PORPHOBILINOGEN DEAMINASE AND ADENOSINE DEAMINASE ACTIVITY AS A POSSIBLE DIAGNOSTIC AID IN LYMPHATIC LEUKEMIAS

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ABSTRACT

It has been demonstrated recently that in addition to morphologic and immunologic methods, enzyme characterisation of lymphoid cells is useful in the study and classification of lympho proliferative disorders. Heme biosynthetic pathway and purine metabolism are reported to be disturbed in such patients. this study was an attempt to estimate two enzymes Adenosine Deaminase (ADA) and Porphobilinogen Deaminase (PBGD) and correlate them with cytological and morphological parameters in lymphatic leukemias. Fasting heparinized venous blood samples were collected from 40 patients and 20 controls. Porphobilinogen Deaminase (PBGD) and Adenosine Deaminase (ADA) assays were carried out in peripheral mononuclear cells. Both enzyme activities were significantly elevated in ALL. In CLL, while PBGD activity was elevated, ADA remained in normal limits. Our study indicates that PBGD and ADA Assays will be useful adjuncts to cytomorphological studies in differentiating ALL from CLL.

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KEY WORDS: ALL; ADA; CLL; PBGD; PMNC.

Introduction

For many years the diagnosis and classification of leukemias were based only on morphological and cytochemical features assessed by light microscopy. In recent times immunological characterisation of leukemic cells and quantitative and qualitative enzyme assays have gained considerable importance in differential diagnosis of leukemias.

Porphyrin metabolism has received a great deal of attention over the last two decades. As early as 1963, Vannotti and Jeunet reported that amino -levulinic acid (ALA) could be converted to porphyrin in leukocytes isolated from patients with leukemias whereas in leukocytes from other patients ALA was converted only to porphobilinogen [1]. Porphobilinogen deaminase (PBGD) (EC 4.3.1.8) is one of the enzymes that transform four molecules of porphobilinogen to uroporphyrin in the biosynthetic pathway of heme. Increased level of PBGD can be induced in lymphocytes by mitogens and by infection with Epstein Barr (EB) virus [2]. Epstein et al observed that determination of PBGD activity in lymphocytes constitutes a sensitive test for diagnosis of malignant lymphoproliferative disorders and may contribute to the clinical management of some patients [3].

The other enzyme which is essential for differentiation of the lymphoid cells, particularly T lymphocytes is adenosine deaminase (ADA) (EC 3.5.3.3). Smyth

and Harrap [4] first reported elevated ADA values in a large series of patients with acute lymphatic leukemia (ALL).

Recent studies performed on a large number of ALL cases revealed a wide overlap between different types of ALL. while the ADA activity was reported highest in T-ALL, it was normal or subnormal in B-ALL and CLL [5,6].

The present study is an attempt to correlate PBGD with ADA activity in peripheral mononuclear cells (PMNC) of patients with lymphatic leukemia with morphologic and clinical diagnostic parameters of the disease

Material and Methods

Forty patients suffering from leukemia taking treatment at Malignant Disease Treatment centre, Command Hospital Pune and Marathe laboratory were studied. Out of these 5 cases were of ALL, 10 CLL and 15 were myeloid leukemia (ML). The diagnosis of the above cases was done by morphological and cytochemical criteria. The control group included 20 healthy subjects. There was no difference in the mean age and distribution of age and gender between two groups. Patients with myeloid leukemia as a group were studied for comparison with lymphatic leukemia. All the cases were studied at the time of diagnosis, before administration of chemotherapy.

10 ml of fasting heparinised venous blood samples were collected for determination of PBGD and ADA activities in PMNC (M-PBGD & M-ADA) and ADA activity in plasma.

The PMNC were separated from heparinised blood according to method described by Boyum [7]. The blast cells and lymphocytes separated at the plasma Ficoll interface were removed with a

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pipette, yielding preparation containing not less than 95% mononuclear cells. Cell populations were checked by preparing Giemsa film of the pipetted cells. The contaminating RBCs were lysed by hemolysing procedures and the pellet obtained after washing and centrifugation was suspended in tris buffer, 0.05 M (pH=8.2). This suspension was stored at -20° C till use.

Before estimation of enzyme activity, the pellet was defrosted and sonicated (10 pulses x 0.4 seconds).

The PMNC PGGD (M-PBGD) activity was measured by modification of method of Magnussen et al [8]. The protein content of PMNC extract was determined by method of Lowry et al [9], with pooled human serum as standard. The M-PBGD activity was expressed as picomols of porphyrin per milligram of lymphocytic protein per hour.

The M-ADA activity and plasma ADA activity were measured by colorimetric method of Giusti [10] based on determination of amount of ammonia released in free reaction mixture. The M-ADA activity was expressed in nanomols of adenosine per milligram of protein per minute (nmol/mg of protein/min) and plasma ADA in IU/L.

Materials:

Histopaque and adenosine were purchased from Sigma (St Louis, MO), uroporphyrin standard was purchased from Porphyrin Products, Loga, Utah. All other reagents were of highest purity commercially available.

Data analysis was done using Analysis of variance technique for comparing the mean values of various parameters.

Results

The M-PBGD, M-ADA and plasma ADA values in 20 controls and 40 patients suffering from different types of leukemia are shown in Table 1.

TABLE 1
PBGD and ADA activities in PMNC and plasma of controls and leukemic patients

Groups	M-PBG pmol of porphyrin/mg of protein/hr	M-ADAnmol of adenosine/mg of protein/min	Plasma ADA IU/L
Control Mean $\pm$ SD (n=20)	10.68 $\pm$ 3.64	9.10 $\pm$ 4.96	17.57 $\pm$ 9.12
ALL Mean $\pm$ SD (n=15)	46.55 $\pm$ 39.65	16.46 $\pm$ 10.90	59.45 $\pm$ 46.45
CLL Mean $\pm$ SD (n=10)	60.93 $\pm$ 20.11	7.4 $\pm$ 5.12	25.2 $\pm$ 10.21
Myelocytic leukemia Mean $\pm$ SD (n=15)	52.11 $\pm$ 38.02	30.72 $\pm$ 22.41	41.1 $\pm$ 21.19

The mean value for M-PBGD activity in control group was 10.68 $\pm$ 3.64 pmol/mg of protein/hour. There is significant elevation of M-PBGD activity in all three groups of leukemia ($p < 0.001$) (Table 1).

The difference of M-PBGD activity amongst the three groups of leukemia is not significant ($p > 0.05$).

M-ADA activity is significantly elevated in ALL ($p < 0.001$) while it is decreased in CLL though not significantly ($p >$

0.05)(Table-1).

The rise in M-ADA activity in patients with myeloid leukemia is highly significant ($p < 0.001$) as compared to controls and patients with ALL. The plasma ADA values also correlate with above findings. The levels are elevated significantly in myeloid leukemia ($p < 0.05$) and in ALL ($p < 0.001$) as compared to normal controls. CLL patients did not show statistically significant changes in plasma ADA activity as compared to normal controls ($p > 0.05$), though the mean value was slightly more than that of controls (Table 1).

In contrast to the M-ADA activity in ALL and ML, the plasma ADA activity is significantly elevated ($p < 0.005$) in ALL patients as compared to those with ML.

Discussion

Present study revealed significant elevation of M-PBGD activity both in ALL & CLL. This finding is in consonance with reports appeared in literature [3,11]. We also found elevated M-PBGD activity in myeloid leukemia. The elevation of M-PBGD activity in leukemias may be attributed to two factors: (i) increased lymphocytic and blast cell counts in peripheral blood of these patients and (ii) overall elevation of the enzymes involved in heme synthetic pathway. Sharabasy et al also showed abnormal changes in porphyrin biosynthesis in patients with ALL and these changes may be attributed to abnormalities of the enzymes of heme biosynthesis [11].

Our observation of elevated M-ADA activity in ML and ALL, more so in the former is in consonance with the findings of other workers [12,13]. The increased ADA activity in leukemic cells may represent a detoxification mechanism preventing accumulation of potentially toxic levels of adenosine and nucleotides arising from increased metabolic activity in the malignant cells [14]. While the plasma ADA activity revealed significant rise in ALL and ML, M-ADA and plasma ADA were either normal or showed insignificant change in CLL. This may be due to abnormal 'B' cell proliferation in CLL [15]. Low ADA activity seems generally to be associated with chronic lymphoid disease of B-cell origin [16].

Considering the above facts it can be assumed that though no differentiation can be made between ALL and AML using PBGD and ADA estimation which show overlapping values, these enzymes may be used as surrogate markers in CLL, as they show reciprocal changes in this condition.

The study was limited only to the basal levels of enzymes in leukemias. The prognostic utility of these enzymes is yet under consideration. Further research to study the above aspects to necessary by follow up of the patients along with the details of their treatment.

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