Leukemia - Original Article

Platelet enzyme abnormalities in leukemias

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Abstract

AIM OF THE STUDY: The aim of this study was to evaluate platelet enzyme activity in cases of leukemia. **MATERIALS AND METHODS:** Platelet enzymes glucose-6-phosphate dehydrogenase (G6PD), pyruvate kinase (PK) and hexokinase (HK) were studied in 47 patients of acute and chronic leukemia patients, 16 patients with acute myeloid leukemia (AML) (13 relapse, three in remission), 12 patients with acute lymphocytic leukemia (ALL) (five in relapse, seven in remission), 19 patients with chronic myeloid leukemia (CML). **RESULTS:** The platelet G6PD activity was significantly low in cases of AML, ALL and also in CML. G6PD activity was normalized during AML remission. G6PD activity, although persistently low during ALL remission, increased significantly to near-normal during remission ($P < 0.05$) as compared with relapse ($P < 0.01$). Platelet PK activity was high during AML relapse ($P < 0.05$), which was normalized during remission. Platelet HK however was found to be decreased during all remission ($P < 0.05$). There was a significant positive correlation between G6PD and PK in cases of AML ($P < 0.001$) but not in ALL and CML. G6PD activity did not correlate with HK activity in any of the leukemic groups. A significant positive correlation was however seen between PK and HK activity in cases of ALL remission ($P < 0.01$) and CML ($P < 0.05$). **CONCLUSIONS:** Both red cell and platelet enzymes were studied in 36 leukemic patients and there was no statistically significant correlation between red cell and platelet enzymes. Platelet enzyme defect in leukemias suggests the inherent abnormality in megakaryopoiesis and would explain the functional platelet defects in leukemias.

Key words: Acute myeloid leukemia, chronic myeloid leukemia, glucose-6-phosphate dehydrogenase, hexokinase, leukemia, pyruvate kinase, platelet enzyme abnormalities

Introduction

Because many of the platelets functions are enzyme dependent, ATP is necessary for ADP-induced aggregation.[1-3] For ATP production human platelets are dependent to a large extent up on the Embden – Meyerhof pathway. As bleeding in leukemia does not always coincide with the number of platelets, many authors have suggested that defective platelet function may play an important role in the production of bleeding in leukemia.[4-6]

The altered metabolism and abnormal platelet functions in leukemias may reflect the inherent defect originating in megakaryocytes.[7-10] This view was substantiated by the study of Wurzel *et al.*,[11] who demonstrated low glucose-6-phosphate dehydrogenase (G6PD) activity in platelets.

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Materials and Methods

Three enzymes, namely G6PD, pyruvate kinase (PK) and hexokinase (HK), were studied in a total of 47 leukemic patients, wherein we included 16 patients of acute myeloid leukemia (AML) (13 relapse, three remission), 12 patients of acute lymphocytic leukemia (ALL) (five relapse, seven remission) and 19 patients of chronic myeloid leukemia (CML), who were selected from the haematology clinic and were diagnosed from their clinical symptoms, peripheral blood counts, bone marrow and bone biopsy.

Twenty five to 30 ml of venous blood was collected in a tube containing EDTA (1-2 mg/ml). Blood was

centrifuged at 4°C at 250g for 20 min. Then, the platelet-rich plasma was taken in another test tube and centrifuged at 1000g for 10 min. The supernatant was discarded and platelets were washed three-times with normal saline. Viability of the platelets was checked with the trypan blue dye exclusion test. Platelet counting was in Neubauer counting chamber.[7,12-15]

Washed platelets were suspended in a small quantity of normal saline. 0.2 ml of these cells were added to 1.8 ml of hemolyzing solution containing b merecaptoethanol, EDTA and NADP and then lysed by repeated three-times freezing and thawing at -80°C. After lysis of the platelets, this was centrifuged at 1000g for 10 min and the supernatant was used for estimating the enzymes. The values were taken as m mole of substrate utilized per min per 10^{11} platelets.[16]

The normal values established in our laboratory are given in Table 1.

Of the 13 patients of relapsed AML two patients were already receiving chemotherapy at the time of study, while others were studied prior to receiving chemotherapy. Thirteen patients were in the age range of 13 – 71 years (median, 30 years) and included nine male and four female. Their hemoglobin ranged between 2.3 and 9.3 gm% (median, 4.8 gm%), total leucocyte count ranged between 1,300 and 156,000/cumm (median, 8,000/cumm) and platelet count between 40,000 and 2,26,000/cumm (median, 80,000/cumm). Myeloblast in the peripheral blood varied between 23 and 95%.

Three patients studied during AML remission belonged to the age group of 17, 14 and 25 years and all three were males. All were receiving cyclical maintenance therapy. Their Hb was 7.2, 11.3 and 13.6 gm%, WBC 3,600, 4,600 and 6,000/cumm and platelet count 230,000, 158,000 and 180,000/cumm, respectively. There were no blasts in their peripheral blood.

Of the five ALL relapsed patients, two patients were already receiving chemotherapy. Three patients were studied before they received chemotherapy. Five patients were in the age group of 15–37 years (median, 17 years), and included four males and one female. Their Hb ranged between 5.9 and 12.6 gm % (median, 10.8 gm%), WBC between 2,100 and 19,000/cumm (median, 10,200/cumm) and platelets between 85,000 and 226,000/cumm (median, 136,000/cumm). Blasts in the peripheral blood ranged between 6 and 80%.

Seven patients of ALL remission belonged to the age group of 18 – 37 years (median, 32 years) and included four males and three females. All were receiving maintenance chemotherapy. The Hb ranged between 10.3 and 13 gm% (median, 10.8 gm%), WBC between 3,400 and 11,600/cumm (median, 8,600/cumm) and platelets between 160,000 and 2,60,000/cumm (median, 190,000/cumm). There were no blasts in the peripheral blood.

Of 19 CML cases, seven patients were receiving chemotherapy and 12 were studied before they received chemotherapy. Nineteen patients were in the age range of 19 – 60 years, included 12 males and seven females. The Hb ranged between 6.9 and 15.2 gm% (median, 12.0 gm%), WBC between 8,000 and 2,40,000/cumm (median, 13,600/cumm) and platelets between 1,30,000 and 5,10,000/cumm (median, 2,00,000/cumm).

Results

Platelets G6PD, PK and HK enzymes were studied in a total of 47 patients, which included 16 AML (13 relapse, three remission), 12 ALL (five relapse, seven remission) and 19 CML [Table 1]. One hundred and

Table 1: Platelet enzymes: Mean ± SD (μ moles of substrate utilized per min per 10^{11} platelets)

Diagnostic group	Status of remission	G6PD	PK	HK
Normal		6.2 ± 3.0 (101)	7.40 ± 5.37 (164)	0.86 ± 0.47 (100)
		Range 2.2 – 10.7	Range 1.7 – 15.3	Range 0.3 – 1.6
AML (16)	Relapse (13)	3.35 ± 5.3	7.85± 16.20	0.67 ± 0.60
	Remission (3)	2.79 ± 5.21	9.03± 17.89*	0.64 ± 0.61
		5.80 ± 6.12	2.73± 0.81	0.80 ± 0.63
ALL (12)	Relapse (5)	3.03 ± 4.44‡	6.18 ± 9.72	0.60 ± 0.76
	Remission (7)	2.68 ± 1.59†	4.14 ± 4.47	0.63 ± 0.46
		3.27 ± 5.85*	7.63 ± 12.4	0.58 ± 0.96*
CML (19)		3.78 ± 3.87†	5.52 ± 4.98	0.76 ± 0.70

*$P < 0.05$ Test of significance was performed applying the Wilcoxon's test (1945)[17]; †$P < 0.01$ Non-parametric test. Figures in parenthesis indicate corresponding number of cases studied; ‡$P < 0.001$. No statistical comparison was done between normal and blastic CML groups as the number of cases studied was only 2

and one normal healthy controls were studied for platelet G6PD enzyme. The mean ± SD was 6.2 ± 3.0 and the normal range was 2.2 – 10.7 m moles of substrate utilized per min per 10^{11} platelets. [Table 1]

The platelet G6PD was lower than normal in 10 patients with AML (nine relapse, one remission), six patients with ALL (one relapse, five remission) and 10 patients with CML. G6PD values were higher than normal in two patients with AML (one relapse, one remission) one patient with ALL remission and two patients with CML. Platelet G6PD deficiency in AML, ALL and CML was statistically significant. AML relapse and remission, when analyzed separately, indicated that platelet G6PD deficiency was highly significant during relapse ($P < 0.001$), while results during remission were not significantly different from controls. The number of patients studied during remission however was small. In ALL, platelet G6PD deficiency was significant both during relapse ($P < 0.01$) and during remission ($P < 0.05$) and also in CML ($P < 0.01$).

One hundred and sixty-four normal healthy controls were studied for platelet PK enzyme. The mean ± SD was 7.40 ± 5.37 and the normal range was 1.7 – 15.3 m moles of substrate utilized per min per 10^{11} platelets [Table 1]. The platelet PK was lower than normal in four patients with AML relapse, in four patients with ALL (one relapse, three remission), in five patients with CML and in one patient with blastic CML. Platelet PK was higher than normal in two patients with AML relapse, one patient with ALL remission and one patient with CML. These results were not significantly different from normals, except in AML relapse, where the platelet PK was significantly high ($P < 0.05$).

One hundred normal healthy controls were studied for platelet HK enzyme. The mean ± SD was 0.86 ± 0.47 and the normal range was 0.3 - 1.6 μ moles of substrate utilized per min per 10^{11} platelets [Table 1]. The platelet HK was lower than normal in five patients with AML relapse, in six patients with ALL (one relapse, five remission) and in five patients with CML, and it was high in one patient with AML relapse, in one patient with ALL remission and in three patients with CML. HK reduction of statistical significance was seen only in ALL remission ($P < 0.05$).

There was a significant positive correlation between G6PD and PK in cases of AML ($P < 0.001$), while in ALL and CML, no statistically significant correlation was found between G6PD and PK. G6PD was not found to be significantly correlated with HK in any of the groups. A significant positive correlation was found between PK and HK in ALL ($P < 0.01$) and in CML ($P < 0.05$). In ALL however, when the correlation was studied separately in relapse and remission significant correlation was found only during remission ($P < 0.01$) [Table 2].

Discussion

Platelet abnormalities are quite common in leukemias. Bleeding in leukemic patients can be due to associated thrombocytopenia. However, no definite correlation was found between bleeding and decline in platelet counts.[4-6] Functional platelet defects have also been demonstrated by several authors in various types of leukemia.

Like any other viable cell normal functioning of the platelet is dependent on intact and effective functioning of the metabolic pathway of platelets. Platelet aggregation is also an energy-dependant procedure.

For ATP production, platelets rely upon, to a large extent, the Embden-Meyerhof pathway.[1-3] Chaudhury *et al.* in 1973 also showed that Krebs cycle and hexose monophosphate (HMP) shunt are quite active in the platelets.[18]

Although a lot of work has been done in the field of

Table 2: Correlation between various enzymes (r' correlation coefficient)

Diagnostic group	Status of remission	G6PD and PK	Platelet enzymes PK and HK	HK and G6PD
AML (16)				
	Relapse (13)	0.79‡	0.20	0.0.3
	Remission (3)	0.95‡	0.20	0.16
		-	-	-
ALL (12)		0.04	0.82†	0.23
	Relapse (5)	0.86	0.02	0.50
	Remission (7)	0.02	0.94†	0.30
CML (19)	-	0.18	0.46*	0.16

*$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$. Platelet enzymes in AML remission were not analyzed statistically because only three patients were included

altered metabolism and abnormal platelet function in leukemia, platelet enzyme abnormalities are not studied in a proper manner at all. The only study performed by Wurzed *et al.* was to demonstrate low G6PD activity in both platelet and erythrocyte of leukemia cells.

In our study, a total of 47 leukemic patients were evaluated for three platelet enzymes G6PD, PK and HK. Platelet G6PD was significantly deficient in acute leukemia both in AML and ALL ($P < 0.001$) and G6PD was also significantly low in CML patients ($P < 0.01$). Interestingly, G6PD deficiency only occurred in AML relapse. During AML remission, this was normal.

In case of ALL, during relapse, G6PD deficiency was notably low ($P < 0.01$) as against in ALL remission, where the deficiency was of a borderline level ($P < 0.05$). Platelet PK values were in a slightly higher range in ALL relapse.

ALL remission demonstrated an intermediate low level of HK deficiency ($P < 0.05$). Platelet G6PD and PK showed a significant correlation in AML cases ($P < 0.001$). There was no such correlation in CML and ALL ($P < 0.01$) and CML ($P < 0.05$), but not in AML. Platelet HK and G6PD had no correlation with each other.

Significant platelet G6PD deficiency during acute leukemia relapse and either no deficiency or significantly lesser level in ALL remission proves indirectly that platelet G6PD deficiency may be from an abnormal megakaryocytic clone at the time of relapse.

Bleeding in leukemias may partly be due to associated thrombocytopenia. There is however no definite correlation between bleeding and platelet counts.[4-6] Platelet functional defects have been demonstrated by several authors in different types of leukemias, including abnormalities of bleeding time, tourniquet test, clot retraction and platelet thromoplastic activity.[14,19-22]

Metabolic energy is essential for most platelet functions, including aggregation. The process of platelet aggregation and maintenance of discoid shape depends on metabolic energy that can be supplied through glucose metabolism. It has been shown that glycolysis and oxidative phosphorylation are important to platelet energy metabolism. The Krebs cycle and HMP shunt are active in intact platelets,[18] and either pathway may compensate for decreased activity of the other.[13] Because many of the platelet functions are energy-dependent, ATP is necessary for ADP-induced aggregation. There is excellent correlation between platelet ATP levels and extent of aggregation. For ATP production, human platelets are dependent to a large extent upon the Embden – Meyerhof pathway,[1-3,7] and it was seen that platelets from the patients of acute leukemia were larger and younger than normal platelets.

The altered metabolism and abnormal platelet functions in leukemia may reflect an inherent defect originating in the megakaryocytes.[7-10] If such is the case, platelet enzymes derived from abnormal megakarocytic clones in leukemia should show enzyme alterations similar to those occurring in leucocytes. Platelet enzymes, however, are not studied properly in the literature. Wurzed *et al.* demonstrated only low G6PD activity in both platelets and erythrocytes of leukemic patients.[11]

We have studied a total of 47 leukemic patients for platelet enzymes (G6PD, PK and HK). Platelet G6PD was significantly deficient in acute leukemias, both AML and ALL ($P < 0.001$). G6PD deficiency was also significant in the platelets of CML patients ($P < 0.01$). Interestingly, G6PD deficiency occurred only during AML relapse, while during AML remission, the G6PD value was normal. In case of ALL, even though G6PD deficiency persisted during remission, the deficiency was only of a border line level ($P < 0.05$), as against ALL relapse, where the deficiency was significantly low ($P < 0.01$). ALL remission demonstrated platelet border line low level of HK deficiency ($P < 0.05$). Platelet PK values were slightly higher in case of AML relapse. Apart from this, platelet PK and HK have been largely normal in all the leukemic subtypes. Platelet G6PD deficiency in leukemia could not be accounted for due to age factors.

Platelet G6PD and PK showed a significant correlation in AML cases ($P < 0.001$). There was no such correlation in CML and ALL. Platelet PK and HK were correlated in ALL ($P < 0.01$) and CML ($P < 0.05$) but not in AML. Platelet HK and G6PD had no correlation in any of the leukemic subtypes.

Significant platelet G6PD deficiency during acute leukemia relapse and either no deficiency or relatively lesser significant deficiency in ALL remission once again strongly indicate that platelet G6PD deficiency in leukemia may be from an abnormal megakaryocytic clone at the time of relapse, while during remission, with reduction of leukemic clone, enzyme values show a tendency toward normalization. The fact that the enzyme alterations have primarily occurred at the time of relapse would further substantiate the abnormalities of more resistant leukemic clone, giving rise to abnormal circulating platelet and enzyme recovery during the remission phase.

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