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Evaluation of Immunoglobulins, Complement and Interleukine-6 in Serum of Iraqi Sepsis Neonates

KEYWORDS: neonates -sepsis-immunoglobulins- Complement components- IL-6.

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ABSTRACT *Back ground: Limited information exists on immune response in Iraqi sepsis neonates. Objective: In this study, we have assessed the humoral immune system in 57 suspected sepsis neonates by measuring their serum concentration of ImmunoglobulinG (IgG), IgM, IgA, C3 and C4 in order to find out a responsible immune defect in these patients in compares to control (healthy) neonates group .Interleukine-6(IL-6) was measured as marker for sepsis. Subjects and methods: blood was taken from (57)full term suspected septicemia neonates, classified into 2 groups. A sepsis group (n = 22) and probable sepsis group (n = 35), and control group(n=23). The parameters studied were: serum immunoglobulins (Ig) including IgA, IgG,IgM, the complement Components (C3, C4) levels were assessed using Single Radial Immunodiffusion (SRID) and IL-6 levels were determined using Enzyme Linked Immunossorbent assay (ELIZA) kit. Results: Serum levels of IgG, IgA, C3 and C4 were significantly lower (p≤0.01) and those of IgM and IL-6 were significantly higher (p≤0.01) in documented sepsis patients than the controls. Considering the result of analytic tests, it was revealed that,sepsis and probable sepsis patients had showed low IgG IgA,C3and C4 levels comber to control` group but no significant differences in IgG and IgA was observed between sepsis group and probable sepsis group. Conclusion: The results of thisstudy points that IgM and IL-6 may be considered as an early diagnostic markers for sepsis andthe need for further research to clarify the role of dysfunction of immunoglobulins, complement systemin sepsis newborn infants and IL-6 correlation with the severity of the disease.*

INTRODUCTION

Sepsis is the inflammatory response of the body to infection, and is one of the major leading causes of death in the first few months of a newborn's life (Sullivan J, 1992). Despite the immune system and immune system components, early development during gestation the newborn still remains vulnerable to infections after they are born because of the immaturity of their immune system(Anna,et al. 2004).

The ability of serum to opsonize invading bacteria is an essential defensive mechanism in infants.The integrity of this function depends mainly on the serum concentrations of certain proteins, collectively known as opsonins. These comprise: immunoglobulins; the C3,C4 complement components; and fibronecrin(Drossou,et al.1995).Immunoglobulin , and with the exception of immunoglobulin G do not cross the placenta and are produced by the fetus in the early stages of gestation(Baker ,1990;Gasque ,et al.2000).

Neonates react to bacterial sepsis with an exaggerated inflammatory response, which may contribute to the high mortality observed in neonatal sepsis (Schultz, et al.2002; Schultz,et al., 2004). The neonatal immune response, however, includes increased production of other inflammatory mediators such as Interleukin-6 (IL-6), IL-1 and TNF-α, the assessment of which may improve diagnostic accuracy in suspected sepsis (Ng, 2004). IL-6 plays many roles in the inflammatory response in relation with the pathogenesis of bacterial infections. Studies have shown that plasma IL-6 levels increase by 64-100% in cases with sepsis (Hack,et al. 1989; Küster,et al. 1998). It is the major regulator of acute protein response. In liver cells, it stimulates the synthesis of C-reactive protein (CRP), serum amyloid A, α1-acid glycoprotein, 1-antitrypsin, and fibrinogen, and is also responsible for myocardial depression (Pathan , et al. 2004). IL-6, stimulated by TNF-α, IL-1, and endotoxin of viral and bacterial infections, acts as a T-cell activation indicator, induces antibody secretion by human B-cells, causes differentiation of cytotoxic T-cells, and also has the ability to inhibit TNF-α production.(Bochud and Calandra ,2003) Researchers started earlier to look into possible rapid competent diagnostic markers of neonatal sepsis to make a clear distinction between neonates who have clinical signs because of serious bacterial sepsis (i.e. Gram-negative infections) and others who also have almost similar signs but because of other non-infectious etiologies. Examples of these markers, which have been investigated with variable sensitivities and specificities, include several interleukins, tumor necrosis factor (TNF), procalcitonin (PCT), C-reactive protein (CRP), immunoglobulins and others (Cairo , 1989;Matthes,et al.1993;Berner , et al., 1998; Martin et al., 2001). Immunoglobulin , complement and IL-6 have not been studied extensively with respect to infection, in our country.

The objective of this study was to investigate the humoral immune status and IL-6 levels in serum of the septic neonates during the early weeks of life.

MATERIAL AND METHOD:

SUBJECTS:

This study was performed at the neonatal care unite in AL-Kadyemia Teaching Hospital in Baghdad from1st of July (2011) to the 1st of February (2012)

patients group:

A total of 57 neonates at age of ≤ 2 months were enrolled inthisstudy and classify into two study groups :group 1 (22 neonates with proven sepsis based on clinical finding of sepsis: Bradycardia ,peripheral circulation impairment ,metabolic acidosis ,hypotonia ,leukocytosis /leukopenia and positive blood culture);group 2 (35 neonates probably sepsis according to clinical sing and symptom but negative blood culture)

Control group:

23 neonates of outpatients of matched age that had illness other than infection

Blood samples:

From each neonate with suspected infection and control group, a blood sample of 1000 µL to 1500 µL was drawn by venipuncture let to clot. Serum was separated by centrifugation at 3000 rpm.for 10 minutes.

Measurement of immunoglobulins(Ig)A,M,G and complement components C3,C4 serum levels :

We determined serum concentration of complement components C3 and C4 and the 3 major classes of immunoglobulin (Ig) A , M , and G by using commercially available mono-

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specific antiserum partigen plates(immuchem,Blegium) by the single radial immunoassay method(Mancini,et al.1965)

Determination of IL-6:
IL-6 serum levels were detected by using human cytokine- enzyme-linked immunosorbent (ELIZA) kit (Biotech Co,LTD),following the manufacture procedure. The detection limit for IL-6 level was 82pg/ml ,measurement rang was 12,5-200pg.

Statistical analysis:
Data were statistically described in terms of mean ±standard deviation (SD)..Then subjected to one way analysis of variance (ANOVA) at ($p<0.05$) &($p<0.01$) .use t-test to find least significant differences (LSD) to compare each two studied groups from (control, septic and probable sepsis) for the tests(IgG,IgM,IgA,C3,C4 and IL-6).at ($p<0.05$) &($p<0.01$) to considered statistically significant. All statistical calculations were done using computer programs SPSS(Statistical Package for the Social Science; SPSSInc., Chicago, IL, USA) version 15 for Microsoft Windows.

Results:
Estimation of IgG, IgM , IgA C3 and C4 Level in the Serum:
Table (1) demonstrated the mean serum levels of IgG,IgM,IgA, C3 and C4 complement components of patients groups in comparison to control group . in this study levels of IgG in the studied groups were significantly lower among sepsis group 417.93± 83.18 pg./ml while in probable sepsis group 447.31 ± 76.95 pg./ml compared with controlled group 676.17 ± 104.88 pg./ml with p = 0.001,on the other hand there was no statically differences in IgG concentration between sepsis group and probable sepsis group(p=0.06) .

The serum levels of IgM, was evaluated in studied groups compared to control group, there were a significant differences in the mean serum levels of IgM in sepsis patients as compared to other groups. The results revealed that patients' groups have higher values of mean serum IgM level (107.2±30.76 mg/dl) with significant difference in comparison to probable sepsis group and control group ($P \leq 0.01$).

Results showed that there were a significant differences in mean IgA levels in sera of patients of sepsis and probable sepsis groups (48.9 ± 18.29 mg/dl)(41.33±10.44) respectively ,and when compared to control group(118.73±33.32) ($P \leq 0.01$)

Statistically significant decrease in serum C3 levels in sepsis group(64.02±11.41pg./ml) while in probable sepsis group (62.17 ± 14.61 pg./ml) compared to control group (85.52±9.69). ($P \leq 0.01$). In patients groups there were no significant differences in C3 serum levels ($p \geq 0.75$).The concentration of C4 was higher in sepsis group (19.81±3.71)than in probable sepsis group (10.32±3.81),in other hand there was no statistically differences between sepsis group and control group($p \geq 0.24$)

Table 1: The difference in mean serum IgG,IgM,IgA,C3 and C4 levels in the study and control group

	Studied groups			
Values mg/dl	Control mean±SD	Septic mean±SD	Probable septic mean±SD	P value
IgG: mg/dl	676.174 ± 104.882	417.936 ± 83.180*	447.314 ± 76.952*	S**
IgM: mg/dl	66.609 ± 21.313	107.218 ± 30.767	40.906 ± 13.644	S**
IgA :mg/dl	118.739 ± 33.321	48.9 ± 18.290	41.331 ± 10.447	S**
C3:mg/dl	85.521 ± 9.690	64.027 ± 11.418*	62.171 ± 14.617*	S**
C4:mg/dl	21.217 ± 3.450*	19.814 ± 3.717*	10.322 ± 3.810	S**

S=Significant at α=0.05&α=0.01**

*** =Not significant**

Estimation of IL-6 Level in the Serum:
Statistical analysis revealed that there was a significant elevation of IL-6 levels in sepsis group 85.86±4.85 pg./ml , in a probable sepsis group 83.92±3.09 pg./ml compared to control group 19.22±3.36pg/ml with $p \leq 0.01$.(Table 2)

Table (2): The difference in mean serum IL-6 levels (pg./ml) between the studied groups

	Studied Groups			
Values Pg./ml	Control No.=23	Sepsis No.=22	Probable sepsis No.=35	P value
Mean	19.226	85.863	83.924	0.01
SD	3.360	4.853	3.092	

Discussion
The role of neonatal immune system's responsiveness to microbial sepsis is now being elucidated. The functional impairments of innate and adaptive immune system in neonate put the neonate at increased risk for developing a serious infection .the objective of this study was to determine the essential components of humoral immunity (complement and circulating immunoglobulin) and IL-6 levels in serum of sepsis neonates.

Complement components and immunoglobulin and with the exception of immunoglobulin G do not cross the placenta and the infected fetus and newborn infant are able to produce IgM antibodies in response to bacterial antigens, but at lower levels than that of adults. However, the synthesis of IgG and IgA is limited (Schelonka and Infante 1998).

The finding in present study that IgG was lower in sepsis and probable sepsis patients improved that, IgG play crucial roles in host defense against pathogens. In a study of preterm infants, patients with IgG concentrations of <4 g/L (400 mg/dL) have been associated with an increased risk of infection in the premature human infant (Clapp et al.1989). Serum IgG levels_100 mg/dL are generally considered seriously increase the risk of infections subclasses responsible for respiratory tract infections ,with 1 study reporting IgG subclass deficiency(mostly IgG3 and IgG4) in approximately 38% of patients with recurrent respiratory infections (Popa, et al. 1993). Infections with encapsulated bacteria, such as Haemophilus influenzae and Streptococcus pneumoniae,are particularly prevalent in patients with IgG2 deficiency (Morell , 1994) Prophylactic immunotherapy with intravenous immune globulin (IVIG) did not reduce infectious episodes or mortality rate in those infants with low IgG levels (Sandberg , et al. 2000). IgM, unlike IgG, does not cross the placental barrier, and its elevation implies the neonate's own postnatal production as a reaction to infective agents .In this study IgM was elevated in the neonates with sepsis. Other researchers also have found an elevation of IgM in neonates with sepsis and have proposed that it May be used, coupled with IL-6, as an early detector of neonatal sepsis (Hodge et al.,2004). In that study, IgM levels were higher in sepsis and suspected infection groups, compared to healthy neonates. The IgG levels were lower in neonates with sepsis, compared to the control subjects. A causative could be speculated between low IgG levels and sepsis, with the reservation that biochemical IgG values were measured rather than functional antibody is useful in the assessment of neonatal infection.

IgA was lower in the sepsis group and probable sepsis group than in the control, the difference was significant. Immunoglobulins provide the body with an important defense mechanism against infectious agent .Yang et al. (1989) studied the mechanism of bacterial opsonization by intravenous immune globulin (IVIG) complement consumption and polymorphonuclear leukocyte membrane receptor mediated phagocy-

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tosis of Staphylococcus epidermidis, Klebsiella pneumoniae, and groups A and B streptococci. IGIV alone did not consume complement and showed no opsonic activity by itself for these organisms. When these bacteria were preopsonized in intravenous immune globulin, significant amounts of complement were consumed (44%-94%) and the uptake and killing of bacteria occurred.

We found that C3 and C4 concentrations in sepsis and probable sepsis were lower than the control group. These finding was not agreed with the study of Singh et al.(1990)who found no statistically significant depression was observed in levels complement component levels of infected babies . poor capacity to control spontaneous complement activation because of the lower levels of circulating IgG,(Frank et al. 2000) makes new born neonates extremely vulnerable to complement-mediated damage of the lung and brain.(Schelonka and Infante.1998; Davies et al. 2001; Watford et al.. 2002)

With increasing survival of high-risk neonates, the involvement of the complementsystem and circulating immunoglobulin in the pathogenesis of lung and brain injury related to prematurity and the higher susceptibility to infection, has emerged as an area of priority in neonatal research. An improved understanding of the humoral immunityin sepsis neonates may offer future solutions to benefit neonatal outcome.

In sepsis, depending on the activation of the complement, contact activation, and the intrinsic coagulation systems, many mediators like interleukin-6 (IL-6), nitric oxide (NO), and tumor necrosis factor-α (TNF-α) are released (Noor,et al.2008) .Although cytokine analyses are far from being diagnostic for bacterial sepsis, serum levels of IL-6, suggested previously as early markers of neonatal sepsis ,with high sensitivity(85.71%),specificity(73.68%),negative predictive value (95%),positive predictive value(80%)(Zakaria and Rafaat , 2012).

In our study, it was observed that IL-6 levels of newborn with culture-proven sepsis and culture-negative sepsis were significantly higher than controls (P <0 .05). This is in agreement with the results of Hotoura et al., (2011) demonstrated in their study an increase in IL-6 in the group of neonates with sepsis to values higher than those in neonates with suspected infection or healthy control subjects with no signs of infection.suggested a cut-off value of IL-6 > 60 pg. / ml proved to be a more sensitive and specific index for early diagnosis of sepsis. Our study revealed a cut- off value of IL-6 > 82 pg./ml. In a prospective study carried out at the neonatology intensive care unit of Cairo University and included 96 full term neonates, IL-6 was significantly higher in the sepsis group than in the control group. IL-6 > 90 pg. / ml was an excellent marker with high sensitivity and specificity(Mostafa and Mona , 2012).

Our findings and the findings of Hotoura et al.(2011) although different in figures, confirm those of earlier researchers who considered IL-6 to be a very precise early marker of neonatal infection (Buck et al., 1994; de Bont, et al., 1994; Harris, et al., 1994). Some investigators have proposed the use of a combination of markers, such as IL-6, which is an acute reactor, and CRP, which increases later in the course of sepsis (Kale et al.2014;Sugithaini ,et al.2013)

CONCLUSION

In conclusion, our results show that concentrations of immunoglobulins, C3, C4 and IL-6 undergo significant changes during the first months of neonate life, dysregulation of this immune/inflammatory response result in sepsis.

Recommendation

A combination of several sepsis biomarkers may be of value in the early diagnosis of sepsis.

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