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Original Article

Increased Turnover of Beta 2 Microglobulin in Circulation serves as Diagnostic and Prognostic Marker for Malignant Lymphoma; A Case Control Predictive Model for Lymphoma Diagnosis

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ABSTRACT

Indolent and aggressive hematopoietic cancer shed lot of Beta 2 microglobulin in interstitial fluid thus increasing the level of B2M in hematological malignancies. **Objectives:** To set forward B2M as potential biomarker for the detection and stage of malignant lymphoma. **Methods:** Serum of newly diagnosed Hodgkin's and non-Hodgkin's lymphoma patients presented to physician prior to any surgical or medicinal treatment were collected and evaluated through sandwich type of ELISA for the possible elevation of B2M. B2M concentrations in healthy individual's serum (control group) were also detected. Mean values of B2M in all three groups were compared by applying one-way analysis of variance to determine the significant difference. **Results:** The serum of Hodgkin's Lymphoma patients depicted 5 folds higher B2M concentration than the healthy subjects, while NHL showed more concentration of circulating B2M where it was 6-fold higher than healthy subjects. Moreover, the advanced stage of the disease with involvement of distant site or organ portrayed increase shedding of MICA 1 chain in circulating blood than localized or regional disease. **Conclusions:** Elevated serum B2M concentrations in blood surely a sign of neoplastic disease involving nucleated cell like lymphoma and its enhanced expression in distantly spreading disease proved it as a prognostic marker as well.

INTRODUCTION

Beta-2 microglobulin (B2M) is a membranous protein of 92 amino acid having small molecular mass (~11.8 kDa). The amino acid sequence of B2M depicted homology to immunoglobulin (www.uniprot.com). Human microglobulin is identical to the light chain of HLA-A, -B, and -C antigen [1-3]. It makes the light chain of class I major histocompatibility complex therefore appear on the surface of all nucleated cells particularly on lymphocytes and shed in blood circulation thus present in blood of healthy persons [4, 5]. The circulating blood is an opulent medium of suspended molecules released from different tissues of the body. When lymph node become aggravated and undergoes abnormal cancerous growth, the shedding of major histocompatibility class I related chain A (MICA) exaggerates leading to intensified release of B2M in extracellular fluid. Thus, the serum levels of B2M are elevated in diseases associated with increased cell turnover such as multiple myeloma, Hodgkin's and non-Hodgkin's lymphoma [6]. The ecto-domain of MICA is shed from tumor cells and may be an important means of

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dodging antitumor immunity proving a sensitive prognostic marker for hematological malignancies. Its concentrations are also intensified in inflammatory diseases, some viral diseases, renal dysfunction, and autoimmune diseases [7]. Though B2M is considered as a preliminary molecule involved in development of lymphoma and can easily be assessed through noninvasive technique, many authors argued about its specificity and compare it with latest technologies like positron emission tomography scan [8]. This state-of-the-art technology is no doubt has great accuracy as it is based on imaging, but the product of cancer cells should be detected in body fluids to confirm the nature of focal lesion as cancerous. Thus, evaluation of serum B2M has its own importance in detection of lymphoma, its grade, stage and extant of disease. The liaison of serum β2 microglobulin with malignant behavior of lymph nodes is still ambiguous since no therapeutic or clinical decision was taken on the value of B2M, however, physician used to determine its levels for diagnosis prior to initiation of treatment or therapeutic use. Many researchers observed specific histological categories in number of patients and reported serum β2 microglobulin as a potent prognostic marker in malignant lymphomas subcategories [9]. In addition, B2M levels found as a supreme and independent variable for predicting time to treatment failure and survival. It was also supposed to relate with tumor burden. The current study was aimed to determine whether serum B2M has any correlation with malignant transformation of lymphocytes or put any impact on disease severity. The hypothesis postulate keeping in mind the fact that correlation is not the cause of disease development.

METHODS

Ethical approval for this manuscript was sought by Govt. College Women University, Madina Town, Faisalabad, Punjab, Pakistan and granted vide letter #GCWUF/IERC/22 Dated 14-04-2022. This study is a case control study. The setting of sample collection was convenient as well as hospital based. The case samples were human beings suffering from either type of lymphoma (n=343) confirmed by Institute of Nuclear Medicine and Oncology Lahore (INMOL). Workup for diagnosis included fine needle aspiration, biopsy, histopathology, radiological imaging etc. The patients were chosen by applying strict exclusion/inclusion criteria. Newly diagnosed lymphoma patients without intervention of any kind of treatment were included, while patients with Epstein bar virus, HIV, HCV or HBV or any other lympho-proliferated disorders were excluded from the study. After careful selection, fifty-one (51) patients were selected for the detection of B2M level in serum to judge whether B2M is a diagnostic and prognostic biomarker for lymphoma or not. STARD [10] and TRIPOD [11] reporting guidelines were used to report diagnostic test accuracy and to access the individual prognosis or diagnosis statement. Sorting of lymphoma on histopathology ground revealed that non-Hodgkin's lymphoma (n=41) prevailed more than Hodgkin's lymphoma (n=10). Effects of gender and age on pathogenesis and morphology was observed closely and described in previous study [12]. For determining the concentration of B2M in healthy individuals, convenient sampling was done, and general population samples were taken to compare the concentration of B2M in their blood with newly diagnosed and untreated patients of lymphoma. Levels of circulating B2M were correlated with disease severity and prognosis. After taking written informed consent, subjects were venipuncture for collection of 3cc blood. Obtained samples were placed in sterile glass tubes at room temperature for 30-60 minute to separate the sera. Serum Samples that sit longer than 60 minutes are likely to experience lyses of cells in the clot, releasing cellular components not usually found in serum samples. Clear serum samples were put to determine beta-2 microglobulin concentrations through solid phase sandwich type of enzyme immuno assay using β-2 microglobulin kit (Genway, GWB-75DA03) (40-374-13010). The assay based on the reaction of a distinct monoclonal antibody with a specific antigen present on the intact β-2 microglobulin molecule. Mouse monoclonal anti-β-2 microglobulin antibody was labeled on the microtiter wells for solid phase immobilization. Horseradish peroxidase was castoff as antibody-enzyme conjugate solution which contains sheep anti-β-2 microglobulin antibody in it. The serum samples were diluted 101 folds and pipetted into microtiter plate encrusted with mouse monoclonal anti-β-2 microglobulin antibody. The samples were allowed to react with the immobilized antibody for 30 minutes at 37°C. The sheep anti-β-2 microglobulin-HRP conjugate is then added and reacted with the immobilized antigen for further 30 minutes at room temperature making β-2 microglobulin molecules sandwiched between the solid phase and enzyme-linked antibodies. Excess antibodies were discarded by washing the wells with distilled water. Further incubation of 20 minute was done at room temperature by adding TMB reagent. A blue color was developed after complete incubation. The reaction was then terminated by adding stop solution that change the color of reaction mixture from blue to yellow. The intensity of yellow color was regarded as directly proportional to the quantity of β-2 microglobulin in samples. Optical density of the sample measured through spectrophotometer at 450 nm and concentration of β-2 microglobulin was calculated through standard curve. Mean values of control, HL and NHL groups were compared, and results were analyzed through one-way

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ANOVA using Graph Pad Prism (Version-6.0).

RESULTS

Enzyme immunoassay revealed a prominent rise in concentrations of β-2 microglobulin in serum of lymphoma patients than healthy people. The normal value of serum β-2 microglobulin was ranged from 0-2 µg/ml and all healthy control subjects had their β-2 microglobulin levels within normal limit (0.814 ± 0.66, Mean ± SEM) indicating no inflammation. The concentration of B2M in serum of HL patients was excessively exceeding than normal values and ranged from 2.41-8.29 µg/ml. The mean value of B2M in HL patient was 5.05 ± 0.65 which was 520 folds greater than the mean value in healthy population. Serum samples of NHL patients were found more enriched with B2M than HL. The average increase of 19.80% was observed in NHL as compared to HL. It is obvious that production of B2M will surely be high in NHL in comparison with control. A profound enhancement of B2M was depicted by NHL serum where its concentration ranged from 2.12-9.86 µg/ml. This concentration was 646 times greater than control (Table 1, Figure 1).

Table 1: Average β-2 microglobulin concentrations in comparable groups

Group	No. of Subjects	Mean ± SEM µg/ml	Group	% age↑ or ↓	p-value	F value	R^2 value
HL	10	5.05 ± 0.65	HL vs. control	****520.39%↑	< 0.0001	79.67	0.65
NHL	41	6.02 ± 0.42	NHL vs. control	****646.91%↑			
Control	38	0.814 ± 0.66	NHL vs. HL	19.80%↑			

****Significant at p<0.0001 at 95% Confidence Interval

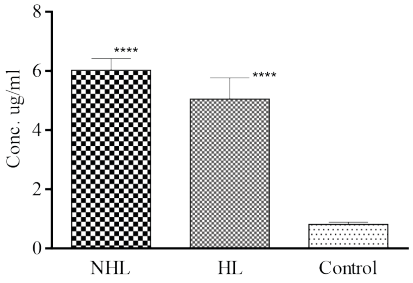

Figure 1: β-2 microglobulin concentration (µg/ml) in comparable groups. Values are Mean ± SEM.

The levels of B2M were also directly related with disease progression as it was successively increased with the spread of malignant cells. Initially, a small rise in level of B2M was observed in stage I of both NHL and HL but concentration was marginally greater in HL than NHL. On average, 3 µg/ml and 3.35 µg /ml of B2M was observed in HL and NHL, respectively. This difference in average value was contributed by exceptional rise in some patients where B2M was exceeded beyond 8.04 µg/ml. Follow up study revealed that the patients with high level of B2M at stage I developed metastatic spread later. Lymphoma at stage II, exhibited B2M concentration up to 4.92 µg/ml in both categories but NHL specifically presented increase shedding of B2M in blood. Patients with B symptoms and spleen involvement (Ann Arbor stage III_E or III_S) portrayed marked rise in B2M levels and again it was more profoundly appeared in NHL than HL patients. When bone marrow or extra hepatic tissue such as liver was invaded by cancerous cells, the turnover of B2M into blood was reached at peak and patients represented highest level ever (up to 9.82 µg/ml). Thus, β-2 microglobulin put great impact on disease stage (involvement of extra-lymphatic solid organs, bone marrow etc.), extent of disease (local, regional, distant) and its mean concentration speckled seriously with disease progression. A direct relation of B2M concentration was observed with stage encroachment and extent of disease. Patient at stage I had mean value of 3.20 ± 0.47 µg/ml that was elevated by 19.37% in stage II. Moreover, β-2 microglobulin levels augmented in stage III and IV (102% and 148.43% as compared to stage I, respectively). Thus, the results predicted a significant association of β-2 microglobulin with stages of lymphoma (Table 2).

Table 2: Average β-2 microglobulin concentrations among various stages of lymphoma

Stage	No. of Subjects	Mean ± SEM µg/ml	Group	% age↑ or ↓	p-value	F value	R^2 value
I	12	3.20 ± 0.47	stage II vs. stage I	19.37%↑	< 0.001	49.58	0.7599
II	8	3.82 ± 0.22	stage III vs. stage I	***102%↑			
III	9	6.49 ± 0.33	stage IV vs. stage I	***148.43%↑			
IV	22	7.95 ± 0.25	stage III vs. stage II	***69.89%↑			
			stage IV vs. stage II	***108.11%↑			
			stage IV vs. stage III	*22.49%↑			

***Significant at p<0.001, * Significant p<0.05 at 95% Confidence Interval

Similar tendency was notice in histopathological differentiation of lymphoma where indolent and aggressive types of histopathologies exhibited successive increase in B2M level. Low and intermediate grade of lymphoma almost have same concentration of B2M (5.048 and 5.48 µg/ml, respectively) while aggressive type of lymphoma has drastic rise in concentration (7.0198 µg/ml). A strong positive correlation was found between B2M and grade of lymphoma (p=0.0039). Similarly, stage of lymphoma was also associated with concentration of beta 2 microglobulin. Individual record revealed that some patients with low grade lymphoma had high value of B2M that was even more than depicted by aggressive type of cancer. This substantial rise may be a predictor of disease progression with or without intervention of treatment and follow up study is needed to evaluate the current disease status.

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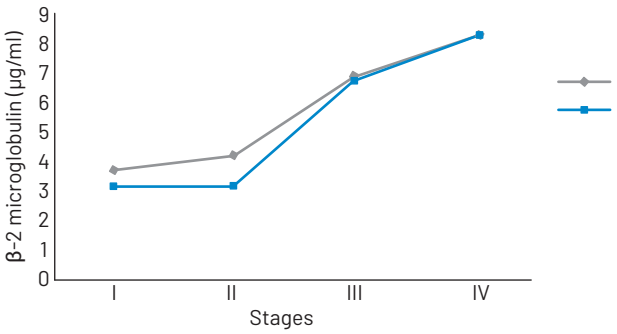

Figure 2: Average β-2 microglobulin concentrations (µg/ml) in various stages of lymphoma. Values are Mean ± SEM, HL Hodgkin's Lymphoma, NHL Non-Hodgkin's Lymphoma

DISCUSSION

Role of B2M in development and progression of lymphoma has been studied extensively and many authors believed that it has great impact in disease etiology [13, 14]. Earliest studies reported its association with treatment response and overall survival in advance stages III or IV [15] but later discrepancies aroused on the canvas revealing disputed impact of serum B2M levels on clinical conclusions [16, 17]. These discrepancies may be due to variety of subcategories of lymphoma on the basis of histopathology as serum level of B2M varied with nature and histology of lymphoma involved and different histopathologies respond in diverse manner to the clinical outcomes [9]. Various studies reported marked rise in B2M in HL [18- 22], however, these studies focused on number of patients depicting B2M greater than cutoff value and relate it with disease stage but provided no data about percentage increase in B2M level. Toth *et al.*, [8] considered it not for diagnosis but staging after diagnosis. Our results are powerfully supported by these studies with an impressive addition of quantitative value of raised B2M. The findings are in accordance with the study of Vassilakopoulos *et al.*, who reported that level of beta 2 microglobulin is significantly related to the stage, b symptoms and other hematological anomalies like anemia. It is further added that high level off b2 M is an indicator of involvement of more anatomical sites [23]. Vassilakopoulos *et al.*, in 9 years follow up study claimed that B2M was an independent prognostic factor for failure free survival [24]. The level of serum B2M also increases with severity of dysplasia [25]. Nakajima *et al.*, [26] examined the prognostic importance of serum B2M in HL and focused on B2M at the time of diagnosis. The patients having low level of B2M showed progression free survival while patients having higher than cutoff values presented less survival rate. Our results are also supported by Miyashita *et al.*, [27] who determined that serum B2M strongly correlated with disease progression and patients with high B2M showed poorer progress free survival than those with low levels of B2M. The findings of current research are also in accordance with other researchers reporting association of B2M with nature and stage of NHL [28-31].

CONCLUSIONS

Our study clearly manifests that during the transformation of normal lymphocytes into malignant cells, B2M levels enhanced prominently, and its level found directly proportional to cancer load and disease stage. Moreover, NHL portrayed high B2M than HL, may be due to large number of subcategories of NHL. Furthermore, Localized, and regional malignancies have slight difference in B2M concentration but distant or stage III/IV lymphoma depicted marked rise in B2M levels. Thus, it can be concluded that B2M is a powerful diagnostic tool for lymphoma and can be related to disease burden. It may serve as prognostic biomarker for both categories of lymphoma and is directly linked with extent of disease, stage, and nature of lymphatic growth.

Authors Contribution

Conceptualization: TM, MWA
Methodology: NA, TM
Formal analysis: NA, MAI
Writing-review and editing: TM, MWA, NA, MAI
All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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