Estimating The Density of Candida Albicans in Children with Acute Lymphoblastic Leukemia (A Pilot Study)

Original Article

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Abstract

Introduction: Oral Candidosis is among the findings of acute leukemia. The aim of this study was to evaluate the changes in the intensity of *C.albicans*, before treatment and at the Induction phase.

Materials and Methods: Twelve patients with Acute Lymphoblastic Leukemia (ALL) aged 2-14 years enrolled in study. Whole Saliva samples were obtained and cultured to determine the mean count of *C.albicans* Colony Forming Units (CFU) before induction of leukemia, and at days 35 and 64 of induction. White blood cell counts were also determined at the same time. Data were transferred to SPSS 19 and analyzed using paired and Independent t-test, Chi square, and stepwise regression.

Results: The mean CFU count was significantly increased before beginning of the treatment [22.41±10.47] to days 35[28.5±9.29] (P=0.006) and 64 of induction[30.5±11.82] (P=0.009) andat the first day of consolidation [49.66±3.01] (P=0.032).The quantity of colonies was sparse (10-100 CFU/ml) without clinical manifestations of oral candidiasis. Main predictors of *C.albicans* colonization were age and white blood cell count (WBC). Children younger than age 10 yrs (OR=-19.7, 95% CL [12.37-15.82 for day 35 evaluation] and (OR=-0.002, 95% CL[-0.004-0.00 inday 64]and those with lower WBC (OR=-13.47, 95% CL [-25.29 -1.65]) showed higher risks of colonization.

Conclusion: *C.albicans* colonization was observed among the leukemia child patients at early phase of treatment without clinical manifestations. The predictors of colonization were age and white blood cell counts.

Key words: **•Acute Lymphoblastic Leukemia- •Candida albicans• Child**

Introduction

Acute Lymphoblastic Leukemia (ALL) is the most common childhood malignancy. During the past two decades, survival rate of patients has increased considerably but treatment of the complications continue to be a source of morbidity and mortality one of the important considerations among leukemic patients is oral complications. The integrity and function of oral tissues are impaired by disease as well as its treatment consequences, hence fungal infections (oral candidiasis) as well as oral ulcers potentialy threat in these patients that might result in a number of complications including pain, discomfort and nutritional deficiency that may also lead to delay in drug administration, longer hospital stays and life threatening infection (septicemia).[1-5]Fungal infections occurring in these patients could be divided to two categories: the pathogenic fungi (cryptococcus neoformans, cistoplasma capsulatum, and coccidioides immitis) and the opportunistic fungi (candida spp, aspergillus spp, and other fungi).

The later types cause infections in the general population but are more likely to cause disseminated infection in the cancer patient. Acute infection during cancer therapy often represents reactivation of latent infection. Opportunistic fungi usually cause only superficial infection in immunocompetent hosts but are the most common cause of systemic fungal infection in patients with impaired host defense mechanisms.[3] At the present time, at least 40% to 50% of fatal infections among cancer patients are caused by fungi. *C.albicans* is the most often candida spp species that is associated with oral lesions, but other candida spp have also been isolated from oral environment.[6] Increased susceptibility to candidiasis may be attributed to general physical debilitation, immunosuppression, prolonged antibiotic therapy, chemotherapy and poor oral hygiene.[4]Although the presence of *C.albicans* has been reported in patients with ALL, there is significant variability in reported prevalence. Soares et al. reported reduced numbers of potentially pathogenic microorganisms, including Candida albicans among ALL patients who rinsed their mouth daily with Chlorhexidine Gluconate 12%.[7] Prevalence of candidosis was also reported low by Fayle et al.[8]

Because of possible role of oral candidiasis in development of systemic infection[1] and due to increased resistance to antifungal agents, prevention of superficial fungal infections[9] is crucial in management of ALL patients. The purpose of the present study was to investigate the intensity of candida albicans, as the main etiological candida species in leukemia pediatric patients,[10] before chemotherapy and after completing the induction phase of cytotoxic treatment to determine the patients at higher risks of candidosis and focus the prevention on high risk subjects.

Materials and Methods

This prospective descriptive cohort study was conducted from September 2013 to February 2014 in two Children's Teaching Hospitals in Rasht and Tehran, Iran.

After gaining parental consent, twelve newly diagnosed leukemic patients aged 2-14 years who were admitted the Hematology/oncology wards enrolled in the study.

Demographic data, diagnosis of disease, cell blood counts at the time of diagnosis, and treatment protocol were extracted from patients' reports. According to study protocol appropriate systemic and local antifungal agents should be initiated if any sign and symptoms of fungal infections were observed. The applied treatment protocol in remission induction protocol included: prednisone, vincristine, DNR, L asparaginase, cyclophosphamide, cystoinearabinoses, 6-

mercaptopurine, and metatroxate for 35 days followed by CNS remission until day 64.[4,7]

Who lesaliva samples were collected before induction and at the days 35 and 64 of induction and first day of consolidation. Saliva samples were gathered 2 hours after breakfast to minimize probable salivary alternations. Patients were asked to split out 1 cc saliva insterile tubes while sitting in a rest position. The saliva was directly transferred to specialized candida albicans culture media (Sabouraued dextrose agar plates containing chloramphenicol) without dilution, incubated for 48 hours and then colonies were counted. The intensity of candida albicans colonies was determined under a well illuminated light source using direct visual inspection method by microbiologist. The quantity was classified into the following categories[8,9] 0 (absent); 1 (very sparse (<10 CFU)); 2 (sparse (10–102 CFU)); 3 (moderate (102-103 CFU)); 4 (rich (>103CFU)).

The prevalence of colonization with *C.albicans* is different among populations, but there are no determined normal values for colony counts.

White Blood Cell (WBC) counts were extracted from patients' records. Normal limit of white blood cells for children 2 to 14 years is considered 4500 to 17000.[7]

Data analysis was carried out using SPSS version 19, Kolmogorov–Smirnov test, Independent t-test, paired t-test, chi-square and stepwise linear regression.

Results

The minimum age of the patients was 25 years; maximum age 77 years and the five out of twelve patients were treated in Rasht and seven in Tehran. There were four boys and eight girls in study with the mean age of 6.68±3.92 years. The normal distribution of the study parameters was confirmed using Kolmogorov–Smirnov test prior to other analysis.

Comparison of *C.albicans* CFU/ml before chemotherapy and at induction days 35and 64, and the first day of consolidation are presented in table 1.There was significantly lower CFUs before induction [22.41±10.47] when compared to induction days 35[28.5±9.29] and 64 [30.5±11.82] and the first day of consolidation [49.66±3.01] using paired sample t-test; p=0.006, p=0.009 and p=0.032 respectively.

Table1. Colony counts before Induction and at induction days 35 and 64 and consolidation day 1 for all study subjects (12 children) and among 10 and 7children at induction day 64 and consolidation day 1 respectively

Evaluation time	N	Colony count Mean(SD)	t	P-value
Before induction	12	22.41(10.47)	3.4	0.006*
Induction day 35	12	28.5(9.29)		
Before induction	10	21.1(10.4)	3.35	0.009*
Induction day 64	10	30.5(11.82)		
Before induction	7	26(3.46)	4.04	0.032*
Consolidation day 1	7	49.66(3.01)		

*Paired sample t-test

Table2. Adjusted data on colony counts before induction and at days 35 and 64 among 10 patients

Evaluation time	N	Colony count Mean(sd)	F	p-value
Before induction	10	21.1(10.4)	11.22	0.009*
Induction day 35	10	27.1(9.48)		
Induction day 64	10	30.5(11.82)		

* Repeated measurement; General Linear model

Data on 64th day of induction and first day of consolidation decreased to ten and seven subjects due to several reasons (table1). Hence, the second analysis was performed to compare the CFU among the ten subjects who participated at 64th day of Induction. The mean CFUs of these patients increased in a similar manner from

21.1±10.4 before chemotherapy to 27.1±9.48 and 30.5 ±11.82 on days 35 and 64 of Induction, using General Linear model repeated measurements with 99% confidence level. The quantities of *C.albicans* from absent to rich before chemotherapy and on days 35 and 64 of induction are presented in table 2. From twelve children, 91.7% were carriers of *C.albicans* before induction. The prevalence of carriers increased to 100% after treatment. Among all patients, the quantity of colonies treatment was sparse (between 10 to 100 CFU/ml) both before and after the treatment except in one case who contained less than 10 CFU/ ml (very sparse).

The mean White Blood Cell counts (WBC) before induction and on induction days 35 and 64, and the first day of consolidation are presented in table 3. The data was available for twelve children at 35th day of induction, but on days 64 and first of consolidation it was decreased to 10 and 7 subjects respectively. The WBC of twelve children before induction was 10181 and decreased to 5308 at day 35(P=0.12). Similar results were found for 10 patients on day 64 (P=0.103).The only significant difference was found between pretreatment status and the first day of consolidation P=0.032; α=0.05(paired sample t-test) (table 3).

Table3. Quantities of *C.albicans* (CFU) isolated from patients from each category from absent to rich

CFU	Before Induction N(%)	Day 35 N(%)	Day 64 N(%)
0	0(0%)	0(%)	0(0%)
10<	1(8.3%)	0(0%)	0(0%)
10-100	11(91.7%)	12(100%)	10(100%)
100>	0(0%)	0(0%)	0(0%)
1000>	0(0%)	0(0%)	0(0%)
total	12(100%)	12(100%)	10(100%)

The distribution of CFUs according to age are shown in table4.There were seven children younger and three older than age 10 years. Density of colonies was not significantly different among children aged under and above 10 years: General linear model repeated measurement; P=0.95(table4).

Table 4. Mean White Blood Cells counts before chemotherapy and at treatment phases among 7 patients

	N	Mean(Sd)	t	P-value
Before treatment	12	10181(3083)	1.6	0.12
Induction day 35	12	5308(2992)		
Before treatment	10	12070(1135)	1.81	0.103
Induction day 64	10	5310(4265)		
Before treatment	7	7728(4251)	2.77	0.032*
Consolidation day 1	7	3542(3105)		

*Paired sample t-test

The predictors of colonization were age and White Blood Cell count. Colonization was increased in children younger than age 10 years [OR: -19.77(-12.37,-15.28), p<0.001] and in those with lower WBC counts [OR: -0.002(-0.004, -0.001), p=0.04). Figure 1and 2 present the transition in colony counts and white blood cells respectively during the treatment.

Table 5. Mean CFUs among children under and over age 10 years

Age (Years)	Time	N	CFU(SD)	Intra group F value	Significant level	Inter-group F value	Significant level
<10	Before treatment	7	26.5(6.5)	33 .7	P=0.053*	0.003	P=0.95*
	Induction day 35	7	32.0(2.3)				
	Induction day 64	7	35.8(9.7)				
> 10	Before treatment	3	8.3(2.8)	6.8	P=0.12*		
	Induction day 35	3	13.6(1.1)				
	Induction day 64	3	18.0(3.4)				

*General linear model (repeated measure)

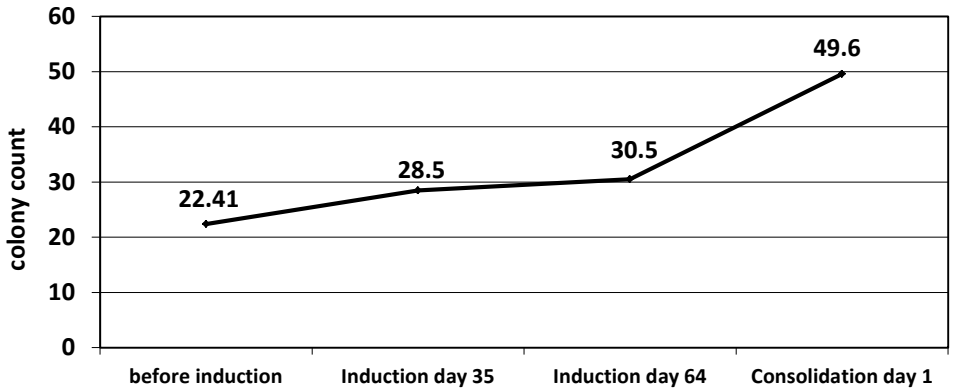

Figure 1: Changes in colony counts during the course of treatment

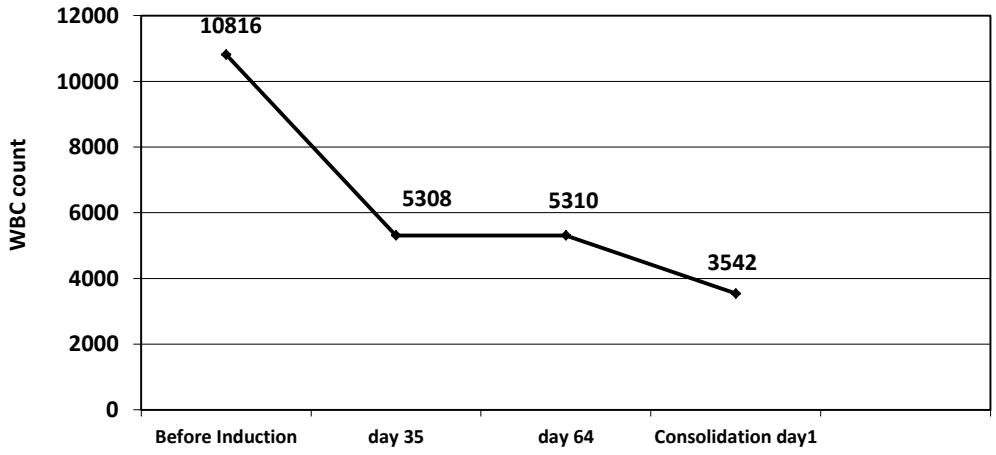

Figure 2: White blood cell changes during the course of treatment

Discussion

Treatment of Acute Lymphoblastic Leukemia was comprised of four phases: remission induction, CNS prophylaxis, consolidation and Maintenance. Our pilot study concentrated mainly on the induction phase. Findings of the study demonstrated that the intensity of *C.albicans* colonies have increased with progression of treatment, however the pattern of fungal growth was sparse (10-100 CFU/ml) and oral candidiasis lesions were not observed during this stage. The phase of chemotherapy is the risk factor for appearing oral candidiasis.[5,11] Manifestations of oral candidiasis become apparent mainly during the consolidation phase when high intensity cytotoxic agents such as methotrexate are used.[7] Hence, the sparse pattern of colonies and lack of oral lesions in remission induction phase, is a usual finding. An association has been reported between oral lesions appearance and colony counts more than 100 CFU/ml.[9] The CFU/ml lower 400 is the cutoff-point for oral manifestations of candidiasis[12], specifically the pseudomembranous form.[13] Williams reported candida oral lesions in 10% of patients. However, candida carriage rates have been reported consistently high in patients with or without oral candidosis.[14]

Our findings indicated that the predictors of *C.albicans* colonization were young age and low WBC count. This finding was consistent with other studies that found a greater incidence of fungal infections associated with younger age, granulo cytopenia[16] and neutropenic episodes of disease.[17,18] Moreover, we did not observe mucositis in our patients during induction phase. An association between oral candidiasis and presence and severity of mucositis has been reported in pediatric ALL.[19, 20]

Infection is the leading cause of morbidity and mortality among leukemia patients. Prevention of superficial fungal infections is important in prevention of systemic infections in ALL patients.[1] Candida species, existing in the mouth, have high probability to infect the digestive pathways and disseminate through the circulation, developing life threatening systemic infections.[5] Detection and identification of Candida spp. involved in oral candidiasis and their sensitivity to antifungal agents are important for the treatment of patients with cancer.[21] Hence, despite the sparse pattern of growth, the findings of present study may be used as a guide to focus the preventive programs on susceptible subjects to avoid antifungal resistance. [22]

One of the limitations of this study was the low number of patients. A number of patients were also discontinued their participation in study due to several reasons e.g. their systemic conditions. Hence, designing a longitudinal multicenter study may be justified to follow the patients until the end of maintenance phase and report more detailed observations.

Conclusion

Low intensity of *C.albicans* colonization was observed in this study. Despite of the sparse pattern of colonization, the subjects may be at risk of later clinical infection and should be monitored in later phases of cytotoxic treatment. More controlled studies in this area are advocated by authors.

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