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Original Research Article

Beta 2 Microglobulin (B2M) As a Marker of Disease Severity and Progression in Acute Myeloid Leukemia (AML)

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Abstract: B2M, a known prognostic factor in multiple myeloma and reflects tumor burden and turnover. Few clinical studies have shown B2M to have prognostic relevance in AML. The study was planned to evaluate the status of B2M in patients of AML before and after chemotherapy. 30 newly diagnosed cases of AML & 30 age and sex matched healthy controls were taken. AML patients were treated with combination chemotherapy of cytarabine and an anthracycline. Routine biochemistry, complete hemogram, serum B2M were performed in newly diagnosed patients before treatment and in controls. The investigations were repeated at first complete remission or after 4 weeks of chemotherapy (whichever is earlier). B2M were measured by ELISA. 60% patients achieved remission, 26.7% did not achieve remission and 13.3% expired. Baseline B2M levels were significantly raised in AML patients (B2M:1.76±0.95µg/mL vs 0.99±0.67µg/mL, p=0.004). B2M levels at diagnosis were significantly higher in patients who expired than patients who achieved remission (p=0.034). B2M levels were significantly correlated with known prognostic factors; total leucocyte count and blast count in peripheral blood (p=0.001 & 0.001).B2M levels decreased significantly after chemotherapy only in remission group (p=0.015). Serum B2M levels have prognostic value in AML patients. Studies are required in larger number of patients.
Keywords: B2M, AML, blast count in peripheral blood

INTRODUCTION

Acute myelogenous leukemia (AML) is characterized by [1] accumulation of abnormal (leukemic) blast cells, principally in the marrow, and [2] impaired production of normal blood cells. Thus, the leukemic cell infiltration in marrow is accompanied nearly invariably by anemia and thrombocytopenia. The absolute neutrophil count may be low or normal, depending on the total white cell count [1, 2].

AML results from a series of somatic mutations in either a hematopoietic multipotential cell or occasionally, a more differentiated, lineage-restricted progenitor cell [3].

Class I human leukocyte antigen (HLA-I) molecules are cell surface-associated proteins that contain two separate polypeptide chains: alpha (heavy) chain encoded by a major histocompatibility complex gene on the short arm of chromosome 6, and a 12-kDa beta-chain (β2-microglobulin, β2M); the β2M gene resides on chromosome 15. Beta-2 Microglobulin (also known as B2M/β2M), is a low molecular weight (11.8kDa) protein on the cell surfaces of all nucleated cells and shed into the blood, particularly by B-lymphocytes and some tumor cells. Its levels are increased in multiple myeloma where it is a known prognostic factor [4].

Increased levels in AML with prognostic relevance were documented by Tsimberidou *et al.;* [5], Mellillo *et al.;* [6], and Vorob'ev *et al.;* [7] Ellegaard *et al.;* also reported increased levels, however with no changes in remission or relapse [8].

AIM AND OBJECTIVE

The study was planned to evaluate the status of B2M in patients of AML before and after chemotherapy.

MATERIAL AND METHOD

The present study was conducted in the Department of Biochemistry in collaboration with Department of Medicine (Clinical Haematology unit);

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Thirty cases of acute myeloid leukemia were taken up for study. The diagnosis was made by history, clinical examination, total and differential leukocyte count, bone marrow examination and cytogenetic studies. Thirty age and sex matched controls were also taken up. Complete history and physical examination was done in controls and cases (before and after treatment).

AML (except APL) patients were treated with a combination chemotherapy of cytarabine and an anthracycline (daunorubicin). APL patients were treated with tretinoin plus concurrent anthracycline chemotherapy followed by maintenance therapy with either tretinoin or chemotherapy [1, 2].

Routine biochemistry, serum B2M were performed in newly diagnosed patients before treatment and in controls. The tests were repeated in AML patients at first complete remission or after 4 weeks of chemotherapy (whichever is earlier).

Fasting early morning venous blood sample was taken in a plain red capped evacuated blood collection tube under all aseptic precautions. Samples were processed within one hour of collection. Serum was separated by centrifugation at 3000 rpm X 10 minutes after clotting. Separated serum was stored at -20^0C (maximum 3 months) for serum TNF and seum B2M estimation.

Serum B2M levels were estimated by a commercial Enzyme Linked Immunosorbent Assay Kit for human B2M. Its reference range is less than 2 µg/mL; the latter is being the lower level of detection.

STATISTICAL ANALYSIS

IBM SPSS ver. 20 was used for various statistical analyses. Comparison of data between groups was done using‘t’ test/ Mann Whitney Test for quantitative data and Chi-square test for qualitative data. Comparison between multiple groups was done using one-way annova / Kruskalwallis test. Paired samples were compared by paired‘t’ test / Wilcoxon sign test. Correlations and regression between groups were analyzed using suitable models. Charts and graphs were prepared using IBM SPSS ver. 20 and Microsoft excels programs.

RESULTS

Both cases and controls had similar age and sex distribution

- Out of 30 patients 16 (53.3%) were males and 14 were females. Median age diagnosis was 35 years.
- Median duration of history of presenting illness was 4 weeks. Fever was present in 80%, 100% had features related to weakness; H/o bleeding was present in 26.7% patients. Splenomegaly & hepatomegaly was found in 13.3% & 13.3%. Lymphadenopathy was found in 13.3% patients. Median haemoglobin levels were 8.5 g/dL, median TLC was 10,000 /cu.mm. Median blasts in peripheral blood were 75%. Median platelet count was 75,000/cu.mm and 6 (40%) patients had platelet count less than 50,000/cu.mm.
- 60% patients achieved remission, 26.7% did not achieve remission and 13.3% expired.
- Baseline B2M levels were significantly raised as compared to controls.
- B2M levels at diagnosis were significantly higher in patients who expired than patients who achieved remission (p=0.034).
- B2M levels were significantly correlated with known prognostic factors; total leucocyte count and blast count in peripheral blood (p=0.001 & 0.001).

Table 1: comparision of beta 2 microglobulin with total leukocyte count and blast count with significance

	R^2	p value
B2M vs TLC	0.558	0.001
B2M vs Blast count	0.579	0.001

B2M levels decreased significantly after chemotherapy only in remission group (p=0.015).

Table 2: value of beta 2 microglobulin in AML patients and in controls

	AML PATIENTS	CONTROLS	p value
B2M (µg/mL)	1.76 ± 0.95	0.99 ± 0.67	0.004*(S) **Significant**

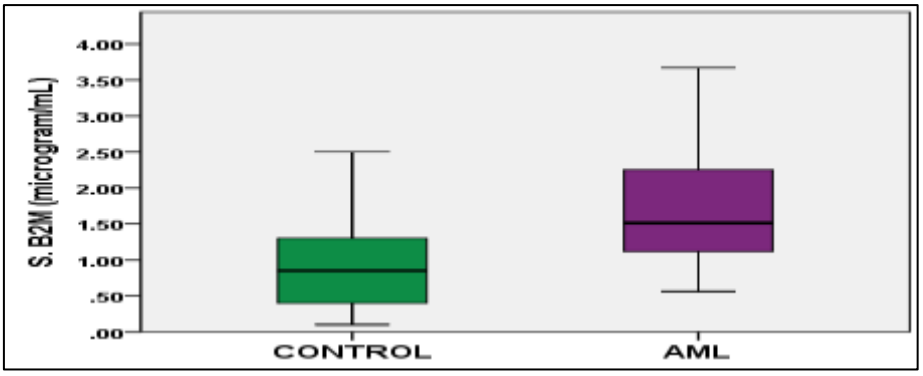

Fig 1: B2M in patients and control

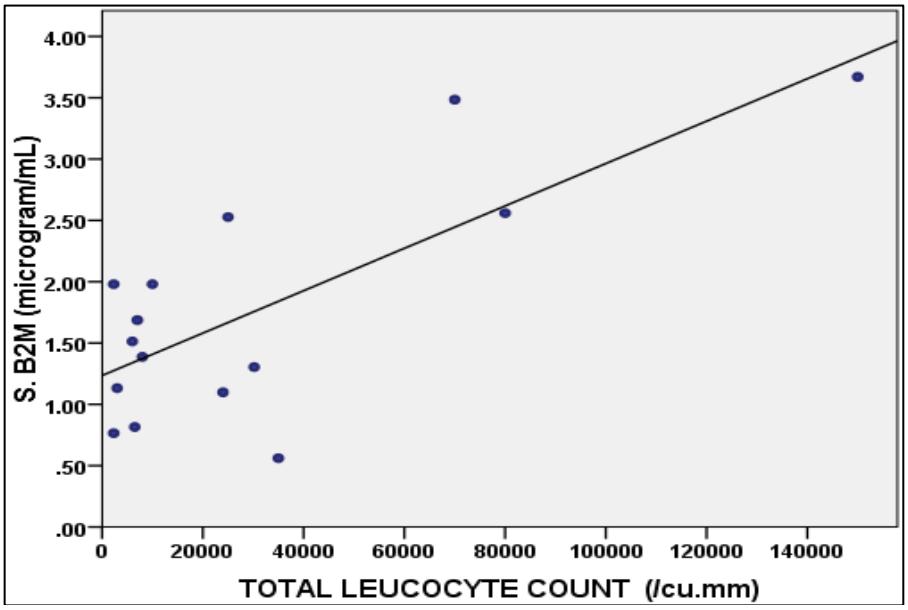

Fig 2: scatter diagram showing correlation of beta 2 microglobulin with total leukocyte count

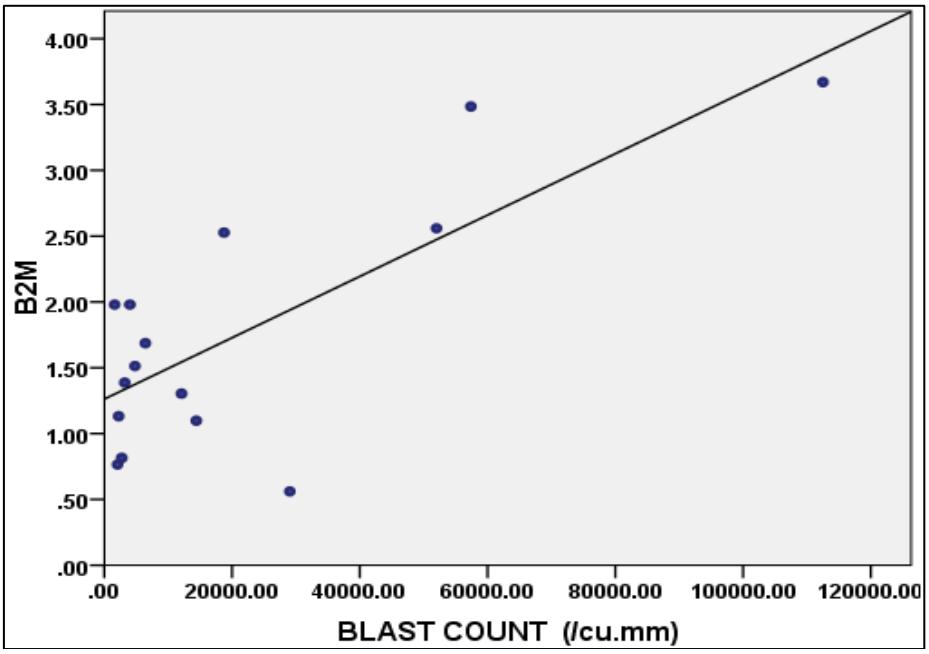

Fig 3: scatter diagram showing relation of beta2 microglobulin with blast count

Table 3: beta2 microglobulin before and after chemotherapy

REMISSION	BEFORE CHEMOTHERAPY	AFTER CHEMOTHERAPY	p value
B2M (µg/mL)	1.49 ± 0.69	0.97 ± 0.42	0.015 (HS) Highly Significant

Table 4: Comparison of b2m levels before and after chemotherapy in patients

NON REMISSION	BEFORE CHEMOTHERAPY	AFTER CHEMOTHERAPY	p value
B2M (µg/mL)	1.46 ± 0.61	1.44 ±0.79	0.465

Table 5: Comparison of beta2microglobulin between patients who had remission and those who did not

	REMISSION	NON REMISSION	MORTALITY	CONTROLS
B2M(µg/mL)	1.49 ± 0.69	1.46 ± 0.61	3.58 ± 0.13	0.99 ± 0.67

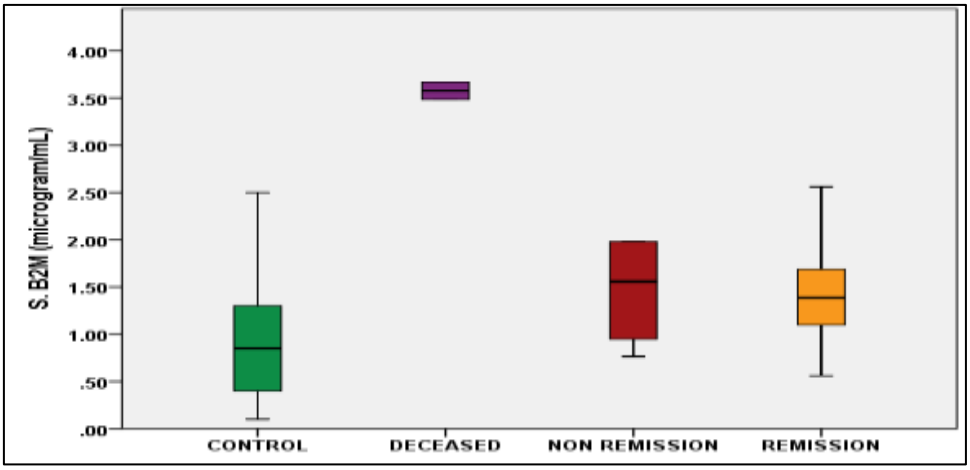

Fig 4: Levels Of Beta2 Microglobulin In Control Deceases , Non Remission And Remission

Table 6: Mann Whitney test showing significance of beta2 microglobulin in different parameters

Mann Whitney test	B2M
Remission vs Controls	0.049 (S)
Non Remission vs Controls	0.120
Deceased vs Controls	0.004 (HS)
Deceased vs Remission	0.034 (S)
Non Remission vs Remission	1.00

Tables and figures showing comparison of B2M levels in remission and non-remission groups and correlation of b2m with TLC and blast count.

DISCUSSION

In the present study the median age at diagnosis was 35 years. There were 16 (53.3%) males and 14 females (46.7%).Ghosh *et al.;* in a series of 192 adults found that a male preponderance was present, with a male to female ratio of 2.5:1 and median age at diagnosis of 27.2 years for Indian patients [9]. Most studies have found a higher incidence of AML in males [10, 11].

Median duration of history of presenting illness was 4 weeks. Weakness and related features were present in all the patients. Fever was present in 80% of patients. Hepato-spleenomegaly was present in 13.3% patients and H/o bleeding was present in 26.7%. Lymphadenopathy (LAP) was found in 13.3% AML patients.

Nearly 50% patients are known to have symptoms for around 3 months before the Leukemia is diagnosed[2]. Clinical features that signal the onset of AML include fatigue, weakness, palpitations, and dyspnea on exertion. H/o of bleeding (easy bruising, petechiae, epistaxis, gingival bleeding, conjunctival hemorrhages, and prolonged bleeding from skin injuries) reflects thrombocytopenia and is frequent early manifestations of the disease. Fever is present in many patients at the time of diagnosis. Palpable splenomegaly or hepatomegaly occurs in approximately one-third of patients. Lymphadenopathy is extremely uncommon except in the monocytic variant of AML [1].

Median haemoglobin levels were 8.5 g/dL. Median total leucocyte count (TLC) was 10,000 /cu.mm.20% patients had TLC < 5,000 /cu.mm.6.7 % had TLC > 1, 00,000 /cu.mm. Median platelet count was 75,000/cu.mm. 6 (40%) patients had platelet count less than 50,000/cu.mm. Patients had median 75% blasts in blood.

Anemia is usually present at diagnosis and can be severe. The degree varies considerably, irrespective of other hematologic findings, splenomegaly, or duration of symptoms. The median presenting leukocyte count is about 15,000/cu.mm. Between 25 to 40% of patients have counts <5000/cu.mm and 20% have counts >100,000/L [2].

B2M

In our study:

- Serum B2M was 0.99 ± 0.67 µg/mL in all controls. Levels were significantly raised in AML patients (1.76 ± 0.95 µg/mL, p =0.004).
- B2M levels were 1.49 ± 0.69 µg/mL in patients achieving remission after induction chemotherapy as compared to 1.46 ± 0.61 µg/mL in patients not achieving remission and 3.58 ± 0.13 µg/mL in patients who expired. Levels were significantly higher in patients who expired than patients who achieved remission (p = 0.034). There was no difference in levels in patients achieving and not achieving remission.
- B2M levels were significantly correlated with total leucocyte count and blast count in peripheral blood.
- B2M levels were significantly decreased (0.97 ± 0.42 µg/mL) after induction therapy as compared to levels at diagnosis (1.49 ± 0.69 µg/mL), p = 0.015.
- B2M levels were (1.44 ±0.79 µg/mL) after induction therapy as compared to levels at diagnosis (1.46 ± 0.61 µg/mL), p = 0.465 in patients not achieving remission.

These findings are compared with the findings in previous studies in table 7.

Table-7: Status of serum B2M in AML in various studies

AUTHOR	FINDINGS	
Tsimberidou *et al.;* [5]	Increased	Associated with poorer prognosis in patients > 60 yrs age
Mellillo *et al.;* [6]	Increased	Associated with TLC, less likelihood of obtaining remission
Vorob'ev *et al.;* [7]	Increased	Correlated with disease stage
Ellegaard *et al.;* [8]	Increased	No change with remission or relapse
Present study	Increased	Significantly higher in mortality group, correlated with TLC and blast count, decreased after remission

Tsimberidou *et al.;* reported that in 1280 patients with AML, B2M was associated with poorer survival in older (>60 years) but not younger patients [12]. Melillo *et al.;* described B2M in 69 cases of acute myeloid leukemias (AML) as a possible prognostic indicator. B2M values paralleled white blood cell count, serum lysozyme levels and expression of monocytic membrane markers at presentation, but no correlation was found with age, renal function or immunological myeloid antigens. Increased levels of B2M were associated with a lower likelihood of obtaining a complete remission (25 versus 58.5%, p less than 0.01) [6].

Vorob'ev *et al.;* examined the concentration of B2M in the serum of 141 patients with acute leukemia and described a statistically significant increase in serum B2M in all varieties of acute leukemia as applicable to the control values. A direct correlation was established between the concentration of serum B2M and the phase of the disease [7].

Ellegaard *et al.;* studied the serum concentration of B2M in 69 patients with acute or chronic lympho- and myeloproliferative disorders. 12 out of 25 patients with acute myeloid leukemia and all of 5 patients with acute myelomonocytic leukemia as well as 4 out of 5 patients with acute lymphatic leukemia had increased serum B2M levels. Also no significant changes in serum B2M were found in either remission or relapse of the acute leukemia [8].

The data obtained indicates that the B2M test can be a prognostic factor as it was significantly higher in mortality group as compared to patients achieving remission and it declined significantly only in patients achieving remission. Further as it was also correlated with TLC and Blast count it may reflect the total amount or turn-over of leukemic cells in the body and that repeated determinations of serum B2M in these patients might be useful as an estimate of the residual leukemic cell mass after therapy.

B2M is expressed on surface of all nucleated cells and shed into blood by B-lymphocytes and tumour cells. It is a known prognostic marker and predictor of long term survival in multiple myeloma and low levels can indicate non-progression of HIV [13, 14]. Thus while elevated levels indicated high turnover of leukemic cells and low levels after chemotherapy may indicate the completeness of remission in terms of the leukemic cell turnover better than the absolute cell counts in blood. Further studies in larger number of

patients with long term follow up are required to validate this role.

Thus serum B2M levels have prognostic value in AML patients. Elevated levels of B2M indicated high turnover of leukemic cells and low levels after chemotherapy may indicate the completeness of remission in terms of the leukemic cell turnover better than the absolute cell counts in blood. Further studies in larger number of patients with long term follow up are required to validate these findings.

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