# IL-1α and IL-8Levels During Gram-Negative and Gram-positive Bacteremia in Leukopenia Leukemic Patients.

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# ABSTRACT:

#### **BACKGROUND:**

A quantitative relationships between circulating leukocytes and infection was established in patients with leukemia, in particular, the probability of being infected is proportional to the severity and duration of leukopenia <sup>(1)</sup>. Infection with gram- negative as well as gram-positive microorganisms may lead to septic shock and death <sup>(2, 3)</sup>. The major functional activities of cytokines are concerned with the regulation of the development and behavior of the immune effector cells. IL-1 is one of proximal cytokines. IL-1 act to stimulate the release of distal cytokines, such as IL-6 and IL-8, IL-8 is most closely related to the severity of the physiological response to infection and systemic inflammation. **OBJECTIVE:** 

This study was conducted to detect the levels of IL-1 and IL-8 during Gram negative and Gram positive bacteremia. That could be useful in determining an appropriate choice for antimicrobial drug depending on the antimicrobial susceptibility pattern.

#### **METHODS:**

IL-1 $\alpha$  and IL-8 was studied in (28) adult leukopenic patient with bacteremia, males and females, more than 15 years old. The study was including (20) healthy control. The bacteremic cases were obtained by culturing blood samples aerobically and anaerobically.

The isolates were identified on the basis of their morphological, cultural and biochemical characteristics. Interleukin-1 $\alpha$  and interleukin-8 cocentration were measured by using a commercially available enzyme-linked immunosorbent assay (ELISA).

## **RESULTS:**

Statistical analysis show no significant difference in IL-1 $\alpha$  levels between Gram-negative and Grampositive bacteremia in leukopenic leukemia patients (P=0.803). While statistical analysis show significant difference between patient with Gram-negative and Gram-positive bacteremia in leukopenic leukemia patients (P=0.037).

## **CONCLUSION:**

In leukopenic leukemia patients with bacteremia we may could determining an appropriate choice for treatment depending on IL-8 levels in the circulation of those patients and the antimicrobial susceptibility pattern.

**KEYWORDS:** IL-1 $\alpha$  and IL-8. bacteremia. leukemia.

#### **INTRODUCTION:**

Cancer patients who are leukopenic due to chemotherapy are susceptible to bacteremia <sup>(1, 2)</sup>. Normally, clinical conditions during blood stream infection are caused by pathogen-associated molecular patterns, which are components that bind to Toll-like receptor (TLR2) and (TLR4) on leukocytes, resulting in the production of inflammatory cytokines <sup>(3)</sup>. The mechanism of this inflammatory response in cancer patients with diminished number of leukocytes is not completely

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clear <sup>(2,4)</sup>. The levels of IL-1 measured in the circulation of leukopenic cancer patients are lower than those measured in that of non leukopenic patients during bacterial infection (5-8). Whereas plasma IL-8 levels show distinct identical increases during bacterial infection in both leukopenic and non leukopenic patients <sup>(2,9,10,11,12,13,14,15,16)</sup>. Normally, these cytokines are mainly secreted by leukocytes <sup>(17)</sup>. In cancer patients with bacterial infections and diminished number of leukocytes, other source of IL-8 production, such as endothelial cell, might be expected. Endothelial cells instead of leukocytes become the most important producers of IL-8 during bacterial infections in patients with chemotherapy-

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induced leukopenia <sup>(18)</sup>. Recently, the involvement of Toll-like receptors (TLRs) as pattern recognition receptors in the innate immune response was demonstrated. The TLRs are characterized by extracellular domain containing leucine-rich repeats and an intracellular domain sharing a high degree of similarity with the il-1 receptors <sup>(19)</sup>. Infections with Gram-negative as well as Gram positive bacteria may lead to septic shock and death. The development of novel strategies to prevent complications of bacterial infections and strategies to diagnose bacterial infections earlier by using inflammatory cytokines <sup>(2,3)</sup>.

## **MATERIAL AND METHODS:**

A total of 84 adult patients with leukemia they were feverish leukopenic patients, males and females more than 15 years old. They were admitted to medical unite in Baghdad teaching hospital, the study include 20 apparently health individuals. The diagnosis is leukemia proved by bone marrow study. 28 leukopenic leukemia patients were with bacteremia the diagnosis were obtained by culturing blood samples aerobically and anaerobically. The isolates were identified on the basis of their morphological, cultural and biochemical characteristics. The IL-1a and IL-8 levels were measured by using a commercially available enzyme-linked immunosorbent assay (ELISA). The principle of these tests was carried out according to the assay procedure given by manufacturing company Immunotech. Sample results were calculated by interpolation from a standard curve that is performed

on the same assay as that of the sample. Data have been analyzed statistically using SPSS program version 10. Analysis of quantitative data was done using ANOVA and t-test. Acceptable level of significant was considered to be below 0.05. **RESULT:** 

Figure (1) shows result of blood culture, 28 (33.33%) were positive on culturing, 53 (63%) were negative and only 3 (3.57%) were contaminated by coagulase negative Staphylococci. The major criteria for contamination were isolation of organism in one bottle only when a similar organism could not be isolated from another major culture site <sup>(20)</sup>. Table (1) shows the type of microorganisms isolated from the blood sample of leukemic patients with leukopenia and bacteremia; Gram negative bacteria constitute the major causative agents of bacteremia. Gram negative organisms were isolated from 18 cases (64.28%), while Gram positive organisms were isolated from 10 cases (35.7%).

Figure (2) shows the distribution of ELISA reading of IL-1 $\alpha$  of the Gram negative bacteremia group and Gram positive one the results was as follows: Gram negative bacteremia cases (46pg/ml SD±25.83) and Gram positive bacteremia cases (43.3pg/ml SD±29.44).

Figure (3) shows the distribution of the ELISA reading of IL-8 of the Gram negative bacteremia group and Gram positive bacteremic one. The results were as follows: Gram negative bacteremia cases (191.3pg/ml SD±152.3) and Gram positive bacteremia cases (83.2pg/ml SD±35.5).



Figure (1): Results of blood culture in febrile cases of leukemia with leukopenia

Microorganisms	No. of isolates	Percentage
Klebsiella pneumoniae	6	21.428
Pseudomonas aeruginosa	4	14.285
Escherichia coli	4	14.285
Proteus penneri	1	3.571
Citrobacter koseri	1	3.571
Salmonella typhi	2	7.142
Staphylococcus aureus	4	14.285
Streptococcus viridans	2	7.142
Staphylococcus epidermidis	2	7.142
Staphylococcus capitis	1	3.571
Staphylococcus xylosus	1	3.571
Total	28	100%



Boxplots of -GB\IL-1 - +GB\IL-1 (means are indicated by solid circles)

GB\IL-1: IL-1 $\alpha$  level in leukopenic patients with Gram negative bacteremia +\GBIL-1: IL-1 $\alpha$  level in leukopenic patients with Gram positive bacteremia Figure (2): The distribution of the ELISA reading of IL-1 $\alpha$  in leukopenic patients with Gram negative

and positive bacteremia

P=0.803

Boxplots of -GB\IL-8 - +GB\IL-8 (means are indicated by solid circles)



-GB\IL-8: IL-8 level in leukopenic patients with Gram negative bacteremia +\GBIL-8: IL-8 level in leukopenic patients with Gram positive bacteremia

Figure (3): The distribution of the ELISA reading of IL-8 in leukopenic patients with Gram negative and positive bacteremia P=0.037

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#### **DISCUSSION:**

As shown in figure (1), it has been observed that, 28 cases of bacteremia were detected during 84 febrile episodes (33.33%) in patients with leukemia and having leukopenia due to chemotherapy and/or disease itself. This study confirms the result of majority studies, in the United States, the incidence of bacteremic infections in patients undergoing chemotherapy for AML during febrile neutropenia has been reported to be 10-30% <sup>(21,22, 23)</sup>. Our finding is in agreement with the results of study done by Kern et al. (2001), in Germany, which included a data of febrile neutropenic adult patients from the both sex whom had hematological malignancies, the bacteremia was 36% as cause of neutropenic fever.

The previous studies confirm the result of our study; 35% in a large American study of fever in leukemic patients <sup>(22)</sup>. Yahya and his colleagues (1985) found that the incidence of bacteremia in patients with acute leukemia was 34%. In some studies about 40% of the patients with hematological malignancies treated by intensive chemotherapy developed bacteremia or septicemia.

The contamination rate 2(2.38%) was acceptable depending on determining rate of blood culture contamination rate of blood culture contamination (lower than 3%) by Forbes et al (2002). This rate reflecting the proper antiseptic and careful techniques in taking peripheral samples which were equally important, otherwise the contamination rate will be un acceptably high and often give false-positive results.

As shown in table (1) Gram negative bacilli constituted the largest percentage of the causative organisms (64.2%), while Gram positive organisms were only isolated from 10 cases (35.7%), this finding is in agreement with the results of previous studies done in western countries in late seventies and early eighties <sup>(26)</sup>, This finding is also in agreement with that had been observed by Yahya et al (1985) and Al-Neemy (1999) in Baghdad. But it disagrees with the finding of Wisplinghoff et al. (2003) who reported that 61% of all episodes were caused by Gram-positive aerobic organisms, and 27% were caused by Gram-negative aerobic organisms. The cause of these results is that, throughout the 1960s and 1970s, Gram-negative organisms were most frequently isolated from neutropenic patients with cancer who had bacteremia. However, during the past 20 years, Gram-positive organisms have become increasingly common<sup>(28,29,30,23).</sup>

But in our country the most telling observation was that, the predominant isolates causing bacteremia in leukemic patients are still Gram-negative bacteria.

The shifting of spectrum from Gram negative to Gram positive in western countries might be due to many therapeutic measures which were not applied to our patients such as the employment of prophylactic antimicrobial therapy to decontaminate the alimentary tract with absorbable antimicrobial agents, such as co-trimoxazole or new fluorinated quinolone like (ciprofloxacin, norfloxacin) which are effective in prevention of Gram negative infection <sup>(31, 32)</sup>

<sup>32)</sup>. In addition the proper antibiotic therapy with new B-lactams and aminoglycosides cover properly the Gram- negative bacterial infection , the wide spreading usage of central venous lines and Hickman catheters in the last few year are commonly associated with Gram positive bacteremia which enhance in that shifting <sup>(26)</sup>.

In figure (2) serum levels of IL-1 $\alpha$  of leukopenic patients with gram negative bacteremia showed a slight increase than that of the leukopenic patients with Gram positive bacteremia but this increase was not significant (P=0.803). Recently, a technique more sensitive than the used ELISA has been introduced, that can detect 1/10 of the routinely detected concentration <sup>(33)</sup>. A better understanding of the role of these cytokines could be an achieved by using a sensitive method.

There was significant difference between patients with Gram negative and Gram positive bacteremia (P=0.037) as showed in figure (3). This result is in agreement with the results of OudeNijhuis et al. (2003). This result may be due to that Gram-negative bacteria and Gram-positive bacteria are known to induce an inflammatory response in the host through binding to pattern recognition receptors on effectors cell. Faure et al. analyzed the patterns of expression of pattern recognition receptors TLR-2 and TLR-4 in human vascular endothelial cells and found that endothelial cells express TLR-4 and, too much smaller extent, TLR-2<sup>(34)</sup>. The blocking of the TLR-4 pathway in Gram negative infection could be significantly reduced IL-8 production. While blocking the TLR-2 pathway in Gram positive infection inhibit the IL-8 production (35).

Therefore, the level of IL-8 in circulation of leukopenic patient with Gram-positive bacteremia is lower than that of Gram-negative bacteremia. This result is in agreement with the result of OudeNijhuis (2003) on a study of IL-8 response in leukopenic

patients with hematological malignancies.

The aim of this study is to detect the levels of IL-1 $\alpha$  and IL-8 during Gram-negative and Gram-positive bacteremia that could be useful in determining an appropriate choice for antimicrobial drug depending on the antimicrobial susceptibility pattern. This goal need further studies about the role of IL-1 $\alpha$  and IL-1 levels in using cytokines as parameter for bacteremia in leukopenic leukemia patients.

### **CONCLUSION:**

In leukopenic leukemia patients with bacteremia we may could determining an appropriate choice for treatment depending on IL-8 levels in the circulation of those patients and the antimicrobial susceptibility pattern.

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