NEW BIOMARKERS AS INDICATORS FOR SEPSIS IN FEBRILE LEUKAEMIC IRAQI PATIENTS

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ABSTRACT

Background: Sepsis in leukaemic patients is a leading killer of both adult and pediatric patients. Analysis of cytokine levels measured around the onset of bactaeremia indicated that IL-8 followed by IL-6 are potentially useful for predicting gram-negative and gram positive bacteremia.**Methodology:** Interleukin-8(IL-8) and Interleukin-6 (IL-6) levels were estimated in 56 cases comprising; 21 bactaeremia by gram-negative bacterial infection ,15 bacteraemia by gram-positive cocci infection and 20 healthy controls.**Results:** The highest serum concentrations of IL-6 and IL-8 were observed in episodes of gram-negative bacteraemia and gram-positive bacteraemia but higher in former (98.7-95 pg/ml) by order. **Conclusions:** IL-8 and IL-6 can be used as sepsis biomarkers their and elevation in serum indicate bacteraemia specially by gram-negative bacteria and less by gram-positive bacteria.

KEYWORDS: Leukaemia, Sepsis, Biomarkers, cytokines IL-6,IL-8.

1.INTRODUCTION

A biomarker is defined as the biological parameter associated with the presence and severity of specific disease states. Biomarkers are detectable and measurable by laboratory assays.Most of the acceptable biomarkers are obtained via blood sample.One of the most traditional laboratory biomarker that still used in medical laboratories and give an indicator for present of infection is C-reactive protein (CRP) (greater than 90 mg/L).Cytokines are serum biomarkers which reflect body's response to the infectious agent and present of bacteraemia. These serum biomarkers include a range of cytokines like IL-6, IL-8, tumor necrosis factor-a, interferon-g, and others which are under investigation and research in

number of studies in Europe.[1] The interleukins have been logical targets of sepsis biomarker investigations related to their role in inflammation and sepsis. Interleukin-6 (IL-6) is a pro-inflammatory cytokine that is produced in response to infection and other conditions of inflammation. IL-6 is an integral part of the cytokine activation cascade. Because discrimination between fever due to bacterial and fungal infections and harmless fever episodes is difficult, circulating proinflammatory mediators such as interleukin-6 (IL-6) and IL-8 have been suggested to be predictive for a systemic microbial bloodstream infection in neutropenic hosts following chemotherapy. The highest serum concentrations of IL-6 and IL-8 were observed in episodes of gram-negative bacteraemia and gram-positive organisms but higher in previous.[1,2,3] Interleukin-8 (IL-8) is an inflammatory cytokine that is released from monocytes, endothelial cells, and neutrophils in response to IL-1 and TNF-α. IL-8 responds by activating T cells, neutrophils and basophils. Increases in circulating IL-8 are seen early in the infectious course. In a study of oncology patients with febrile neutropenia,IL-8 levels were elevated earlier and higher in patients with gram-negative sepsis from *Escherichia coli* or *Klebsiella pneumoniae*. Levels were likewise elevated in gram-positive sepsis, but less-so than with gram-negative infection.[4,5,6]

2. MATERIALS AND METHODS

Patients

A total of 97 patients with leukaemia who were attended the Nanakaly Hospital Blood diseases which is a centre for haematological malignancy in North Iraq-Erbil during the period between January 2012 to October 2012 were included in this study. Their age ranged between 4-76 years. Clinical examination was done by specialized haematologist at the above-mentioned hospital.

Healthy controls (exclusion criteria)

A total of 20 apparently healthy volunteers (non leukemic) were included in the study. They were 11 males and 9 females. They were not received any medications, with no history of any chronic or acute illnesses, and had normal complete blood picture (CBP) and erythrocyte sedimentation rate (ESR).

Blood samples

10 ml of venous blood was taken from the patients through a sterilized syringe added to screw capped bottles containing at least 20 ml of Brain Heart infusion broth and incubated at 37°C for 24 hours .After the incubation period, the signs of growth appeared in the blood

culture e.g.; gas production, turbidity, haemolysis and flocculation then inoculation of samples from bottles on cultures plates of, MacConky agar, Blood agar, Eosine methylene blue, Mannitol salt agar and Pseudomonas Agar Base+CN(Cetrimide, Nalidixicacid),Sabouraud`s dextrose agar-Chloramphenicol .Plates were incubated for 1-2 days at 37°C. Identification of bacteria was done as carried elsewhere.[7]

Estimation of serum IL-8 and IL-6 level by ELISA method

It was done folowing two immunological steps sandwich type assay. In the first step the IL-8 was captured by a monoclonal antibody bound to the wells of a microtiter plate. In the second step a biotinylated monoclonal anti-body was added together with streptavidine–peroxidase conjugate.The biotinylated antibody binds to the solid phase antibody–antigen complex, in turn, bound the conjugate. After incubation, the wells were washed and antigen complex bound to the well and detected by addition of a chromogenic substrate (Al-j and haeel,2014). The intensity of the coloration is proportional to the IL concentration in the sample or standard.[8,9]

Statistical analysis

Statistical analysis was done using SPSS version 18 computer software (Statistical Package for Social Sciences). The mean value with the standard deviation (SD) for each value was determined. The statistical significance of difference in mean of a certain continuous outcome (dependent) variable between more than 2 groups (independent variable) was assessed by ANOVA test.

3.RESULTS

Distribution of infections caused by gram-positive and gram- negative microorganisms causing 36 bacteremia

The results of the surveillance cultures for bacteraemia are presented in Tables 1.Of 97 blood cultured 36 were positive for bacteremia and 21 (58.33%) were caused by gram-negative bacterial infection and 15(41.66%) were due to gram-positive cocci infection.

Table 1.Distribution of infections caused by gram-positive and gram-negative microorganisms causing 36 bacteremia

Bacteremia	No.of isolates	Percentage %
By Gram-negative bacterial infection	21	58.33%
By Gram-positive cocci infection	15	41.66%
Total	36	100%

12 Cytokines used as a sepsis biomarkers in febrile leukemic patients

IL-8 and IL-6 levels were estimated in 56 cases, comprising 21 bacteraemia by gram-negative bacterial infections, 15 bacteraemia by gram-positive cocci infections and 20 healthy controls. There were differences between mean levels of baseline IL-8 (pg/ml) among the two bacteraemic studied groups according to bacterial isolates and healthy control .The present results showed a significant elevation in serum IL-8 level among leukaemic patients with bacteraemia by gram-negative bacterial infection (78.65±22.64pg/ml) and bacteraemia by gram-positive cocci infection (71.27± 14.54) comparing to healthy control(1.77± 0.76pg/ml) and the differences were highly significant (p<0.001) as shown in Table 2.

Table 2: The difference in mean levels of baseline IL-8 (pg/ml) among the three studied groups

P(ANOVA)				
Study group	**Bacteremia caused by**		**Healthy control**	**P (ANOVA)**
	Gram-negative bacterial infection	**Gram-positive cocci infection**		
Baseline serum IL-8				
Minimum	22	41	0.7	
Maximum	98.7	96	3.1	
Mean	78.65	71.27	1.77	< 0.001
SD	22.64	14.54	0.76	
SE	4.62	4.19	4.62	
NO.	21	15	20	

Bacteraemia by gram-negative bacterial infection X Healthy control <0.001 t=16.46

Bacteraemia by gram-positive cocci infection X Healthy control <0.001 t =23.69

Also there were differences between mean levels of baseline IL-6 (pg/ml) among the two bacteraemic studied groups according to bacterial isolates and healthy control .The present results showed a significant elevation in serum IL-6 level among leukaemic patients with

bacteraemia by gram-negative bacterial infection(68.17 ± 25.88pg/ml) and bacteraemia by gram-positive cocci infection(58.68 ± 26.03)in comparison to healthy control (24.7 ± 8.56pg/ml) and the differences were significant ($p<0.005$); (Table 3).

Table 3: The difference in mean levels of baseline IL-6 (pg/ml) among the three studied groups

Study group	Bacteremia caused by		Healthy control	P (ANOVA)
	Gram-negative bacterial infection	Gram-positive cocci infection		
Baseline serum IL- 6				
Minimum	20	8	11	
Maximum	95	90	41.7	
Mean	68.17	58.68	24.7	< 0.005
SD	25.88	26.03	8.56	
SE	8.56	7.51	1.74	
NO.	21	15	20	

Bacteraemia by Gram-negative bacterial infection X Healthy control <0.005   t =6.91

Bacteraemia by Gram-positive cocci infection X Healthy control <0.005   t =3.53

4. DISCUSSION

The present study revealed that the number of leukaemic patients with gram-positive cocci bacteraemia occurred more in children 86.67%,while bacteraemia by gram-negative occur more in adult 95.24% . Statistical difference ($p<0.05$) was demonstrated between these two age groups.Similar result found by Santolaya etal.[10] who reported in his result that gram-positive bacteria was the most common pathogens causing bacteraemia among children with cancer having fever, and neutropenia. In a large European trial comparing the outcome of febrile infections in children with cancer to that of adults with malignancies ,gram-positive bacteria were the causes of bacteremia in 60% of the cases.[11] The more intensive treatment of cancer leading to more severe damage to mucosal barriers and increased risk for infection from resident gastrointestinal flora.[12,13]

Sepsis in leukaemic patients is a leading killer of both adult and pediatric patients, and delays in recognition and treatment significantly increase the risk of morbidity and mortality. The concept of early detection serves as the foundation of the search for clinically viable indicators of severe sepsis – hence the interest in sepsis biomarkers. Biomarker is defined as the biological parameter associated with the presence and severity of specific disease states.

An ideal sepsis biomarker would accurately detect the presence of infection in a patient with it. A traditional laboratory biomarker associated with high risk of infection include C-reactive protein, additional serum biomarkers for sepsis include range of cytokines like Interleukin-8 (IL-8), Interleukin-6 (IL-6), tumor necrosis factor-a, interferon-g, and others which are under research and investigation in a number of studies in Europe and other areas. Because classical inflammatory signs of infection are often absent and fever is frequently the only sign of infection, the aim of this study was to assess the significance of serum interleukin-6 (IL-6), IL-8 in identifying bacterial infections during start of fever and suggest to use these in laboratory diagnosis as sepsis biomarkers. The triggering molecule for these cytokines appear to be LPS lipopolysacchride in gram negative bacteria and lipoteichoic acid and capsular prteoglycan in gram positive bacteria.

In present study as showed in tables2 and 3 ,the level of IL-8 and IL-6 in sera of patients with bacteraemia was significantly higher than the healthy control group(p<0.001) and (p<0.005) and the highest serum concentration of IL-8 and IL-6 were observed in episodes of gram-negative bacteraemia(98.7-95 pg/ml) respectively. This present result was similar to a study carried out in Germany by Engel etal[14], Diepold etal[15] and Lehrnbecher etal[16] They found that IL-8 levels were elevated earlier and higher in leukaemic patients with gram-negative sepsis by *E. coli* or *Klebsiella pneumoniae* and they also found that levels were likewise elevated in gram-positive sepsis, but less-so than with gram-negative infection. Another studies done by Enguix etal[17] and Magudumana etal[18] showed that the highest serum concentrations of IL-6 were observed in episodes of gram-negative bacteraemia and gram-positive organisms but higher in former.Furthermore, the present findings were almost similar to these results.However, the present findings were different from those of Buyukberber etal[19] who found that IL-8,IL-6 and other cytokines concentrations are not predictive of bacteremia in febrile neutropenic patients.

5. CONCLUSIONS

Gram-negative microbes were the predominant findings in blood. Bacteremia by gram-positive cocci occurred more in children while in adult gram negative bacteria were more predominant in association with leukaemia. IL-8 and IL-6 can be used as sepsis biomarkers as their elevation in serum indicated bacteraemia specially by gram-negative bacteria and less by gram-positive bacteria.

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