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RESEARCH ARTICLE

Hematological findings in favism among children admitted to Karbalateaching hospitalof children (in Iraq).

Mohammed Sadiq AL-Mosawy[1] and Mohammed Hussien Hamadi AL- Dawwd[2]

1. **MBChBMSc Hematopathology**
2. **FIBMS of Pediatric.**

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Corresponding Author

MohammedSadiq AL-MosawyMBChB.

Abstract

Back ground:-Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency is a hereditary condition in which red blood cells break down when the body is exposed to certain drugs, stress of infection or oxidant effect of certain substances like that which find in fava bean .

Aim of study:-To identify common laboratory findings in children complain from hemolytic crisis due to fava bean ingestion admitted to hospital.

Methods:-This cross-sectional study was conducted in Karbala teaching hospital of children in holy Karbala city in Iraq during the period from February 2014 through July 2014. We toke 100 child complained from acute hemolytic anaemia from them we selected forty patients their age range from 8 month to 12 years

Result:-Cases were 40, 31 were males and nine were females, there age range from 8 months to 10 years. Most of them were from urban areas and mostly were recorded with in April and March, Most of them were O + blood group and the lowest frequencies was AB + blood group, Allcomplain from reduced in RBCs mass and reduced hemoglobin level also we found in most of cases there is significant leukocytosis with predominant neutrophil. Platelets count was within normal range in both male and female while reticulocytes count were significantly increased. In blood film there were many blister, horn cells and nucleated RBCs. G6PD enzyme assaywas deficient in 36 out of 40.

Conclusion:-Favism affect male more than female especially pediatric age and more common during March and April. All cases complain from significant changes in complete blood picture and reticulocytosis.Morphological changes in RBCs as blister, horn cells and Heinz body cells. Enzyme qualitative evaluation was deficient in 90% of the cases

Introduction:-

Glucose 6-phosphate dehydrogenase (G6PD) deficiency, an X-linked disorder, is the most common enzymatic disorder of red blood cells in humans, affecting 200 to 400 million people worldwide [(1,2,3)] .

The gene for G6PD is located on the X chromosome at Xq28.[(4)]Complete inactivity of the enzyme in nucleated cells would not be compatible with life.[(5)] The clinical consequences of G6PD deficiency are virtually confined to the red blood cell, with occasional evidence of leucocyte malfunction in some variants.[(6)]

The likelihood of developing hemolysis and the severity of disease are determined by the magnitude of the enzyme deficiency, which in turn is determined by biochemical characteristics of the G6PD variant. The World Health Organization has classified the different G6PD variants according to the magnitude of the enzyme deficiency and the severity of hemolysis [(7)] . Classes IV and V are of no clinical significance.

- Class I variants have severe enzyme deficiency (less than 10 percent of normal) and have chronic hemolytic anemia
- Class II variants also have severe enzyme deficiency, but there is usually only intermittent hemolysis
- Class III variants have moderate enzyme deficiency (10 to 60 percent of normal) with intermittent hemolysis usually associated with infection or drugs
- Class IV variants have no enzyme deficiency or hemolysis
- Class V variants have increased enzyme activity

Clinical features:-
There are four main syndromes associated with G6PD deficiency. In all four, haemolysis is aggravated or promoted by exposure to oxidative stress through infection or ingestion of oxidative foods or drugs, but the clinical presentations differ. Age always modifies the clinical effects, not always as might be expected.

The four syndromes are neonatal jaundice, favism ,chronic non –spherocytichaemolyticanaemia and drug – induced haemolyticanaemia . [8]
Epidemiology
G6PD deficiency is widely disseminated throughout Africa, the Mediterranean basin, the Middle East, Southeast Asia and indigenous populations of the Indian subcontinent.[4] Iraq is situated within a region of a high frequency of G6PD deficiency genotype with a carrier frequency in the population of 6.3 % .[9]

Expression in males and females:-

Males, who have only one copy of the X chromosome, are either normal or homozygous for the variant G6PD gene. Thus, G6PD deficiency is expressed in males carrying a variant gene on their X chromosome that produces sufficient enzyme deficiency to lead to symptoms. All of the red cells in affected males are vulnerable to hemolysis. [7]

Females, who have two copies of the X chromosome, are either normal, heterozygous, or homozygous for the variant gene .[7]

Heterozygous females are usually clinically normal. However, their mean red blood cell enzyme activity may be normal, moderately reduced, or grossly deficient depending upon the degree of X chromosome inactivation (lyonization) and the degree to which the abnormal G6PD variant is expressed .[10,11] A female with 50 percent normal G6PD activity, due to inactivation of one X chromosome in each cell via lyonization, has 50 percent normal red cells and 50 percent G6PD-deficient red cells. [12]

Homozygous females are as severely affected clinically as hemizygous males. All of their red cells are vulnerable to hemolysis. [12]

Laboratory diagnosis include[13] : *Diagnosis of hemolytic anemia* as CBC , blood film and reticulocytes count
Screening: Qualitative assessment of G6PD enzymatic activity
Confirmatory test: Quantitative measurement of G6PD enzymatic activity
Molecular test: Detection of G6PD gene mutation

Materials and Methods:-

This cross-sectional study was conducted in Karbala teaching hospital of children in holy Karbala city during the period from February 2014 through July 2014.

Selection of cases:-
Study group:-
we took40child complained from acute hemolytic anaemia (31 males and 9 females) according to the criteria below and their age range from 8 month to 12 years.
Criteria for selection of patients in this study were:

- All cases were diagnosed clinically and laboratory as favism in Karbala teaching hospital words .
- The inclusion of all favism cases with available results and sufficient blood sample for hematological investigation.

Relevant information were noted on prepared sheet including age, gender, history, clinical findings (including body weight) and laboratory findings (CBP , peripheral blood film , blood group and G6PD enzyme assay) .

Equipment and materials:-

A- The following equipments and materials were used throughout the research:

Water bath, light microscopy, micropipettes with tips, timer, gloves , slides, cover slides, staining jars, calibrated test tube, buffer solution, distilled water and lieshman stain

Evaluation of all the study cases including:-

a. Complete blood count using automated blood counter (RUBY), blood film and reticulocytes count done for all cases in our study

b. Blood group , G6PD enzyme assay (by use reducing nitrate method) were done to all cases .

c. Patient data like address , age and weight were registered from patient family .

The company of the apparatuses that were used?

Statistical analysis

Statistical analysis was performed with **SPSS** 20 (statistical package for social sciences) and **Excel 2010** programs. Data analysis was done usingindependentt- test & chi –square test for tables with frequencies, percentages, range mean &standard deviation.

Result:-

Cases which included in the study were 40 , 31 of them were males and nine of them were females , there age range from 8 months to 10 years (120 months) with mean nearly 50 months for males and 60 months for females .Our cases were from urban areas in about 25 out of 40 while about 15 out of 40 were from rural areasalso cases of the study were recorded mostly with in April and March as show in table 1

Table 1 show gender distribution , address and months of admission among study cases

		Frequency	Percent	Valid Percent
Gender	Male	31	75.6	77.5
	Female	9	22.0	22.5
	Total	40	97.6	100.0
Address	Urban	25	61.0	62.5
	Rural	15	36.6	37.5
	Total	40	97.0	`100
Month of admission	March	18	43.9	45
	April	22	53.7	55
	Total	40	97.6	100

Most of them were O + blood group and the lowest frequencies was AB + blood group as in table 2

Table 2 show ABO system distribution in study cases

		Frequency	Percent	Valid Percent	Cumulative Percent
Blood group	A+ve	6	14.6	15.0	15.0
	A-ve	2	4.9	5.0	20.0
	B+ve	9	22.0	22.5	42.5
	O+ve	19	46.3	47.5	90.0
	O-ve	3	7.3	7.5	97.5
	AB+ve	1	2.4	2.5	100.0
	Total	40	97.6	100.0	

To study hemolytic effects of G6PD deficiency we found in all cases in our study complain from reduced in RBCs mass with mean value 2.12 $*10^6$ /cmm and complain from variable degree of reduced Hb level (anaemia) also we found in most of cases there is significant leukocytosis with mean value 16.3 cells / cmm mostly there are absolute neutrophilia .

Platelets count was within normal range in both male and female while reticulocytes count which mostly reflects bone marrow response to hemolysis were significantly increased in both males and females with mean more in male than in females as reticulocytes count was 31 % with mean nearly 17 % in male and 12 in femaleas show in table 3 .

Table 3 show CBC changes in study group

	Gender	N	Mean	Std. Deviation	Std. Error Mean	P value
RBC	Male	31	2.0287	.67746	.12168	0.096
	Female	9	2.4433	.48903	.16301	
Hb	Male	31	6.1968	2.04409	.36713	0.406
	Female	9	6.8444	2.00382	.66794	
WBC	Male	31	16.5548	6.99094	1.25561	0.676
	Female	9	15.4778	5.72140	1.90713	
Neutrophil	Male	31	61.7419	14.38974	2.58447	0.566
	Female	9	64.8889	14.21658	4.73886	
Plat	Male	31	360.8065	95.73485	17.19449	0.331
	Female	9	326.6667	74.33034	24.77678	
Retic	Male	31	17.5806	6.63211	1.19116	0.041*
	Female	9	12.1111	7.47403	2.49134	

*: means significant difference

RBC count lower in male than in female and Hb. Level was better in female than male so the anaemia was milder in female than male but both of them lower than normal as in above table .
The blood film findings were also correlate with changes in C.B.C. and there were characteristic cells commonly seen which are blister and horn red blood cells as show in table 4

Table 4 percent of blister and horn cells in male and females in study group

				Gender		Total	p
				male	Female		
Blister cell		Present	Count	26	7	33	0.67
			% within Blister	78.8%	21.2%	100.0%	
		Absent	Count	5	2	7	
			% within Blister	71.4%	28.6%	100.0%	
Total			Count	31	9	40	
			% within Blister	77.5%	22.5%	100.0%	
Horn cell	Present		Count	20	6	26	0.9
			% within Horn	76.9%	23.1%	100.0%	
	Absent		Count	11	3	14	
			% within Horn	78.6%	21.4%	100.0%	
Total			Count	31	9	40	
			% within Horn	77.5%	22.5%	100.0%	

We study G6PD enzyme assay in study group and was deficient in 36 out of 40 and nearly same spread in both males and females as show in table 5

Table 5 show enzyme assay in study group

			Gender		Total	p
			male	female		
Enzyme	Deficiency	Count	28	8	36	0.9
		% within Enzyme	77.8%	22.2%	100.0%	
	Normal	Count	3	1	4	
		% within Enzyme	75.0%	25.0%	100.0%	
Total		Count	31	9	40	
		% within Enzyme	77.5%	22.5%	100.0%	

Discussion:-

Male were predominant in our study group and this due to fact that G6PD deficiency is inherited in an X-linked recessive pattern. The gene associated with this condition is located on the X chromosome, which is one of the two sex chromosomes. In males (who have only one X chromosome), one altered copy of the gene in each cell is sufficient to cause the condition. In females (who have two X chromosomes), a mutation would have to occur in both copies of the gene to cause the disorder. So male were predominant in our study group this result agree *with (CHRISTOS A. KATTAMIS, MARIA KYRIAZAKOU, and STAVROS CHAIDAS From the Universitv Department of Paediatrics, St. Sophie's Children's Hospital, Athens)* [13]

Favism is primarily a disease of childhood.[1] this correlate with study results which were below 10 years and mainly between 2- 5 years children above this age have less incidence as incidence of favism falls as the children get older simply because affected children avoid consuming fava beans after a haemolytic episode and also children after this age can tolerate oxidant stress more than younger child .[14, 15]

The disease occurred mainly throughout March and April as the disease is considerably higher during the spring as this seasonal predilection coincides with the ripening of the beans.[16]

Most of cases were O + blood group and the lowest frequencies was AB + blood group as this blood group is already is common blood group among the population [17] and un related to disease itself .

This disease is classified as one of common hemolytic anaemia in our country [18] so as part of hemolytic process it will lead to hemolysed RBC and as consequence lead to reduce in its count and reduced in Hb. Concentration these ideas correlate with study findings in which there is variable reduction in RBC count and Hb. . as mean RBC count was $2.0 * 10^9$ / cmm in male and $2.4 *10^9$ cmm in female while Hb. Concentration was 6.1 g/l in male and 6.8 g/l in female in both even there is no statistical significance but clinically there is significance differences , this explained as female usually less effected than female .

As a result the normal bone marrow response to this hemolysis reticulocytes count increased in a degree that correlate this hemolysis status . In this study maximum reticulocytes count was 31 % with mean nearly 17 % in male and 12 in female and recorded statistical significant differences .

Leukocytosis had been recorded in most of the cases with mean value 16.5 cells / cmm in male and 15.4 cells /cmm in female with absolute neutrophilia in both gender as part of WBCs response to hemolysis . Platelets count remain within normal range in mean 320000 and 360000 / cmm in both female and male respectively .

All the above investigation results in our study are consist with finding that recorded by (Seyyed Mohamed Hassan Aletayeb and his college in their study The high incidence of acute hemolysis due to favism in Ahvaz in Iran clinical and laboratory) . [15]

Blood film finding in this type of hemolytic anaemia characterized by finding of blister (red cells with contracted haemoglobin in ‘ ghost ’ membranes) , horn and nucleated RBCs respectively in addition to finding of heinz body included cells in few cases (about 40 % of cases) .[19]

In related to G6PD enzyme assay which used as qualitative enzyme evaluation we used the most widely used tests brilliant cresyl blue decolorization test . This test can reliably distinguish between deficient and non - deficient individuals, but are not reliably quantitative. Hemizygous deficient males and homozygous deficient females will be identified, the threshold being a G6PD activity of about 30% of normal in this test , our results were 36 out of 40 deficient and only 4 cases were normal .[19]

The normal G6PD activity found in 4 of the patients is not surprising; it could be explained even in the presence of only a small percentage of normal cells, sinceduring crisis, when all the old deficient cells are destroyed, the measurable activity depends on young deficient erythrocytes, on all normal un hemolysed old cells, and on an increased number of normal young erythrocytes (reticulocytes)with a very high activity.[20]

Conclusion:-

Favism affect male more than female specially pediatric age group less than 12 years old age , more common during March and April . *O positive* blood group recorded in about 50% among study patients .

As part of hemolytic crisis all cases complain from dramatic reduction in red blood cells , hemoglobin concentration with white blood cells changes in form of increased in count and absolute neutrophilia , with normal platelets count and significant reticulocytosis .

By use blood film evaluation we found red blood cells characteristic morphological changes as blister , horn cells and Heinz body cells . Enzyme qualitative evaluation was deficient in 90% of our cases .

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