Erythrocyte Enzyme Abnormalities in Leukemias

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Abstract
Red cell enzymes were assayed in a total of 67 patients, including 24 patients of acute myeloid leukemia (AML) including 19 relapse, 5 in remission phase, 16 patients with ALL including 10 relapse, 6 in remission phase, 22 patients of chronic myeloid leukemia (CML) in chronic phase and 5 with blastic CML (Table 1). These patients were in the age range of 13-71 years (Median 30 years) these included both male and female.

Collection of Blood and Isolation of Red Cells:
10 ml of venous blood was collected from each patient after taking informed consent from them in EDTA (1-2mg/ml) and kept at 4°C for 10-15 minutes. The supernatant and buffy coat layers were removed after centrifugation. Packed red cells washed in normal saline and centrifuged at 4°C at 1000g for 10 minutes and repeated three times in order to free the packed red cells from plasma, leucocytes and platelets.

Haematological details of these patients at the time of enzymes study:
AML relapse (19 patients):
These patients were in the age range of 13-71 years, (Median – 32 years) and included, 14 males and 5 females. Their hemoglobin (Hb) ranged between 2.8 – 9.2 gm% (Median – 5.8 gm%) White blood cell counts (WBC) between 1,300-156,000/cumm. (Median – 12,400/cumm) and platelet counts between 50,000 – 236,000/cumm (Median – 70,000). Myeloblasts in the peripheral blood studied in a total of 67 patients, including 24 patients of acute myeloid leukemia (AML) including 19 relapse, 5 in remission phase, 16 patients with ALL including 10 relapse, 6 in remission phase, 22 patients of chronic myeloid leukemia (CML) in chronic phase and 5 with blastic CML (Table 1). These patients were in the age range of 13-71 years (Median 30 years) these included both male and female.

Introduction
Various acquired erythrocyte enzyme abnormalities have been observed in patients with acute leukemia,2,3 preleukemia3 and after exposure to chemotherapy.4 Although these facts have been recognized for several decades, the correlation among the erythrocyte enzyme level with various phases of acute leukemia has not been studied in detail. The present study was done to correlate the erythrocyte enzyme abnormality namely glucose-6-phosphatase dehydrogenase, pyruvate kinase and hexokinase enzymes in red cells of acute leukemia both during relapse and remission and in CML and CML blast crises patients along with healthy controls.

Material and Methods
Patient’s diagnosis was made based on clinical presentation, peripheral blood and bone marrow smear and cytochemistry as per the FAB criteria. This study was approved by the AIIMS Ethics Committee. Three enzymes namely glucose-6-phosphate dehydrogenase (G6PD), pyruvate kinase (PK) and hexokinase (HK) were assayed in a total of 67 patient including 24 patients with AML (19 relapse, 5 remission), 16 patients with ALL (10 relapse, 6 remission), 22 patients with CML and 5 patients with blastic CML.

Diagnosis of leukemia was based on clinical presentation, peripheral blood smear and bone marrow examination (as per FAB classification). PK activity was significantly high in case of CML and blastic CML (p<0.01). Red cell HK was high in all leukemia subtypes. There was no alteration in red cell G6PD. Notably there was no PK deficiency in AML or G6PD deficiency in ALL. Activities of G6PD and PK could be correlated in cases of CML, AML, (p<0.05) and ALL (p<0.01) i.e. when there was increased activity of G6PD, PK activity also tended to be higher. HK activity showed a positive correlation with PK and G6PD activity in cases of CML (p<0.05), however in acute leukemia there was no such correlation. Alteration of enzyme activities among red cells in leukemia occurred only during relapse. At the time of remission there has been no significant alteration in any of the enzyme activities. It would therefore, appear that enzyme alterations seen in leukemia patients is due to abnormal pluripotent stem cell that has given to a leukemia cell. The fact that enzyme alterations have primarily occurred at the time of relapse would further substantiate that abnormalities of red cell enzymes may be the result of a derivation some circulating red cells from the abnormal pluripotent stem cell. With the recovery of normal stem cells function during remission, enzyme abnormalities tend to become normal.
ranged between 8-95%.

With the exception of 4 patients, who received 2-4 units of blood transfusion on 1 day, 6 days, 1 month and 2 months prior to the study respectively, the remaining patients were studied before they received any blood transfusion. Two patients had already been initiated on induction chemotherapy, while the remaining were studied before chemotherapy was started on them. Induction chemotherapy consisted of a combination of Cytosar continuous infusion along with Daunomycin given for 3 days.

AML remission (5 patients):

Five patients who achieved remission following this therapy were restudied. They ranged between 13 – 65 years (Median – 17 years) of age and all 5 were males. During this time all the patients were undergoing cyclical maintenance chemotherapy. Their Hb ranged between 7.0 – 12.0 gm% (Median – 11.4 gm%) WBC ranged between 2,100 – 9,600/cumm. (Median – 4,300/cumm) and platelets between 150,00 – 242,000/cumm. (Median – 204,000/cumm). Peripheral smear demonstrated no blasts.

ALL relapse (10 patients):

Ten patients ranged between 14 – 52 years of age (Median – 18.5 years) and included 7 males and 3 females. Their Hb ranged between 1.2 – 11.5 gm% (Median – 5.4 gm%), WBC between 1,000 – 41,000/cumm (Median – 9,300/cumm) and platelets between 60,000 – 138,000/cumm. (Median – 114,000/cumm). Peripheral blood blasts varied between 9 – 90%. None of the patients had received any blood transfusions prior to the study. 2 patients were already on induction chemotherapy at the time of study, while others were studied, before they were started on chemotherapy.

Induction therapy consisted of 3 drug combinations including adriamycin, vincristine and prednisolone for a period of 4-8 weeks.

ALL remission (6 patients):

Six patients who achieved remission were receiving maintenance chemotherapy continuously, they were in the age range of 14-37 years (Median – 19.5 years) and included 3 males and 3 females. Their Hb ranged between 8.8 – 14 gm% (Median – 10.6 gm%), WBC between 6,100 – 11,600/cumm (Median – 8,900/cumm) and platelets between 160,000 – 338,000/cumm. (Median – 254,000/cumm). Peripheral blood smear had no blasts.

CML (22 patients):

These patients were in the age range of 19 – 60 years (Median – 35 years) and included 14 males and 8 females. 10 patients at the time of study were already receiving chemotherapy (busulphan – 9, hydroxyurea – 1). 12 patients were studied before they were put on chemotherapy. None of the patients had received blood transfusions prior to the study. Their Hb ranged between 6.0 – 16.0 gm% (Median – 10.8%), WBC between 1,000 – 240,000/cumm (Median – 166,000/cumm) and platelets between 30,000 – 520,000/cumm (Median – 218,000/cumm).

CML in blast crisis (5 patients):

Five patients were in the age range of 23 – 61 years and all 5 were males. All these patients had more than 20% blasts in their peripheral blood. Their Hb ranged between 7.3 – 16.0 gm% (Median – 10.5 gm%), WBC between 4,000 – 50,000/cumm (Median – 5,600/cumm) and platelets 122,000 – 270,000/cumm (Median – 180,000/cumm). All the patients were receiving chemotherapy at the time of study and none had received blood transfusion.

Enzyme Assays:

Enzymes (G6PD, PK and HK) were estimated as described by Beutler (1975).6 0.2 ml of washed packed red cells was added to 1.8 ml of hemolyzing solution. After adding the hemolyzing solution, the tubes were covered with the paraffin and kept at 4° C for 10 minutes, than centrifuged at 1000-2000 g x 10 minutes. Supernatant was utilized for the assay of HK, PK, and G6PD, as well as for Hb estimation.

RESULT

The normal value for each enzyme was established in our laboratory prior to the enzyme assays done in leukemic subjects. The normal range for each enzyme was taken as percentiles P10-P90. Statistical analysis for testing the significance of the difference in the values of various parameters between different groups were carried out by employing Wilcoxon’s non-parametric test (Wilcoxon’s 1945).6

Red Cell G6PD: (Table 1)

The mean ± SD for red cells G6PD among 38 healthy controls was 7.07 ± 1.75 (the normal range was 5.2-9.44) IU/gm. of Hb.

Although 7 patients with AML (6 relapse, 1 remission), 6 patients with ALL (5 relapse, 1 remission), 5 patients with CML and one with blastic CML had lower G6PD values as compared to normal and 5 patients with AML (3 relapse, 2 remission), 5 patient with ALL (3 relapse, 2 remission), 9 with CML and one with blastic CML had increased G6PD activity, none of these enzyme alterations were significant statistically.

Red Cell PK: (Table 1)

Normal red cell PK value as studied in 42 healthy control, was 7.64 ± 2.35, with the normal range of 4.7 – 11.1 I.U./gm of Hb.

Red cell PK values were lower than normal in 5 patients with AML (relapse), 5 patients with ALL (4 relapse, 1 remission), and 3 patients with CML and PK values were higher than normal in 3 patients with AML (relapse) and 4 patients with ALL (3 relapse, 1 remission). These enzyme alterations were not
statistically significant. Higher red cell PK activity in 11 patients with CML and 3 patients with blastic CML as shown in Table 1, were statistically significant (p<0.01).

Red Cell HK: (Table 1)

Normal values of red cell HK in 27 healthy controls were 0.34 ± 0.15 and normal range was 0.15-0.61 IU/gm of Hb.

HK values were lower than normal in two patients with AML relapse and one patient with blastic CML, HK values were higher than normal in 4 patients with AML relapse, 3 patients with ALL relapse, in 5 patients with CML and 2 patients with blastic CML, on an average HK values were significantly higher than normal in AML, ALL (p<0.05) and CML (p<0.01) cases.

Correlation of Various Red Cell Enzymes: (Table 2)

There has been a significant positive correlation between G6PD and PK in cases of AML (p<0.05), ALL (p<0.01) and also in CML (p<0.05), while in ALL and AML, during remission, no statistically significant correlation was found between G6PD and PK. HK did not show any significant correlation with PK and G6PD, in AML and ALL cases but in CML however, HK also showed a significant positive correlation with PK and G6PD (p<0.05). If there was a tendency for rise in PK value, there was a tendency for HK also to be higher.

### DISCUSSION

We have studied red cell enzymes in a total of 67 leukemic patients. Similar to the finding of Tanpaichitr and Eys (1975)\(^7\), the present study revealed significantly high red cell PK activity in cases of CML (p<0.01). Red cell HK values were significantly higher than normal in cases of acute leukemia, both AML and ALL as well as in CML. Boivin et al (1975)\(^8\) and Miwa (1979)\(^9\), found higher red cell HK activity in both acute leukemia and CML, which is in agreement with our findings; these authors however in addition noted increased activity in G6PD in cases of acute leukemia, along with HK. In the present study red cell G6PD has shown no significant alterations in any of the leukemia subtypes. Since enzyme deficiencies described by other authors have occurred in approximately 1/3 of cases only, and because the enzyme deficiency described was usually mild, it is possible that if we had studied larger number of patients, we could have found significant enzyme deficiency in our cases also.

Observation of high PK activity in CML and high HK activity in all leukemic subtypes is unlikely to be due to the effect of age of the red cell, because red cell enzymes have shown no defects during remission.

In the present study, PK activity was significantly high in CML and CML blast crisis (p<0.01). HK activity was significantly high in ALL relapse (p<0.05) and CML (p<0.01).
Several reports described red cell enzyme abnormalities in the form of increased or decreased activity in acute leukemia as well as chronic leukemia (Vives et al, 1979\textsuperscript{10}; Boivin et al 1975\textsuperscript{4}, Hopkins and Tudhope, 1973\textsuperscript{11}; Boivin et al 1974\textsuperscript{4} and Valentine et al, 1973\textsuperscript{12}). Majority of the studies have been carried out in cases of acute leukemia. Although different enzymes have been found to be abnormal, red cell PK deficiency appears to be the most consistent defect at the time of relapse (Miwa, 1981\textsuperscript{15} and Khan 1981\textsuperscript{16}). Reduced levels of red cell PK were found in 7 of 23 patients of acute myeloid leukemia (AML) by Boivin et al (1970\textsuperscript{13}), 14 of 36 patients by Boivin et al (1975\textsuperscript{8}), 6 of 17 patients by Kouchoppilai et al (1983\textsuperscript{17}) and in 47 patients (34 AML, 13 ALL) by Tanpaichitr and Eys (1975\textsuperscript{7}). Decreased activity of PFK, 2,3 DPGM, AK (Boivin et al, 1975\textsuperscript{8}; Miwa 1979\textsuperscript{8}) and Pyrimidine nucleotidase (Lieberrmann and Gordon-Smith 1980\textsuperscript{18}) have also occurred in acute leukemia. Increased activities described in acute leukemia include those of HK, ALD, endase (ENOL), 6PGD, G6PD (Boivin et al, 1975\textsuperscript{8-9}), and Hopkins and Tudhope (1973\textsuperscript{11}), glutathione peroxidase (GSH-Rx) in four patients with AML and one with erythroleukemia. One patient with AML achieved a partial remission with normalization of GSH-Px activity.

In chronic leukemia red cell enzyme alterations in the form of increased activity of HK 6PGD and GSH-Px and decreased activity of acetyl cholinesterase (ACHE), GR and Glucose Phosphate isomerage (GPI) have occurred in a case of chronic myelo-monocytic leukemia (GR and Glucose Phosphate isomerage (GPI) have also been described in case of chronic myelomonocytic leukemia (CMML). (Vives et al, 1979\textsuperscript{10}). Deficiencies of PFK, G3PD, GR, GSH-Px, ACHE and PK have been described in case of CML. Activity of ACHE was low in cases of CLL also (Boivin et al 1975\textsuperscript{8} and 1975\textsuperscript{9}), Increased activities described in CML include those of PK (Tanpaichitr and Eys 1975\textsuperscript{7}) and G6PD (CaO 1970\textsuperscript{9}).

Enzyme deficiency seen in leukemia was usually mild to moderate and are similar to the activity observed in heterozygous subjects, who are clinically and hematologically normal (Boivin et al, 1973).\textsuperscript{8}

Altered enzyme activity in leukemia may either be due to intracellular stress with temporary inhibition of red cell glycolysis, (Mohler and Crockett 1964\textsuperscript{19}), post-translational molecular modification (Boivin et al, 1975\textsuperscript{8}) or cyto and Karyokinetic abnormalities with loss of functional genetic material governing enzyme synthesis, leading to enzyme abnormality (Valentine et al, 1973\textsuperscript{3}).

The red cell enzyme changes may reflect production of an abnormal clone of red blood cell and that these may arise from altered stem cells, has been suggested by some authors (Jacobson et al, 1978\textsuperscript{20}; Wiggans et al,1978\textsuperscript{21}).

The present work demonstrates that the enzyme alterations occur primarily during relapse and tend to get, either normalized or show only minor defects during remission. This observation strongly supports the contention that the defective enzyme activities during relapse may be the result of derivation of abnormal cell lines form an abnormal pluripotential stem cell. The presence of such a pluripotent cell has been demonstrated by the presence of a Ph chromosome not only in myeloid precursors but also in erythroid precursors in CML.\textsuperscript{7,8} Occurrence of chromosomal abnormalities not only in myeloid precursors but also in erythroid precursors in acute leukemia suggests the presence of a similar abnormal pluripotent stem cell in the latter condition also. That enzyme alterations demonstrated by us in the present study are from this abnormal stem cell is further potentiated by the demonstration that in majority of the cases, enzyme alterations occurring during relapse, have normalized during remission, following an eradication of the leukemic cell done with the help of chemotherapy.

Acknowledgement

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REFERENCES

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API Announcement

Award Sessions
1. Dr. DP Basu Young Award in Cardiology.
2. E Merck Award.
3. Dr. JN Berry Memorial Award and
4. Dr. MJ Shah Memorial Award in Tropical Medicine

There will be four award sessions at the 2007 Annual Conference of API at Goa. The rules and regulations of these awards are as under.

1. Papers that are accepted for presentation in the Award Session at the Annual Conference will be divided subject - wise into four groups.

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<tr>
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<td>Cardiology</td>
<td>DP Basu Young Award</td>
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<td>Group II</td>
<td>Chest Diseases</td>
<td>E Merck Award</td>
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<td>Group III</td>
<td>Other Specialties</td>
<td>JN Berry Memorial Award</td>
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<tr>
<td>Group IV</td>
<td>Tropical Medicine</td>
<td>MJ Shah Memorial Award</td>
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The Award of Dr. JN Berry Memorial Award and E Merck Award are given in alternate years in Group II and III papers. At the 2007 Annual Conference at Goa, Dr. JN Berry Memorial Award will be for "Other Specialties" and E Merck Award for "Chest Diseases". Dr. DP Basu Young Award will be for "Cardiology" and Dr. MJ Shah Memorial Award for "Tropical Medicine".

2. The competitor must be the first author of the paper submitted for presentation at the API sessions of the Annual Conference. A testimonial must be submitted from the head of the institution that the major work has been done by the competitor. Papers which are previously presented or published will not be considered. The competitor should also give a written pledge stating that the work has not been presented or published before. He should be a member of API.

3. Dr. JN Berry Memorial and DP Basu Young Awards are worth Rs. 1000/- each E Merck Award Rs. 2000/- and Dr. MJ Shah Memorial Award is worth Rs. 2500/-

4. The upper age limit of the competitor is 40 years.

5. The decision will be taken by a panel of judges appointed by the Governing Body of API.

6. The candidate must apply for the award and full manuscript of the paper will have to be submitted. The paper will be presented in separate award session.

7. Eight copies of full manuscript will have to be submitted Dr. RK Singal, President - Elect and Chairman Scientific Committee, APICON 2007, "C/o Initials, E-39, Flatted Factory Complex, Jhandewalan, New Delhi 110 055; e-mail : rksapicon2007@yahoo.co.in/drSingal2005@yahoo.co.in; mobile : 9811472555 by 31st July, 2006. One copy of the paper should be sent to Dr. Sandhya Kamath, Hon. General Secretary of API Unit No. 6 and 7, Turf Estate, Opp. Shakti Mill Compound, Off. Dr. E Moses Road, Near Mahalaxmi Station West, Mumbai 400 011. Tel. 022-5666 3224; Fax : 2492 0263.

8. The decision of the panel of judges will be final and binding to all concerned.

Prestigious Awards of API
2. Distinguished Member (2007)