Assessment of Serum Beta-D-Glucan Levels for Early Detection of Fungemia in Febrile Neutropenic Patients

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Abstract
Blood Stream infection (BSI) is the major causes of morbidity and mortality in leukemic patients with neutropenic fever. Early diagnosis of fungemia is a challenge to the clinician and the laboratory. There is a growing attention in the use of aβ-D-Glucan(βDG) biomarker for rapid detection of fungemia. To evaluate the diagnostic role of β-D-Glucan as early diagnostic markers of fungemia in febrile neutropenic patients (FN),β-D-Glucan (βDG) levels were determined in 75 leukemic patients with neutropenic fever. They were measured in serum samples obtained from those patients at the same time when blood was drawn for blood culture.Out of the 75 patients with febrile neutropenia enrolled in this study, 10 patients had a positive blood culture (13.3%), eight of the isolates were bacteria (5 Gram negative and 3 Gram positive) and only two of the isolates were fungi.β-D-Glucan(βDG) levels of >0.1020pg/ml were considered as positive, current study revealed that out of 75 patients,10(13.3%) patients had levels greater than 1020pg/ml, ROC analysis revealed maximum sensitivity of 81.3%,and a specificity of 66.7%. In conclusion β-D-Glucan showing acceptable sensitivity and can provide rapid detection for fungemia in FN patients, which can lead to avoid prolonged and empirical used of treatment.

Introduction
Blood stream infection (BSI) is defined as a positive blood culture in combination with symptoms of infection, which is most common caused by bacterial and fungal microorganism (1). It considered as most frequent cause of morbidity and mortality in Immunocompromised patients especially leukemic patients how are under chemotherapy management (2)Those patients are liable to get infection due to damages in external barriers as a result to use of indwelling catheters or in internal barriers including humeral and cellular immunity as a consequence of disease as well as treatment (3).Rapid diagnosis of BSI in immunocompromised patients is a very important step in limiting mortality and precise determining the appropriate treatment, though many leukemic patients receive systemic antibiotic or antifungal while diagnosis still in progress (2)The primary source to blood stream infection including Catheter-associated bloodstream infection (BSI) , oral flora and gut flora(4).

Infectious Diseases Society of America (IDSA) had defined neutropenic fever as a single oral temperature of >38.3°C or persistent temperature of ≥38.0°C continued for 1 hour in presence of an absolute neutrophil count (ANC) of < 1.5x109/L, or decreasing to less than <0.5x109/L within 48 hours(5). It is a major complication of chemotherapy and should be taken into attention in assessment of treatment choice(6).

Materials and methods
A cross-sectional study was conducted from October 2017 to January 2018. Blood samples were collected from seventy-five febrile neutropenic patients who attended and admitted to Baghdad Teaching Hospital and Al - Imammain Al-Kadhumain Teaching Hospital. They were randomly selected. Clinical manifestations were determined by consultation of a hematological specialist and verification of the information in the medical record. A consent letter or verbally was taken from all patients. This was done in microbiology Laboratories College of Medicine of Al-Nahrain University and it was approved by the Institutional Review Board (IRB) at College of Medicine of Al-Nahrain University. Inclusion criteria included: age group (14-75year) with FN, with underlying hematological malignancies whether receiving prophylaxis antibiotic and chemotherapy treatment or not. While those with following features were excluded:Age group less than 12year,All neutropenic patients without episodes of fever or with local infection,Patients with solid tumor,Thyroid malignancies and Aplastic anemia.

Venous blood sample (7 ml) from peripheral venipuncture was usually drawn from each patient after carefully disinfect the skin with an appropriate disinfectant. It was divided into two parts,first part of the blood sample equivalent to 5 ml directly inoculated into blood culture bottle containing brain heart infusion broth. The blood culture bottle was incubated at 30°C under aerobicfor 14 days. An aliquot of the blood-broth mixture was then subcultured. The first subculture was done after 37 hours of incubation second on the sevendays, and the final on the 14 days, subculture has been used Blood agar, and Sabouraud dextrose agar with Chloramphenicol, Plates were incubated aerobically at 30°C, observed once daily for 7 days, and then examined every other day for 14 days. The second one of blood (2) ml transferred to plane tube to obtain the serum which stored at -20 until used in ELISA for (βDG) according to manufacturing instructions kit from Abbexa Ltd., Cambridge Science Park, CB4 0EY, UK – England.

Statistical Analysis

All data were analyzed with SPSS software for Windows (version 19). Continuous variables were expressed as median and range while dichotomous variables were expressed as frequency and percentage. The ROC curve was used to find the area under the curve (AUC), sensitivity, specificity and cutoff values for the studied biomarker. The level of significance was set at $P<0.05$.

Results

Characteristics of the study patients:

A total of 75 neutropenic patients, (44males and 31 females) were recruited in this study with male to female ratio of 1.41:1. Their ages range from (14–75) years with a mean of ages (36.56±16.22) years. Concerning occupation, housekeeper is the most frequently reported in this study 25 (33.33%), followed by both students and free business with 16 (21.33%) for both. AML is the most leukemic type 36 (48%) of hematological malignancies. All patients in the current study were febrile neutropenic, mean oral temperature was (38.75±0.75) ^{0}C, while WBC mean was (1.92±6.02) $\times10^3$/ml and neutrophil counts mean was (0.337±0.346)$\times10^3$/ml, most patients were anaemic as haemoglobin concentration mean was (7.98±1.57) g/dl. Table(1)

Table (1) Characteristics of the study subjects

Variables	Result
Age (Mean ±SD) years (Range)	36.56±16.22 (14-75)
Sex Male Female	 44 (58.67%) 31 (41.33%)
Occupation Students Free business Housekeeper Soldier Employee Retired	 16 (21.33%) 16 (21.33%) 25 (33.33%) 2 (2.67%) 6 (8%) 10 (13.33%)
Hematological malignancy AML ALL NHL CLL	 36 (48%) 23 (30.76%) 15 (20%) 1 (1.33%)
Hematological parameters WBC (Mean ±SD) $\times10^3$/ml Neutrophil (Mean±SD) $\times10^3$/ml Hb. (Mean ±SD) g/dl	 1.9±6.02 0.337±0.346 7.98±1.57
Temperature (Mean ±SD) ^{0}C	38.75±0.75

SD: Standard Division, AML: Acute Myeloid Leukemia, ALL: Acute Lymphoblastic Leukemia, NHL: Non Hogdkin Lymphoma, CLL: Chronic Lymphocytic Leukemia.

Blood culture for diagnosis fungemia

Seventy-five blood samples of neutropenic patients were implanted for a blood culture, positive blood cultures were detected in 2 samples (2.6%), both fungal isolates were*Candida albicans*as provedby the germ tube technique, figure (1).

Figure (1) Germ tube of Candida albicans

Measurement of β-D-Glucan concentration:
The βDG level was measured by using the Sandwich ELISA assay, the receiver operating characteristic (ROC) curve was constricting to assess the accuracy of serum βDG levels in discrimination between patients with or without fungemia, by applying optimal cut-off value created by ROC analysis, which was1020.33pg/mL, the results showed that βDG was positive in 10 (13.33%) from 75 neutropenic patients in the present study. The sensitivity of the cutoff value was 81.3% and its specificity was 66.7%, (95% Confidence Interval 0.562–0.908).

The area under the ROC curve (AUC) was 0.735 (P <0.001) which entitles a highly significant threshold of the accuracy for βDG in diagnosis of fungemia in neutropenicpatients.Figure(2)

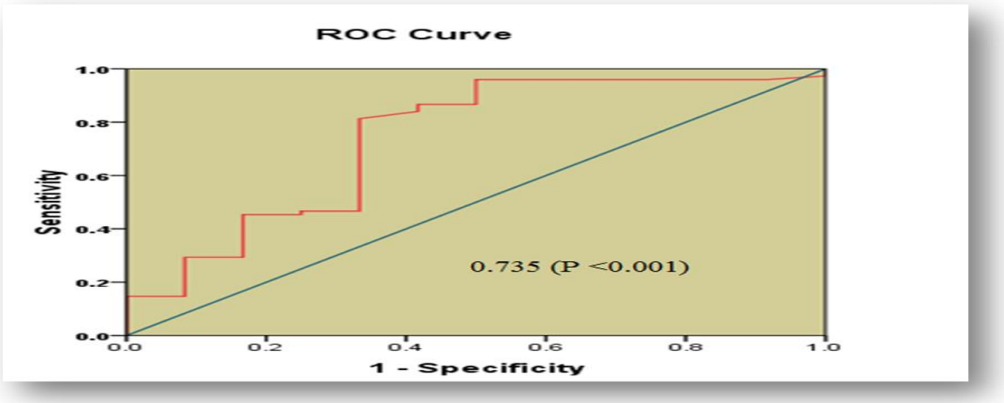

Figure (2) ROC curve analysis of serum βDG for fungemia in febrile neutropenic patients

Association of βDG with different age groups
The association between the marker (βDG) with different age groups showed that there is no significant association with the agegroups, table (2).

Table (2): Association of βDG with different age groups

Age groups	No. (%)	βDG pg/ml (mean±SD)	F-test	P-value
≤ 20 years	15 (20%)	335±571.9	0.555	0.647 NS
21-40 years	34 (45.33%)	362.9±345.2		
41-60 years	20 (26.66%)	257.3±233.3		
> 60 years	6 (8%)	118.8±41.79		

Association of (βDG) with hematological parameters and Temperature.
It was found that neutrophils count showed significant association with the concentration of βDG, while temperature doesn't have association with it, table (3).

Table (3) Association of βDG withhaematological parameters and Temperature.

Parameter/Units (mean ±SD) (Range)		**βDG pg/ml**
WBC x10^3/ml (1.92±0.60)	Person correlation	-0.071
	(P value)	0.548
NEU. x10^3/ml (0.337±0.346)	Person correlation	-0.227
	(P value)	0.050*
Hb. g/dl (7.98±1.57)	Person correlation	0.148
	(P value)	0.205
TEM.^{0}C (38.75±0.75)	Person correlation	0.218
	(P value)	0.061

Association type of leukaemia with (βDG)
Distribution of neutropenic patients in this study, according to the types of leukemia were shown in table (4), it is found there is no significant association of Beta-D-Glucan with different types of hematologic malignancies.

Table (4) Association of βDG with different types of hematologic malignancies

Malignancies	**No. %**	**βDG pg/ml (mean±SD)**	**F-test**	**P-value**
ALL	23 (30.6%)	383.2±525.3	0.698	0.501 NS
AML	36 (48.3%)	311.2±308.7		
NHL	15 (20%)	143.6±83.5		

ALL: Acute Lymphoblastic Leukemia, AML: Acute Myeloid Leukemia, NHL: Non Hodgkin Lymphoma..

Relationship between blood culture results and (βDG)
The level of βDG in the blood-positive culture was higher than in the blood-negative patients. By using statistical analysis (t- test) the results show a significant association between the biomarker with Positive blood culture p= 0.006 for βDG as shown in table (5).

Table (5) Association of BDG with blood culture

Biomarkers	**Positive culture (10)**	**Negative culture (65)**	**T-test**	**P-value**
βDG pg/ml (mean±SD)	646.9±425.1	336.9±292.1	2.833	0.006*

*Significant

Discussion

In this investigation the incidence of leukemia in males was 44 (58.67%) which was slightly higher than in females 31 (41.33%),this agree with other Iraqi researchers like Naba'a A. *et al*, who found that the epidemiology of leukemia in male more than female, with percent 60% in males and 40% in

females(7).Another study conducted by Sevan, mentioned that the incidence of leukemia was higher in males than in females(8),and this agree with World Cancer Declaration, progress Report (2016); that leukemia mortalities in males higher than females(9).

The mean ages of leukemic patients in this study were 36.56 ±16. 22 which is less than in the Iraqi study done by Nabaå A*et al* who reported that mean age was 41±1.24 (7).This suggests that by the time leukemia increase in younger persons, the highest mean of leukemia incidence was happening in age group located between (21-40) years with percentage 45.3%, followed by age group (41-60) years, ≤ 20, > 60 with lower percentages 26.6%, 20%, 8% respectively,which was contrary with the Iraqi study done by Hussein Ali (10),who found that the mean age range of 14 to 62 years and in agreement with a study results done by Al-Mulla *et al*(4).

Age has been perceived as a critical prognostic factor of both occurrence and survival in many types of leukemia, different investigations have directed to pinpoint particular genetic and biological processes taking place in various ages to represent the prognostic value of age at diagnosis, most researchers have reasoned in this field that leukemia does not discriminate between people of any age group, It is the leading cause of malignant neoplastic disease-related deaths among youngsters and young adults(11).

Regarding the type of leukemia, AML percent was 48 % in patients enrolled in this study followed by ALL, NHL and CLL with percentages 30.76%, 20% and 1.3% respectively. The possible explanations for such results are that the majority of patients in current study range from 21 to 40 years. As is known,AML is commonly known to affect adults in the later stages of life(12). This observation agrees with a study done in Iraqby Kashmoola*et al* (13)and in Canada by Shysh*et al* (14) who reported that the incidence rate of acute myeloid leukemia (AML) more than other types of leukemia.

In this investigation, blood culture for fungal infection was positive in 2 (2.6%) out of (75) samples, the percentage of patients with fungemicstate in the current study was lower than the percentage that reported in various studies.

Regarding fungal infection associated with febrile neutropenic patients, current study reported two cases (2.6%) infected with Candida species, which is considered in this study a small proportion compared with the results of other studies such as Deepak *et al*. (15)who reported account for 9-13% of Candida species causes of bloodstream infections and Daniel K. *et al*(16) Who found account for 9% Candida species causes of bloodstream infection.

Isolation fungi from blood cultures in febrile neutropenic patients can be more troublesome,as the optimal growth conditions and media used in recovering of fungi from blood samples varies.Most blood culture methods enable the growth of fungi, because the acquisition fungal growth conditionsare variable and dependable on laboratory methods.

The role of β-D-Glucan in febrile neutropenic patients

During the past years, there has been dramatically increased in the frequency of invasive fungal infection in immune compromised patients, so there is an urgent need to use the method in diagnosis be noninvasive, relatively easy and can detect the most common opportunistic invasive fungal pathogen(17).

In the current study, the level of the biomarker was estimated in 75 febrile neutropenic patients by ELISA methods, several studies have determined the β-D-Glucan value that would be predicted in the diagnosis of

The data presented in the current study was selected a β-D-Glucan cutoff value of 1020.33pg/mL on the basis of the ROC curve, the results of the current study showed out of 75 febrile neutropenic patients 10 (13.33%) were positive,the sensitivity was 81.3% and its specificity was 66.7%. He S *et al* mentioned that most cutoff values have a good diagnostic test accuracy and very high diagnostic accuracy (19).

The performance of βDG for the diagnosis of IFI has been estimated in several meta-analyses(20).The importance role ofβDGin diagnosis of invasive fungal infection has been increased after European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG)criteria definitions includes this biomarker as a diagnostic tool in predicted mortality of invasive fungal infection(21). Additionally,the patients with positive blood culture in this study had significantly higher serumβDG in corresponding to negative BCs with a p value (0.006) which referred to high accuracy of this biomarker in diagnosis of IFI.

An Association of (βDG) with hematological parameters and Temperature

The results in this investigation showed that among the different hematological parameters , absolute neutrophil count (ANC) has a significant correlation with βDG concentration at the time of febrile neutropenia, this similar to the study of(22).Who mentioned that there are strong correlations between the concentration of βDG with decrease of neutrophil count. While the temperatures have not correlated withβDGconcentration. That might be due to a variation in estimating of temperature due to sex, physical activity, age and body sites used to assess body temperature.

The association between the type of leukaemia and concentration of βDG

The study results statistics indicated that there's no significant relationship between β-D-Glucan concentration with types of leukemia these results in line with many other studies that deal with βDG concentration in febrile neutropenic patients such as Koltze A (23) who reported the same results with regard to βDG level in patients with febrile neutropenia.This result disagreement with Senn*et a.l* who reported that β-D-glucan level more significant in acute leukemia(18).

This discrepancy in such results may be due to that the process of patient's selection in each study subject to criteria and controls are vary from one study to another in addition to that the number of patients entering each research and study design (cohort vs. case-control studies) has a significant impact in the final data for each study.

Conclusion

This investigation concluded that β-D-Glucan showing acceptable sensitivity and can provide rapid detection for fungemia in FN patients which can lead to avoid prolonged and empirical used of treatment.

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