

## **Original Research Article**

# **Prediction of Disease Severity by Beta 2 Microglobulin Levels In Hodgkin Lymphoma**

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**Abstract:** Hodgkin's lymphoma (HL) also known as Hodgkin lymphoma or Hodgkin's disease is a type of lymphoma, in which cancer originates from a specific type of white blood cells, called B lymphocytes. When lymph nodes of Hodgkin's lymphoma are examined microscopically, multinucleated Reed–Sternberg cells (RS cells) are the characteristic histopathological finding. In this study we studied the role of beta 2 microglobulin (B2M) as a marker of disease severity in Hodgkin lymphoma. 30 patients, diagnosed with Hodgkin lymphoma and 30 age and sex matched healthy controls were included in this study. All patients were given 6 cycles of ABVD regimen. Sample was analysed for routine biochemistry and B2M. B2M levels were estimated by a commercial Enzyme Linked Immunosorbent Assay kit for human B2M (DRG β2-MG ELISA). Mean serum B2M was  $0.40 \pm 0.38$  µg/ml in controls. Levels were significantly raised in HL patients ( $4.20 \pm 1.88$  µg/ml,  $p < 0.01$ ). In this study 13(43.34%) patients presented with late stage (III + IV) and 17(56.66%) patients presented with early stage (I + II). It was found that patients with late stage had comparatively higher mean B2M levels ( $7.02 \pm 0.023$  µg/ml) as compared to those earlier stages ( $3.2 \pm 0.065$  µg/ml). This correlation was found to be statistically significant ( $p < 0.01$ ). Male patients suffering from HL had higher B2M ( $4.83 \pm 1.24$  µg/ml) levels as compared to females ( $3.89 \pm 1.32$  µg/ml) but the difference was statistically not significant. Patients with mediastinal large lymphnodes (bulky lymphadenopathy) had higher B2M levels ( $5.11 \pm 0.22$  µg/ml) as compared to other HL patients ( $3.65 \pm 0.16$ ,  $p < 0.05$  µg/ml). HL patients who presented with B symptoms had higher B2M levels ( $4.81 \pm 0.20$  µg/ml) as compared to those without B symptoms ( $4.11 \pm 0.65$ ,  $p > 0.05$  µg/ml). The conclusion in our study, B2M was found to be significantly associated with the severity of disease. Though, B2M has been discovered long time back but its role as a marker for the severity of disease has not been studied extensively, so still larger studies with longer follow up are required to validate its role in Hodgkin lymphoma as a disease severity marker.

**Keywords:** Hodgkin lymphoma, beta2 microglobulin, stages, lymphnodes.

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### **INTRODUCTION:**

Hodgkin's lymphoma [1] (HL) also known as Hodgkin lymphoma or Hodgkin's disease [2] is a type of lymphoma, in which cancer originates from a specific type of white blood cells, called B lymphocytes. A previous episodes of infectious mononucleosis due to infection by Epstein–Barr virus (EBV) may increase risk of HL, but the precise association of Epstein–Barr virus remains largely unknown [3, 4].

When lymphnodes of Hodgkin's lymphoma are examined microscopically, multinucleated Reed–Sternberg cells (RS cells) are the characteristic histopathologic finding. The overall five-year survival rate in the United States for 2004–2010 is 85% [5]. The disease has bimodal age presentation so it shows two peaks: the first in young adulthood (age 15–35) and the second in those over 55 years old [6]. It was named

after the English physician Thomas Hodgkin, who first described abnormalities in the lymph system in 1832 [7, 8]. Treatment of Hodgkin's disease has been improving over the past few decades. Recent trials that have made use of new types of chemotherapy have indicated higher survival rates than have previously been seen. In one recent European trial, the 5-year survival rate for those patients with a favourable prognosis was 98%, while that for patients with worse outlooks was at least 85% [9].

In 1998, an international study identified seven prognostic factors that accurately predict the success rate of conventional treatment in patients with locally extensive or advanced stage Hodgkin's lymphoma. Freedom from progression (FFP) at 5 years was directly related to the number of factors present in a patient [10].

The adverse prognostic factors identified in the international study are:

- Age >45 years, Stage IV disease, Hemoglobin < 10.5 g/dl, Lymphocyte count < 600/ $\mu$ l or < 8%, Male, Albumin < 4.0 g/dl, White blood count > 15,000/ $\mu$ l. In this study we studied the role of beta 2 microglobulin as a marker of disease severity in Hodgkin lymphoma[10].

**MATERIALS 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 Hodgkin lymphoma cases 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. All patients were given 6 cycles of ABVD regimen. Five ml of venous blood sample was collected from patients at the time of diagnosis in red capped evacuated vacutainers and also in purple capped EDTA vacutainers 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  $\beta$ 2-MG ELISA). The reference range for B2M according to this kit is less than 2 $\mu$ g/ml [12].

**Statistical 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. Any p value less than 0.05 was considered significant.

**RESULTS:**

In this study 60 subjects were included, among them 30 were Hodgkin lymphoma patients and 30 were age and sex matched controls. The patients belonged to various age groups ranging from 15-60 years. Mean age of the patient group is 26  $\pm$  7.5 years and in control group mean age was 30 $\pm$  6.25 years. Among these 30 cases 20 were males and 10 were females, which show a relatively higher prevalence in males. B symptoms were present in 26.66% of HL patients. 100% of HL patients had features related to weakness. Splenomegaly and hepatomegaly was found in 10% and 30% HL patients respectively. Only lymphadenopathy (LAP) was found in 60% HL patients (Table 1). Mean haemoglobin levels were 10.85 g/dl in HL patients.

Mean total leucocyte count (TLC) was 6000/cu.mm at presentation in HL patients. Mean platelet count was 4 lakhs/cu.mm in HL patients. Mean serum albumin was 4.2gm/dl in HL patients. Mean serum B2M was 0.40 $\pm$  0.38  $\mu$ g/ml in controls. Levels were significantly raised in HL patients (4.20 $\pm$ 1.88 $\mu$ g/ml, p<0.01). In this study 13(43.34%) patients presented with late stage (III + IV) and 17(56.66%) patients presented with early stage (I + II). It was noted that patients with late stage had comparatively higher mean B2M levels (7.02 $\pm$ 0.023 $\mu$ g/ml) as compared to those whose stage is earlier(3.2 $\pm$ 0.065 $\mu$ g/ml)(TABLE 2). This correlation was found to be statistically significant (p<0.01). Male patients suffering from HL had higher B2M (4.83 $\pm$ 1.24  $\mu$ g/ml) levels as compared to females (3.89 $\pm$ 1.32  $\mu$ g/ml) but the difference was not statically significant. Patients with mediastinal large lymphnodes (bulky lymphadenopathy) had higher B2M levels (5.11 $\pm$ 0.22  $\mu$ g/ml) as compared to other HL patients (3.65 $\pm$ 0.16  $\mu$ g/ml, p <0.05). HL patients who presented with B symptoms had higher B2M levels (4.81 $\pm$ 0.20) as compared to other patients (4.11 $\pm$ 0.65, p>0.05).

**Table 1: Patients characteristics**

CASE	30
MALE	20(66.66%)
FEMALE	10(33.33%)
B SYMPTOMS PRESENT	8(26.66%)
WEAKNESS	30(100%)
HEPATOMEGALY	9(30%)
SPLENOMEGALY	3(10%)
ONLY LYMHADENOPATHY	18(60%)
EARLY STAGE (1 AND 2)	17(56.66%)
LATE STAGE(3 AND 4)	13(43.34%)

**Table 2: Comparison of B2m in Early (Stage I + II) and Late Stages (Stage Iii + IV) In HI Patients**

N	STAGE	B2M (ug/ml)	P value <0.05
17	Stage I+II	3.2 $\pm$ 0.065	
13	Stage III+IV	7.02 $\pm$ 0.023	

**DISCUSSION:**

Beta-2-Microglobulin is a single polypeptide chain that is linked noncovalently to the major histocompatibility complex Class I cell surface antigen [11]. Its specific function remains unknown [11]. Membrane turnover is the principal source of B -2M in the blood [11]. Serum B2M is excreted almost exclusively by the kidneys. As a result, increased levels are found in patients with a decreased glomerular filtration rate [12]. Despite normal renal function in patients, elevated serum levels of B2M, have been associated with a large tumor burden and a poor prognosis in patients with several lymphoid malignancies, including multiple myeloma, B-cell non-Hodgkin lymphoma, and chronic lymphocytic leukaemia [12-15]. A number of groups have also reported that serum B2M levels correlate with Ann

Arbor stage and the presence of B symptoms in patients with Hodgkin disease and that elevated levels of this polypeptide predict a less favourable prognosis. Dimopoulos *et al.*; reported that after a median follow-up of 2.5 years, an elevated serum B2M level was an independent predictor of a lower likelihood of response to therapy and a shorter time to treatment failure in Hodgkin disease patients [16]. Gregory M. Chronowski in a study also concluded that B2M was an independent adverse prognostic factor for overall survival [17]. Yuki Nakajima conducted a retrospective analysis of 67 patients with HL diagnosed and treated at seven institutes of the Yokohama City University Hematology Group between 1998 and 2011. The patients included 40 males and 27 females with a median age of 41 years (range 16–81 years) [18].

Patients with B2M levels  $>2.5$  mg/L ( $n = 18$ ) showed inferior progression-free survival (PFS; 4-year PFS rate, 42 %) and inferior OS (4-year OS rate, 60 %) compared to patients who had B2M levels  $<2.5$  mg/L ( $n = 49$ ; 4-year PFS rate, 87 %; 4-year OS rate, 98 %;  $P < 0.001$ ). In multivariate analysis, only a serum B2M level  $>2.5$  mg/L was a significant adverse prognostic factor in regard to PFS [18]. So in our study we concluded that B2M is an important prognostic factor in HL patients as its higher levels are correlated with bulky mediastinal disease, presence of B symptoms, higher stage and male sex. These findings are correlated with previously conducted studies.

## CONCLUSION

B2M has been discovered long time back but its role as a prognostic marker has not been studied extensively, so still larger studies with longer follow up are required to validate its role in Hodgkin lymphoma.

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