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Original Research Article.

Frequency of Candida Infection in Post Chemotherapy Acute Leukemia Patient with Febrile Neutropenia

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ABSTRACT

Objective: In this study our main goal is to evaluate the frequency of candida infection in post chemotherapy febrile neutropenia patient with acute leukemia.

Method: This prospective type of observation study carried out at Department of Haematology of Bangabandhu Sheikh Mujib Medical University (BSMMU) from September 2016 to August 2017. A total 63 patients of acute leukaemia (AML and ALL) were admitted in the Department of Haematology in BSMMU. Patients were selected by purposive sampling. A typed questionnaire was supplied to all patients and those who gave the written consent were selected as cases.

Results: In the study, it was found that 19 (30.2%) patients belonged to age ≤20 years followed by 19 (30.2%) of 21-30 years, 13 (20.6%) 31-40 years, 6 (9.5%) 41-50 years and 6 (9.5%) >50 years of age. Immunophenotypically AML was found in 35 patients which was 55.6% of study population, B ALL in 9 (14.3%), T ALL in 5 (7.9%) and APL in 2 (3.2%) patients. Majority of the patients 28 (44.4%) were found in consolidation phase of chemotherapy followed by 26 (41.3%) in induction phase, 5 (7.9%) in relapse, 3 (4.8%) in re-induction and 1 (1.6%) in palliative phase. 6 (9.5%) patients were found positive for throat swab for Candida in this study and blood culture were negative for candida. Among the AML patients 4 (66.7%) patients were found positive for candida in throat swab and 36 (64.3%) were found negative.

Conclusion: In the study, it was found that among candida in throat swab were more common in AML than ALL. Cause was unknown but might be due to Reduce duration of neutropenia by applying G-CSF and empirical local and systemic antifungal therapy.

Keyword: Candida Infection, Leukemia, Lymphoma, Myelodysplastic Syndrome.

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INTRODUCTION

Hematological malignancy is primary cancers originating from cells of the bone marrow and lymphatic system and they comprise a broad range of diseases, with significant variation in their presentation, natural history, treatment and prognosis (Fey 2007).[1] There are three main categories of hematological malignancy including forms of leukemia, lymphoma, and multiple myeloma (MM). Other includes myelodysplastic syndrome (MDS), Myeloproliferative disorders are also considered under hematological malignancies (Hossain et al. 2014).[2] Acute Leukemia is clonal evolution of cancer where gain of function of oncogene and loss of function of tumor suppressor gene coincide which causes disease (Thomson and Dave, 2014 Wintrobe 13th).[3] The four common types of leukaemia are: acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL), chronic myeloid leukaemia (CML), chronic lymphocytic leukaemia (CLL) (Sharma andLokeshwar,2005).[4]

Patients with acute leukemia are highly susceptible to infectious diseases due to factors related to the disease itself, factors attributed to treatment, and specific individual risk factors in each patient. Patients with chemotherapy-induced neutropenia are at

particularly high risk, and microbiological agents include viral, bacterial, and fungal agents. The etiology is often unknown in infectious complications. The commonest fungal infections in neutropenia are candidosis, aspergillosis and Mucor mycosis. Invasive and local fungal infection are major complication of neutropenic patient and BMT recipients (Warnock 1998).[5] Systemic fungal infections occur in Acute Leukaemic patient with neutropenia associated with high rate of morbidity and mortality (Donowitzet al. 2001).[6] Invasive fungal infection occur in 20% of patients with neutropenia (Walsh and Pizzo, 1989) but in case of autopsy report it is 40% (Bodey et al. 1992).[7,8] Most common opportunistic mycosis are candida albicans, cryptococcus neoformans, Aspergillus fumigatus. In invasive fungal infection Candida is 42%, Aspergillus is 29%, Cryptococcus is 4% and others molds are 19% (Pfaller et al. 2006).[9] Among fungal Pathogens Candida is the most common during neutropenia and Aspergillus species to be a distant second (Wingard, 2004).[10] Major risk factor for candida infection are Neutropenia, Central venous catheter, renal support, steroids, colonization, antimicrobials, and surgeries. Presentations of local and systemic Candidiasis in neutropenia are non-specific. In Case of local Candida infection, Skin and mucous membranes of GI and GU tracts involved in immunocompetent and immunosuppressed patient (Guarner et all,2011).[11] Oral candidiasis most commonly found in leukaemia patients is acute pseudomembranous candidiasis which often appears as white plaques on the mucosa of tongue, cheeks, gingiva, pharynx (Dreizen, 1984).[12] In systemic candidiasis presentation is persistent or recurrent fever resistant to treatment with broad-spectrum antibacterial agents (Warnock, 1998).[5] Polyarthralgia, polymyalgia, azotemia, and elevated alkaline phosphatase levels are the important clues to the diagnosis of candidiasis (Wingard, 2004).[10]

However frequency of Candida infection in post chemotherapy acute leukaemia patient with febrile neutropenia is not known in Bangladesh. With combine diagnostic approach performed a prospective study over 63 patients of AML and ALL to diagnose local and systemic Candida infection after chemotherapy when ANC<500/cmm which persist at least 3 days to know the frequency of local and systemic candida infection. The aim of this study was to observe local and systemic candida infection in 63 AML and ALL patients in Bangladesh.

OBJECTIVES

General Objective

To see the frequency of candida infection in post chemotherapy febrile neutropenia patient with acute leukemia.

Specific Objectives

1) To assess local candida infection from throat swab by culture and microscopy.

2) To assess systemic candida infection from blood by culture.

3) To correlate local and systemic candida infection.

METHODOLOGY

Type of Study: Prospective type of observational study

Place of Study: Department of Haematology of Bangabandhu Sheikh Mujib Medical University (BSMMU).

Study Period: September 2016 to August 2017

Study Population: 63 patients of acute leukaemia (AML and ALL) were admitted in the Department of Haematology in BSMMU.

Sampling Technique: A purposive sampling technique was used to identify the participants. This study used a non-random sampling technique.

Method: Patients were selected by purposive sampling. A typed questionnaire was supplied to all patients and those who gave the written consent were selected as cases.

Statistical Analysis: Data was collected on preformed data collection sheets. Normality assumption was done for continuous variables. Continuous variables were expressed as mean and standard deviation (SD) whereas categorical variables were summarized using frequencies and percentages. Pair and independent t-test were performed to compare quantitative variables and Chi-square test was done to see the association among qualitative variables between groups. p value < 0.05 considered level of significance. Statistical analyses were carried out by using the Statistical Package for Social Sciences version 23.0 for Windows (SPSS Inc., Chicago, Illinois, USA)

Sample Size Estimation

Formula:

Sample size for measuring any proportion, $n = Z^2pq/d^2$, where

Z = area under the normal curve corresponding to the desired confidence level and for 95% confidence level value for Z is 1.96

p = 0.701

q = 1-0.701 = 0.299

d = 0.1 (Power of study is 90%)

So, $n = 1.96^2 \times 0.701 \times (1-0.701)/0.1^2 = 0.8048)/.01=80.48$

So estimated sample size is 80 cases.

SELECTION CRITERIA

Inclusion Criteria

- Age 10 -70 years.
- Diagnosed case of Acute Leukaemia (AML and ALL).
- Post-chemotherapy patient in any phase.
- Absolute neutrophil count (ANC) ≤500/cmm.
- Febrile patient.
- Giving informed written consent to participate.

Exclusion Criteria

- Patient refuse to participate.
- Age less than 10 years and more than 70 years.
- Suffering from DM, CKD and HIV.
- ANC >500/cmm.
- Neutropenia without fever.

Data Collection Procedure: All the diagnosed case of acute myeloid leukaemia and acute lymphoid leukaemia patient on the basis of BM morphology with or without immunophenotype, cytochemistry and cytogenetic results admitted in the department of haematology of BSMMU were recruited in this study. Patient was treated according to standard protocol.

RESULTS

Table I shows that 19(30.2%) patients belonged to age ≤20 years followed by 19 (30.2%) of 21-30 years, 13 (20.6%) 31-40 years, 6 (9.5%) 41-50 years and 6 (9.5%) >50 years of age.

Figure I observed that more than half (50.8%) patients were male and 31(49.2%) patients were female. Male female ratio was 1.03:1.

Table II shows that bone marrow aspiration report of 40(64.5%) patients were AML, whereas 18(29.0%) were ALL and 4(6.5%) were APL.

Table III observed that Immunophenotype typically AML was found in 35 patients which was 55.6% of study population, B ALL in 9(14.3%), T ALL in 5(7.9%) and APL in 2(3.2%) patients. In 12(19.0%) cases immunophenotype was not done.

Table IV shows that AML was found in 45 cases among them 17 (37.8%) were under DA protocol, whereas HiDAC was on 20(44.4%), DAE on 3 (6.7%) and 5(11.1%) cases got others chemotherapy. ALL was found in 18 cases. Among them 10 (55.6%) were under Hyper CVAD followed by 8 (44.4%) on modified BFM.

Table V observed that majority of the patients 28 (44.4%) were found in consolidation phase of chemotherapy followed by 26 (41.3%) in induction phase, 5 (7.9%) in relapse, 3 (4.8%) in re-induction and 1 (1.6%) in palliative phase.

Table VI observed that mean absolute neutrophil count was found 182.7±110.7 in 1st enrolment when ANC<500/cmm and 164.84±88.29 in 1st follow up done after 3-5 days. The difference was statistically significant (p<0.05) between two group.

Table VII showed that 6(9.5%) patients were found positive for throat swab for Candida in this study.

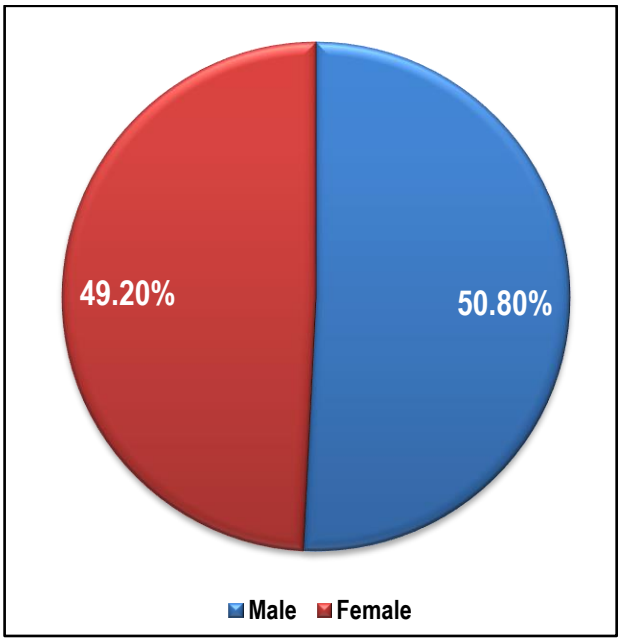

Figure I: Sex distribution of the study population

Table I: Age distribution of the study population (n=63)

Age (in years)	n	%
≤20	19	30.2
21-30	19	30.2
31-40	13	20.6
41-50	06	9.5
> 50	06	9.5

Table II: Bone marrow report of the study population (n=62)*

Bone marrow report	n	%
AML	40	64.5
ALL	18	29.0
APL	04	06.5

*Note: one patient was diagnosed as a case of AML by immunophenotype from blood. So initial bone marrow aspiration was not done.

Table III: Immunophenotype type report of the study population (n=63)

Immunophenotype type	n	%
AML	35	55.6
B ALL	9	14.3
T ALL	5	7.9
APL	2	3.2
ND	12	19.0

Table IV: Type of chemotherapy of study population (n=63)

Type of chemotherapy		n	%
AML			
	DA	17	37.8
	DAE	03	06.7
	HiDAC	20	44.4
	Others	05	11.1
ALL			
	Modified BFM	08	44.4
	Hyper CVAD	10	55.6

Note: Others (subcutaneous cytosar and decitabine)

Table V: Phase of chemotherapy of study population (n=63)

Phase of chemotherapy	n	%
Induction	26	41.3
Consolidation	28	44.4
Relapse	05	07.9
RE-induction	03	04.8
Palliative	01	01.6

Table VI: Absolute neutrophil count in different follow up

	Absolute neutrophil count		p value
	1st enrolment	1st follow up (after 3-5 days)	
Mean±SD	182.7±110.7	164.84±88.29	0.001s

s= significant; p value reached from paired t-test

Table VII: Throat swab for candida of the study population (n=63)

Throat swab for candida	n	%
Positive	06	9.5
Negative	57	90.5

Table VIII: Blood culture for candida of study population (n=63)

Blood culture for candida	n	%
Positive	00	0.0
Negative	63	100.0

Table IX: Association between bone marrow report with throat swab for candida (n=62)

Bone marrow report	Throat swab for candida				P value
	Positive (n=6)		Negative (n=56)		
	n	%	n	%	
AML	04	66.7	36	64.3	
ALL	02	33.3	16	28.6	0.788ns
APL	00	0.0	04	7.1	

ns= not significant; P value reached from chi square test

Table VIII observed that blood culture were negative for candida.

Table IX observed that among the AML patients 4(66.7%) patients were found positive for candida in throat swab and 36(64.3%) were found negative. In ALL cases it was 2(33.2%) for positive result and 16(28.6%) for negative. Four (7.1%) patients of APL were negative for candida infection. The difference was not statistically significant (p>0.05) among positive and negative groups.

DISCUSSION

In this observational study people were diagnosed as a case of acute myeloid leukaemia and acute lymphoid leukaemia on the basis of BM morphology with or without immunophenotype, cytochemistry and cytogenetic results admitted in the department of haematology BSMMU getting specific chemotherapy in any phase who underwent to neutropenia on the basis of peripheral blood count (ANC <500/cmm).

In this present study it was observed that 19(30.2%) patients belonged to age ≤20 years followed by 19(30.2%) 21-30 years, 13(20.6%) 31-40 years, 6(9.5%) 41-50 years and 6(9.5%) >50 years of age. In study carried out by Ma et al. (2008) it was observed that the median age was 45.5 years (range from 17 to 58 years).[13] Schaffner and Schaffner (1995) study showed the mean age was found 39±13 years.[14] In this current study it was observed that more than half (50.8%)of the patients were male and 31(49.2%) patients were female. Male female ratio was 1.03:1.Phikulsod et al.[15]

In this study it was observed that 40(64.5%) patients were AML, 18(29.0%) were ALL and 4(6.5%) were APL. In the study carried out by Ma et al. (2008), study revealed that out of 163 patients, 114 patients received chemotherapy among them 79 patients (69%) were AML and 35 patients (31%) were ALL.[13]

In this series it was observed that immunophenotypically AML were found in 35 patients which were 55.6% of study population, B ALL in 9(14.3%), T ALL in 5(7.9%) and APL in 2(3.2%) patients. However, in the study of Gupta et al. (2015), they showed their observation according to immunophenotyping profile into AML (83 cases: 51%), ALL (66 cases:42%) and mixed lineage leukemia (11 cases: 7%).[16] That results was dissimilar with our study. In the study of Gupta et al. (2015), the immunophenotypic diagnosis of pre B ALL were seen in 83% and T-cell ALL were seen in 16% cases which was also similar to observations of Shanta et al. (1996); Magrath et al. (2005) but less than the observations of Schrappe et al. (2000); Tsuchida et al. (2000).[16,17,18,19] This difference in the incidence of distribution of various subtypes of ALL might have some regional influences which needed further evaluation.

In this study it was observed that AML was found in 45 cases among them 17(37.8%) were under DA protocol, whereas HiDAC were on 20(44.4%), and DAE on 3(6.7%). Five (11.1%) cases got others chemotherapy. ALL were found in 18 cases. Among them 10(55.6%) were under Hyper CVAD followed by 8 (44.4%) on modified BFM. Similar observation was found by Phikulsod et al. (2017), they showed that out of 233 febrile patients with CIN (Chemotherapy induced neutropenia), 188 patients (80.7%) had diagnosis of hematological malignancies among them 84 (36%) with acute myeloid leukemia (AML), 22 (9.4%) with acute lymphoblastic leukemia (ALL), 72 (30.9%) with lymphoma, 7 (3.7%) with blastic-phase of chronic myeloid leukemia (CML), 2 (0.9%) with Myelodysplastic Syndrome (MDS) and 1 (0.9%) with multiple myeloma.[15]

In this series it was observed that majority of the patients (44.4%) were found in consolidation phase of chemotherapy followed by 26(41.3%) in induction phase, 5(7.9%) in relapse, 3(4.8%) in re-induction and 1(1.6%) in palliative phase.

In this study it was observed that mean absolute neutrophil count was found 182.7±110.7 in 1stenrolment when ANC<500/cmm and 164.84±88.29 in 1st follow up done after 3-5 days of 1st enrolment when ANC<500/cmm. The difference was statistically significant (p<0.05) between two groups. Study of Bhatt et al. (2011) observed that the higher rate of neutropenia in acute myeloid leukemia could be related to a unique intrinsic functional defect or to a relative reduction in the absolute numbers of neutrophils at the start of treatment (Prentice et al. 2000).

Six (9.5%) patients were found positive for throat swab for candida in this study showed similar result in his study. Among 105 cases only 38 cases were positive for oral candida infection. Bhatt et al.(2011) study showed that most of the yeast infections were caused by Candida species (91%); Candida non albicans species were responsible for over half the episodes of candidemia (57%). This increase is believed to be secondary to the extended survival of AL patients as well as to advances in supportive care, improved control of bacterial infections (Leventakos et al. 2010), and the use of intensive chemotherapy (Sung et al. 2009), immunotherapy regimens, and hematopoietic stem cell transplantation (HSCT) (Bow, 2008).[20,21,22]

In this study it was observed that blood culture were negative for candida. Similar result was found in the study of Albert et al. (2006) and Stinnett et al. (1992).[23] In the study of Stinnett et al. they showed out of 42 patients no patient were found positive for blood culture.[23]

Exact cause is unknown. Probable cause in my study might be 1) Reduce duration of neutropenia 2) use of prophylactic antifungal therapy 3) use of GCSF 4) Culture is positive in late course of infection 5) Lack of update diagnostic method like candida PCR, candida ELISA, MALDI, DNA based method.6) Small Sample size.

LIMITATIONS

There are some facts to be considered which might affect results

1. The study population was selected from one selected hospital –BSMMU, Dhaka. So, the results of the study might not reflect the exact picture of the country.
2. All of the patients of AML and ALL were under prophylactic antifungal therapy, otherwise we might have more number of positive cases.
3. Small sample size was also a limitation of the present study

CONCLUSION

In this study it was observed that candida was present in throat swab of 9.5% of patients and rests were negative. None of the patients suffered from systemic candidiasis. We observed that candida in throat swab were more common in AML than ALL. Cause was unknown but might be due to Reduce duration of neutropenia by applying G-CSF and empirical local and systemic antifungal therapy. If not treated timely, local candida infections may disseminate and produce systemic candida infection. There will be increased chance of mortality even.

RECOMMENDATIONS

- Local and systemic candida infection should be confirmed by candida PCR or ELISA or DNA-DNA hybridization.
- Candida infection should be suspected in all patients with neutropenic fever and investigation should be sent routinely.
- Further studies can be undertaken including large number of patients at different major tertiary hospitals.

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