Original Article

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Red Cell Enzymopathies in Patients with Hemolytic Anemia in Southern Iran: Case Series

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Abstract

Background: Hereditary red cell enzyme disorders are a group of Non-immune/Spherocytic Hemolytic Anemia, although these disorders are rare and they have not public health problems, the detection of these defects could help to physician in treatment and differential diagnosis. This study evaluated 5 enzymopathies in patients with Hereditary Non –immune/Spherocytic Hemolytic Anemia (HNSHA) during one year.

Materials and Methods: This cross-sectional study, evaluated 5 erythrocyte enzymes in 22 patients (mean age of 10 ± 9.3) with Hereditary Non-immune/ Spherocytic Hemolytic Anemia in Southern Iran from Jan 2014- Feb 2015. Evaluated erythrocyte enzymes consisted of pyruvate kinase (PK), G6PD, Catalase, Glutathion Proxidase(GP) and Glutathion Reductase(GR), all of these enzymes checked by quantitative assay except G6PD that evaluated by qualitative activity assay. The clinical and para clinical data were gathered from patient's documents.

Results: Results showed that 2 patients were PK deficient (9.1 %), 4 patients were G6PD deficient (18.2%), 1patient was GP deficient (4.5%), 1 patient was Catalase deficient (4.5%) and there is no patient with GR deficiency.

Conclusion: This study showed that enzymopathies should be into consideration in patients with non-immune hemolytic anemia, if other common causes of hemolysis such as hemoglobinopathies and membranopathies have been excluded.

Key Words: Hereditary enzymopathy, Hemolysis, Hemolytic anemia, Enzyme deficiency

Introduction

Hereditary red cell enzymopathies are a group of Non-Spherocytic Hemolytic Anemia (HNSHA) arising from mutation in red cell metabolic enzymes. Many of these enzymopathies are inherited as autosomal recessive and some of these disorders are X-linked, and hemolysis occurs only in homozygous or compound heterozygous situations [1, 2]. The degree of hemolysis is variable, ranging from mild to fatal anemia at birth and dependent on the severity of enzyme deficiency, in addition to anemia, other clinical finding are jaundice, reticulocytosis, and splenomegaly [3], red blood cells (RBC) morphology in enzyme disorders is commonly normocytic normochromic but some morphological changes may be seen such as anisocytosis, polychromasia and basophilic stippling without specific spherocytic and elliptocyte changes, so these groups of anemia called Hereditary Non- Spherocytic Hemolytic Anemia (HNSHA) .Some enzyme deficiencies are associated with non- hematological manifestations such as neurological dysfunction, mental retardation, and myopathy. Diagnosis of RBC enzymopathies is based on specific enzyme assays and molecular testing [4, 5]. Patients with mild anemia usually do not require any treatment but in severe cases may be needed to supportive therapy (blood transfusion) and in some cases, splenectomy may be considered [6]. G6PD is an essential enzyme in erythrocytes that protect their proteins from oxidative

damage and G6PD deficiency is the most common enzymopathy with more than 400 million person affected worldwide [7, 8]. The most prevalent enzyme deficiency of anaerobic glycolysis is Pyruvate Kinase (PK) deficiency, this enzyme convert the phosphoenol pyruvate to lactate to generating ATP in the cells, so lack of this enzyme may cause to hemolysis due to ATP insufficiency [9]. There are a number of other rare enzyme deficiencies causing HNSHA such as glucose-6-phosphate isomerase (GPI), phosphofructo kinase (PFK), aldolase, and enzymes involved in antioxidant defense system such as glutathione reductase, glutathione peroxidase and catalase [10-17]. This study was design to evaluate RBC enzymopathies in patients with non-immune hemolytic anemia of unknown origin during one year in southern Iran.

Materials and Methods

This cross sectional study, was assessed 5 specific RBC enzymes in all patients (n=22) with Non- Immune hemolytic anemia of unknown origin referred to tertiary hospital in Shiraz, Southern Iran from Jan 2014- Feb 2015. Because this type of the disease is rare we evaluated all patients with immune hemolytic anemia of unknown origin that other common causes of hemolysis such as hemoglobinopathies and membranopathies have been excluded during a specific time period (one year). The study was approved by medical ethics committee of Shiraz University of Medical Sciences and consent form was taken from patients or their legal parents, none of the patients had any history of blood transfusion during this study. Through this study with consideration of age and sex, Complete Blood Count (CBC), Peripheral blood smear, Reticulocyte count , total, direct and indirect bilirubin, Combs test, Hemoglobin Electrophoresis, Osmotic Fragility Test, LDH, AST,ALT were assessed in study group. Exclusion criteria were patients with immune hemolytic anemia, hemoglobinopathies, and membranopathies. Blood sample for enzymatic assays were collected into heparinized tubes and then centrifuged at 3000 rpm for 15 minutes and then erythrocyte washed three times with isotonic saline solution. After this step, sample preparation was done according to the kit manual.

Enzyme assays

PK activity was measured by lactate dehydrogenase coupled spectrophotometric assay (all substrates provided by Sigma USA) and G6PD activity assay also was done by Kimiya Pajohan G6PD activity assay kit, all results were expressed in U/g hemoglobin (Hb). GPx activity was measured by continuous monitoring of the regeneration of reduced glutathione (GSH) from oxidized glutathione (G-S-SG) upon the action of GR and NADPH according to the spectrophotometeric method of Fecondo and Augusteyn at 340 nm at 37 □ C. GR activity was assayed using the method described by Carlberg and Mannervik with some modifications, following this procedure: cuvette contained 270 µl incubation buffer (60 µM), 40µl BSA (Sigma USA) (10 mM), 100 µl GSSG(Fluka Swiss) (33% M), 100 µl NADPH (Sigma USA) (2 mM) as substrate and 150 µl sample in a final volume of 660 µl, and then decrease in the absorbance was monitored spectrophotometrically at 340 nm. Specific enzyme activity was calculated in terms mU/mg protein. In addition, Catalase activity was assayed spectrophotometerically by monitoring the decomposition of H2O2 (Sigma USA), using the procedure of Aebi. Descriptive data were demonstrated in an appropriate table and summarized as mean, standard deviation, and percentages.

Results

All clinical and para clinical data are shown in table-1. Osmotic fragility test and Combs test were negative for all patients and also Hb electrophoresis showed normal pattern in these patients.

Among 22 patients with HNSHA (10 Female, 12 Male) with mean age of 10 ± 9.3 , who were referred to hospital, 2 (9.1%) patients were PK deficient, 4(18.2%) patients were G6PD deficient, 1(4.5%) patient was GP deficient, 1(4.5%) patient was Catalase deficient and there is no patient with GR deficiency.

Table-1: Clinical and para clinical data of patients with hereditary non-immune hemolytic anemia

patient	age	Sex	Splenomegaly	LDH(IU/L)	AST(IU/L)	ALT(IU/L)	T.Bili(mg/dl)	D.Bili(mg/dl)	Specific Enzymopathy
1	8	F	Yes	642	30	35	2	0.3	Non
2	11	M	Yes	1486	38	35	4	0.3	G6PD deficiency
3	1	F	Yes	1262	155	72	2.5	0.31	Non
4	4	M	Yes	485	45	25	2.5	0.3	Non
5	9	M	Yes	1078	58	35	3.8	0.4	G6PD deficiency
6	5	F	Yes	766	57	33	3.9	0.4	Non
7	9	F	Yes	2567	65	30	4.5	0.5	Non
8	25	M	Yes	775	89	35	8	0.8	GP deficiency
9	18	M	Yes	539	70	29	3.9	0.3	Catalase deficiency
10	5	M	Yes	421	65	25	4	0.5	Non
11	18	M	Yes	817	70	30	6	0.3	G6PD deficiency
12	1	M	Yes	2205	55	30	4	0.3	G6PD deficiency
13	10	F	Yes	2348	65	28	3.5	0.4	Non
14	11	M	Yes	792	59	33	4.5	0.4	Non
15	6	M	Yes	470	70	30	5	0.3	Non
16	8	F	Yes	643	66	28	7.5	0.5	PK deficiency
17	14	F	Yes	336	85	40	3.5	0.5	Non
18	6	M	Yes	660	50	30	3	0.4	Non
19	3	M	Yes	796	59	28	4.2	0.4	PK deficiency
20	2	F	Yes	241	130	60	2	0.28	Non
21	42	F	Yes	510	37	35	5.5	0.4	Non
22	4	F	Yes	260	35	21	2.2	0.2	Non

Discussion

G6PD deficiency is one of the most prevalent enzymopathies in the world and the frequency of this enzymopathy in Iranian population is about 11.5 percent (18) and G6PD is an essential enzyme in erythrocytes that protect their proteins from oxidative damage. Previous investigations have reported that about 80% of HNSHA are due to PK and G6PD deficiencies.

The most prevalent enzymopathy of anaerobic glycolysis in red cells is PK deficiency, this enzyme convert the phosphoenol pyruvate to lactate to generating ATP in the cells. Hemolysis degree in PK deficiency is variable, ranging from mild compensated hemolysis to severe transfusion-dependent hemolytic anemia. Some studies showed an increasing frequency for PK deficiency from Europe to Asian population and from

central area of Iran to the Arabian Peninsula [18]. Glutathione reductase (GR) is an important enzyme for protecting red cell proteins against oxidative agents by regeneration of reduced glutathione (GSH) from oxidized glutathione (GSSG) and hereditary GR deficiency is very rare disorder characterized by susceptibility to oxidative damage [19]. Affected individuals being generally asymptomatic but hemolysis may occur with exposure to oxidative drugs or fava beans. Glutathione reductase requires flavonoids for enzymatic activity then acquired GR deficiency may also occur due to in sufficient flavin intake, but this acquired deficiency is associated with no clinical presentations. Glutathione Proxidase deficiency and other enzymopathies involved in RBC antioxidant defense system such as Catalase have been reported in some studies but they don't cause hemolysis [20]. This study showed these enzymopathies in patients with non-immune hemolytic anemia, should be into consideration if other common causes of hemolysis such as hemoglubinopathies and membranopathies have been excluded. This study investigated 5 erythrocyte metabolic enzymes in patients with HNSHA who were referred to tertiary hospital in Shiraz, Southern Iran. According to gathered data in this study, estimated frequency of PK deficiency among our 22 patients was 9.1 % (2 patients), and for G6PD deficiency was 18.2% (4 patients), for GP deficiency was 4.5% (1 patient), for Catalase deficiency also was 4.5% (1 patient) and there is no patient with GR deficiency. However, the exact frequency of these enzymopathies needs more comprehensive investigation.

Conclusion

In conclusion, present study showed that as like as worldwide distribution the most of these ezymopathies related to G6PD deficiency and then related to PK deficiency. Other enzymopathies evaluated in this study have same frequency in patients. Indeed, to detection of exact causes of hemolytic anemia in other patients and to determine the certain frequency of these enzymopathies, more comprehensive investigation with large sample size and complete assay such as molecular testing is needed.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

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