MICROBIOLOGICAL SPECTRUM AND OUTCOME OF FEBRILE NEUTROPENIA IN PAEDIATRIC PATIENTS WITH LEUKAEMIA

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ABSTRACT

BACKGROUND

Febrile neutropenia is the most common morbidity in leukaemia and one of the most common causes of mortality. The knowledge of pathogens causing bacteraemia and the sensitivity pattern in febrile neutropenia is essential in order to start empirical antimicrobial therapy.

MATERIALS AND METHODS

We studied the bacterial spectrum and the antibiotic susceptibility pattern of organisms causing infections in children with febrile neutropenia admitted to Govt. Medical College, Kozhikode, Paediatric Oncohaematology Ward, over a period of one and a half years Jan 2011 to June 2012. Common Pathogens were isolated from blood cultures using Automated (Bactec) methods done in the Dept. of Microbiology.

RESULTS

Blood samples were collected from 325 febrile patients with neutropenia. Growth was obtained in 101 blood cultures, the rest 224 were sterile. Bacteraemic episodes occurred in 92 cases, of which 27 were the maximum number of isolates of Gram positive bacteria and 21 Gram negative bacteria. Among the Gram-positive cocci, Coagulase-Negative Staphylococci (CoNS) were the predominant isolates (n = 13) followed by Methicillin Resistant Staphylococcus aureus (MRSA) (n = 9). Klebsiella and Acinetobacter species (n = 6) were the predominant pathogens among Gram negative rods followed by Pseudomonas aeruginosa (n = 5). Among the nine fungal pathogens of Candida isolated, one was Candida tropicalis and other significant isolates being Candida glabrata and Candida albicans. The culture grew 33 skin flora being diphtheroids and micrococci and 11 Aerobic spore bearers (contaminants) that were of no significance. All gram-positive isolates were susceptible to vancomycin. Among Gram negative rods, Ceftazidime/Netilmicin was the first line antibiotic in 69% cases.

CONCLUSION

The microbiological profile in febrile neutropenia in children with leukaemia in our population appears to be towards gram positive bacteria.

KEYWORDS

Febrile Neutropenia; Leukaemia; Fever.

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BACKGROUND

Fever with neutropenia in cancer patients was considered a medical emergency, requiring prompt in-hospital evaluation and administration of broad-spectrum intravenous antibiotics. Approximately, 60% of febrile neutropenic episodes were caused by bacterial infection with or without bacteraemia.^[1]

Febrile neutropenia is the most common cause of morbidity in patients with leukaemia. The most important sign of infection in neutropenic patients is fever and frequently may be the only evidence of infection.^[2]

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The major causes of febrile neutropenia in leukaemia are chemotherapy, systemic infections, immunosuppression and immunodeficiency associated with cancer.^[3] Increasing use of cytotoxic chemotherapy for different cancers has led to more number of neutropenic patients. Acute leukaemia is the most common of all childhood malignancies accounting for about 30% of its total incidence. Approximately, 1 in 2000 children will develop it before the age of 15 years. The cure rate of acute leukaemia is 80% - 90% in developed countries.^[4] A systemic review of literatures showed that age, nutritional status, chemotherapy dose and intensity, low baseline blood counts are all risk factors for greater morbidity and mortality.^[5]

Fever is defined as a single oral measurement of 38.3° C or 2 measurements of 38° C separated by at least one hour.^[6] Mild neutropenia is defined as absolute neutrophilic count (%PMN + %band forms) < 1500, moderate neutropenia as ANC 500 - 1000, severe neutropenia ANC < 500.^[7]

Traditionally, children with malignant disease who present with fever and neutropenia are hospitalised for parenteral antibiotics. Based on the study by Bodey in 1966 indicates that an ANC < 500 cells/mm³ conferred a high risk of bacteraemia.^[4]

Very few studies have been conducted in India based on febrile neutropenia,^[8] such a study was not conducted in Kerala to evaluate the outcome. The available studies had several limitations lacking independent validation or differences in predictive factors considered.

This study was undertaken to know the susceptibility patterns of bacteria causing bacteraemia in febrile neutropenic children with leukaemia in our hospital and to assess the usefulness of antibiotic regime. It is also to study the common organisms associated with infections in febrile neutropenic patients and its outcome.

MATERIALS AND METHODS

The study was carried out at the Clinical Microbiological Laboratory and Department of Paediatrics of Govt. Medical College, Kozhikode, oncohaematology ward, Institute of Maternal and Child Health. No discrimination was made based on age or gender.

Population

All febrile neutropenic children admitted to IMCH leukaemia ward with fever $> 38.5^{\circ}\text{C}$ or 101.5°F and $\text{ANC} < 500$.

Study Period

One and a half years (January 2011 to June 2012).

Inclusion Criteria

All leukaemic children (ALL or AML) with febrile neutropenia (fever $> 38.5^{\circ}\text{C}$ and $\text{ANC} < 500$ cells/mm³) admitted to IMCH leukaemia ward from Jan 2011 to June 2012.

Exclusion Criteria

Patients who received prior bone marrow transplantation.

Descriptive Study

Children presenting with febrile neutropenia from January 2011 to June 2012 admitted to IMCH, Kozhikode who satisfy the inclusion criteria.

Method of Study

All children (AML/ALL) with leukaemia admitted to paediatric wards with fever (A single axillary measurement of 38.5°C or 2 measurements of 38°C separated by at least one hour) and severe neutropenia ($\text{ANC} < 500$ cells/mm³) are included in the study.

The patients are followed up throughout the hospital stay till discharge and the outcome is noted in terms of return of counts to normal, resolution of fever, discharge of the patient or death.

A pre-written proforma is filled in based on clinical examination and questionnaire which includes age, sex, type of leukaemia, date of diagnosis of leukaemia, fever, ANC, number of neutropenic episodes, stage of leukaemia, details of chemotherapy, systemic involvement, IV site infection, PEM, microbiologic flora, antibiotic sensitivity, ICU admissions and the outcome.

Blood is collected in Paediatric Bactec bottles prior to antibiotic therapy and culture is done accordingly. The blood culture bottles were incubated at 37°C for up to 7 days. Positive culture bottles after the beep are subcultured to appropriate media. The isolates were identified by doing a gram stain and biochemical tests. Blood culture results are

noted to study the common organisms. If cutaneous ulcers are present, swabs are taken from the deeper part and inoculated into routine bacterial culture media and Sabouraud's dextrose agar. The antibiotic susceptibility testing of isolates was done by the modified Kirby-Bauer disk diffusion technique and the results were interpreted according to the recommendations of National Committee for Clinical Laboratory standards (NCCLS).^[9]

The patients are followed up throughout the hospital stay till discharge and measures taken to overcome neutropenia.

RESULTS

A total of 325 children with febrile neutropenia were included in the study. Acute Lymphatic Leukaemia was the most common diagnosis. Growth was obtained in 101 blood cultures, rest 224 were sterile.

Bacteraemic episodes occurred in 92 cases, of which 27 were the maximum number of isolates of Gram positive bacteria and 21 Gram negative bacteria.

Among the Gram-positive cocci, Coagulase negative staphylococci (CoNS) were the predominant isolates ($n = 13$) followed by Methicillin Resistant Staphylococcus aureus (MRSA) ($n = 9$). Staphylococcus aureus ($n = 5$).

Klebsiella and Acinetobacter species ($n = 6$) were the predominant pathogens among Gram negative rods, followed by Pseudomonas aeruginosa ($n = 5$). E. coli ($n = 3$). Salmonella enteritidis ($n = 1$).

Among the nine fungal pathogens of Candida isolated, one was Candida tropicalis and other significant isolates being Candida glabrata and Candida albicans. The culture grew 33 skin flora being diphtheroids and micrococci and 11 aerobic spore bearers (Contaminants) that were of no significance.

	Bacteria	Percent
Negative	224	68.9
Positive	101	31.1
Total	325	100

Table 1. Blood Culture Yield

Bacterial Spectrum	Number	(%)
Gram Positive Cocci	27	29.3
Coagulase Negative Staphylococci	13	48
Staphylococci aureus	5	19
Methicillin Resistant Staphylococcus aureus	9	33
Gram-Negative RODS	21	%
Klebsiella pneumoniae	6	29
Acinetobacter species	6	29
Pseudomonas aeruginosa	5	24
Escherichia coli	3	14
Salmonella enteritidis	1	4
Fungal Isolates Candida Species	9	%
C. albicans	7	78
C. glabrata	1	11
C. tropicalis	1	11

Table 2

Bacterial spectrum of isolates ($n = 92$) from blood of neutropenic patients.

Culture was found to be positive in 31.1% of the cases. The culture was sterile in 68.9%; in positive cultures CONS was grown in 48%.

Culture was positive for 48 significant bacteria, which included Gram negative and Gram positive bacteria.

n = 325

Age Group	No. of Children	Percentage
< 1 yr.	0	0
1 - 5 yrs.	129	40
5 - 10 yrs.	120	37
> 10 yrs.	76	23
Total	325	100

Table 3. Age Distribution

There was no subject of age < 1 yr. There was almost an equal distribution between those less than 5 yrs. (40%) and more than 5 yrs. (37%) admitted with febrile neutropenia.

n = 325

Sex	No. of Children	Percentage
Male	67	21
Female	258	79
Total	325	100

Table 4. Sex Distribution

The incidence of febrile neutropenia was more among females [79%] as compared to males [21%].

n = 92

Type of Leukaemia	Frequency of Neutropenic Episodes	Percentage
ALL	69	75
AML	23	25
Total	92	100

Table 5. Types of Leukaemia

Among the 92 neutropenic episodes, almost 75% had ALL.

n = 325

	No. of Children	Percentage
Expired	9	3%
Relieved	316	97%
Total	325	100.0

Table 6. Mortality

Out of the 325 children admitted due to febrile neutropenia, 9 expired which contributed to 3% mortality.

Antimicrobiotics	CoNS	MRSA	Staphylococcus
Trimethoprim/ Sulfamethoxazole	38.46	20	20
Cefazolin	46.15	20	20
Amikacin	46.15	0	50
Vancomycin	100	100	100
Erythromycin	30.76	0	100
Penicillin	38.46	0	0
Cefoxitin	46.15	0	100
Gentamicin	53.84	20	20

Table 7. Percentage of Antimicrobial Susceptibility of Gram-Positive Isolates

CNS- Coagulase Negative Staphylococci; MRSA- Methicillin Resistant *S. aureus*.

All gram positive isolates were sensitive to vancomycin 100%. Resistant gram-positive pathogens such as MRSA has

become more common and are the most prevalent resistant isolates in some centres.

Emergence of MRSA in positive blood cultures from patients with febrile neutropenia- a cause for concern.

Antimicrobiotics	Klebsiella SP	P. aeruginosa	Acinetobacter	E. coli
Amikacin	100	100	66.6	66.6
Chloramphenicol	41.6	0	0	0
Ciprofloxacin	66.6	20	66.6	0
Pip-tazobactam	50	60	33.6	66.6
Cefotaxime	0	50	50	33.3
Gentamicin	83.3	20	83.3	66.6
Ceftazidime	33	69	0	0
Cefoperazone-sulbactam	66.6		66.6	66.6
Ampicillin	0	0	20	20

Table 8. Percentage of Antimicrobial Susceptibility of Gram Negative Isolates

DISCUSSION

Febrile neutropenia is one of the most common cause of mortality and morbidity in children with leukaemia. This study on febrile neutropenia included the population with acute leukaemias excluding those who received prior bone marrow transplantation. The present study included 325 patients with febrile neutropenia, bacteraemic episodes occurred in 92 cases, of which 27 were Gram positive bacteria and 21 Gram negative bacteria documented over a period of one and a half years between Jan 2011 - June 2012. A retrospective study conducted by Timothy et al¹⁰ in Trinidad and Tobago involved 36 subjects with 71 episodes of febrile neutropenia documented between 2001 and 2008.

The present study and the study conducted by Timothy et al on comparison showed almost equal number of subjects with more than one febrile neutropenic episodes among subjects.

There was no subject of age < 1 yr. There was almost an equal distribution between those less than 5 yrs. (40%) and more than 5 yrs. (37%) admitted with febrile neutropenia. Only 23% were of age > 10 yrs.

The incidence of febrile neutropenia was more among females [79%] as compared to males [21%].

75% of the cases were ALL and 25% AML contributed to around a quarter of the cases. Out of the 325 children admitted due to febrile neutropenia, 9 expired which contributed to 3% mortality. Maximum mortality was observed in children who had febrile neutropenia in the relapse phase and this was statistically significant.

In a study conducted by Santolaya et al,¹¹ it was found that 60% of febrile neutropenic episodes were caused by bacterial infections with or without bacteraemia. In another study conducted by Hughes W T, Armstrong D, Bodey G P et al¹² showed that clinically documented infections occurred in 20% - 30% of febrile episodes; common sites of tissue-based infection include the intestinal tract, lung and skin and bacteraemia occurred in 10% - 25% of all patients with most episodes occurring in the setting of prolonged or profound neutropenia (ANC, 100 neutrophils/mm³). Pain et al¹³ concluded that early in the development of cytotoxic chemotherapy, during the 1960s and 1970s, gram negative pathogens predominated. Then during the 1980s and 1990s, gram-positive organisms became more common because of

increased use of indwelling plastic venous catheters, which can allow for colonisation by and entry of gram-positive skin flora as documented in the Hughes, Bodey et al¹⁴ study. Currently, coagulase-negative staphylococci are the most common blood isolates in most centres (Kanamaru et al).¹⁵ CoNS are the commonest organism isolated in the Western countries followed by staphylococcus aureus.

In our study CoNS was the commonest organism (48%), followed by Methicillin sensitive staphylococcus aureus (18.5%) of the gram positive bacteria.

Among Gram-negative bacilli, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella* sp. are the commonest pathogens. The predominant isolates were *Klebsiella* (28.5 %) and *Acinetobacter* sp. (28.5%). Culture was sterile in 224 cases. Common fungal pathogens included candida species, of which *Candida albicans* was the predominant one (78%).

Other studies also had similar results with maximum cultures being sterile. A study by Alison G. Freifeld, Eric J. Bow, Kent A. Sepkowitz et al¹⁶⁻¹⁷ said majority of patients who develop fever during neutropenia have no identifiable site of infection and no positive culture results. Increased mortality was observed in children who had culture positivity. This was clinically significant even though statistically insignificant.

Even though the most common organism grown was CONS, maximum mortality was seen with MRSA and pseudomonas infections. Among the culture positive cases, majority grew CONS. Among them 2 cases of mortality were recorded; 1 case of MRSA expired and another case of mortality was associated with the growth of gram negative bacilli. 5 deaths had a sterile culture result.

Most of the children had febrile neutropenia while they were on cytosine arabinoside and daunorubicin. Majority of patients who develop fever during neutropenia have no identifiable site of infection and no positive culture results. This was shown in the Hughes et al study. Our patients received Ceftazidime/Netilmicin as the first line antibiotic in 69% cases. In our country, Celkan et al showed 50% of *S. aureus* isolates and 18% CNS isolates had a methicillin resistance in paediatric haematology-oncology department between 2000 and 2004.

In our study methicillin-resistant *S. aureus* accounted for 33.3% of staphylococcal isolates. A national surveillance programme in Norway showed more than 99% susceptibility to aminoglycosides in blood culture isolates of *E. coli* and *Klebsiella* spp.^[18] In our study, 100% susceptibility to aminoglycosides in blood culture isolates of *Klebsiella* spp. only was reported. Butt et al^[19] showed an increasing resistance to third generation of cephalosporins. In this study, resistance rates were reported for Gram-negative pathogens, reaching nearly 50% for ceftazidime, 33% for and ciprofloxacin.

For the effective empirical antibiotic treatment in febrile neutropenia, it is essential to know the resistance pattern of the hospital and region. Continuous surveillance in local hospitals is also important for the monitoring of rates of resistant organisms.

In conclusion, the shift toward gram-positive organisms and the continuing need to provide Gram-negative coverage demands the use of an agent or agents that provide coverage for the spectrum of potential infecting organisms. Studies reporting local microbiological findings are necessary for

appropriate antibiotic choice. As the aetiology of febrile neutropenic infections has shifted from gram-negative to gram-positive organisms in many centres. Our results also show that infections with gram positive bacteria predominates in febrile neutropenic attacks in our centre.

CONCLUSION

From this study, we analysed the various parameters associated with febrile neutropenia in children.

There was however a higher mortality among children more than 5 yrs. of age admitted with febrile neutropenia.

Although, most of them were suffering from ALL in the induction phase, mortality rates were higher among patients with ALL in relapse. Respiratory complaints were the common manifestations in leukaemic children presenting with febrile neutropenia, though in more than 60% the blood cultures were sterile.

However, among the positive cultures CONS was the most commonly grown organism with MRSA, GNB, micrococci, aerobic spore bearers and pseudomonas contributing for the rest. Among the common fungal isolates, the predominant fungus was *Candida albicans*. Children with severe anaemia had an increased mortality rate. Children who developed febrile neutropenia in the relapse phase had a higher mortality. Gram negative bacteria were susceptible to Ceftazidime, Ciprofloxacin, Amikacin, Piptazobactam and Imipenem. Gram positive bacteria responded to Vancomycin.

The incidence of febrile neutropenia was more among females [79%] as compared to males [21%].

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