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Original Article

Biochemistry Section

Erythrocytic Pyruvate Kinase and Malondialdehyde Levels in Acute Leukaemia Patients

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ABSTRACT

Introduction: Many acquired enzymopathies have been reported in patients with acute leukaemia. Oxidative stress is also found to be increased in acute leukaemia, which can be indicated by the increased malondialdehyde (MDA) levels. Malondialdehyde itself may lead to the alteration of the enzyme, pyruvate kinase. Therefore, this study was planned to estimate the levels of erythrocytic pyruvate kinase and malondialdehyde in patients of acute leukaemia before and after chemotherapy.

Methods: This study was conducted on 20 patients of acute myeloid leukaemia, 20 patients of acute lymphoblastic leukaemia and 20 healthy controls. The levels of pyruvate kinase and malondialdehyde were estimated in the haemolysate in the controls and in the patients at the time of diagnosis. These levels were also estimated in the patients after six weeks of chemotherapy or remission, whichever was earlier. The results were compared statistically.

Results: The levels of erythrocytic pyruvate kinase were found to be decreased significantly in the acute leukaemia patients as compared to controls and to be increased after chemotherapy, while the levels of erythrocytic malondialdehyde were found to be increased significantly in these patients as compared to the controls and to be decreased after chemotherapy. The levels were not statistically significantly different in acute myeloid and acute lymphoblastic leukaemia.

Conclusion: The erythrocytic pyruvate kinase and the malondialdehyde levels may help in the diagnosis, for asessing the severity and for the monitoring of the acute leukaemia patients.

Key Words: Erythrocyte, Pyruvate kinase, Malondialdehyde, Acute leukaemia, Chemotherapy

INTRODUCTION

Leukaemias are caused by the clonal neoplastic proliferation of immature cells of the haematopoietic system, which are characterized by aberrant or arrested differentiation. These are divided into acute or chronic, depending upon the clinical course of the disease and into myeloid and lymphoid, depending upon the cell of origin [1].

Abnormalities have been reported in the activity of a number of enzymes in haematological malignancies, which include glutathione reductase [2], glucose-6-phosphate dehydrogenase [3], phosphofructokinase [4], leucocytic alkaline phosphatase [5], adenosine deaminase [6] and 5' nucleotidase [7]. Pyruvate kinase (PK) deficiency has also been reported in patients of acute leukaemia by some authors [8,9].

Pyruvate kinase (PK) catalyzes the third irreversible step in glycolysis i.e. conversion of phosphoenolpyruvate to pyruvate, with the synthesis of one molecule of adenosine triphosphate (ATP). It requires K^+ and Mg^{2+}/ Mn^{2+} for its activity [8]. PK has four different isoenzymic forms which are L, R, M_1 and M_2. The L-type PK is present in the liver cells, the R-type is present in the mature red blood cells and M_1 and M_2 are the muscular forms. Immature erythrocytes have both M_2 PK and R-PK [10]. R-PK has a typical four domain subunit structure. Several mechanisms including genetic and post-translational ones have been considered to be responsible for the enzyme defects in the erythrocytes of various blood disorders [11].

Malondialdehyde (MDA) is the by product of free radical mediated reactions which lead to the formation of lipid peroxides, alcohol and aldehydes. Normally, MDA is quickly oxidized to acetate or malonate and then to carbondioxide by the Kreb's cycle. If it is accumulated in excess, MDA can combine with different serum proteins and cell membrane components to form altered determinants [12]. It can interact with deoxyribonucleic acid (DNA) and inhibit the biosynthesis of the DNA, ribonucleic acid (RNA) and proteins. The chemical structure of MDA closely resembles that of carcinogenic compounds like glycidaldehyde and beta propiolactone and it thus may itself, have carcinogenic properties [13].

Erythrocytic lipids are more susceptible to auto-oxidation under coditions of oxidative stress, more so, in the acute leukaemia patients. Erythrocytic MDA (eMDA) has been found to be increased in leukaemia [14]. As MDA has the propensity to attack the sulfhydryl group, it may be involved in the alteration of the erythrocytic PK (ePK) levels in acute leukaemia patients [15]. Therefore, the present study was planned to estimate the levels of ePK and eMDA in patients of acute leukaemia before and after chemotherapy.

MATERIAL AND METHODS

This study was conducted on 40 patients diagnosed with acute leukaemia, who were admitted in wards or were attending the Clinical Haematology Department of our institute, after obtaining informed consent and approval from the institutional board of studies. The diagnosis was made by doing a clinical examination and a complete haemogram, bone marrow examination and cytochemistry. All the patients were divided into two groups: group I (20 patients of acute myeloid leukaemia, AML) and group II (20 patients of acute lymphoblastic leukaemia, ALL). The patients

were monitored both clinically and haematologically. Heparinised blood samples were collected at the time of diagnosis i.e. before starting chemotherapy and after 6 weeks of chemotherapy or remission, whichever was earlier. The patients were considered to be in remission if the tumour cell mass was decreased below 10^9-10^{10} cells, if there were <5% blasts in the bone marrow, or there was absence of leukaemic cells in the blood, or there was restoration of the normal peripheral blood count, or there was absence of the physical findings which were attributable to the extramedullary involvement of leukaemia and or if there was the absence of Auer rods in acute myeloid leukaemia [16]. Twenty age and sex matched healthy subjects were taken as the controls (group III).

The heparinised blood was centrifuged for 10 minutes at 3000 rpm. After removing the supernatent plasma and the buffy coat, the red blood cells (RBCs) were washed thrice with normal saline. The washed RBCs were lysed by adding distilled water and by keeping the cell pellet in a refrigerator for 15 minutes [17]. After estimating the haemoglobin (Hb) concentration of this haemolysate [17], the levels of ePK [18] and eMDA [19] were estimated in both the controls and the patients by using standard colorimetric methods and a semi-autoanalyser (Techno 168). The results were expressed as per gram of Hb and they were compared statistically.

RESULTS

The mean age of presentation was 37.6 years (range 21-88 years) in group I, 39.40 years (range 21-82 years) in group II and 38.5 years (range 20-85 years) in group III. The male to female ratio was 2:3 in all the three groups. The presenting complaints of the patients included generalised weakness, swelling, bleeding tendencies, pallor and fever. All the patients had anaemia (mean Hb 5.99 g/dL) and hepatosplenomegaly. Lymphadenoapthy was observed in approximately 30% of the patients of both the groups.

The levels of ePK and eMDA, before and after chemotherapy, in the patients of AML and ALL and the controls, is shown in [Table/Fig-1]. After chemotherapy, most of the patients i.e 17 out of 20 in AML and 18 out of 20 in ALL were found to be in complete remission. The levels of ePK and eMDA were compared between the AML and ALL patients who achieved remission and who were not in remission n [Table/Fig-2 and 3] respectively.

DISCUSSION

In the present study, the ePK levels were found to be decreased significantly in the patients of acute leukaemia (both AML and ALL) as compared to the healthy controls and they were observed to increase after chemotherapy ($p<0.001$). These results were in accordance with those of earlier studies [8,9]. The mechanism which has been put forward has been explained at two levels. It was considered that the defect was true molecular and that the decreased concentration of the ePK-related antigen was irreversible. In the other mechanism, the defect in the ePK was considered to be primarily functional and the enzyme activity was hoped to be be restored with treatment. Various posttranslational modifications may be responsible for this kind of defect [11]. The intra-cellular stress of the leukaemic blasts on the RBCs may also lead to the temporary inhibition of the erythrocytic glycolytic enzymes [20]. But in another study, no correlation could be established between the number of blast cells and the ePK levels [21]. Another author has suggested a selective abnormality in the erythroid precursor stem cells [2]. The ePK levels were found to be higher in ALL as compared to those in the AML patients, but the difference was not statistically significant. So, these levels could not be used for the differentiation between the AML and the ALL patients.

The eMDA levels were found to be increased in the patients of AML and ALL as compared to the controls and a statistically significant

	Controls	AML patients		ALL patients	
Number	20	20		20	
		Before Chemotherapy	After Chemotherapy	Before Chemotherapy	After Chemotherapy
ePK (U/gHb)	15.17±4.01	10.11±3.03*	12.67±2.48**	11.25±2.85*	12.84±3.04**
eMDA (nmol/ gHb)	0.56±0.27	2.26±1.57*	1.29±0.67**	2.38±0.86*	1.31±0.65**

[Table/Fig-1]: Comparison of ePK and eMDA levels in patients of AML and ALL before and after chemotherapy and controls
*p value <0.001 as compred to controls
**p value <0.001 as compared to levels before chemotherapy

	In remission		Not in remission	
Number	17		3	
	Before Chemotherapy	After Chemotherapy	Before Chemotherapy	After Chemotherapy
ePK (U/gHb)	10.39±2.78	13.23±2.04*	9.56±4.64	10.54±2.83
eMDA (nmol/ gHb)	2.40±1.67	1.33±0.71*	1.94±0.22	1.68±0.31

[Table/Fig-2]: Comparison of ePK and eMDA levels in patients of AML in remission and not in remission before and after chemotherapy
*p value <0.001 as compared to levels before chemotherapy.

	In remission		Not in remission	
Number	18		2	
	Before Chemotherapy	After Chemotherapy	Before Chemotherapy	After Chemotherapy
ePK (U/gHb)	11.31±2.86	12.86±3.84*	11.18±2.64	12.28±3.13
eMDA (nmol/ gHb)	2.41±1.07	1.35±0.82*	2.34±0.88	1.78±0.68

[Table/Fig-3]: Comparison of ePK and eMDA levels in patients of ALL in remission and not in remission before and after chemotherapy
*p value <0.001 as compared to levels before chemotherapy.

decrease was observed after chemotherapy. The difference in the eMDA levels between the AML and the ALL patients was not statistically significant. This increase in the eMDA levels in the acute leukaemia patients may be due to the increased oxidative stress in leukaemia [22]. There is an increased cellular turnover in leukaemia which leads to the increased breakdown of purine to uric acid. This reaction produces hydrogen peroxide as a byproduct. Increased oxidative stress may lead to cytotoxicity, mutagenecity and alteration in the gene expression, which may culminate in the initiation and propagation of carcinogenesis [22].

In has been reported that certain lipid peroxidation products like MDA can attack the sulfhydryl groups and the amino group in proteins [15]. The sulfhydryl group of PK is prone to this action by MDA [15]. Thus, increased eMDA may also be a causative factor for the decrease of the ePK activity in acute leukaemia patients.

No significant difference was observed in the ePK and the eMDA levels in patients who attained remission and in patients who were not in remission. The difference in these levels after chemotherapy was statistically significant in patients in remission, but it was not significant in patients who were not in remission. But the number of patients who did not achieve remission was too small in both the AML and ALL groups and so it is difficult to comment on the findings. A larger study group with a greater number of such patients is required to confirm this observation.

Therefore, it was concluded that the estimation of the ePK and the eMDA levels in patients of acute leukaemia may be helpful in assessing the disease activity and in predicting the response to chemotherapy. It could serve as a prognostic indicator in these patients. The results need to be substantiated by further research which comprises of larger study groups.

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