 8 articles (50%) had evaluated IL6 and 1 article (6.25%) had evaluated IL8, 1 article (6.25%) had reviewed IL10 and 6 articles (37.5%) had used combined markers.

3. Results

3.1. Prevalence studies conducted on IL

The review of studies that have been conducted from 1994 to 2016 show that IL6 is a biomarker that has been studied more than any other interleukin (8 articles, 50% of articles). 6 articles were related to the combination of interleukins, one article was about IL8 and one study was about IL10.

3.2. Heterogeneity of studies

The searched studies on the relationship of interleukins and diagnosis of neonatal sepsis were different in terms of inclusion criteria of infants, defining the subject group, research methodology, sample size, interleukins boundary limit and interleukins diagnostic value. One retrospective study, three cross-sectional studies and 12 prospective studies were conducted. The cutoff for IL6 was between 10 and 181 pg/ml, IL8 was between 54 and 900 pg/ml, IL10 was between 14 and 40 pg/ml. Sensitivity, specificity, positive predictive value and negative predictive value of ILs in searched studies were different (Table 1).

3.3. Global distribution of studies related to interleukins

Most studies related to diagnosis of neonatal sepsis is conducted using interleukins in Iran (6 studies, 37.5%) and then in India (4 studies, 25%), China (1 study, 6.25%), Greece (1 study, 6.25%), Bangladesh (1 study, 6.25%), Denmark (1 study, 6.25%), Brazil (1 study, 6.25%) and Germany (1 study, 6.25%).

3.4. Articles related to the assessment of IL6 (8 article)

Sonawane et al., (2015) in a prospective study evaluated the efficiency of IL6 as a primary diagnostic marker of sepsis. 40 infants with risk factors, clinical signs and symptoms of sepsis as the subject group and 40 healthy infants without risk factors of sepsis were studied as the control group. IL6 had 100 pg/ml, 95.83% sensitivity, 87.50% specificity, 92% positive predictive value, 93.33% negative
Table 1  Summary of conducted articles about diagnosis of neonatal sepsis by interleukins.

<table>
<thead>
<tr>
<th>Author/year</th>
<th>Study method</th>
<th>Location</th>
<th>Subject group</th>
<th>Control group</th>
<th>Markers</th>
<th>Measurement time</th>
<th>Turning point</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
<th>Results</th>
<th>QUADAS scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhao et al., 2015</td>
<td>Prospective</td>
<td>China</td>
<td>140 infants susceptible of having infection (49 infants with sepsis and 91 with Local infection)</td>
<td>61 infants without infection</td>
<td>IL6, 8</td>
<td>Before and 3 days after treatment</td>
<td>IL6: 32 pg/ml</td>
<td>IL6: 87.8%</td>
<td>IL8: 77.6%</td>
<td>IL8: 71.4%</td>
<td>IL6: 79.6%</td>
<td>IL8: 63.8%</td>
<td>IL6: 86.2%</td>
</tr>
<tr>
<td>Boskabadi et al., 2011</td>
<td>Prospective</td>
<td>Iran</td>
<td>36 infants</td>
<td>41 infants</td>
<td>IL10</td>
<td>Within the first 6 h after occurring infection signs</td>
<td>IL6: 14 pg/ml</td>
<td>77.7%</td>
<td>87.8%</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sonawane et al., 2015</td>
<td>Prospective</td>
<td>India</td>
<td>40 infants</td>
<td>40 infants</td>
<td>IL6</td>
<td>During the first 24 h of life</td>
<td>IL6: 100 pg/ml</td>
<td>95.83%</td>
<td>87.5%</td>
<td>92%</td>
<td>IL6 level in patients with sepsis is increased and its' increase rate depends on sepsis severity.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arani et al., 2013</td>
<td>Analytic description</td>
<td>Iran</td>
<td>142 infants</td>
<td>36 infants</td>
<td>IL6</td>
<td>12 h after hospitalization</td>
<td>IL6: 100 pg/ml</td>
<td>95.83%</td>
<td>87.5%</td>
<td>92%</td>
<td>IL6 level in patients with sepsis is increased and its' increase rate depends on sepsis severity.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basu et al., 2012</td>
<td>Prospective</td>
<td>India</td>
<td>36 signed infants with positive blood culture</td>
<td>36 infants without sign and risk factor</td>
<td>IL6</td>
<td>Soon after birth</td>
<td>IL6: 40.5 pg/ml</td>
<td>92.3%</td>
<td>90.48%</td>
<td>IL6 Umbilical cord blood can be used as an early diagnostic marker with high sensitivity and specificity and with 40.5 pg/ml boundary limit.</td>
<td></td>
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</tr>
<tr>
<td>El-Sonbaty et al., 2016</td>
<td>Prospective</td>
<td>Greece</td>
<td>36 infants susceptible of having clinical sepsis, 48 infants with confirmed sepsis by culture</td>
<td>32 infants without infection</td>
<td>IL6, 8</td>
<td>6 h after hospitalization</td>
<td>CRP: 12 mg/l</td>
<td>91%</td>
<td>CRP: 100%</td>
<td>CRP: 47%</td>
<td>CRP: 83%</td>
<td>CRP: 100%</td>
<td>CRP: 47%</td>
</tr>
<tr>
<td>Kumar et al., 2016</td>
<td>Prospective</td>
<td>India</td>
<td>41 infants with susceptible sepsis</td>
<td>42 healthy infants without clinical and laboratory signs of infection</td>
<td>IL6 and CRP</td>
<td>The first day of occurring symptoms or the first day of hospitalization</td>
<td>IL6: 181 pg/ml</td>
<td>80/1%</td>
<td>IL6: 85/7%</td>
<td>IL6: 100%</td>
<td>IL6: 84/6%</td>
<td>IL6: 81/8%</td>
<td>CRP: 90/5%</td>
</tr>
<tr>
<td>Khaled Noor et al., 2008</td>
<td>Prospective</td>
<td>Bangladesh</td>
<td>45 infants susceptible of having sepsis</td>
<td>30 healthy infants</td>
<td>IL6</td>
<td>At the day of sepsis diagnosis</td>
<td>IL6:10 pg/ml</td>
<td>55.6%</td>
<td>83.3%</td>
<td>IL6 is a new biomarker with high sensitivity and good specificity for sepsis. IL6 has higher diagnostic value than CRP</td>
<td>Determining the rate of IL6 at the beginning of signs and symptoms of infection can be helpful for diagnosis of neonatal sepsis.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bender et al., 2008</td>
<td>Retrospective</td>
<td>Denmark</td>
<td>Sepsis group: A1 with positive blood culture in terms of bacteremia and B1 high susceptible of having sepsis (25 infants)</td>
<td>IL6, IL8, IL10, IL18, TNF-α, INF-γ, PCT</td>
<td>CRP, IL6, IL8, IL10, IL18, TNF-α, INF-γ, PCT</td>
<td>At the day of sepsis diagnosis</td>
<td>IL6: 900 pg/ml</td>
<td>IL6: 71%</td>
<td>IL6: 88%</td>
<td>IL6: 88%</td>
<td>IL6: 88%</td>
<td>IL6: 88%</td>
<td>IL6: 88%</td>
</tr>
<tr>
<td>Campos et al., 2010</td>
<td>Cross-sectional</td>
<td>Brazil</td>
<td>55 infants with sepsis laboratory and clinical evident in first 72 h who have been alive at least to first 7 days.</td>
<td></td>
<td>CRP, Glucose, IL6, IL8 and TNF-α</td>
<td>At the day of sepsis diagnosis</td>
<td>IL6: 97%</td>
<td>IL8: 97%</td>
<td>In infants with high levels of cytokines while birth, abnormality of these parameters will be continued in whole infectious process.</td>
<td>Combination of an early marker with a late marker may reduce the period without paraclinical diagnostic result.</td>
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</tr>
</tbody>
</table>

Diagnostic value of IL6 was more than IL8. IL10 is an early predictive marker for neonatal infection and its' high values are related to severe infection. IL6 level in patients with sepsis is increased and its' increase rate depends on sepsis severity. Inflammatory markers are effective in diagnosis of neonatal sepsis.
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Country</th>
<th>Number and Classification of Subjects</th>
<th>IL6, IL8, CRP</th>
<th>IL6, IL8 Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boskabadi et al., 2013</td>
<td>Prospective</td>
<td>Iran</td>
<td>41 with sepsis clinical and laboratory findings or positive blood culture or cerebrospinal fluid</td>
<td>IL6, IL8</td>
<td>At screening time of neonatal sepsis: IL6: 10/85 pg/ml, IL8: 64/05 pg/ml</td>
</tr>
<tr>
<td>Boskabadi et al., 2010</td>
<td>Prospective</td>
<td>Iran</td>
<td>38 infants with specified infection or clinical sepsis</td>
<td>IL8, CRP</td>
<td>During the primary assessment: IL8: 60 pg/ml, CRP: 6 mg/dl</td>
</tr>
<tr>
<td>Ganesan et al., 2016</td>
<td>Prospective</td>
<td>India</td>
<td>40 infants susceptible of having sepsis</td>
<td>IL6, CRP and hs-CRP</td>
<td>IL6: 114 pg/ml, CRP: 13.49 mg/l</td>
</tr>
<tr>
<td>Buck et al., 1994</td>
<td>Prospective</td>
<td>Germany</td>
<td>11 infants with positive blood culture, 15 infants with clinical sepsis, 41 infants with infection, 54 infants without clinical and laboratory evidence and 101 remaining infants as a complex group</td>
<td>CRP, CBC and IL6</td>
<td>At the time of admission and 24 h after admission: CRP: 13.49 mg/l</td>
</tr>
<tr>
<td>Adib et al., 2007</td>
<td>Cross-sectional</td>
<td>Iran</td>
<td>First group 19 infants with positive blood culture having sepsis symptoms, second group 31 infants with negative blood culture but having 2 or 3 sepsis symptoms, third group 10 healthy infants without sepsis symptoms.</td>
<td>IL6, CRP</td>
<td>At the time of admission: IL6: 30 pg/ml, CRP: 10 mg/ml</td>
</tr>
<tr>
<td>Gharehbaghi et al., 2015</td>
<td>Prospective</td>
<td>Iran</td>
<td>141 preterm infants including: 1) group A, 12 infants with early sepsis signs and symptoms based on positive blood culture in first 72 h of birth. 2) group B, 24 infants with diagnosis of clinical sepsis. 3) group C, 61 infants with probable infection and negative blood culture and 4) group D, 44 infants without clinical and laboratory symptoms of infection in first 72 h of life.</td>
<td>CRP, IL6</td>
<td>Immediately after birth and after cutting umbilical cord: IL6: 18 pg/ml</td>
</tr>
</tbody>
</table>

Simultaneous assessment of IL6, IL8 and IL 10 can improve diagnostic sensitivity and specificity of early neonatal sepsis.

IL8 is a valid and primary predictive marker for neonatal infection and is related to the infection severity.

IL6 is a very sensitive marker and CRP is a very specific marker for diagnosis of neonatal sepsis.

Measuring IL6 for diagnosis of neonatal sepsis especially before the onset of full symptoms of sepsis within the first 24 h of infection is more useful than CRP.

IL6 has fairly good sensitivity and medium specificity for detecting early sepsis and non-infectious ill infants.
predictive value and 92.50% accuracy. The results showed that IL6 has maximum sensitivity and specificity compared with other septic markers (CRP, Micro-ESR). It was also found that IL6 level is increased in patients with sepsis and its increase rate is depended on the severity of sepsis. In the study of Arani et al., (2013), infants were divided into three groups: Group 1) infants who were hospitalized with positive blood culture. Group 2) infants who were hospitalized with negative blood culture and susceptible to neonatal sepsis. Group 3) healthy and term infants with similar age and weight who were hospitalized due to jaundice (control group). The average plasma level of IL6 for sepsepsis group with signs and symptoms of sepsis and positive blood culture was 1545.65 pg/ml and it was 14.79 pg/ml with sepsis sign and symptoms but with negative blood culture (susceptible to sepsis group) and 11.04 pg/ml in control group. Comparison of tests showed that there is a significant difference between IL6 levels in the three groups (p = 0.001). Basu et al., (2012) checked 87 infants with risk factors of neonatal sepsis before birth for 72 h in terms of sepsis. Sensitivity and specificity of IL6 with 40.5 pg/ml cutoff was respectively 92.3% and 90.48%. In a study by Kumar et al. (2016), IL6 with a turning point of 181 pg/ml had sensitivity, specificity, positive predictive value and negative predictive value of 80.1%, 85.7%, 84.6% and 81.8% respectively. In a study by Khaled Noor et al., (2008), IL6 had 55.6% sensitivity and 83.3% specificity in diagnosis of neonatal sepsis. IL6 had 100% sensitivity, 47.61% specificity, 12% positive predictive value, 100% predictive value and 51.11% accuracy compared with CRP. In the study of Buck et al., (1994), IL6 was more sensitive than CRP in diagnosis of neonatal sepsis at the time of admission (73% towards 58%). Adib et al., (2007) reported sensitivity, specificity, positive predictive value and negative predictive value of IL6 (with 30 pg/ml cutoff) respectively 78%, 95%, 100% and 87% for diagnosis of neonatal sepsis. Gharehbaghi et al. (2015), in a prospective study reviewed 141 preterm infants at 26–35 weeks in terms of the relation between early sepsis and increasing the levels of CRP and IL6 in plasma of umbilical cord. They reported the turning number of 18 pg/ml, 72% sensitivity and 55% specificity for diagnosis of early sepsis.

The average values of IL6 in the proposed studies showed that an IL6 above 68 pg/ml has 85% sensitivity and 80% specificity.

3.5. Articles related to the assessment of IL8 (1 item)

Boskabadi et al., (2010) in their study reported sensitivity, specificity, positive predictive value and negative predictive value for IL8 boundary limit above 60 pg/ml respectively 95%, 10%, 97% and 10%.

3.6. Articles related to the assessment of IL10 (1 item)

Boskabadi et al., (2011) showed that the values above 14 pg/ml of IL10 has sensitivity, specificity, positive predictive value and negative predictive value of respectively 77.7%, 87.8%, 73.6% and 90%.

3.7. Articles related to the assessment of combined interleukins (6 articles)

Zhao et al., (2015) evaluated the importance of IL6 and IL8 in diagnosis of neonatal sepsis. IL6 with 32 pg/ml boundary limit had sensitivity, specificity and accuracy of respectively 87.8%, 79.6% and 81.4% for diagnosis of neonatal sepsis. IL8 with 54 pg/ml boundary limit had 77.6% sensitivity, 63.8% specificity and 67.2% accuracy for diagnosis of neonatal sepsis. Combination of IL6 and IL8 had also sensitivity, specificity and accuracy of respectively 71.4%, 86.2% and 82.6%. El-Sonbaty et al., (2016) conducted a study to assess IL6, TNF, CRP and IL1 to confirm or reject the diagnosis of neonatal sepsis in the early stages before receiving antibiotics in Greece. CRP with 12 pg/ml boundary limit had 91% sensitivity and 100% specificity, TNF above 113.2 pg/ml had 83% sensitivity and 100% specificity, IL6 above 16.8 pg/ml had 100% sensitivity and 47% specificity and IL1 above 15 pg/ml had 100% sensitivity and 47% specificity for infection diagnosis before starting treatment with antibiotics. According to the results of the study, TNF and CRP markers were superior towards determining the amount of IL6 and IL1 values in sepsis diagnosis. Bender et al., (2008) evaluated early and late markers for diagnosis of neonatal sepsis in Denmark. Levels of IL6, IL8, IL10, IL18, TNF-a, and INF-y, procalcitonin (PCT) and CRP were measured in a 8-hour intervals (at 0, 8, 16, 24, 48 and 72 h after suspicion of neonatal sepsis). IL8, IL10 and IL6 were from new markers and PCT, I/T and CRP were from old markers of diagnosis of neonatal sepsis. PCT above 25 pg/ml for diagnosis of neonatal sepsis had 70% sensitivity and 86% specificity. IL6 above 250 pg/ml had 71% sensitivity and 88% specificity; IL8 above 900 pg/ml had 50% sensitivity and 88% specificity; IL10 above 40 pg/ml had 43% sensitivity and 87% specificity. The ratio of immature neutrophils to total (I/T) above 0.35 had 59% sensitivity and 88% specificity. The results of TNF, IL18 and INF couldn’t predict neonatal sepsis. In this study, PCT and CRP were late diagnostic marker for neonatal sepsis. Thus, combination of IL6 with PCT is a good way to evaluate neonatal sepsis at susceptible time of infection. The old primary marker (I/T) is effective almost as much as IL6. Combination of an early marker and a late marker may reduce paraclinical diagnostic time. Campos et al., (2010) evaluated the serum levels of cytokine and clinical and laboratory parameters. IL6, IL10, IL18 and TNF-a were measured by ELISA method. Although not much changes were created in the values of these cytokines during the study, but, infants having the highest levels of cytokines during sepsis already had their increased levels in umbilical cord blood which their levels were already high up to 96 h after sepsis diagnosis. Clinical and laboratory parameters between infants with sepsis varied widely. Abnormal parameters persisted throughout the infectious process in infants with high levels of cytokines at birth. Boskabadi et al. (2013) in a study assessed the serum levels of interleukins 6, 8 and 10 as diagnostic markers for infant infection and mortality. The study results showed that there is a significant statistical difference between the two groups of subject and control in terms of serum levels of IL8, IL6 and IL10. IL6 with 10.85 pg/ml is proposed
for differentiating the two groups of subject and control and 78.2 pg/ml for prediction of neonatal death. IL8 with 60.05 pg/ml is valuable for differentiating specified infection from unspecified. In this study, however, IL10 level in the subject model was more than the control group; but it was not helpful as IL6 and IL8 in predicting and diagnosing neonatal infection. Measuring IL8, IL6 and IL10 in infants suspected of having sepsis with mentioned boundary limit values may predict neonatal sepsis and prevent receiving unnecessary antibiotics. The researchers concluded that simultaneous assessment of IL8, IL6 and IL10 can improve sensitivity and specificity of diagnosis of neonatal sepsis. These combined markers had 89% sensitivity and 100% specificity in differentiating subject and control groups.15

## Table 2

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Boundary values (pg/ml)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Boundary range</th>
<th>Sensitivity range (%)</th>
<th>Specificity range</th>
<th>Average of boundary limit</th>
<th>Average of sensitivity</th>
<th>Average of specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL6</td>
<td>32</td>
<td>87.8%</td>
<td>79.6%</td>
<td>10–100 pg/ml</td>
<td>71–100%</td>
<td>47–95%</td>
<td>67.31 pg/ml</td>
<td>84.10%</td>
<td>79.25%</td>
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<tr>
<td></td>
<td>100</td>
<td>95.83%</td>
<td>87.5%</td>
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<td></td>
<td>40.5</td>
<td>92.3%</td>
<td>90.48%</td>
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<td>16/8</td>
<td>100%</td>
<td>47%</td>
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<td>181</td>
<td>80/1%</td>
<td>85.7%</td>
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<td></td>
<td>10</td>
<td>55.6%</td>
<td>83.3%</td>
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<td></td>
<td>250</td>
<td>71%</td>
<td>88%</td>
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<td></td>
<td>10/85</td>
<td>92/5%</td>
<td>97/3%</td>
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<td></td>
<td>51.29</td>
<td>100%</td>
<td>62.86%</td>
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<td></td>
<td>30</td>
<td>78%</td>
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<td></td>
<td>18</td>
<td>72%</td>
<td>55%</td>
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<tr>
<td>IL8</td>
<td>54 pg/ml</td>
<td>77.6%</td>
<td>63.8%</td>
<td>54–900 pg/ml</td>
<td>50–95%</td>
<td>10–88%</td>
<td>269.51 pg/ml</td>
<td>79.8%</td>
<td>51.45%</td>
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<tr>
<td></td>
<td>900 pg/ml</td>
<td>50%</td>
<td>88%</td>
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<tr>
<td></td>
<td>64/05 pg/ml</td>
<td>93/7%</td>
<td>44%</td>
<td></td>
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<tr>
<td></td>
<td>60 pg/ml</td>
<td>95%</td>
<td>10%</td>
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<tr>
<td>IL10</td>
<td>14 pg/ml</td>
<td>77.7%</td>
<td>87.8%</td>
<td>14–40 pg/ml</td>
<td>43–77.7%</td>
<td>87–87.8%</td>
<td>27 pg/ml</td>
<td>60.35%</td>
<td>87.4%</td>
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<td></td>
<td>40 pg/ml</td>
<td>43%</td>
<td>87%</td>
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<tr>
<td>IL6, IL8, IL1, IL10</td>
<td>10/85</td>
<td>IL6: 10/85</td>
<td>IL6: 97/3%</td>
<td>IL6: 97/3%</td>
<td>IL6: 97/3%</td>
<td>IL6: 97/3%</td>
<td>186/83 pg/ml</td>
<td>75/63%</td>
<td>71/49%</td>
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<td></td>
<td>IL8: 4/05</td>
<td>IL8: 93/7%</td>
<td>IL8: 44%</td>
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<td></td>
<td>IL6: 32</td>
<td>IL6: 87.8%</td>
<td>IL6: 79/6%</td>
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<td></td>
<td>IL8: 54</td>
<td>IL8: 77/6%</td>
<td>IL8: 63.8%</td>
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<td></td>
<td>IL6: 16/8</td>
<td>IL6: 100%</td>
<td>IL6: 47%</td>
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<td></td>
<td>IL1: 15</td>
<td>IL1: 100%</td>
<td>IL1: 47%</td>
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<tr>
<td></td>
<td>IL8: 900</td>
<td>IL8: 71%</td>
<td>IL8: 88%</td>
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<tr>
<td></td>
<td>IL6: 250</td>
<td>IL8: 50%</td>
<td>IL8: 88%</td>
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<td></td>
<td>IL10: 40</td>
<td>IL10: 43%</td>
<td>IL10: 87%</td>
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challenge due to non-specific clinical signs, lack of standard boundary limit values of sepsis markers and difficulty of differentiating it from non-infectious conditions such as respiratory distress syndrome. So, a reliable test is required for diagnosis of neonatal sepsis. Because, delay in beginning treatment with antibiotics can lead to early death during the hours of onset of symptoms of infection. Despite that blood culture is a gold standard for diagnosis of neonatal sepsis, but, it is not actually helpful in early diagnosis of neonatal sepsis. IL-assessment has been approved in recent studies in order to reduce the time of diagnosis and increase the accuracy of diagnostic tests in the early of infection. Increasing cytokines may be created in normal status after childbirth and this can limit the application of cytokines as a diagnostic marker in newborns care section especially immediately after childbirth. Also, there are numerous other variables such as hypoxia, fetal distress, and prematurity, steroid use before calving and meconium aspiration which increase cytokines levels and limit their application in diagnosis of early neonatal sepsis. Chemokine and pro-inflammatory cytokines are essential for host defense against microbial infection, but, increase of activated pro-inflammatory mediators can cause harmful results and lead to extensive damages of small blood vessels, dysfunction of multiple organs and death.

Several interleukins with different boundary limit, sensitivity and specificity were evaluated for diagnosis of neonatal sepsis. But, neonatal sepsis is still a major challenge in medicine of newborns due to the lack of a standard in the values of boundary limit of interleukins. IL6 is used in most studies for diagnosis of neonatal sepsis.

IL6 is a marker that recently has been taken into consideration for early diagnosis of neonatal sepsis. IL6 is created by monocytes, endothelial cells, fibroblasts and lymphocytes T and B and is much more sensitive than CRP. But, it cannot be used for sepsis as a single marker due to its’ short half time. The study results of Buck et al., showed that IL6 level reach its’ peak at the time of admission and is non-quantifiable after 24 h. Since IL6 has a vital role in inducing the creation of CRP in liver, there is the hypothesis that this cytokine is identified in the blood in earlier levels of bacterial infection compared with CRP. The results of the studies showed that the average of serum level of IL6 in infants with sepsis is higher than healthy infants. So, IL6 can be applied as an important marker for early neonatal sepsis in neonates care sections. Since studies on adults with sepsis have shown that increasing IL6 level is along with higher mortality, it seems that this mediator is important in pathogenesis of sepsis. One advantage of IL6 assessment is that its’ level is increased at the onset of infection, while CRP reaches its’ maximum concentration with delay. IL6 of umbilical cord blood is a better predictor for starting treatment immediately after birth in infants having risk factors of infection before birth compared with CRP. So, increasing concentration of IL6 and CRP is a risk factor of preterm delivery before 32 weeks. IL6 has the highest sensitivity (89%) and negative predictive value (91%) at the onset of infection compared with other chemical markers such as TNF and CRP. Hu et al., (2015) evaluated the diagnostic value of IL6 for neonatal sepsis using meta-analysis and reviewed 33 studies including 3135 infants. Sensitivity and specificity of IL6 for diagnosis of neonatal sepsis was calculated respectively 79% and 83%. Shakkar et al., (2011) conducted a systematic review and evaluated the role of IL6 to predict neonatal sepsis using meta-analysis method. They reviewed 13 studies including 353 infants with sepsis and 691 infants of control group. Sensitivity and specificity of IL6 were respectively 79% and 84%. According to the results of this study, IL6 is a valid marker for prediction of neonatal sepsis and can be applied for sepsis early diagnosis in neonates care units. Based on the results of studies, IL6 higher than 68 pg/ml has 85% sensitivity and 80% specificity. IL8 is a valid and primary predictive marker for neonatal infection which is relevant with severity of infection.

IL8 is a pro-inflammatory cytokine which is often created by monocytes, macrophages and endothelial cells and causes the release, activation and chemotaxis of neutrophils and its’ level is increased in early stages of neonatal bacterial infections. It is so that based on the results of a study, IL8 concentration in died infants with sepsis is increased 3.3 times compared with survived infants. Boskabadi et al., in their study showed that serum concentration of IL8 in infants with confirmed sepsis is significantly higher than in healthy infants before blood culture become positive. Also, the serum level of this marker in the infants with sepsis who died was much higher than survived infants. Sensitivity, specificity, positive predictive value and negative predictive value for IL8 was respectively 95%, 10%, 97% and 10% and for CRP, it was respectively 83%, 86%, 83% and 69%. Boundary limit of IL8 was above 60 pg/ml and boundary limit of CRP was above 6 pg/ml. Results of a study showed that IL8 in diagnosis of definite infection is more effective than IL6 and IL10. IL8 above 60 pg/ml as an accurate marker is considered with a sensitivity of 80 to 91% and a specificity of 76 to 100%. In a study, IL8 with 54 pg/ml boundary limit had 77.6% sensitivity, 63.8% specificity and 67.2% accuracy for diagnosis of neonatal sepsis. In the study of Kocabaset al, the increase of IL8 in early and late neonatal sepsis had 80 to 91% sensitivity and 100% specificity. Zhou et al., (2015) in a meta-analysis study reviewed the role of IL8 in diagnosis of neonatal sepsis. 8 studies including 548 infants entered the study. IL8 had moderate accuracy for diagnosis of neonatal sepsis. IL8 above 269.51 pg/ml had 80% sensitivity and 50% specificity in sepsis diagnosis.

IL10 is an early predictive marker for neonatal infection and its’ high values are relevant to very severe infection. Gram-negative infections are associated with higher concentrations of IL10 compared with Gram-positive infections. Boskabadi et al., showed that quantitative measurement of IL10 can help the neonates’ specialists to predict the infection severity. In addition, IL10 values in died infants was higher than survived infants. IL10 above 27 pg/ml had 60% sensitivity and 87% specificity in diagnosis of neonatal sepsis.

Various markers such as IL6, PCT, CRP and IL8 are applied both for early diagnosis (24–48 h) of neonatal sepsis and for controlling antibiotic treatment due to not having delay in
Neonatal sepsis by measuring ILs

diagnosis compared with time consuming results of blood culture. IL6, IL8 and oxidative parameters in umbilical cord blood can predict neonatal sepsis in neonates with known risk factors. IL6 and IL8 increase in inflammatory responses and values of these factors will be changed based on the severity of infection. In a study, combination of IL6 and IL8 had 71.4% sensitivity, 86.2% specificity and 82.6% accuracy. Diagnostic value of IL6 was more than IL8. Combination on IL6 and IL8 can increase the diagnostic accuracy of neonatal sepsis.

Simultaneous assessment of IL6, IL8 and IL10 can improve sensitivity and specificity of early diagnostic of neonatal sepsis. These combined markers have 89% sensitivity and 100% specificity in differentiation of subject and control groups. In the study of Franz et al., combination of IL8 and CRP for early diagnosis of neonatal sepsis had 91% sensitivity and 73% specificity.

According to the results of conducted studies, combined interleukins above 186.83 pg/ml have 75.63% sensitivity and 71.49% specificity.

One of the strengths of this study is that it was found within the searches that this study is the only study which has examined the role of interleukins in diagnosis of neonatal sepsis. Limitations of this study include lack of access to all articles and unpublished reports, lack of correct and quality and usable report of some articles, limited number of articles in the field of each interleukin and impossibility of accurate judgment about their effect, lack of measuring variables with same method, lack of similar definition for subject group in studies and lack of a standard in boundary limit of interleukins.

5. Conclusion

Wide effort has been done in order to find studies related to diagnosis and prediction of neonatal sepsis. The found studies were different in terms of methodology, method, boundary limit of interleukins and diagnostic value of interleukins. The results of the studies showed that IL6, IL8 and IL10 are primary markers for diagnosis of neonatal sepsis. IL8 above 269.51 pg/ml had 80% sensitivity and 50% specificity in sepsis diagnosis. IL10 above 27 pg/ml had 60% sensitivity and 87% specificity in diagnosis of neonatal sepsis. Based on the results of studies, IL6 above 68 pg/ml had 85% sensitivity and 80% specificity. However, IL6 had the most sensitivity and IL10 had the most specificity for sepsis diagnosis.

Combination of IL6 with other interleukins and diagnostic markers will have higher sensitivity and specificity due to its’ short half time. Despite primary diagnostic markers of neonatal sepsis, this disease is yet a major challenge in newborn medicine; it may be due to not having standard values of boundary limit of interleukins and high cost of testing. Therefore, it is helpful to conduct extensive studies to identify more interleukins and standardize the values of boundary limit of interleukins in early diagnosis of neonatal sepsis.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

Hereby, the authors of the article appreciate the Deputy of Research, Research Director and other officials and all people who helped us in this project.

References


