Tumor Necrosis Factor Alpha and Interleukin-6 in Infants with Sepsis

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*Thirty-two infants above one month of age admitted to a tertiary care hospital with signs of infection and presumptive diagnosis of sepsis were included. Cytokine levels of tumor necrosis factor alpha (TNFμ) and interleukin-6 (IL-6) were estimated at admission and after 48-72 hr, and their relationship examined to the outcome. Significantly higher TNF*a *and IL-6 levels were seen in infants with sepsis compared to control. The TNF*a *levels significantly decreased in patients with sepsis, septic shock and the survivors, while the patients who did not survive, the levels showed no significant change after 48 hr. The initial levels of IL-6 were comparatively higher in patients with septic shock and non-survivors, and increased at 48 hr of admission in patients with sepsis, septic shock and non-survivors.*

Key words: *Cytokines, Interleukin-6, Tumor necrosis factor.*

DESPITE improved antibiotic therapy and advances in cardiorespiratory monitoring mortality rates in systemic sepsis are high. Infection begins with the invasion of the host by bacteria or other pathogens and progress to systemic inflammatory response syndrome, septic shock and multiorgan failure. The most prominent cytokines tumor necrosis factor alpha (TNF∝), interleukin (IL)-1, and IL-6. Various studies have shown that in response to sepsis, immune system initiates a cytokine cascade characterized by sequential production of TNF∝, IL-1, IL-6(1-6).

TNF∝ is first to appear with its primary response being upregulating vascular adhesion molecules, activating neutrophils and stimulating monocytes to secrete IL-1 and IL-6. It activates coagulation system, suppresses bone marrow stem cell maturation, myocardial depression, hypotension and DIC. IL-6 acts on the hepatocytes to increase acute phase reactants and β-cells growth factor, promoting antibody formation and release(7). The concentration of TNF∝ and IL-6 as an early indicator of sepsis and their correlation with outcome has been studied in adults(2-6, 8-10).

It is proposed that cytokine estimation is an early indicator of sepsis and its prognosis, it might help in improving the outcome with the early starting of therapy. This study was planned to evaluate blood levels of TNF∝ and IL-6 in infants more than one month of age with sepsis and its relationship with outcome.

Subjects and Methods

A prospective study was carried out on 32 infants above one month of age admitted with the presumptive diagnosis of sepsis. Clinical parameters of infection, including fever (temperature >38ºC), hypothermia (temperature <36ºC), tachypnea (adjusted for age) and tachycardia (adjusted for age) were noted;

features suggestive of severe sepsis, septic shock like change in mental status, agitation, lethargy and obtundation, hypotension, cold extremities, cyanosis and pallor were also recorded. Features of focal infection *eg.* meningitis, pneumonia or arthritis were noted. Investigations included either a positive blood culture alone, or a C-reactive protein (CRP) [more than 4 mg/dL], with more than one of the following: (*a*) total leukocyte count (TLC) <6000 to >17,000 cells/cu mm; (*b*) metabolic acidosis (pH <7.25); (*c*) erythrocyte sedimentation rate (ESR) >20 mm/hr. Septic shock was defined as patients having sepsis with hypotension (systolic pressure in infants less than 65 mm Hg), despite adequate fluid resuscitation and presence of perfusion abnormalities(7). Patients with immunodeficiencies and on immunosuppressive medications were excluded.

Other relevant investigations like lumbar puncture, urine and stool examination, kidney and liver function tests, coagulation profile were done wherever required. These patients were then managed appropriately with intravenous fluids and oxygen; initial antibiotics were cefotaxime and gentamycin, with change depending on culture reports and clinical parameters.

Blood samples for cytokine determination were drawn first at admission and again after 48 hrs (if patient survived); sera was separated and stored at –20ºC. Cytokine determination was performed using a solid phase sandwich enzyme linked immunosorbent assay (ELISA). Biosource cytoscreen TM hTNF∝ and IL-6 kits were used for the cytokine levels and duplicate determination was performed for those samples, with results on either extremes. The minimal detectable levels were 2 pg/mL for IL-6 and 1.7 pg/mL for TNF∝. Fifteen age matched healthy control infants were sampled for baseline cytokine levels. The Ethics Committee of the Institute approved the study.

For statistical analysis the risk factors associated with mortality in infants Chi square test, Fisher exact test, Paired 't' test and Wilcoxon signed ranks test were applied to see the significance of results obtained, with $P < 0.05$ taken as statistically significant.

Results

Of 32 patients, 23 were boys; the mean age was 4 months. Of the patients, 25 had pneumonia, 11 each had meningitis and diarrhea and 5 had no obvious focus of infection. Blood cultures showed *Klebsiella spp., E. coli.* Pseudomonas and *S. aureus* in 10 patients. CRP was positive in 84.3%, deranged TLC in 56.3%, ESR >20 mm/hr in 46.8% and pH <7.25 in 59.4% cases. Ten patients (32%) developed septic shock and 7 (21.8%) patients died during the study, two died within 48 hours. The hospital stay of patients who expired varied from few hours to 4 days, with the average stay being 2.15 days. High mortality rate was observed in patients with shock (7/10), followed by bacteremia (4/10), and no focus of infection (2/5).

Table 1 shows the TNF∝ levels for all patients with sepsis. At admission TNF∝ levels were noted to be significantly higher than after 48 hr. Patients with septic shock had very high TNF∝ levels, which fell significantly after 48 hr. Similarly, among patients without septic shock the levels also fell significantly though their initial levels were not high. However, in patients who did not survive there was no significant change in levels, while survivors showed a significant decline in TNF∝ levels. TNF∝ levels both in blood culture positive and negative showed significant fall.

The results of IL-6 levels (*Table-II*) showed that in sepsis there was an increase in

levels, which further rose after 48 hrs, though it was not significant. Significantly, higher levels of IL-6 were observed in patients with septic shock compared to patients with no shock, both initially (P <0.002) and later (P <0.001). Due to high initial levels in shock the rise was not significant, while the rise in nonshock patients was significant (P <0.001). The subjects who did not survive, with high levels at admission had no significant rise, though in the survivor group this rise was significant (P = 0.002). There was a significant rise in levels of IL-6 in blood culture positive cases as compared to culture negative cases. Fifteen ages matched healthy control babies showed no detectable cytokine levels.

Discussion

Many studies in adults have reported high TNF∝ and IL-6 in cases of septic shock, a persistent elevation of TNF∝ in sepsis with poor outcome and raised IL 6 levels with increased risk of bacteremia and death(2-4,8,9). The studies in children are few and none in infants(7,11). Our findings suggest that trends in the levels of TNF∝ and IL-6 may help in early diagnosis of sepsis. We speculate that cytokines levels may be useful for early detection and prognosis in patients with septicemia.

Our results showed that the cytokine levels in sepsis were high, TNF∝ at admission were higher compared to levels at 48-72 hr. Fong

TABLE I–*TNF∝ Levels (Pg/ML) in the Study*

Group	At admission	After 48 hours	P
All patients (n=32)	227.2 ±197.3 (10-910)	99.4 ± 135 (0-450)	0.001
Septic shock (n=10)	338 ± 144 (90-505)	238.7 ± 168.7 (18-325)	0.006
Non shock (n=22)	175 ± 199 (10-910)	48 ± 74.6 (0-450)	0.001
Expired (n=7)	391.4 ± 97.8 (240-505)	330 ± 127.9 (125-450)	NS
Survivors (n=25)	180 ± 194.3 (10-910)	53 ± 77 (0-300)	0.02
Blood culture positive (n=10)	267 ± 138.7 (90-455)	146.7 ± 133.1 (20-450)	0.02
Blood culture negative (n=22)	207 ± 219 (10-910)	79.1 ± 133.8 (0-300)	0.01

TABLE II– *Il-6 Levels (Pg/ML) in the Study*

Group	At admission	After 48 hours	P
All patients (n=32)	268.1 ± 215 (20-700)	329 ± 245 (5-750)	0.08
Septic shock (n=10)	453.6 ± 201(100-700)	556.8 ± 111.3 (100-700)	NS
Non shock (n=22)	138.8 ±164.7 (20-575)	246.3 ± 227.9 (5-750)	0.001
Expired (n=7)	514 ±176 (258-700)	532± 104.9 (125-700)	NS
Survivors (n=25)	119.2 ± 171.1 (20-575)	288.5 ±246 (5-750)	0.002
Blood culture positive (n=10)	299 ±219 (20-625)	480 ± 107 (290-635)	NS
Blood culture negative (n=22)	254± 216.8 (20-700)	264 ± 260 (5-750)	0.02

Key Messages

- TNF∝ levels rise early and their persistence indicates poor outcome.
- IL-6 levels rise later, their levels both initially and later is related with poor prognosis.

and Lowry have previously shown that TNF∝ levels rise 30 to 90 minutes after endotoxin challenge, followed by a decrease(1). Sullivan, *et al.* showed declining levels of TNF∝ in patients with sepsis(3). Moscovitz, *et al* detected TNF∝ levels only in severely ill patients and a consistent rise of the cytokine in sepsis was not found(4). Others showed that TNF∝ levels remain constant with no fluctuations or peaks(5). We found that IL-6 levels at admission and 48-72 hr showed a rise, which was not statistically significant. Others have shown high IL-6 in patients with sepsis and a decline in levels over next 2-4 days(3,12,13).

In septic shock patients the cytokine levels *i.e.*, TNF∝ at admission was higher compared to their value for all the patients with sepsis. A significant decrease in TNF∝ levels at 48-72 hrs intervals was observed (P = 0.006). In various other studies, it was observed that cases with septic shock had high TNF∝ levels and remained so over next few days before their decline (5,8,14). While the IL-6 levels were very high at both the intervals with no significant change in levels after 48-72 hr, which was also observed in other studies (4,6,8,12).

The variations in TNF∝ and IL-6 in the study over 48 hrs. could be explained by the fact that TNF∝ rises early in sepsis *i.e.*, at presentation and IL-6 rises later and has sustained increase(1,3,6). Therefore, the release of TNF∝ as central mediator in pathogenesis of sepsis and septic shock appears true and IL6 though appears later also has a role in septic shock and sepsis. Our results suggest that measurement of plasma TNF∝ and IL-6 concentration might be helpful in differentiating sepsis with the risk of septic shock and mortality. If facilities are available in the emergency department these may be useful to predict the severity of illness and outcome.

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