Original Article

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BETA 2 MICROGLOBULIN AS A MARKER OF DIAGNOSIS AND DISEASE SEVERITY IN NON HODGKIN LYMPHOMA

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ABSTRACT:

Lymphoma, which can be described as proliferations of lymphoid cells arising as discrete tissue masses**,** is broadly divided into Non – Hodgkin Lymphoma (NHL) and Hodgkin disease. Majority of malignant lymphoma are found to be NHL. Beta-2 microglobulin (B2M) is synthesized in all nucleated cells and forms the light chain subunit of the major histocompatibility complex class I antigen. Despite its potential role as a convenient and non-invasive prognostic indicator in malignant lymphomas, the influence of serum B2M is currently underestimated, and therapeutic decision making is rarely affected by this marker.

Objective: To find out the levels of B2M in patients of NHL and to observe if the levels are significantly correlated with the stage of the disease, performance status as denoted by ECOG performance scale, International prognostic index(IPI) evaluated at the time of diagnosis, and thereby, to the severity of disease.

Methods: 30 patients with confirmed diagnosis of NHL and 30 age and sex matched controls were selected. The levels of B2M in their serum were estimated. B2M was estimated using ELISA technique.

Results: Serum B2M levels were found to be significantly elevated in patients of NHL (4.60±2.24 µg/ml**)** ($p< 0.01$). Levels were significantly higher in patients with later stages (stage III and IV) (8.30±0.099µg/ml) than those with early stages (stage I + II) ($p<0.01$). Patients presenting with poor performance status (ECOG scale) were having a higher B2M levels (5.510±2.35 µg/ml) ($p<0.01$). There was also a significant difference between B2M levels in each IPI groups. Low B2M levels were found in low risk and low intermediate risk groups as compared to high intermediate and high risk IPI groups ($p<0.01$).

Conclusion: On the basis of observations, it can be concluded that B2M is a good marker of tumour burden and also is a potential marker for disease severity as indicated by higher serum B2M levels in patients with advanced disease stage, higher IPI group and poor performance status.

Keywords: lymphoma, non hodgkins lymphoma, beta 2 microglobulin

INTRODUCTION:

Malignancies of lymphoid cells range from the most indolent to the most aggressive human malignancies.[1]Lymphoma, is described as proliferations of lymphoid cells arising as discrete tissue masses**.** In

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general, lymphomas are divided into 2 large groups of neoplasm, namely non-Hodgkin lymphoma (NHL) and Hodgkin disease. About 85% of all malignant lymphomas are NHLs.[2] The incidence of NHL in India is around 2.93 in 1000 females and 4.45 in 1000 males [3]. The morbidity of NHL is increasing worldwide in the past 3 decades [3].

A patient with NHL may present with localized and generalised peripheral lymphadenopathy [4-6].NHL includes diverse B cell malignancies of lymph node follicle and several less common T-cell proliferations and macrophage malignancies. NHL is mainly diagnosed through lymph node biopsy and histology. Once histological diagnosis is established, it is necessary to determine the disease extent and severity, in order to plan the treatment regimen. The prognosis of patients with NHL is best assigned using the International Prognostic Index (IPI). It is a predictor of outcome in all subtypes of NHL [1].

Investigational approaches, such as, assessment of biological markers at the time of diagnosis and during the course of treatment may benefit patients at high risk for failure with conventional therapy. The clinical importance of biological markers in NHL is based on their support of morphological diagnosis, their role in staging and prognostic assessment. There have been so many serological markers discovered for NHL, beta 2 microglobulin being one of the earlier recognised markers and it reflects the tumour load [7]. Higher B2M levels are associated with a advanced stage, B symptoms, bone marrow involvement.[8] B2M value in addition to the International Prognostic Index (IPI) may help in selection of the patients with NHL at higher risk for treatment failure, and in identification of those who may require specifically tailored therapeutic approaches.[9]

B2M is a low molecular weight protein, synthesised in all nucleated cells. It occurs in small quantities in normal urine, plasma and cerebrospinal fluids.[10] B2M is homologous in sequence to the constant portion of immunoglobulin light chains and to the homology regions of the constant portion of γ1 (heavy) chains of immunoglobulin G. It is found abundantly on the surface of lymphocytes. Increased production or destruction of the cells causes B2M levels in the blood to increase.[11]

In this study, we aim to find out the levels of beta2 microglobulin in patients of NHL and to observe if the levels are significantly correlated with the stage of the disease and performance status as denoted by ECOG performance scale, IPI and, thereby, to the severity of disease.

MATERIAL AND METHODS:

The present study was conducted in the Department of Biochemistry, in collaboration with the Department of Medicine (Clinical Haematology Unit), in PT. B.D.SHARMA PGIMS, Rohtak. The study protocol was approved by the institutional ethics committee. 30 diagnosed NHL patients and 30 age and sex matched healthy controls were

included in this study. The diagnosis of patients was made by careful history and physical examination, complete blood count, routine biochemistry tests, bone marrow biopsy and lymph node biopsy. A written consent was obtained from all the patients, participating in this study. The clinical stage of patients was determined according to Ann Arbor staging.

Five ml of venous blood sample was collected from patients at the time of diagnosis in red capped evacuated vaccutainers and also in purple capped EDTA vaccutainers for complete hemogram, under all aseptic precautions. Samples were processed within one hour of collection. Serum was separated by centrifugation (2000 rpm X 10 minutes) after clotting. Sample was analysed for routine biochemistry and B2M. EDTA sample was analysed for complete haemogram. Serum B2M levels was estimated by a commercial Enzyme Linked Immunosorbent Assay kit for human B2M (DRG β2-MG ELISA). The reference range for B2M according to this kit is less than 2µg/ml.[12]

Stastistical analysis: Statistical package for the social sciences (SPSS ver. 20) was used for various statistical analyses. Comparison of data between groups was done using 't' test. Comparison between multiple groups was done using anova test. Paired samples were compared by paired 't' test. Any p value less than 0.05 was considered significant.

RESULTS:

Participants with the habits were more common in 4th and 5th decade where as In this study 60 subjects were included, among them 30 were Non- Hodgkin lymphoma patients and 30 age and sex matched controls. The patients belonged to various age groups ranging from 38-67 years. Mean age of the patient group is 52.10 ± 8.10 years and in control group mean age was 52.83± 7.04 years. Among these 30 cases 23(77%) were males and 7(23%) were females, which shows a relatively higher prevalence in males. B symptoms were present in 33.33% of NHL patients. 100% of NHL patients had features related to weakness. Splenomegaly and hepatomegaly was found in 40% and 16.6% NHL patients respectively. Lymphadenopathy (LAP) was found in 100% NHL patients [Table 1]. Mean haemoglobin levels were 10.32 g/dl in NHL patients. Mean total leucocyte count (TLC) was 7100/cu.mm at presentation in NHL patients. Mean platelet count was 107933.33/cu.mm in NHL patients. Mean serum albumin was 3.3gm/dl in NHL patients.

Mean serum B2M was 0.47± 0.30 µg/ml in controls. Levels were significantly raised in NHL patients (4.60±2.24 µg/ml, p <0.01). In this study 23(76.66%) patients presented with early stage(I + II) and 7(23.33%) patients presented with late stage(III + IV). It was noted that patients with late stage had comparatively higher mean B2M levels (8.30±0.099µg/ml) as compared to those whose stage is earlier(3.4±00.852µg/ml).This correlation was found to be statistically significant (p<0.01).

In this study 11(36.6%) cases initially presented with ECOG 0 or 1 performance

status and 19(63.33%) patients initially presented with ECOG 2 or 3 performance status. It was seen that NHL cases associated with poor performance(ECOG 2 or 3) status had higher B2M levels(5.510±2.35 µg/ml) as compared to those patients who presented with good performance status(ECOG 0 or 1) (3.0409±0.68 µg/ml).There were 26 B cell lymphoma and 4 T cell lymphoma cases. In this study the mean value for B2M (4.88±2.55µg/ml**)** in B cell lymphoma patients was higher, as compared to T cell NHL patients (3.44±1.13µg/ml), but due to lesser number of T cell NHL patients, it was difficult to establish a significant correlation.

B symptoms were present in 10(33.33%) NHL cases. It was found that those patients who initially presented with B symptoms had higher B2M levels (4.89±2.32 µg/ml) as compared to those who initially presented without B symptoms(4.18±2.5µg/ml). 10 patients had haemoglobin less than 10g/dl while 20 patients had haemoglobin more than 10 g/dl. B2M levels (6.00±2.68µg/ml) were higher in patients having haemoglobin value less than 10g/dl and the correlation was significant (p=0.003) 14 patients had albumin <3.5 g/dl and 16 patients had albumin >3.5g/dl. B2M levels were higher in patients having albumin levels <3.5g/dl(6.122±2.93µg/ml) as compared to those who had albumin >3.5g/l(3.43±0.75µg/ml) in NHL cases. This correlation was found to be statistically significant (p=0.001)

B2M levels in different IPI groups were low risk (LR) (3.2533± 0.72 µg/ml), low intermediate risk (LI) (3.0730±0.70 µg/ml), high intermediate (HI) (8.2417±1.20 µg/ml), high risk (HR) (4.6127±2.31 µg/ml)

TABLE 1 – PATIENTS CHARACTERISTICS

Cases	30
Age	52.10 ± 8.10 years
Male	23(76.7%)
Female	7(23.3%)
B symptoms present	10(33.33%)
B symptoms absent	20(66.67%)
Weakness	100%
Splenomegaly	12(40%)
Hepatomegaly	5(16.6%)
Only Lymphadenopathy	11(36.6%)
Early Stage I+II	23(76.66%)
Late Stage III+IV	7(23.33)
ECOG 0 or 1	11(36.6%)
ECOG 2 or 3	19(63.4%)

TABLE 2: COMPARISON OF B2M IN EARLY (Stage I + II) AND LATE STAGES (Stage III + IV) IN NHL PATIENTS

N	STAGE	B2M(µg/ml)	p value
23	Stage I+II	3.4804±.85257	<0.01 significant
7	Stage III+IV	8.3000±.09993	

TABLE 3: COMPARISON OF B2M IN BETWEEN THE SUBTYPES OF NHL CASES

N	Subtype of NHL	B2M(µg/ml)	P value
26	B cell lymphoma	4.8815±2.55858	0.090
4	T cell lymphoma	3.4400±1.13616	(Non – significant)

TABLE 4: COMPARISON OF B2M LEVELS IN DIFFERENT IPI CLASSES IN PATIENTS

IPI	N	B2M(µg/ml)	p value
LR	3	3.2533± 0.72	p-value is <0.01(significant)
LI	10	3.0730±0.70	
HI	6	8.2417±1.20	
HR	11	4.6127±2.31	

LR – Low risk, LI- Low intermediate risk, HI- High intermediate risk, HR- High risk

DISCUSSION:

B2M has been discovered three decades before and it is very easy to measure but still its role in prognosis and disease extent has been underestimated. Among the 30 cases of NHL taken, 23(77%) were males and 7(23%) were females, which shows a relatively higher prevalence in males. This is in accordance with the studies done by Johnson et al (60% males)[13]. The mean value of B2M levels was found to be significantly higher in NHL patients as compared to control group which reflects the increase in cell turnover . Melillo [14] in his study concluded that B2M reflects tumor burden of malignant cells.

The mean value of B2M in stage I + stage II and stage III + stage IV were 3.4804±.85 µg/ml and 8.3000±.099 µg/ml respectively. So we found that mean value of B2M was significantly higher in later stages(stage III and stage IV) as compared to earlier stages(stage I and stage II). Similar observations were made by Hagberg [15] who reported B2M level greater than 3.0 µg/ml in 15% of patients with stage I and II and in 65% of those with stage III and IV. Study done by Massimo Fedrrico and Naghmana Mazher [16,17] also found that later stages had higher B2M as compared to early stages. Besides the late stages, we also found that patients presented with poor performance status as evaluated by ECOG numbering, B symptoms, those who were older than 60 years, low haemoglobin(<10

gm/dl), and low albumin(<3.5 gm/dl) had significantly higher mean B2M levels. Massimo Fedrrico [16] in his study also found the similar results.

It was seen in this study that there was significant difference between the B2M levels in each IPI groups. Low B2M levels were found in low risk and low intermediate risk groups as compared to high intermediate and high risk IPI groups. Study done by Massimo Federico [16] also showed results similar to our study that B2M levels were significantly higher in high risk IPI groups.

CONCLUSION:

According to our study B2M levels were found to be higher in advanced stage, poor performance status, B symptoms positive patients, older patients(>60 years)patients and patients with higher risk group in IPI. These results suggest that B2M can be a potential marker of disease extent and tumor load and that repeated determinations of serum B2M in these patients might be useful as an estimate of the residual malignant cell mass . Further studies in larger number of patients with long term follow up are required to validate this role.

REFERENCES:

1. Longo DL. Malignancies of Lymphoid cells. In: Longo, Fauci, Kasper editors. Harrison's Principles of internal medicine. 17th edition. USA. McGraw-Hill 2011. p. 677-90.
2. Shustik J, Quinon M, Connors JM. Follicular non Hodgkins lymphoma grades 3A and 3B have similar outcome and appear incurable with anthracycline based therapy. Ann oncol. 2011; 22: 1164-9.
3. Zhang JP, Wang YL. The advancement of biotherapy for malignant lymphoma. Journal of Leukemia & Lymphoma 2006; 15: 474-6.
4. Karin ZC, Jose SR, Antonio CA, Maria RR. Prognostic factors in non-Hodgkin's lymphoma. Sao Paulo Med J 2000; 118 : 7-12.
5. Skunca Z, Gveric-Krecak V, Dominis M, Planinc-Peraica A, Jaksic B. Non-Hodgkin's lymphoma: clinical symptoms, therapy and prognosis in 37 patients. Acta Med Croatica 2003; 57 : 261-7.
6. Adesuwa Olu-Eddo N, Egbagbe EE. Peripheral lymphadenopathy in Nigerian children. Niger J Clin Pract 2006; 9 : 134-8.
7. Morra E. The biological markers of non-Hodgkin's lymphomas: their role in diagnosis, prognostic assessment and therapeutic strategy. Int J Biol Markers. 1999; 14: 149-53.
8. Gui W, Wang T, Wang J, Wang L, He J, Yang B et al . An improved prognostic parameter for non Hodgkins lymphoma based on the combination of three serum tumor markers. Int J Biol Markers. 2008; 23: 207-13.

9. Conconi A, Zucca E, Roggero E, Bertoni F, Bernasconi A, Mingrone W et al. Prognostic models for diffuse large B-cell lymphoma. Hematol Oncol. 2000; 18: 61-73.

10. Berggard I, Bearn AG. Isolation and properties of a low molecular weight beta 2 globulin occuring in human fluids. J Biol Chem. 1968; 243: 4095-103.

11. Bernier GM. Beta 2-Microglobulin: structure, function and significance. Vox Sang 1980; 38: 323-7.

12. Terrier N, Bonardet A, Descomps B, Cristol JP, Dupuy AM. Determination of beta-2 microglobulin in biological samples using an immunoenzymometric assay (chemiluminescence detection) or an immunoturbidimetric assay: comparison with a radioimmunoassay. Clin Lab. 2004; 50: 675-83.

13. Johnson PW, Whelan J, Longhurst S, Stepniewska K, Matthews J, Amess J et al. Beta-2 microglobulin: a prognostic factor in diffuse aggressive non-Hodgkin's lymphomas. Br J Cancer. 1993; 67: 792-7.

14. Melillo L, Musto P, Tomasi P, Cascavilla N, BodenizzaC, Ladogana C, Carotenuto M. Serum beta 2-microglobulin in malignant lymphoproliferative disorders. Tumori 1988; 74: 129-35.

15. Hagberg H, Killander A, Simonsson B. Serum beta 2-microglobulin in malignant lymphoma. Cancer. 1983; 51: 2220-5.

16. Federico M, Guglielmi C, Luminari S, Mammi C, Marcheselli L, Gianelli U et al. Prognostic relevance of serum beta2 microglobulin in patients with follicular lymphoma treated with anthracycline-containing regimens. A GISL study. Haematologica 2007; 92: 1482-8.

17. Naghmana Mazher, Zafariqbal, Naumaan Aslam, Seema Mazehar. Beta 2 Microglobulin as a marker of extent of disease in non Hodgkin lymphoma. Biomedica. 2010; 26: 1-4.