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Original Research Article

Assessment of serum zinc level in childhood malignancy at time of initiation of chemotherapy and to study its association with survival during initial first month of the treatment

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ABSTRACT

Background: Survival in childhood malignancies continues to be a challenge in developing than in developed countries. Multiple factors have been implicated for this gap, like poor health infrastructure, over-population and malnutrition. Malnutrition and associated zinc deficiency may be implicated as one preventable factor for mortality in childhood malignancy. The main aim of this study was to assess serum zinc level in childhood malignancy and to study its role in survival of the subjects.
Methods: This prospective observational study was conducted from August 2014 to July 2015 on 219 patients of vari ous childhood malignancies, from age 2 months to 15 years (151 males, 68 females). Cases were enrolled at the initiat ion of chemotherapy and followed for 1 month.
Results: Serum zinc level (mean ± SD) was 78.70±32.83 µg/dl in the cases and it was found to be significantly lower than in controls ($p<0.05$). During follow up, 51 patients died due to various complications and disease progression. Significant relationship between low serum zinc level and unfavourable outcome was found ($p<0.05$). Logistic regression analysis revealed zinc level to be a significant predictor of the final outcome.
Conclusions: This study states that low serum zinc level has an association with mortality in pediatric malignancy. As this study did not include any intervention in form of zinc supplementation, a future study with intervention of zinc supplementation might help in implicating the role of zinc as a predictor of outcome in childhood malignancy.

Keywords: Zinc, Childhood malignancy, Haematological malignancy, Pediatric

INTRODUCTION

Childhood malignancies constitute a small fraction of the global cancer burden but the access to healthcare facilities is limited in developing countries which comprise of the greater chunk of pediatric population. On an average, 2% of cancers in developing countries and 0.5% of cancers in developed countries are childhood cancers.[1] Various trace elements have been a focus of research for over 5 decades regarding their role in carcinogenesis and possible use of their assays in biological fluids as diagnostic, prognostic, and novel therapeutic aid. Till now, zinc has been studied extensively among them. Decreased intake of zinc due to inadequate feeding practices during rapid growth phase put children, especially from lower socio-economic level, at an elevated risk of zinc deficiency. Zinc plays a critical role in regulation of DNA repair mechanisms, cell proliferation, differentiation and apoptosis including the action of various transcriptional factors.[2] Zinc deficiency manifest at the time of onset of cancer but levels usually normalize during remission and deficiency recurs with

relapse of cancer.[3] Malnutrition is a well-established prognostic factor in children with haematological malignancies and multiple micronutrient deficiencies are associated with malnutrition. Thus, it seems reasonable to assess the role of zinc in childhood malignancy.[4]

Previous Indian literature has reported the prevalence of zinc deficiency in children, but its estimate in the patients of paediatric malignancy is still understudied. It would be of interest to know the level of zinc deficiency and complications attributed to it, in childhood malignancy.

METHODS

This prospective observational study was conducted in a tertiary healthcare centre of North India from August 2014 to July 2015. Children, aged 2 months to 15 years, admitted in oncology unit of department of paediatrics, King George Medical University, Lucknow and diagnosed as new cases of childhood malignancy were selected for the study. All patients fulfilling the inclusion criteria were included in the study (sample of convenience). All enrolled cases were followed up daily for 1 month. All enrolled cases had diagnosis of malignancy confirmed by histology or bone marrow examination in our centre. They had no history of chemotherapy initiation before presentation to our centre. Children who were already on chemotherapy were excluded from the study. At the time of enrolment, a baseline serum zinc level was observed. Ethical clearance for the study was obtained from ethics committee of King George's medical university, Lucknow (ref code-72nd ECM thesis II-B-Thesis/p2).

Five ml blood sample (for zinc levels) was withdrawn along with other necessary blood sampling for the patient care. Blood was centrifuged, serum separated and preserved at-200C. Repeat blood sampling was done after one month of chemotherapy initiation. Serum was analyzed for Zinc levels at the end of 1 month of chemotherapy by ICP-OES (Inductive coupled plasma-optical emission spectrometry) method in the department of biochemistry.

Method of serum zinc analysis

Standards and reagents

The standard for ICP-OES was prepared from stock solutions of Zn at 1000 mgL-1 concentration. Working solutions were prepared from the stock as per necessity. All other reagents and solvents used in this study were analytical grade obtained from Fischer Scientific, USA. Milli-Q water was used in washing laboratory glassware and in the preparation of sample and standard solutions.

Sample preparation

1.0 ml of serum sample was diluted to 5.0 ml by Mili-Q water and analysed by ICP-OES. Blank was prepared in similar conditions replacing serum by 1.0 ml of Mili-Q water. Each sample was analysed in triplicate.

ICP-OES analysis

Determination of zinc was carried out by inductively coupled plasma-optical emission spectrometer (Optima 8000, Perkin Elmer).

The calibration standard was prepared by diluting the stock standard solution (1000 mg/L-1) in 0.2% (v/v) nitric acid. Calibration curve was prepared by different concentrations of standard in the range 0.01 to 1.0 mg/L.

Data analysis

Data was entered in the Microsoft excel sheet under pre-decided variables. For the purpose of analysis EPI Info software version 7 was used. The result is presented as frequencies and percentages for categorical data and mean ± SD for parametric data.

Definitions used

Zinc deficiency is defined as serum zinc level below 65 µg/dl (9.9 µmol/L).[5]

Socioeconomic classes are defined according to the updated Kuppuswamy's socioeconomic scale 2014.[6]

RESULTS

A total of 219 patients (151 males, 68 females) and 50, age and gender matched healthy controls (children coming for immunization and other routine visits) were recruited. Mean age of the patients was 6.04±3.84 (Mean ± SD, in years). Demographic characteristics of the cases have been shown in Table 1.

Table 1: Demographic characteristics of the cases, (n=219).

Demographic characteristics	No. of patients, n (%)
Gender	
Male	151 (68/.9)
Female	68 (31.1)
Age (years)	
Mean ± SD	6.04±3.84
<5	94 (42.9)
5-10	72 (32.9)
≥10	53 (24.2)
Socioeconomic status	
Upper	2 (0.9)
Upper middle	15 (6.8)
Lower middle	58 (26.5)
Lower	144 (65.8)
Residence	
Rural	174 (79.5)
Urban	45 (20.5)

Most common malignancy in the study group was acute lymphoblastic leukemia (ALL) followed by Wilm's tumour and acute myeloblastic leukemia (AML). Table 1 shows clinico-pathological characteristics of our subjects. Out of a total of 219 patients, only 117 patients suffered from haematological malignancy, accounting to a prevalence of 53.4% (Table 2).

Table 2: Clinico-pathological characteristics of patients, (n=219).

Clinico-pathological characteristics	No. of patients, (n) (%)
Diagnosis	
ALL	70 (32.0)
AML	22 (10.0)
Non-Hodgkin's lymphoma	13 (5.9)
Hodgkins's lymphoma	12 (5.5)
Wilm's tumor	23 (10.5)
Retinoblastoma	20 (9.1)
Neuroblastoma	12 (5.5)
Rhabdosarcoma	9 (4.1)
Ewing' sarcoma	10 (4.6)
Germ cell tumor	5 (2.3)
RCT	5 (2.3)
Others	18 (8.2)
Haematological malignancy	117 (53.4)

Table 3 shows the comparison of zinc levels between the two groups. The mean zinc level was significantly lower (15.0%) in cases as compared to controls (p<0.05). More cases than controls, with zinc deficiency suggest that there is a relation between malignancy and serum zinc level.

Table 3: Comparison of zinc levels between two groups.

Variables	Controls, (n=50)	Cases, (n=219)	P value
Serum zinc (µg/dl): Mean ± SD	90.49±31.92	78.70±32.83	0.022*
Zinc deficiency, n (%)	11 (22%)	84 (38.3%)	0.029*

*p<0.05, Significant

Cases with haematological malignancy were found to have significantly higher incidence of zinc deficiency than those having non-haematological malignancy (p=0.005). Malnutrition was present in 83.5% of the study patients but its association with zinc deficiency was found to be insignificant (Table 4).

After registration for treatment, the patients were followed up for one month. During one month of follow-up 51 patients died due to the disease process (23.3%). The mean survival duration of patients ranged from 2 to 30 days (median=30 days). A total of 36 patients were lost due to refusal for treatment and treatment abandoned. Mean serum zinc level in alive group (83.3±36.4 µg/dl) was significantly higher than expired patients' group (68.8±23.53µg/dl) (p=0.01). One hundred and thirty-two children who survived till the end of first month follow up were evaluated clinically and biochemically. The before and after body weights were not significantly different while zinc levels showed a significant rise (83.38±36.40 and 90.82±29.86 µg/dl**)** at the end of 1 month (p=0.023). Significant association was found for zinc status (χ^2=5.02, p=0.035) and negative final outcome. The logistic regression analysis also found zinc levels as significant predictor of the final outcome.

Table 4: Association of zinc status with biodemographic and clinical variables, (n=219).

Variables	N	Serum zinc status		P value
		Deficient, (n=84) (%)	Normal, (n=135) (%)	
Age (years)				
<5	94	32 (34.0)	62 (66.0)	0.275
5-10	72	33 (45.8)	39 (54.2)	
>10	53	19 (35.8)	34 (64.2)	
Sex				
Male	151	62 (41.1)	89 (58.9)	0.220
Female	68	22 (32.4)	46 (67.6)	
Haematological malignancy				
Yes	117	55 (47.0)	62 (53.0)	0.005
No	102	29 (28.4)	73 (71.6)	
Malnutrition				
Normal	36	17 (47.2)	19 (52.8)	0.246
Moderate	59	18 (30.5)	41 (69.5)	
Severe	124	49 (39.5)	75 (60.5)	
Respiratory infection*				
Absent	139	50 (36.0)	89 (64.0)	0.054
Present	44	23 (52.3)	21 (47.7)	

*evaluated on 91 cases and ¥evaluated on 183 cases.

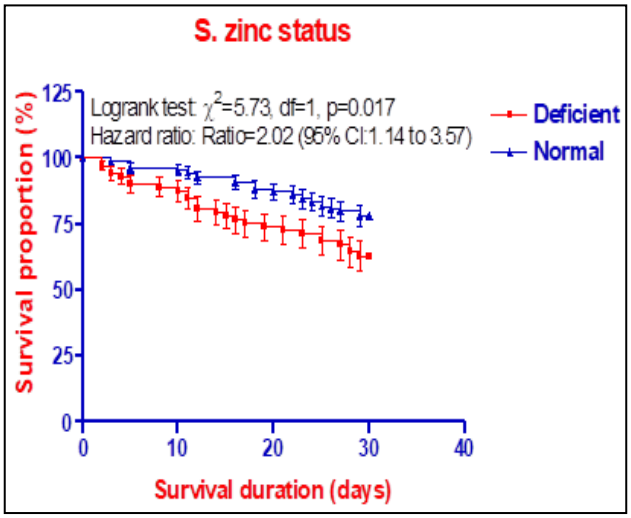

Figure 1: One-month overall survival of patients with respect to zinc status (deficient or normal).

One-month overall survival study was done between zinc status and final outcome of the patients. It was found that zinc levels can predict survival chance significantly. The patients who had deficient zinc status, their survival rate became significantly low as compared to those who had normal S. zinc status (χ^2=5.73, p=0.017) and their rate of death was 2.02-fold more per day (Hazard ratio=2.02, 05% CI=1.14 to 3.57) (Figure 1).

DISCUSSION

The present study aimed at evaluating the prevalence of zinc deficiency in north Indian children diagnosed with childhood cancer and its association with final outcome. Nutritional depletion can be present in a significant number of children with leukemia before treatment, thus influencing the course and prospects for patients' improvement. This large variation is because of several factors such as socioeconomic status and educational and cultural background of children's family, beyond the disease itself. In malnourished children a decreased tolerance to chemotherapy is well known. It comprises an increased infection rate, which contributes to a poor outcome of the disease. Zinc deficiency comprises an important aspect of malnutrition.

Our study reported zinc deficiency in 38.4% cases (significantly higher numbers as compared to the healthy controls) (p=0.02) with mean serum zinc significantly lower than control group. A study from North India by Kapil et al observed the prevalence of zinc deficiency in children (6-60 months age) to be 43.8%.[7] In contrary to our data, they reported no significant difference in the prevalence of zinc deficiency in children with malignancy on comparison to the normal population. A study by Consolo et al revealed low zinc levels in 38 patients of leukemia. They concluded that supplementary zinc exerts a positive effect on nutritional status as positive weight gain and significant reduction in the number of infection episodes possibly because of the immune stimuli.[8] Various studies have shown the estimates of zinc deficiency as 48.1%, 49.4% and 73.3%.[9-12] The later study reported an unusual high prevalence of zinc deficiency that was probably due to the higher cut-off taken for diagnosis of zinc deficiency i.e., <70 µg/dl.[11,12]

In our study, children with haematological malignancy were found to have significant higher incidence of zinc deficiency than those having non-haematological malignancy (p<0.05). This corroborated well with earlier studies where children with haematological malignancy were also found to have lower zinc levels. This might occur because of increased consumption of zinc by immune cells due to increased turnover. Previous studies also observed significant decrease in serum zinc in haematological malignancy in comparison to normal subjects and other malignancies.[11,12] Our study showed an association between episodes of respiratory infection and zinc deficiency (p<0.05) (Table 3). This finding was found to be similar to previous observation by investigator.[13,14] They observed low serum zinc level in patients affected with acute lower respiratory tract infection. A total of 36 (16.4%) patients were lost to follow up due to various reasons (which was a significant lot) so excluded from study for further evaluation. Out of remaining 183 children, 51 expired within 1 month of admission regardless of starting the chemotherapy. In this study, we observed that low serum zinc level has an association with mortality in paediatric malignancy. There was a significant difference between serum zinc levels at presentation of alive and expired group.

We found that negative outcome of children was significantly associated with low serum zinc level (p<0.05). It can be inferred that serum zinc level is related to nutritional status of children and finally to overall immunity and capability of them to cope up with infections, chemotherapy related complications and advanced stages of malignancy. Zinc levels in remaining alive patients after 1 month of enrolment had increased significantly in serial samples. This might have happened due to better nutritional counselling and food security in the hospital although no designated zinc supplementation was done during the period of the study.

Strengths

Inclusion and exclusion criteria were defined properly and strictly followed while recruiting the cases. Children with many different malignancies were enrolled to have a better pool of patients. Comparison of serum zinc level between cases and control for adequate assessment of prevalence was done.

Limitations

Although zinc was found to have a significant association with negative outcome, other confounding factors such as low serum folate, intensity of chemotherapy, stage of the disease complicates this hypothesis. Follow up was done for 1 month only which was a very short duration to conclude upon the role of zinc. A longer period of follow up was needed to have a better understanding of morbidity, clinical complications and death**.** No zinc supplementation done during study period.

CONCLUSION

The present study observed a significant association of low serum zinc levels with mortality in paediatric malignancy. A future interventional study involving zinc supplementation would be needed to generalize this finding and to have a better understanding of the role of zinc in childhood malignancy.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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