SERUM β2 MICROGLOBULIN AND LACTATE DEHYDROGENASE 2 ISOENZYME AS MARKERS OF BONE MARROW INFILTRATION IN NON-HODGKIN LYMPHOMA

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ABSTRACT

Objective: To determine the levels of serum lactate dehydrogenase 2 (LD2) Isoenzyme and β2 microglobulin (β2m) in patients of non-Hodgkin's lymphoma (NHL) and to correlate these levels in NHL patients with and without bone marrow infiltration.

Study Design: Cross sectional study.

Place and Duration of Study: Post Graduate Medical Institute, Lahore and Centre of Excellence in Molecular Biology (CEMB), Lahore, from 2008 to 2010.

Material and Methods: The study was conducted on 80 subjects irrespective of age and sex and divided into three groups i.e. group A comprising 20 normal healthy controls, group B 30 patients of NHL without bone marrow infiltration while group C consisted of 30 NHL patients with bone marrow infiltration. Serum β2m and LD2 isoenzyme levels were determined in the subjects and the values were compared with healthy age and sex matched controls. The estimations were made prior to the institution of chemotherapy.

Results: Serum β2 microglobulin and LD2 levels were significantly raised in NHL patients compared with controls. There was also significant difference when the values were compared between the patients of NHL with and without bone marrow infiltration.

Conclusion: The levels of β2m and LD2 showed positive correlation with the extent of the disease, so we conclude that non-invasive parameters are useful indicator of the extent of the disease.

Keywords: Beta 2 microglobulin, Lactate Dehydrogenase, Non-Hodgkin Lymphoma.

INTRODUCTION

Lymphoma is a tumor of the lymphoid tissue[1,2]. Lymphomas represent clonal malignancies in which the majority of the cells are frozen at a single stage of normal differentiation[3,4]. Two broad types of lymphomas are named as Hodgkin lymphoma and Non-Hodgkin lymphoma[5]. Among which about 90% of are non-Hodgkin's type while about 10% are Hodgkin's[6].

In non-Hodgkin lymphoma (NHL) primary manifestations of disease occur outside the bone marrow at the site of normal lymphocytes homing lymph nodes, spleen, MALT (mucosa associated lymphoid tissue) or anywhere. Non-Hodgkin lymphoma can cause many symptoms, such as swollen, painless lymph nodes in the neck, armpits or groin, unexplained weight loss, fever, soaking night sweats, coughing, trouble breathing or chest pain, weakness and tiredness that don't go away, pain, swelling or a feeling of fullness in the abdomen.A patient with NHL may present with localized and generalized peripheral adenopathy[7-10].

Once histological diagnosis of malignant lymphoma has been established, the next is to determine extent of disease so that treatment protocol may be decided. Patients at high risk for failure with conventional therapy may benefit from investigational approaches. The biological markers of NHL are distinguished in three categories: serological, immunophenotypic and molecular markers. The clinical importance of biological markers in NHL is based on their support of morphological diagnosis, their role in staging and prognostic assessment. Among the most important serological β2m reflects the

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tumor load and LD indicates invasive potentials of lymphoma[11].

β2m is a low molecular weight polypeptide, non-covalently linked to the heavy chain of class 1- histocompatibility antigens which are shed with cell turnover. It is plentiful on the surface of lymphocytes. Increased production or destruction of the cells causes β2m levels in the blood to increase. LD has molecular weight of 135,000 Daltons and is a zinc containing enzyme. LD catalyzes the reversible oxidation of lactate to pyruvate. It is expressed at higher levels when lymphocytes are dividing or when cells are distressed or damaged. Elevating LD is an indication of disease progression. Sharp increase can indicate transformation. LD has five isoenzymes which differ slightly in structure. LD2 is concentrated in lymphocytes[10].

The objective of this study was to determine the levels of serum LD2 isoenzyme and β2m in patients of NHL and to correlate these levels in NHL patients with and without bone marrow infiltration.

MATERIAL AND METHODS

It was a cross sectional study conducted on 80 subjects irrespective of age and sex and were divided into 3 groups as follows

- Group A (Controls): 20 Normal healthy subjects.
- Group B: 30 patients of NHL without bone marrow infiltration.
- Group C: 30 patients of NHL with bone marrow infiltration.

The cases were selected from Lahore General Hospital, Lahore, Institute of Nuclear Medicine

Table-I: Demographic and clinical data of patients.

Variable		Control (n=20)	NHL without Infiltration (n=30)	NHL with Infiltration (n = 30)
Age (years) mean ± sd	Male	35.1 ± 22.1	35 ± 19.8	35.6 ± 18.7
	Female	34.3 ± 15.4	34.6 ± 13.6	34 ± 17.2
Blood complete examination (mean ± sd)	Hemoglobin (hb) g / dl	12.53 ± 0.57	11.58 ± 1.30	9.61 ± 0.80
	Esr (mm / hr)	11.95 ± 3.28	38 ± 9	54 ± 25
	Platelets count (× 109 / l)	216.1 ± 59.08	238.8 ± 32.4	177.47 ± 69.86
	Total leucocyte count (× 109 / l)	8.51 ± 0.85	8.69 ± 0.92	10.96 ± 8.22
Clinical data (%)	Weight loss	NIL	8 (26.67)	10 (33.33)
	Lymphadenopathy	NIL	30 (100)	30 (100)
	Spleenomegaly	NIL	15 (50)	23 (76.67)
	Hepatomegaly	NIL	8 (26.67)	14 (46.67)

Table-II: Comparison of β2 microglobulin and ld2 levels in controlsand nhlpatients without bone marrow infiltration.

Parameters		Controls (n=20)	Non-Infiltration (n=30)	*p*-value
β2m	(µg / ml)	1.52 ± 0.435	2.44 ± 0.596	<0.001*
LD2	(%)	28.85 ± 4.107	38.87 ± 3.491	<0.001*

*Very highly significant (Using t-test).

Table-III: Comparison of β2- microglobulin and ld2 levels in controls and nhl patients with bone marrow infiltration.

Parameters		Controls (n=20)	Infiltration (n=30)	*p*-value
β2m	(µg / ml)	1.52 ± 0.435	3.93 ± 0.710	<0.001*
LD2	(%)	28.85 ± 4.107	52.53 ± 4.967	<0.001*

*Very highly significant (Using t-test).

and Oncology (INMOL), Lahore, Services Hospital, Lahore and Mayo Hospital, Lahore from 2008 to 2010.

A detailed clinical history was taken and physical examination of all subjects was performed.

Inclusion Criteria

Newly diagnosed cases of NHL by lymph node biopsy prior to the institution of chemotherapy of both sexes and all age groups were selected for the present study.

Exclusion Criteria

The patients with the history of myocardial infarction, renal failure, hepatic dysfunction, skeletal muscle disease, hemolytic anemia, malignancy of any other system, cerebrovascular accident, Infectious mononucleosis and intestinal infarction were excluded.

Bone marrow aspiration and trephine biopsy were performed bilaterally from right and left posterior superior iliac spine. Serum LD2 isoenzymelevel was estimated by agarose gel electrophoresis at Centre of Excellence in Molecular Biology (CEMB), Lahore. Analysis was carried out on fresh sera, or stored for up to one week at room temperature (15-30oC). Hemolyzed samples were discarded. Ten µl serum was applied on SEBIA Hydragel K20 and placed the gel into an electrophoresis chamber by plugging the chamber to the power supply.

Migration Conditions	**Sebia K20**
Volume of buffer per compartment	150mL
Total buffer volume	300mL
Migration time	25 minutes
Constant voltage	80V
Initial current (pergel)	10 ± 2mA

Then incubation with ISO-LDH substrate was done by applying 2.25 ml of ISO-LDH substrate solution and by placing the gel in the incubator-dryer at 51°C for 25 minutes. Substrate was eliminated and blocking solution was applied. After eliminating the blocking solution gel scanning was done.

Serum β-2m was measured by enzyme linked immunosorbent assay (ELISA) technique.

Immunoassay Procedure

Pipetted 100 µl calibrator, control or patient sample, incubated for 30 minutes at room temperature, discarded the contents of the wells and washed 3 times with 300 µl wash, solution, pipetted 100 µl enzyme conjugate, incubated for 15 minutes at room temperature, discarded the contents of the wells and washed 3 times with 300 µl wash solution, pipetted 100 µlsubstrate solution, incubated for 15 minutes at room temperature, added 100 µlstop solution, left untouched for 5 minutes and read at 450 nm.

A specially designed, well-structured and pre-tested questionnaire were used to collect desired information. Data were collected after getting consent of the patients and ethical issues were considered during the entire study duration. Statistical analysis was carried out using SPSS version 19.0. All study variables were presented as frequency and percentages. Mean ± S.D was calculated for quantitative variables. Quantitative variables were compared by independent sample t-test. Correlation was calculated by using Pearson's correlation coefficient between control group, non-infiltration group and infiltration group. Analysis of variance (ANOVA) technique was applied for comparing Control group and patients of NHL groups. A *p*-value 0.05 was considered as significant.

RESULTS

The mean age for controls was found to be 35.1 ± 22.1 SD years (males) and 34.3 ± 15.4 SD years (females), in NHL patients without infiltration it was 35 ± 19.8 SD years (males) and 34.6 ± 13.6 SD years (females) and in NHL patients with infiltration the mean age was 35.6 ± 18.7 SD years (males) and 34 ± 17.2 SD years (females) (table-I). The number of males and females in each group was equal. The complete blood examination shows hemoglobin levels (Hb) as 12.53 ± 0.57 SD g/dl for controls, 11.58 ± 1.30 SD g/dl for NHL patients without infiltration

and 9.61 ± 0.80 SD g/dl for NHL patients with infiltration (table-I). The ESR count for controls was 11.95 ± 3.28 SD mm/hr, 38 ± 9 SD mm/hr in group B and 54 ± 25 SD mm/hr in group C. The platelets count (× 109) for controls, NHL patients without infiltration and NHL patients with infiltration was 216.1 ± 59.08 SD, 238.8 ± 32.4 SD and 177.47 ± 69.86 SD respectively. The total leucocyte count (× 109/l) for controls, NHL patients without infiltration and NHL patients with infiltration was 8.51 ± 0.85 SD, 8.69 ± 0.92 SD and 10.96 ± 8.22 SD respectively (table-I). Clinical data for NHL without infiltration patients (n=30) showed 8 (26.67) had weight loss, all had lymphadenopathy, 15 (50%) had splenomegaly and 8 (26.67%) were suffering from hepatomegaly. Clinical data for NHL with infiltration patients (n=30) showed 10 (33.33%) had weight loss, all had lymphadenopathy, 23 (76.67%) had splenomegaly and 14 (46.67%) were suffering from hepatomegaly (table-I). In controls mean serum β2m level was 1.52 ± 0.43 µg/ml. Mean serum β2m level in group B patients Bone marrow was aspirated from both right and left posterior superior iliac spine was 2.41 ± 0.48 µg/ml while in group C it was 3.93 ± 0.71 µg/ml. The difference between the mean levels of the controls and patients groups, as well as between the NHL patients with and without bone marrow infiltration were highly significant (p<0.001)

Table-IV: Comparison of β2m levels in patients of non-hodgkin's lymphoma (NHL) and control group.

β2m			
Group	**Comparison Gr oups**	**Mean ± Std. Deviation**	***p*-value**
Control Group	NHL Without Bone Marrow Infiltration	1.5160 ± 0.43477	<0.001*
	NHL With Bone Marrow Infiltration		<0.001*
NHL Without Bone Marrow Infiltration	Control Group	2.4453 ± 0.59593	<0.001*
	NHL With Bone Marrow Infiltration		<0.001*
NHL WithBone Marrow Infiltration	Control Group	3.9333 ± 0.71050	<0.001*
	NHL Without Bone Marrow Infiltration		<0.001*
Total		2.7710 ± 1.14595	

*Very highly significant (ANOVA-LSD).

Table-V: Comparison of Id2 levels in patients of non-hodgkin's lymphoma (NHL) and control group.

LD2			
	Comparison Groups	**Mean ± Std. Deviation**	***p*-value**
Control Group	NHL Without Bone Marrow Infiltration	28.85 ± 4.10744	<0.001*
	NHL With Bone Marrow Infiltration		<0.001*
NHL Without Bone Marrow Infiltration	Control Group	38.8667 ± 3.4912	<0.001*
	NHL With Bone Marrow Infiltration		<0.001*
NHL WithBone Marrow Infiltration	Control Group	52.5333 ± 4.96702	<0.001*
	NHL Without Bone Marrow Infiltration		<0.001*
Total		41.4875 ± 10.34224	

*Very highly significant (ANOVA-LSD).

(tables-II-IV). In 6 cases with lymphocytosis β2m value ranged from 4.5-5.23 µg/ml with mean value of 4.91 ± 0.27 µg/ml. Highest values (5.23 & 5.2 µg/ml) were noted in 2 (7%) cases with peripheral spill over. The cut off limit of serum β2m among group B & C derived from the data was 3.0µg/ml. The cases with infiltration showed values above this cut off limit (table-II).

While in controls mean serum LD2 level was 29.1 ± 4.08%, in group B was 39.83 ± 2.09% while in group C it ranged from 52.53 ± 4.47%. The difference between the mean levels of the controls and patients groups, as well as between the NHL patients with and without bone marrow infiltration was highly significant (p=0.001) (tables-II-V). In 6 (20%) cases with leukocytosis LD2 values ranged from 54-60% with mean value of 57.5 ± 2.07%. Highest values (60 & 59%) were noted in 2 (7%) cases with peripheral spill over. The cut off limit of serum LD2 among group B & C derived from the data was 45%. The cases with infiltration showed values above this cut off limit.

DISCUSSION

Beta 2 microglobulin being a low molecular weight polypeptide shed with cell turnover. β2m seems to reflect tumor burden of malignant cells Pathological specifications and clinical data showed a significant change between controls and NHL with and without bone marrow infiltration. Mean β2m level was significantly raised in NHL patients with and without bone marrow infiltration ascompared to controls (p<0.001). So β2m level was elevated with progression of disease and its level showed an increase in mean values with the advancing stage of NHL[11-14,16].

β2m seems to reflect tumor burden of malignant cells[15] and is significantly associated with NHL and greatly effects the overall survival of the cancer patients and is among the most important serological markers which reflect tumor load[17-19]. β2m can be helpful in the diagnosis of NHL. In this study β2m level ranged from 1-1.88 µg/ml in four patients of stage I and was 2.0-2.42 µg/ml in eight patients of stage II while in eighteen patients from stage III, its range was 1.99-3.29 µg/ml[20-23].

Some characteristics of serum LD isoenzyme profiles in patients with NHL and some of the specific alterations may help to refine the prognostic value of total serum LDH suggesting it to be a reliable indicator of malignant potential in NHL. Highest LD2 isoenzyme activity was found to be helpful to evaluate the tumor burden and prognosis in patients of NHL and predominance of LD2 was related to the early and only sign of occult malignant lymphoma[22].

Thus Levels LD2 and β2m showed an increase in mean values with the advancing stage of NHL. In stage III and IV β2m and LD2 levels were significantly higher than normal. Our results resemble the observations made by Tong 2009. Changhoon Yoo 2014 stated current evidence supporting the role of serum β2m and LD2 as prognostic factors in patients with NHL.

CONCLUSION

In view of above observations it was concluded that β2m being a non-invasive parameter can be considered as a marker of tumor burden and can be used to assess the tumor burden in NHL patients. While LD2 isoenzyme can serve as an indicator of extent of disease. Being non-invasive parameters, these two parameters can be used to assess the proliferative activity and invasive potentials of lymphoma.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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