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Role of complement system, C-reactive protein and white blood cell counts in the diagnosis of neonatal septicemia in Gaza city hospitals

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ABSTRACT

Objective: to evaluate the serum levels of complement, CRP and WBCs count for diagnosis of neonatal septicemia in Neonatal Intensive care units (NICU) in Gaza City Hospitals. Methodology: This prospective descriptive study was carried out in Al-Nasser and Al-Shifa hospitals in Gaza City, between January 2004 to January 2005. Blood Samples were collected at admission and during infection. Results: five hundred seventy nine enrolled babies, 193 (33%) were classified as early-onset septicemia, 135 (23%) as late-onset septicemia and 251 (44%) as a nosocomial septicemia. WBCs count were low at 7% of cases, and high at 23% of cases at admission while leucocytosis were recorded in 30% of cases at follow up. Values of C-reactive protein were higher is septicemic neonates with a positive blood culture exhibiting 72%. The concentrations of C3 & C4 levels were low in (99%) of cases at admission, while after infections took place, (33%) had a normal level, 67% had increased levels of C3, whereas C4 shows low levels (64%) at admission and increased in (79%) of cases after proven infection. Conclusion: our study suggests that no individual test can diagnose infected neonates, and that although the combination of WBCs count, C-reactive protein values and C3 & C4 levels exhibits a high specificity.

KEYWORDS

WBCs;
C-reactive protein;
C3 and C4;
Neonatal sepsis.

INTRODUCTION

Sepsis is ranked as the sixth leading cause of death among neonates and the eighth leading cause of death for infants through the first year of life[1]. The incidence of neonatal sepsis is 1 to 5 per 1000 live births[2].

Neonatal sepsis is classified as either early or late based on the timing of presentation. In the literature, however, there is no definitive consensus as to what age limits apply, with early-onset sepsis ranging from 48 hours to 6 days after delivery[3]; late-onset sepsis generally occurs beyond the first week of life.

The clinical relevance of this distinction is that early-onset disease is often due to organism acquired during delivery. Late-onset disease is only occasionally a acquired after delivery (nosocomial or community sources)[3].

Bacterial infections in the neonatal intensive care unit are a major cause of morbidity and mortality[4,5].The early and reliable diagnosis of sepsis in the neonate remains an important goal, but to date no single marker of infection, apart from culture and isolation of the relevant microorganisms, has been identified. Microbiological screening has the disadvantage of requiring 24 to 48 hours to provide results, while potentially life threatening neonatal infections must be treated immediately. Other more indirect markers of infections, such as the white cell, platelet, and neutrophil counts and C reactive protein concentrations, have been shown to provide some diagnostic help[6,7] but do not vary solely in response to the presence of infection[8-10].

Neutrophils are inflammatory cells with potent oxidative and proteolytic potential are usually the first line of defense against invading pathogens. Activated neutrophils produce cytotoxic factors leading to deleterious inflammatory processes, including tissue injury while lymphoid cells are undergoing accelerated apoptosis, spontaneous neutrophils apoptosis associated with septicemia[11].

C-reactive protein is an acute phase protein released by the liver as a consequence of inflammation. CRP is frequently used to assess the presence and severity of inflammatory response. Although CRP is often used as a marker of bacterial infection, it is induced by a variety of non-bacterial stimuli, e.g. after surgery, during autoimmune and rheumatic disorders, or even myocardial infarction and malignant tumors. Despite a relatively high sensitivity, its predictive value was less than cytokines for the diagnosis of infection in febrile patients with episodes of Gram-negative bacteremia[12]. The CRP is another marker of infection, but appears more useful in monitoring response to treatment of infection rather than in its diagnosis[13].

The complement system is comprised of a series of over 30 proteins which are an essential component of host protection against a range of pathogenic organisms[14]. The complement cascade is activated directly by bacteria and antigen-antibody complexes, and the degree of complement activation could, therefore, provide early and specific evidence of bacterial infection. To examine the possibility that triggering of the complement cascade reflects the presence of infection in the neonatal period, we studied the concentrations of complement fragments released during complement activation using techniques recently developed[15-17]. The efficacy of complement activation in distinguishing infected from non-infected neonates is compared with other widely used indicators such as the platelets, neutrophils, and white cell counts and the concentration of C reactive protein.

Therefore, the present study was designed and aimed to determine the levels of white blood cell counts, neutrophil counts, C- reactive protein, C3, and C4 levels in neonatal septicemia in Neonatal Intensive Care Units (NICU) in the two hospitals (AL-Nasser and AL-Shifa hospitals) in Gaza city. The objective of the present study was to determine the diagnostic accuracy of a variety of possible markers of infection and inflammation during disease and after recovery.

METHODOLOGY

Study population

Five hundred and seventy nine prospective newborn infants, who were admitted to NICU (AL-Nasser and AL-Shifa hospitals) in Gaza city and diagnosed clinically as septicemia were enrolled in the study, blood samples were collected from neonates during the period of January 2004 to January 2005.

Blood samples

Blood samples were collected with all a septic precautions and divided into two tubes, the first were collected in silicone vacuum filled tubes (Vacutainer, SST model) after centrifugation at 3500 rpm for 3 minutes, the obtained sera samples were frozen and stored at -20°C until processing, with the exception of the samples for CRP, which were analyzed immediately. The second tubes contains EDTA for CBC counts.

CBC, were analyzed using CBC machine (Cell-dyne Abbott corporation). CRP was determined using CRP-Avitex latex test. Reference values for healthy neonates, using quantitative techniques, are less than 6mg/dl. Radial immunodiffusion test were done according to standard methods (Bindard, 2003). Complement factors 3 (C3) and C4 were measured using RID by measuring

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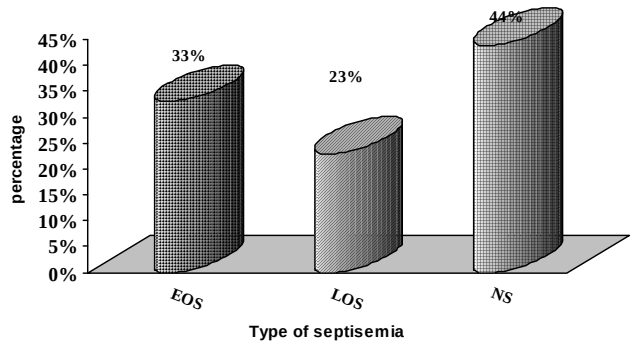

Figure 1 : Distribution of the study population according to the type of septisemia

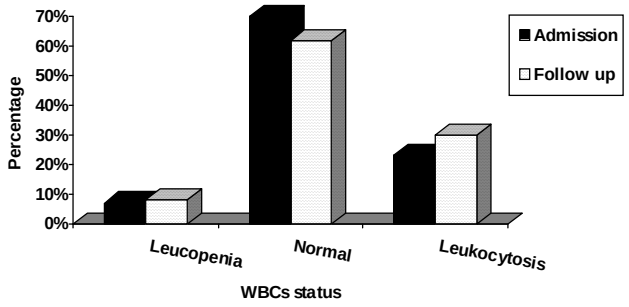

Figure 2 : WBCs status om neonates at admission and follow up

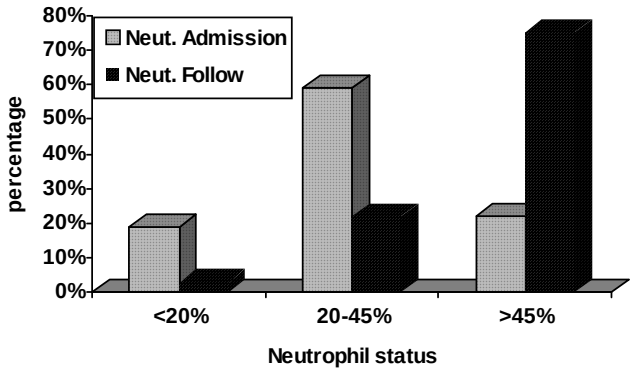

Figure 3 : Neurtophil status at admission and follow up

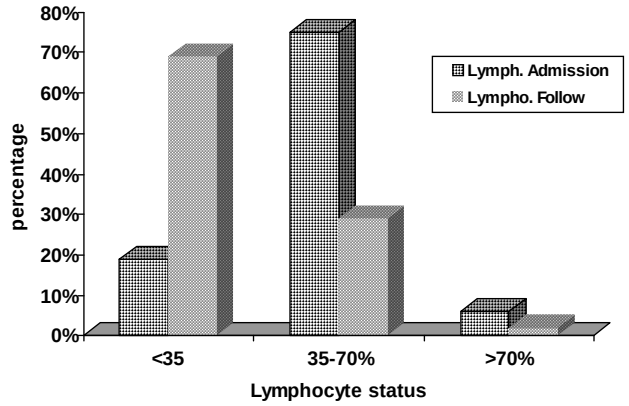

Figure 4 : Lymphocyte status at admission and follow up

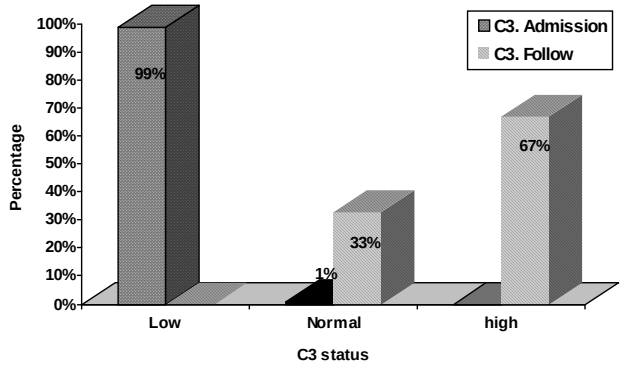

Figure 18 : C3 status at admission and follow up

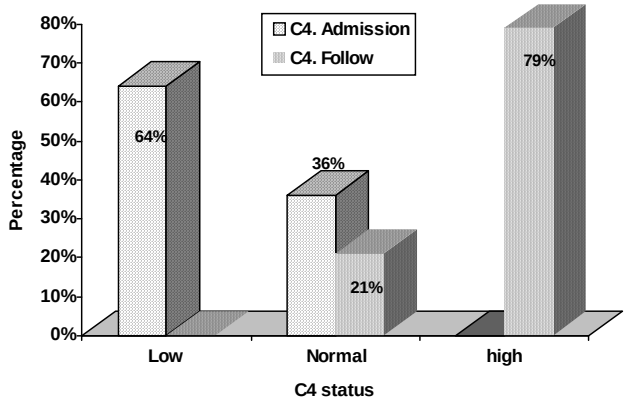

Figure 19 : C4 status at admission and follow up

the diameters nearest to 0.1mm, using eyepiece or RID plate reader. The normal serum levels for C3, 103 – 150mg/dl and C4, 17 – 39mg/dl. Variation Coefficients were less than 0.7 for C3 and less than o.9 for C4.

RESULTS

Among the 579 enrolled babies, 193(33%) were classified as early-onset septicemia, 135(23%)as late-onset septicemia, 251(44%) as a nosocomial septicemia (TABLE 1).

White blood cell count (WBCs)

The average count of WBCs for cases was 12.6×10 cell/μl (average range from 1.3-44.2 ×10 cell /μl). Figure 1 shows the number of WBCs in all cases at admission and follow up, 7% of cases with leucopenia (WBCs < 5×10 cell /μl), 23% of cases with leucocytosis (WBCs > 15×10 cell /μl), while in the follow up in 396 cases still a life in NICU shows (8%) of cases with leucopenia and (3%) with leucocytosis, (34.4%) of cases with leucocytosis were died (Figure 2).

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TABLE 1 : Distribution of study population according to type of sepsis

Sepsis	Frequency	Percentage
Early-onset sepsis	193	33
Late-onset sepsis	135	23
Nosocomial sepsis	251	44
Total	579	100

Figure 2,3 shows an increase in neutrophile count in (22%) of septicemic cases at admission, while (75%) of cases showed a normal lymphocyte count, however after infections took place neutrophile counts were increased in (75%) of cases, while lymphocytes showed a decrease in (2%) of cases.

C- reactive protein(CRP)

CRP levels were higher in infected neonates with a positive blood culture, exhibiting 72% positive and 28% negative (TABLE 2).

Complement measurements

The levels of C3 and C4were measured at the time of neonates admission to the NICU, 99% of the admitted cases shows low C3 level, while after the infections took place (33%) of cases had a normal (103-149mg/dl), (67%) had increased levels of C3 (> 150 mg/dl). Whereas C4 shows (64%) had low level at admission, while after proven infection (79%) of cases had high levels figure 4.

DISCUSSION

Various panels of septic screening tests and/or septic scores have been proposed in order to rationalize managements, including antibiotic administration for newborn infants suspected of being septic[18-20]. This study was based on three laboratory which are readily performed in the laboratory and the results of which can be rapidly available to the clinician.

Previous studies have shown that screening tests and septic scores are insufficiently reliable to allow delay in the initiation of antibiotic use in cases of suspected sepsis. As in previous studies elsewhere[21-23], the WBC count showed a low detection sensitivity in neonatal infection. In our study WBCs count showed an increase in 23% of cases at admission, while during infection 30% of cases had leucocytosis, neutrophil shows an increase in septicemic neonates at admission, whereas lymphocytes counts were in normal range and increases after infections took place as an immune defense against infections with wide range of microorganisms.

TABLE 2 : C-reactive protein in cases

	Frequency	Percentage
Negative<6mg/l	161	28
Positive>6mg/l	400	72
Total	561	100

CRP has been thoroughly studied as a diagnostic tool in neonatal sepsis and also as an indicator of response to therapy[24-26]. In the present study 72% of cases had an elevated values of CRP and only 28% of cases had a normal values.

As complement forms an important arm of the innate immune system, it provides protection against infection during neonatal period, when the specific, acquired immune system is still relatively immature[27]. In the present study, neonates with a presumptive diagnosis of infection made on laboratory hospitals had a low level of C3 and C4 at admission, while after infection there where an increased level of C3 and C4 in 67% and 79% respectively.

In conclusion,no parameter proved useful as a solitary tool to identify infected neonates. The best diagnostic value as an individual test was achieved by CRP.Owing to its unacceptably low sensitivity in identifying newborns with a highly combination (CRP, WBC count and levels of complement).

Recommendation: We recommend the use of combination (CRP, WBC count and levels of complement) to ensure the diagnostic power for early onset sepsis. We hope that future research will disclose a more sensitive and cheaper tests.

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