Phagocytosis in Pediatric with Septicemia

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Abstract

This study focused on the determine the frequency of microorganisms isolated from blood samples of patients with septicemia admitted to Pediatric Hospital in Kirkuk from March to May 2015. The (41) patients were grouped according to the result of blood culture, 22 case show positive blood culture named as infected group represented (53.65%) other 19 cases show negative blood culture(46.37%) which were taken as control group. The most frequent causative agents of septicemia were Staphylococcus aureus 16(39%) followed by Escherichia coli 6(22%). Phagocytosis results of these 41 clinically suspected septicemia appeared significant difference between infected group and control group (P<0.01), while there were no significant difference in the average of the leukocyte counts between groups but it show significant difference between infected groups and control in neutrophil count p-value P<0.01 There were no significant difference between studied groups in the monocyte count and lymphocyte count except in the lymphocyte count between infected group by the Escherichia coli & control groups.

Keywords: Bacteria; Phagocytosis; septicemia.

1. Introduction

Septicemia refers to generalized infection with positive blood culture in the early28 days of neonates [1]. Advances in early diagnosis and treatment have led to better prognosis of new born, various diseases of new born including septicemia, meningitis, arthritis, pneumonia, osteomyelitis and urinary tract infection [2].
When pathogenic bacteria gain access into the bloodstream, they may cause overwhelming infection without much localization (septicemia) or may get predominantly localized to the lung (pneumonia) or the meninges (meningitis). Neonatal septicemia is a life-threatening emergency and delays in diagnosis and treatment may have adverse consequences, surveillance is needed to identify the common symptoms and signs as well as the antibiotic sensitivity patterns for the agents [3]. Most cases of neonatal sepsis in the community are caused by Staphylococcus aureus and Escherichia coli. In hospitals, Klebsiella pneumoniae is also a common organism [3]. Hypothermia is a common manifestation of sepsis, whilst fever is infrequent. Diarrhea, vomiting and abdominal distension may occur. Episodes of apneic spells or gasping may be the only manifestation of septicemia. In sick neonates, the skin may become tight giving a hide-bound feel (sclerema) and the perfusion becomes poor [4]. Phagocytes are the white blood cells that protect the body by ingesting (phagocytosing) harmful foreign particles, bacteria, and dead or dying cells. Their name comes from the Greek phagein, "to eat" or "devour", and "-cyte", the suffix in biology denoting "cell", from the Greek kutos, "hollow vessel. They are essential for fighting infections and for subsequent immunity [5]. The phagocytes move by a method called chemotaxis. When phagocytes come into contact with bacteria, the receptors on the phagocyte's surface will bind to them. This binding will lead to the engulfing of the bacteria by the phagocyte [6,7,8]. Some phagocytes kill the ingested pathogen with oxidants and nitric oxide[7,9]. After phagocytosis, macrophages and dendritic cells can also participate in antigen presentation, a process in which a phagocyte moves parts of the ingested material back to its surface. This material is then displayed to other cells of the immune system. Some phagocytes then travel to the body's lymph nodes and display the material to white blood cells called lymphocytes. This process is important in building immunity [1,2,8,9]. However, many pathogens have evolved methods to evade attacks by phagocytes [5,9,11].

1.1 Aims of the study

To study the spectrum of the significant bacterial isolates from positive blood culture causing septicemia in Kirkuk Governorate in neonates And study the relationships between phagocytic ratio with differential leukocyte counts.

2. Materials and Methods

Sample Collections:

The 41 patients studied were referred to the Pediatric Hospital of Kirkuk from March to May 2011. The isolated bacteria both of Staph. aureus & E.coli were isolated from children suffering from septicemia. The specimen obtained was blood taken with sterile disposable syringe 0.5 ml of the blood used for phagocytosis other part of Blood was drawn for culture, whenever possible before antimicrobial therapy was started. Sterile equipments and strict aseptic technique were employed. Vein puncture was performed and 3-5 milliliters of blood were drawn, the sampling needle was discarded and another sterile needle fitted. The sample was injected into bottle using aseptic condition. Inoculated in brain heart infusion broth which for cultivation of aerobic bacteria [11,12]. Gram stain was performed on respective to the macroscopic evidence of growth, and random subcultures were carried out every day up to 21 days. All specimens used for subcultures were aspirated from
the blood culture bottles, using one milliliters disposable syringe after cleaning the cover with 2% iodine and 70% alcohol. Smears were prepared directly from specimen. each specimen taken from every patient were inoculated in the Blood agar plates which were incubated aerobically for 24 hrs. at 37C. Other blood agar plates were incubated under CO2 for 24 hrs. at 37C° in a candle jar. Chocolate -agar plates were incubated under CO2 for 24 hrs. at 37C° in a candle jar. MacConky agar plates were incubated aerobically for 24 hrs. at 37C°, all isolates were identified using the conventional methods [13]. Immunological tests done by measuring of phagocytosis according to the method [14]. Hematological tests done by counting total leukocytes according to method [15].

Statistical analysis

Complete Randomized Design (C.R.D.) was used as an experimental design. Data were analyzed using SAS [16] to study the effect of different factors on the diameters of inhibition zones. Least significant difference (LSD) was used to compare the significant difference between means at P≤ 0.05.

3. Results and Discussion

Isolation and Identification of bacterial strains:

Suspected bacterial colonies were picked up from blood agar and MacConkey's plates and identified by microscopic examination and biochemical tests. the gram-positive cocci were identified by microscopic examination and catalase and coagulase tests. Among the 38 children included in this study 22(58%) was positive other are negative.

![Figure 1: Percentage of isolated pathogens of septicemia under study](image)

Phagocytosis:-

In this study Table (1) shows the phagocytosis results of these 41 clinically suspected septicemia were reviewed and showed 58% positive result which appeared significant difference between infected group and control group (P<0.01) using t-test.
Table 1: Percentage phagocytosis rate in septicemic patients.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Phagocytosis rate%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected with <em>Staph aureus</em></td>
<td>%52.96+0.090</td>
<td>0.072% 43.81%+</td>
<td>0.0003</td>
</tr>
<tr>
<td>Infected with <em>E.coli</em></td>
<td>%40.30+0.0677</td>
<td>0.071% 42.85+</td>
<td>0.0003</td>
</tr>
<tr>
<td>Infected group both <em>Staph aureus &amp; E.coli</em></td>
<td></td>
<td></td>
<td>0.005</td>
</tr>
</tbody>
</table>

Leukocytes:

In table (2) show no significant difference in the average of the leukocyte counts between groups but table (3) show significant difference between infected groups and control in neutrophil count p-value P<0.01 by using t-test. while the table(4,5) show no significant difference between studied groups in the monocyte count and lymphocyte count except in infected group by the *E.coli* with control groups show significant difference p-value P<0.01 by using t-test.

Neonatal sepsis is a life threatening emerging infection in the developing countries and it is estimated about million neonatal death occur every year worldwide. Therefore, differences in the ethnicity and socioeconomic status may contribute to the varying incidence of septic infection among neonates in different populations[17,18].

In present study the culture positivity was (58.5%), which is quite high to the study of( 19)Which show only 28.30% positive culture while in the study of (20) positive cultures represented about 41% of the cases. 39% of organisms isolated from septicemia cases were *Staph. Aureus* this agreement with other study [19]. Which show *Staphylococcus* were mostly predominant then other organisms also *Staphylococcus aureus* appeared as the most common bacterial agent causing septicemia in the neonatal units in 1950. Followed by *E.coli* which represented 22% disagreement with [20].became a major cause of nursery outbreaks throughout the world.

Table (1) showed significant difference in the phagocytosis between infected groups both *staph & E.coli* with control groups' p-value<0.001 the study showed that after bacteria challenge, A number of factors contribute to the efficient function of phagocytic system. These factors include the presence of adequate numbers of monocytes and neutrophils in the peripheral blood, the ability to respond to signals from sites of inflammation, the migration to these sites and the capacity to ingest and kill the invaded microorganisms. The intake of bacteria is compromised in neonates either due to decreased levels of opsonized proteins or/and to low levels of receptors involved in phagocytosis. Numerous studies have shown that complement components are reduced in both premature and full term neonates [21,22].

Table (2) show no significant difference between infected group and control group in the average of leukocyte count it founded in a study of 49 cases of neonatal septicemia and 18 cases of asymptomatic neonatal septicemia total leucocyte count <5000/cmm [11,18,20].
Table 2: Percentage of phagocyte ratio according to infected bacteria in septicemic ratio

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control %</th>
<th>Phagocytosis rate%</th>
<th>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected with \textit{Staph. aureus}</td>
<td>8.475+3.41</td>
<td>7.975+4.66211683</td>
<td>0.73</td>
</tr>
<tr>
<td>Infected with \textit{E.coli}</td>
<td>8.74+3.9929939</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>Infected group both \textit{Staph. aureus &amp; E.coli}</td>
<td>8.009091+4.395984</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: The average of Neutrophil count between infected group and control group under study.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control %</th>
<th>average of Neutrophil count</th>
<th>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected with \textit{Staph. aureus}</td>
<td>12.91+44.16</td>
<td>28.715+22.66</td>
<td>0.002</td>
</tr>
<tr>
<td>Infected with \textit{E.coli}</td>
<td>12.56+ 25.66</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>Infected group both \textit{Staph. aureus &amp; E.coli}</td>
<td>26.34+18.95</td>
<td>0.0033</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: The average of Monocyte count between infected group and control group under study.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control %</th>
<th>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected with \textit{Staph aureus}</td>
<td>11.15+10.87958</td>
<td>0.655</td>
</tr>
<tr>
<td>Infected with \textit{E.coli}</td>
<td>11.15+10.87958</td>
<td>0.27</td>
</tr>
<tr>
<td>Infected group both \textit{Staph aureus &amp; E.coli}</td>
<td>19.95+14.42</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Table 5: The average of Lymphocyte count between infected group and control group under study.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control %</th>
<th>average of Lymphocyte count</th>
<th>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected with \textit{Staph aureus}</td>
<td>13.97+14.34</td>
<td>15.43+10.63</td>
<td>0.002</td>
</tr>
<tr>
<td>Infected with \textit{E.coli}</td>
<td>29.066+19.28</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>Infected group both \textit{Staph aureus &amp; E.coli}</td>
<td>19.15+14.42</td>
<td>0.0033</td>
<td></td>
</tr>
</tbody>
</table>

Table (3) show significant difference between infected group and control group in the average of neutrophils count while as shown in table (4) and (5) no significant difference between studied groups in monocyte and
lymphocyte except in the lymphocyte count between infected group by *E. coli* and control groups. In the study of 100 babies evaluated that the use of sepsis band to neutrophil count was found to be most sensitive 80% [23], also in study of 49 cases of neonatal septicemia noted that band neutrophil counts were elevated most frequently in proven cases of sepsis. And elevation occurred usually within 24 hours of onset of signs of disease [24,25].

The elevated absolute numbers of neutrophils noted in children might partially compensate the defect found in the ingestion of bacteria by these cells [19, 26, 10].

4. Conclusions

From the results of this paper we concluded that theirs high frequency of *S.aureus* isolated from blood samples of patients with septicemia admitted to Pediatric Hospital in Kirkuk and theirs correlation with phagocytosis

5. Recommendations

We should avoid infection the neonate with septicemia and control the infections.

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