TRACE ELEMENTS IN MALIGNANT LYMPHOMA

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Abstract

Human malignant lymphomas are heterogeneous groups of tumors that differ in regard to clinical behaviour and survival. Many histological classifications had categorize the degree of malignancy of the neoplasm by histological, cytochemical, immunological and genetic criteria, but additional indices can be useful for a better definition of the aggressiveness of lymphoid tumors. Trace elements particularly copper (Cu) and Zinc (Zn) which are essential metals which are required for growth and proliferation of healthy cells as well as for normal lymphocyte maturation and regulation of immune function therefore the estimation of the intracellular concentration of these metals may be used as a marker alongside other biological indices to define the aggressiveness of the malignant lymphomas.

Aim: To determine the intracellular lymphocyte Cu and Zn and its relation with the grades of NHL and the histotypes of HD.

Patients & Methods: Trace elements Cu, Zn and Cu/Zn ratio was estimated in 42 patients with malignant lymphoma. Of them 23 patients had non Hodgkin's lymphoma (NHL) [age range 5-75 years] and 19 patients had Hodgkin's disease (HD) [age range 6-70 years]. They were compared with 19 age matched healthy control subjects [age range 7-70 years]. All patients were newly diagnosed and did not receive any medication. Intracellular lymphocyte Cu and Zn were estimated using flame atomic absorption spectrophotometer, Perkin-Ewer 400 and the classification and grading of the lymphoma were based on the Rye classification and N.C.I. system for HD and NHL respectively.

Results: intracellular Cu and Cu/Zn ratio were significantly high while intracellular Zn was insignificantly low when compared to control group. Moreover, only lymphocyte Cu had correlated with the grades and the histotypes of NHL and HD respectively.

Conclusion: Therefore, we may propose that in malignant lymphoma Cu and Cu/Zn ratio rather than Zn may be used as a diagnostic markers and for following up the patients alongside other biological indices, and that only intracellular Cu reflect the disease severity in NHL and therefore can be used as a prognostic marker.

Keyword: Malignant Lymphoma, Trace elements Cu, Zn and Cu/Zn ratio

Iraqi J Med Sci, 2004; Vol. 3(1):8-13

Introduction

Malignant lymphomas are a heterogenous group of malignancies of B cells and T cells that usually originate in the lymph nodes but may originate in any organ of the body[1]. It is divided into two main categories:

1. Non – Hodgkin's lymphoma (NHL). 2. Hodgkin's lymphoma (HD)[1,2].

According to the results of Iraqi Cancer Registry (1991-1997), NHL and HD were the fourth (6.2% of the total) and the tenth (2.5% of the total) most common cancer in Iraq respectively[3].

Copper (Cu) and Zinc (Zn) are biological elements that are called as trace elements because small amount of them are found in human body[4], however they are essential metals that are required for growth and proliferation of healthy cells and for normal lymphocytes maturation and regulation of the immune function[5], Moreover changes in the level of these elements may impair cellular and physiological functions[6], through changes in the activities of metalloenzymes which require a small and constant number of metal per mole to attain full activity. Therefore a minute variation in these elements cause significant changes in the activity of these enzymes[7].

Copper and zinc have been critically examined in the etiology of various diseases especially cancer. They may serve as useful indices, independent to any other hematological and biochemical tests[8].

The aim of the study includes:

1. To determine the intracellular lymphocytes Cu and Zn in patients with malignant lymphoma.
2. To evaluate the relation between the lymphocytes Cu and Zn and the grades of NHL and the histotype of HD.

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Received 22nd September 2002: Accepted 19th January 2004.

Patients & Methods

This study was conducted on 42 patients who attended the institute of Nuclear Medicine, Baghdad and Al-Kadhimiya Teaching Hospital. They include 19 patients with HD (11 males, 8 females. Age range is 6-70 years) and 23 patients with NHL (12 males, 11 females. Age range 5-75 years). All patients were newly diagnosed and did not received any medication. Additionally, 19 healthy subjects had served as control (16 males, 13 females. Age range 7-70 years) (Table 1).

Blood was collected in EDTA containing tube and was treated immediately with Ficoll-Hypaque (lymphocyte separation medium) for the separation of lymphocytes[9,10]. Intracellular lymphocyte Cu and Zn was estimated using flame atomic absorption spectrophotometer [Flam (AAS)], Perkin-Ewer 400.

The diagnosis of lymphoma was confirmed by two independent pathologists and the classification and grading of lymphoma was based on the Rye classification for the HD[2,11] and the N.C.I. system for the NHL[2,12].

The results are expressed as mean±SE of number of cases. The data was analysed by unpaired student "t" test and by the confidence interval of control taking $p<0.05$ as the lowest limit of significance.

Table 1: The demographic charactersitics of cases with malignant lymphoma

Character	Control	NHL	HL
No.	19	23	19
Sex:			
♂	10	12	11
♀	9	11	8
Age (year)			
Range	7-70	5-75	6-70
Median	27	50	24

Results

Forty-two cases with malignant lymphoma who were newly diagnosed and nineteen healthy subjects served as control were included in this study (Table 1).

On estimation of lymphocyte Cu in both NHL and HD, it was significantly high when compared with control value [$p<0.001$ and < 0.05 for NHL and HD respectively] (Figure 1) and (Table 2).

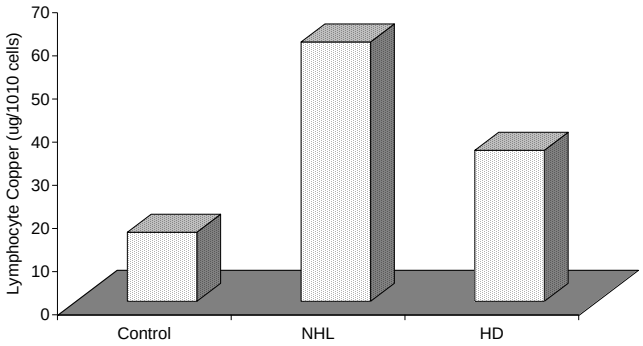

Figure 1: Concentration of lymphocytes copper (ug/10^{10} cells) of cases enrolled in this study.
NHL= Non Hodgkin's lymphoma, HD = Hodgkin's disease.

Table 2: The changes in lymphocytes trace elements of control and patients with malignant lymphomas

	Control	NHL	HL
Cu µg/10^{10} cell	15.8±1.8	60.48±9.78*	40.5±8.38***
Zn µg/10^{10} cell	75.42±9.63	68.34±12.64	73.8±14.4
Cu/Zn ratio	0.32±0.048	1.038±0.182*	0.53±0.082**#
Number of seperated lymphocytes x 10^6/ml	3.15±0.28	2.61±0.302	3.27±0.51

* = P<0.001 in comparison with control, ** = P<0.01 in comparison with control, *** + P<0.05 in comparison with control, # =P<0.01 in comparison with NHL.

Further analysis was done to evaluate the level of intracellular Cu in different grades of NHL in comparison with the control confidence interval [95% C.I.= 19.328-12.272]. It revealed that lymphocyte Cu in the high and intermediate grade had a high significant value. Moreover, the high grade had higher value than that of the intermediate which was higher than the lower grade (Table 3, Figure 2).

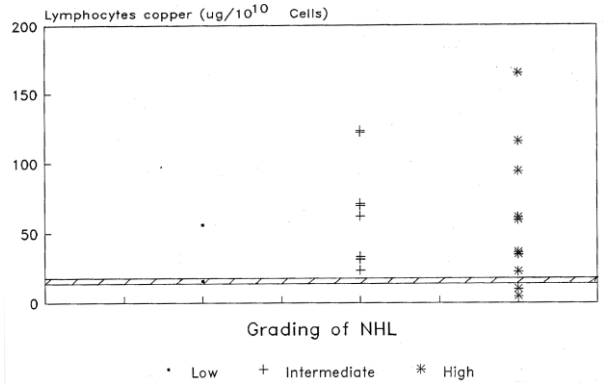

Figure 2: Distribution of lymphocytes copper (ug/10^{10} cells) according to the grading of non Hodgkin's lymphoma.
Shaded zone represents Mean±SE of controls value.

Table 3: Mean levels of lymphocytes trace elements of patients with NHL according to the grades

Grades	Cu µg/10^{10} cell	Zn µg/10^{10} cell	Cu/Zn ratio
Low (n =2)	15.55	14.2	1.095
Intermediate (n=10)	x =68.61 median =62	x =73.2 median =50.6	x =1.648 median =1.296
High (n=11)	x =104.96 median =55.83	x =81.24 median =33.8	x =1.134 median =1.14

But in HD although all the histological subtypes values were significantly high when compared to control confidence interval [95% C.I.= 19-32-12.272] ($p < 0.05$) (Table 4), but the value of the mixed cellularity was lower than that of nodular sclerosis and lymphocyte depleted subtype (Figure 3, Table 4).

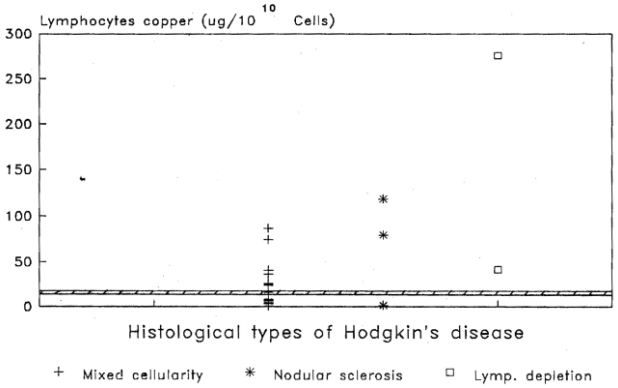

Figure 3: Distribution of lymphocytes copper level according to the histological types of Hodgkin's disease.
Shaded zone represents Mean±SE of controls value.

Table 4: Mean levels of lymphocytes trace elements of patients with HD according to the histological type

Histological type	Cu µg/10^{10} cell	Zn µg/10^{10} cell	Cu/Zn ratio
Lymphocyte predominance (n=0)	---	---	---
Mixed cellualrity (n=14)	x =27.61 median =23.66	x =63.4 median =33.24	x =0.469 median =0.39
Nodular sclerosis (n=3)	x =66.083 median =78.83	x =64.2 median =92.8	x =2.25 median =0.769
Lymphocyte depletion (n=2)	x =158.41 median =158.41	x =92 median =92	x =1.44 median =1.44

On estimating lymphocyte Zn in both HD and NHL there was an insignificant reduction in Zn level when compared with control value (Table 2, Figure 4).

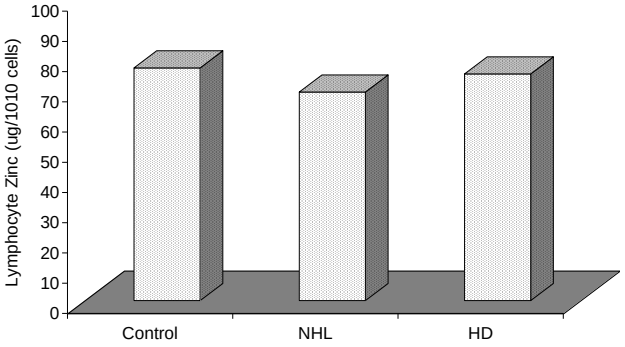

Figure 4: Concentration of lymphocytes zinc (ug/10^{10} cells) of cases enrolled in this study.
NHL= Non Hodgkin's lymphoma, HD = Hodgkin's disease.

Figures 5 & 6 and tables 3 & 4 showed a non significant correlation between lymphocyte Zn level and the grades of NHL and histotypes of HD respectively.

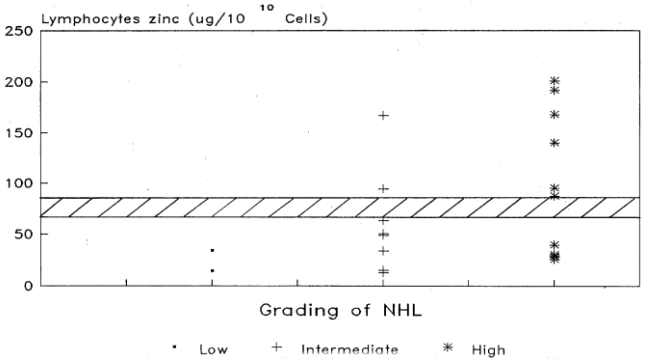

Figure 5: Distribution of lymphocytes zinc (ug/10^{10} cells) according to the grading of non- Hodgkin's lymphoma.
Shaded zone represents Mean±SE of controls value.

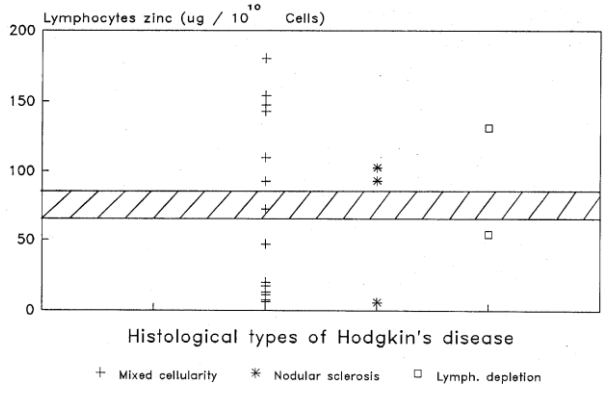

Figure 6: Distribution of lymphocytes zinc (ug/10^{10} cells) according to the histological types of Hodgkin's.
Shaded zone represents Mean±SE of controls value.

Furthermore Cu/Zn ratio was estimated in HD and NHL where there was a significant high value when compared with the control (Table 2 and Figure 7). Moreover the ratio in NHL was

significantly higher than that of HD. However there was no significant correlation between the lymphocyte Cu/Zn ratio and the grades of NHL or the histotypes of HD as shown in tables (3 and 4) and Figures (8 and 9).

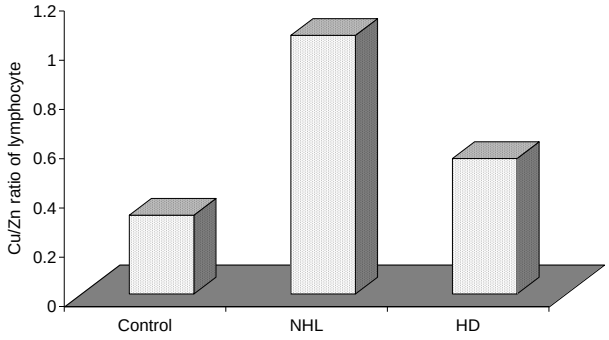

Figure 7: The values of Cu/Zn ratio of lympocytes of cases enrolled in this study.
NHL= Non Hodgkin's lymphoma, HD = Hodgkin's disease.

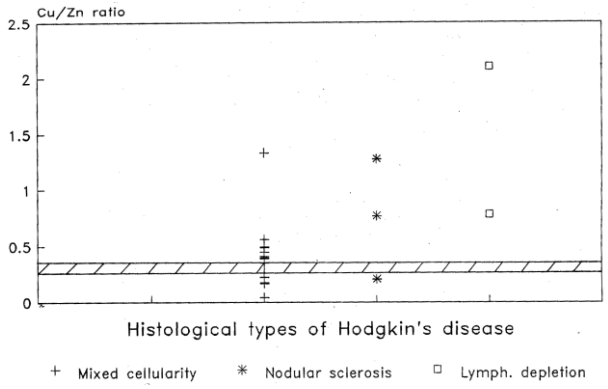

Figure 8: Distribution of lymphocytes Cu/Zn ratio according to the grading of non- Hodgkin's lymphoma.
Shaded zone represents Mean±SE of controls value.

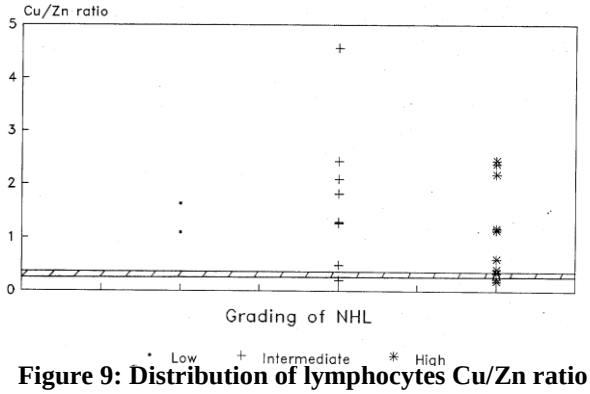

Figure 9: Distribution of lymphocytes Cu/Zn ratio according to the histological types of Hodgkin's disease.
Shaded zone represents Mean±SE of controls value.

Discussion

In the present study there was a significantly high and an insignificant low intracellular Cu and Zn respectively in both HD and NHL (Table 2, figures1 & 4).

Similarly Carpentieri *et al* had found that malignant lymphocyte whether they were separated from patient with acute lymphocytic leukemia[5] or they were grown in a media with optimum concentration of Cu and Zn[13], would show a significant high and insignificant low intracellular Cu and Zn respectively[5,13].

This may be explained by the effect of the increase in serum Cu particularly caeruloplasmin which was observed in various specific and non specific pathological condition and was attributed either to the increase in the release of the synthesized protein caused by the high turnover of the cells or to the induction of the protein synthesis or to both[14,15].

This increase in serum Cu had a deleterious effect on the cell membrane by oxidizing the sulphhydryl group in the membrane protein which will seriously impair the membrane flexibility, deformability and permeability[16].

Since Zn may act as a stabilizer of various biological membranes by interacting with the extrinsic macromolecule components of the membrane mainly the enzymes and/or directly with the intrinsic structure of the plasma membrane[17], therefore the low Zn level will affect the membrane stability as well.

This modification of the cell membrane may be a cause for the great changes in the concentration of many trace elements particularly Cu and Zn which was observed in malignant cells[5]. Whereas in normal cells there was an active regulation in the intracellular metal concentration at the membrane level[5].

Furthermore, two studies were done on lymphocyte Zn in malignant lymphoma [HD and NHL][18] and in acute lymphoblastic leukemia (ALL)[5]. Both of them showed an insignificant reduction in the intracellular Zn level when compared with control value[5,18]. This reduction was not associated with reduction in Zn serum level and was not corrected by oral Zn supplement or by changes in T and B cell ratio[5,18]. Therefore they had concluded that this cellular hypozincaemia was resulted from the defective uptake of Zn by the malignant cells and that mitogenic stimulation has no effect on zinc binding protein within the cells[5,18]. These changes was attributed to the modification in the cell membrane of the malignant lymphocytes[5].

Further analysis of the results of this study had revealed that there was a positive correlation

between the intracellular Cu and the grades of NHL (Table 3 and Figure 2). This was in agreement with many studies done on NHL which revealed that serum Cu directly correlated with the disease activity and the grades of the tumor[19-22].

Since serum Cu correlate with cellular Cu in malignant lymphocytes[5], and that cellular Cu and Zn reflect more accurately body copper status[23-25], therefore we may propose that intracellular Cu like serum Cu can be used as an auxiliary marker in the diagnosis, follow up and as a prognostic marker in NHL. Moreover, the exposure to copper dust had been considered as a predisposing agent since it had a close association with the incidence of NHL[26].

On the other hand in HD, this study is similar to many other studies[14,26,27] had revealed that there was a positive and a negative correlation between Cu and Zn level and the disease activity (regardless of their histological subtypes) respectively (Table 4, Figure 3 and 6). Therefore they had speculate that Cu as well as Zn level can be used to differentiate between active and inactive disease and in detecting early relapse (Figures 1, 2, 3, 4, 5 and 6).

Additionally, similar to many studies there was a significant rise in Cu/Zn ratio in both HD and NHL (Table 2, Figure 8). Therefore they had proposed that Cu/Zn ratio can be used as a tumor marker alongside other biochemical indices[28,29]. However, this increase in Cu/Zn ratio did not correlate with the grades or the histotypes of NHL and HD respectively. Similarly a study was done on patients with melanoma where they had a significant rise in serum Cu and serum Cu/Zn ratio and a non significant reduction in serum Zn, however neither Zn nor Cu/Zn ratio correlate with the disease activity, therefore they had speculate that the Cu/Zn ratio had only reflect the changes in serum Cu[30] (Figures 7, 8 and 9).

Conclusion

1. Intracellular Cu and Cu/Zn ratio rather than Zn may provide a simple and reliable auxiliary tumor marker for the diagnosis and the follow up of patients with malignant lymphomas.
2. Only intracellular Cu had reflect the disease severity in NHL, therefore may be used as a prognostic marker.

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