Original Article

Current Spectrum of Aerobic Bacterial Infection in Patients with Hematological Malignancy.

Jaba Roy[1], Salahuddin Shah[2], Yakub Jamal[3], Sharmeen Ahmed[4], M. A. Bashar[5]. Md. Ruhul Amin Miah[6]

[1]Assistant Professor, Department of Microbiology, Dhaka National Medical College; [2]Assistant Professor, Department of Hematology, BSMMU; [3]Professor, Department of Paediatric Hemato-Oncology, BSMMU; [4]Professor, Department of Microbiology & Immunology, BSMMU; [5]Professor & Head, Dept. of Cardiology, Dhaka National Medical College & Hospital. [6]Professor & Chairman, Department of Microbiology & Immunology, BSMMU

Abstract :

Bacterial infections are a major cause of illness in patients with hematological malignancy and can lead to mortality, if not treated early and properly. The incidence of bacterial infection in these patients and the spectrum of causative organisms are liable to change according to region & time. This observational study was designed to isolate and identify the aerobic bacteria causing infection in patients with hematological malignancy. The Study was done in the Department of Microbiology and Immunology, BSMMU from March 2012 to August 2012. Forty diagnosed patients of hematological malignancies who were admitted in the Hematology Department and in The Paediatric Hemato-oncology Department of BSMMU with symptoms of sepsis &/ or UTI or RTI were enrolled in this study. Blood, throat swab and urine were collected from each patient and sputum was collected from four patients. Infection was microbiologically detected in 37.5% of patients. Gram negative bacteria were most frequently isolated from various specimens except throat swab from which only S. pyogenes were isolated.

Introduction :

Bacterial infection is the most common complication encountered in patients with hematological malignancies. Several conditions like immunodeficiency associated with primary malignancy and neutropenia, B & T cell deficiency caused by use of chemotherapeutic drugs make these patients vulnerable to   infections[1]. A recent survey showed that pneumonia is the predominant infection (38%) in these patients followed by blood stream infection (35%), urinary tract infection (11%) and skin & soft tissue infection (6%)[2]. The spectrum of bacteria causing infection in these patients is quite big. In the past few decades major changes have occurred in the type and range of bacteria causing infection in such patients[3]. Upto mid-1980s, Gram negative bacteria accounted for majority of bacterial infections in these patients[4]. But it seems that this pattern of causative bacterial agents has changed significantly. Rolston reviewed that Gram-positive organisms account for 47% of documented infections, Gram-negative pathogens for 30%, and polymicrobial infections for 23% in these patients[5]. As infections in these immuno-suppressed patients leads to increased morbidity & mortality[1], so, prompt treatment of infection is very important.

But as the currently available laboratory tests are not sufficiently rapid to isolate and identify the causative bacterial agents of infection, so administration of specific antimicrobial agents is delayed[6]. In this situation, prompt empiric antibiotic treatment with broad spectrum antibiotic can reduce morbidity and mortality[6]. Clinical trials revealed that antibiotic prophylaxis in neutropenic patients reduced infection-related mortality by 42%[7]. Selection of appropriate empiric antimicrobial regimen for these patients in a particular institution requires that the spectrum of commonly isolated bacteria to be known[7].

Study population :

Diagnosed patients of hematological malignancies who were admitted in the Hematology Department and in The Paediatric Hemato-oncology Department of BSMMU. Forty Patients with hematological malignancy with suspected sepsis &/ or UTI or RTI were included in the study.

Materials & methods :

This observational study was carried out in the Department of Microbiology & Immunology, BSMMU, Dhaka, from March to August 2012 after approval of The Institutional Review Board of BSMMU. Blood, throat swab and urine were

collected from each patient and sputum was collected from four patients who had productive cough.

Blood was collected maintaining strict aseptic precaution after taking verbal consent from patient or attendant. Collected blood was injected immediately into the automated blood culture bottle, respective of age at the bed side. The blood culture bottles were kept in automated BACTEC 9240 machine. When the machine gave positive signal, bottle was withdrawn from machine for subculture[8]. Subculture was done onto Blood agar, Chocolate agar and MacConkey agar plates. Gram staining from the bottle was done to see the morphological characteristic of organisms . From each patient two throat swabs were collected in the morning before any mouth wash & ingestion of food. Swabs were taken with sterile cotton tipped swab sticks in sterile test tubes. One swab was used to make two smears for Gram stain and Albert's stain for microscopy. With the other swab inoculation of media was done. For sputum collection, patient was asked to take a deep breath & then expel the expectorate directly into a sterile plastic container. With the purulent portion of sputum, gram staining & Zeihl-Neelsen staining were done & seen under microscope. All four sputum specimens were accepted for culture as they fulfilled the necessary criteria for culture. Blood agar, Chocolate agar and MacConkey agar plates were used for culture of throat swabs & sputum. About 20ml of freshly passed clean-catched midstream urine was collected in a sterile clean plastic tube. Urine was plated on Blood agar & MacConkey agar using a calibrated wire loop that holds 0.001 ml of urine sample. The bacterial colonies were counted & multiplied by 100 to give an estimate of the number of bacteria present per ml of urine. Significant growth was determined as $\geq 10^5$ colony forming units. The results of culture were correlated with finding of stained smears & isolated bacteria in pure culture was considered as pathogen. Isolated Gram negative bacteria were identified by colony morphology & various biochemical tests and Gram positive bacteria were identified by colony morphology, time required to grow, morphology on stained slides, hemolytic property on Blood agar, biochemical tests, Bacitracin sensitivity tests[9] etc.

Results:

A total of 40 diagnosed patients of hematological malignancy with signs of infection were investigated in this study. Among the different specimens, rate of isolation of bacteria was highest from blood (20%), followed by throat swab (10%). Three sputum specimens out of four collected (75%), showed positive culture. Urine had least isolation rate (7.5%) of bacteria. (Table I). Gram negative bacteria were the predominant isolates (75%) from blood. E. coli was the most

frequently isolated organism (37.5%), followed by *P. aeruginosa* (25%). Only *S. pyogenes* was isolated from throat swab, whereas from sputum specimens, *E. coli*, *Klebsiella* spp. & *P. aeruginosa* were isolated. *Klebsiella* spp. were the predominant bacteria (66.67%) isolated from urine & no Gram positive organism was isolated in sputum & urine. (Table II)

Table I Isolation rate of bacteria in different clinical specimens and among patients

Type of malignancy	No. of clinical specimens showing positive culture				No. of patients showing positive culture
	Blood n= 40	**T/S** n= 40	**sputum** n= 04	**Urine** n=40	**N = 40**
ALL	04	02	00	02	7
AML	03	01	03	01	6
NHL	00	01	00	00	1
MM	01	00	00	00	1
Total (%)	08 (20)	04(10)	03 (75)	03(7.5)	15 (37.5)

Note: Figure in parenthesis indicates percentage.

ALL = Acute lymphoblastic leukaemia

AML = Acute myeloid leukaemia

NHL = Non- Hodgkin's lymphoma

MM = Multiple myeloma

N = total number of patients

Table II Distribution of bacterial isolates from various culture positive specimens

Name of bacteria	**Blood** n = 8	**T/s** n = 4	**Sputum** n = 3	**Urine** n = 3
Gram negative				
E. coli	03(37.5)	00	01 (33.33)	00
Klebsiella spp	00	00	01(33.33)	02(66.67)
P. aeruginosa	02 (25)	00	01 (33.33)	00
Acinetobacter spp	01(12.5)	00	00	01(33.33)
Total Gram negative (%)	06(75)	00 (00)	03(100)	03(100)
Gram positive				
S pyogenes	00	04(100)	00	00
S. aureus	01(12.5)	00	00	00
C. Jeikeium	01(12.5)	00	00	00
Total Gram positive %	02(25)	04(100)	00(00)	00(00)

Note: Figure in parenthesis indicates percentage.

Discussion :

Causative bacterial agents were microbiologically detected from various clinical specimens in 37.5% of patients having infective episode. In majority of patients the causative bacterial agents could not be detected. The causes may be that patients were already on antibiotic therapy before collection of specimens, or they were infected with anaerobic bacteria, virus or fungus. In this study, blood stream infection was microbiologically documented in 20% of patients. This rate is higher than a previous study in Bangladesh done on AML patients in which isolation rate of bacteria from blood was only 5%[10]. The cause of lower isolation rate in that study may be that lysis centrifugation method of blood culture was used & the lysing solution is toxic to some organisms[11]. In the current study, 75% of isolated bacteria from blood was Gram negative. Among them *E. coli* was more frequently isolated (37.5%), followed by *P. aeruginosa* (25%). This finding correlates with that of some recent works [12, 13], though workers from India reports predominantly Pseudomonal blood stream infection[14]. The present study, including various other previous studies[12-14] clearly indicates that a shift of bacterial epidemiology from Gram positive to Gram negative causing blood stream infection has occurred in patients with hematological malignancy. The current shift of bacterial epidemiology may be due to widespread use of antibacterial prophylaxis mainly targeted against Gram positive micro-organisms[15]. Though in this study, sputum could be collected only from 4 patients, 3 specimens (75%) yielded growth of pathogenic bacteria. Only Gram negative bacteria were isolated. Previous workers reported various Gram positive bacteria along with Gram negative from sputum but with Gram negative predominance[13,16]. A much lower (7.5%) isolation rate of bacteria was obtained in urine in the current study, probably because the patients had no associated favoring factors of UTI, like, hypertension or diabetes mellitus or catheterization[1]. Enterobacteriaceae group of organisms were the predominant isolates in urine in this study. On the other hand, *S. pyogenes* was the only isolate from throat swab, no other Gram positive or Gram negative bacteria were isolated in this study. In contrary, various Gram positive & Gram negative bacteria from throat swab has been reported in recent studies[13,16].

Conclusion:

This study gives us an idea to the current spectrum of aerobic organisms causing infection in patients with hematological malignancy. This study indicates that infection by Gram negative organisms predominate and blood stream infection is the major site of infection in such patients .

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