Glucose-6 Phosphate Dehydrogenase Deficiency in terms of hemolysis indicators and management

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Abstract:

Background: Glucose-6 Phosphate Dehydrogenase (G6PD) Deficiency is one of the commonest inherited enzyme abnormalities in humans, caused by many mutations that reduce the stability of the enzyme and its level as red cells progress in age.

Objectives: To determine the useful hematologic indicators of hemolysis, observe an early detection of G6PD enzyme deficiency (if any), and the available therapeutic measures.

Patients and Methods: 123 patients with G6PD deficiency and hemolysis after exposure to fava beans whom visited AL-Elwiya Pediatric Teaching Hospital from the 1st of February 2016 till 31st of May 2016 were entered this study retrospectively. Hemolysis laboratory indicators were observed. Management supportive measures were put in consideration also.

Results: We found that 10-20% levels of hematocrit and normochromic normocytic anemia were the most frequent on presentation, while a range of 15.1-20% of reticulocyte counts was the most common with lower rates in females group. Hyperbilirubinemia was seen with nil patients had abnormal renal function tests. About three quarters (76.4%) of the total number of involved cases had glucose-6-phosphate dehydrogenase (G6PD) deficiency.

Only 4 patients required no blood transfusion, 102 patients (82.9%) needed transfusion once, and the rest 17 (13.8%) had more than one blood transfusion. Most of cases (91.1%) recovered within the first 3 days. However; all cases were recovered by the fourth day of admission.

Conclusion: Hemoglobin and blood morphology with hyperbilirubinemia were useful hematologic indicators of hemolytic process, while blood transfusion was the most used therapeutic measure, and recovery was expected within 2-3 days.

Key words: G6PD enzyme, hemolysis, blood transfusion, and therapeutic measures.

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Introduction:

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common genetic abnormality known to predispose to acute hemolytic anemia (AHA), which can be triggered by certain drugs or infection. However, the commonest trigger is fava beans *(Vicia faba)* ingestion, causing AHA (favism), which may be life-threatening especially in children. (1) A clinical symptom of G6PD deficiency closely linked to drug induced haemolysis is the hemolytic anemia resulting from the ingestion of the fava bean. (2,3)

Patients with favism are always G6PD deficient, but not all G6PD deficient in- dividuals develop haemolysis when they ingest fava beans. It is assumed that some other factors, such as genetic and metabolism of the active ingredients in the beans, which causes oxidative damage in red blood cells, are involved. (3) During the second half of the twentieth century, severe hemolytic anemia in individuals ingesting fava beans, more commonly in children, was reported. (4)

In Mediterranean area, the major type of allele that exists is called the "Mediterranean" variant, among the population, whereas in other countries such as Japan there is a different variant with a different type of mutation prevalent within that population. This type of mutation is called the "Japan" variant. The Mediterranean variant, found in Southern Europe, the Middle East and in India, is characterized by very low enzyme activity (0-10%) in RBCs using spectrophoto-metric and potentiometric methods. (5)

Deficiency of glucose-6-phosphate dehydrogenase (G6PD), an X-linked recessive enzymatic defect in the hexose monophosphate shunt that protects cellular damage from oxidative stress, is a common haematological problem worldwide. (6)

At a public health level, preventive measures may be useful. Subjects with G6PD deficiency may not even know which fava beans are until they collapse with favism. (7)

Nevertheless; ingestion of fava beans, certain drugs, infections, and metabolic conditions can cause

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hemolysis. Inadequate management of those G6PD-deficient individuals who develop acute hemolytic anemia can lead to permanent neurologic damage or death. (8,9)
Number of factors can precipitate hemolysis in G6PD-deficient subjects; such as certain drugs, infections, and some metabolic conditions, like diabetic ketoacidosis. (10–17)
Clinical signs and symptoms of hemolysis typically arise within 24 to 72 hours of drug dosing, and anemia worsens until about day (7). This makes it difficult for the health practitioner to identify a hemolytic crisis in patients who undergo outpatient or short hospital stay (less than 24 hour) procedures. Therefore, the practitioner should inform the high-risk patient and his or her caretaker to look for signs and symptoms of a hemolytic crisis (headache, dyspnea, fatigue, lumbar/substernal pain, jaundice, scleral icterus, and dark urine). (4)
Treatment consists of discontinuation of the offending agent and maintenance of urine output by infusion of crystalloid solutions and diuretics such as mannitol and/or furesomide, with blood transfusion when needed. (4,18)
Also, treatment of hyperbilirubinemia in G6PD-deficient neonates, when indicated, is with phototherapy and exchange transfusions. Prophylactic oral phenobarbital does not decrease the need for phototherapy or exchange transfusions in G6PD-deficient neonates. (19,20)

Aim of the study:
To determine the useful hematologic indicators of hemolysis, observe an early detection of G6PD enzyme deficiency (if any), and the available therapeutic measures.

Patients and Methods:
Cases with history of fava beans ingestion reaching the emergency room of AL-Elwiya Pediatric Teaching Hospital during the period from the 1st of February 2016 till 31st of May 2016 were targeted retrospectively in this study, and their total number was 238 patients.
The important investigations tracked from medical records includes: complete blood count with blood film and Reticulocyte count, direct Coomb's test, renal function tests, serum bilirubin (total and fractionated), as well as general urine examination (with urobilinogen) and G6PD enzyme assay screening test.
Supportive measures in the form of intravenous fluids with or without blood transfusion were recorded from hospital files.
Only fully recovered patients whom discharged in a good condition were allowed to enter this study.
Any file of any patient that did not contain any of the above information was excluded.
Because of that; only 123 patients were involved out of 238, 101 of them were males and 22 were females.
All of them were between 1-13 years old at time of diagnosis.
Z-test to measure P-value was used for statistical evaluations.

Results:
Investigations:
A. Hematocrit level (PCV):
The most frequent range of hematocrit levels that our patients got on presentation at the hospital was 10-20%, while less than 10% levels became next in frequency, as shown in Figure 1.

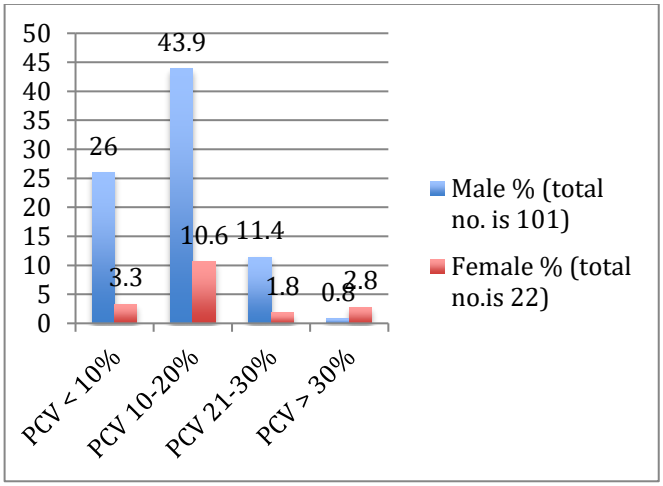

Figure 1 The range of hematocrit levels (during their first presentation at emergency department)

Doing simple calculations based on figure (1) and results, we have 86 male patients (69.9%) out of the total (123 patients) whom had hematocrit levels ≤ 20%, compared to 17 females (13.9%).
Z-test between 2 proportions = 3.54→ P-value = 0.022.
B. Type of anemia*:* Most of patients had normochromic normocytic anemia (118 patients; 95.9%) and only 5 patients (4.1%) had hypochromic microcytic red blood cells (1 male and 4 females). Nevertheles; 100 males (81.3%) out of the total (123 patients) had mean cell volume (MCV) levels > 70 femtoliter (fl) compared to 18 females (14.6%), which was the cut-line for microcytic red blood cells, while a level of mean cell hemoglobin concentation (MCH) less than 27 picogram (pg) was considered as hypochromic red blood cells.

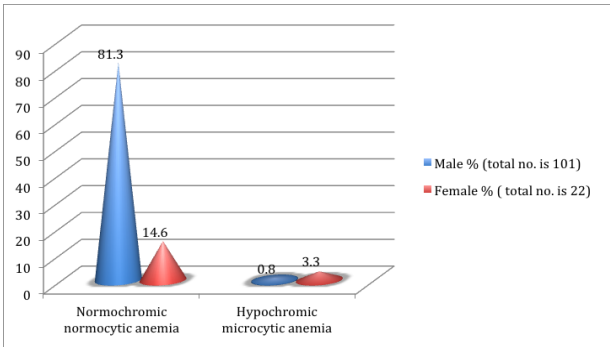

Figure **2** shows all that pecentages.
Z-test between 2 proportions = 3.23→ P-value = 0.0223

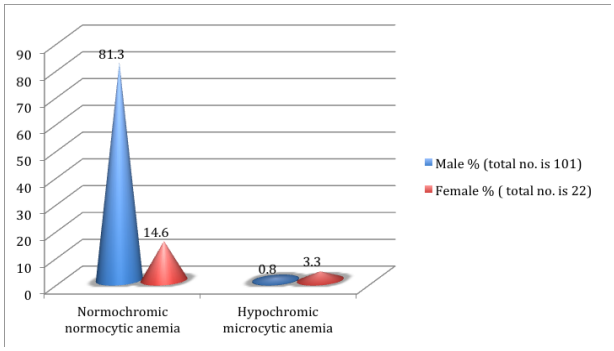

Figure 2 The types of presenting anemia associated with favism

Table ***1*** with a similar trend in case of reticulocyte production index (RPI)(21) . A value of 2-3 (of RPI) was the most frequent in both sex groups with a high

C. Reticulocyte count &RPI (reticulocyte production index): We found generally that reticulocyte counts (%) of 15.1 – 20% were most common, but females favoured lower counts inside their group, as illustrated in
percentage of 65% of total no. of patients (49.6% males and 15.5% females).

Table 1 The reticulocyte count (%) and reticulocyte production index (RPI)

Reticulocyte count (%)	Total		Male		Female	
	No. of patients	%	No. of patients	%	No. of patients	%
2 – 6	19	15.4	9	7.3	10	8.1
6.1 – 10	26	21.1	16	13.1	10	8.1
10.1 – 15	25	20.3	23	18.7	2	1.6
15.1 – 20	30	24.4	30	24.4	0	0
> 20	23	18.7	23	18.7	0	0
RPI	Total		Male		Female	
	No. of patients	%	No. of patients	%	No. of patients	%
< 2	13	10.6	10	8.1	3	2.4
2-3	80	65	61	49.6	19	15.5
3	30	24.4	30	24.4	0	0

$$RPI = reticulocyte\% \times \frac{observed\ hemoglobin}{normal\ hemoglobin} \times 0.5$$,
where normal hemoglobin (Hb) = 12g|dl.

D. The shape of the erythrocytes:
Blister cells appeared in most of blood films in both sexes, this is clarified in Figure 3.

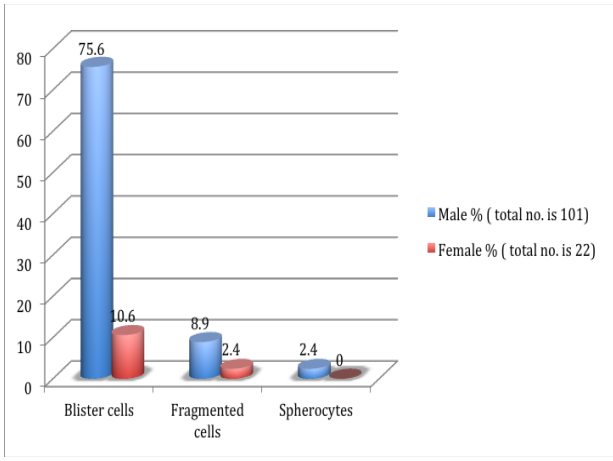

Figure 3 Distribution of various shapes of red blood cells (RBCs) in favism

E. Serum bilirubin level: Hyperbilirubinemia was found in all patients, which is considered a usual result

of hemolysis with the highest readings of 25-30 mmol/L, as seen in
Table ***2***.
F. Blood urea nitrogen and serum creatinine: Renal function (blood urea nitrogen and serum creatinine) was normal in all patients (100%), and so the function was not significantly impaired.

Table 2 Range of total serum bilirubin

Total serum bilirubin mmol/L	Total		Male		Female	
	No. of patients	%	No. of patients	%	No. of patients	%
25-50 (>1.5-3 mg/dl)	52	42.3	44	35.8	8	6.5
51-100 (3.1-6 mg/dl)	49	39.8	41	33.3	8	6.5
101-200 (6.1-12 mg/dl)	21	17.1	16	13	5	4.1
>200 (>12 mg/dl)	1	0.8	0	0	1	0.8

G. G6PD enzyme assay: The deficiency was screened through de-coloration of methylene blue.
Around 94 patients (76.4%) out of the total cases had deficient G6PD enzyme; most of them were males (90 patients) that stands for (73.4%) compared to 4 female patients, which represents (3.3%). This is obvious in Figure 4.
Z-test between 2 proportions = 2.56→ P-value = 0.02.

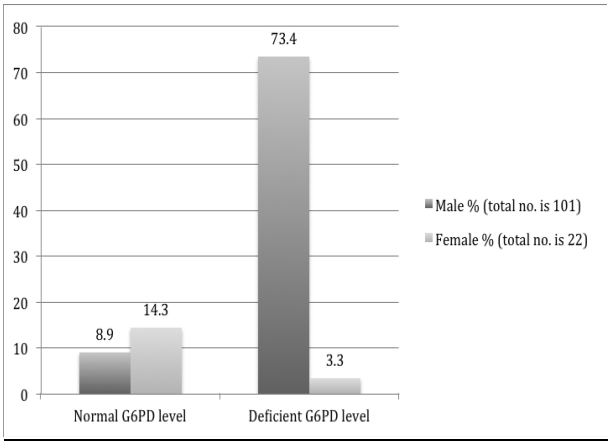

Figure 4 G6PD enzyme

Treatment: Nearly all patients required blood transfusion, only 4 of them were considered with no need to blood as per the opinion of treating physician, also some patients required more than one time of blood transfusions as shown in Table 3.
This might be due to continuous hemolysis even with high initial hemoglobin levels.
Recovery: It was dependent on regaining normal hematocrit levels (around 30%) for at least 12-24 hours. As it is stated in table 4 below, all of the cases (100%) recovered from the disease within the 1st 4 days, and (91.1%) recovered within the 1st 3 days.

Table 3 Treatment regimen used

TREATMENT	Total		Male		Female	
	No. of patients	%	No. of patients	%	No. of patients	%
Blood transfusion (once)	102	82.9	83	67.5	19	15.5
Blood transfusion (> one time)	17	13.8	17	13.8	0	0
NO Blood transfusion	4	3.3	1	0.8	3	2.4
Intravenous Fluid transfusion	123	100	101	82.1	22	17.9

Table 4 Duration of stay in the hospital

Recovery (days)	Total		Male		Female	
	No. of patients	%	No. of patients	%	No. of patients	%
1	26	21.2	19	15.4	7	5.7
2	33	26.8	21	17.1	12	9.8
3	53	43.1	50	40.7	3	2.4
4	11	8.9	11	8.9	0	0

Discussion:
Hematocrit level was ≤ 20% in majority of cases ; this level may be nearly similar to the results of a study from Mosul (22), but lower than that mentioned by Segel GB. (23). This may give us a clue of a more lower hematocrit levels in Iraqi patients rather than western populations. In 118 cases (95.9%), the anemia was of normochromic normocytic type, while the rest (5 cases) had hypochromic microcytic anemia. Nearly similar results were reported by Sawsan S. Abbas (24), NelsonDA (25), and ErbagclAB. (26) Reticulocyte count generally was high, precisely the RPI (Reticulocyte Production Index), which was between 2-3 in most of the involved cases; this indicates that hemolysis exceeded the ability of bone marrow to compensate, so the true reticulocyte count may be low for the first 3-4 days and this consistent with Hassan MK (27) and ErbagclAB. (26)

Blister erythrocytes were seen in the blood film of most cases, followed by fragmented cells, and spherocytes in the last rank. Nearly similar picture was reported by Luzzatto L (28), Yilmaz N (29) and Frank JE. (30) Hyperbilirubinemia was found in all patients, which is considered as a usual result of hemolysis. (22) The peak level of total serum bilirubin was between 25-100 mmol/L (>1.5-6 mg/dl). This agrees with many other studies like those done by Omar SK (22), Sawsan S. Abbas (24), and Beutler E. (31) Renal function (blood urea nitrogen and serum creatinine) was normal in all patients (100%), and clinically they were well. This is consistent with Belsy MA (32) and IbidS. (33) G6PD enzyme assay was done early in the first day of admission and showed early detection of deficient enzyme in 94 cases (76.4%). This is consistent with other authors such as ErbagclAB (26), Abbas SS (34), Mehta A (35), and S ML. (36) Since most of patients had moderate to severe anemia, and continuous hemolytic process was suspected even with relatively mild anemia, 119 patients (96.7%) were given blood transfusion as a major constituent of therapy. This relatively aggressive approach might point out to the severity of variant(s) existing in our locality, while only 4 cases (3.3%) did not need blood transfusion. Approximate results were also reported by Omar SK (22), ErbagclAB (26) and S ML. (36) Finally, all patients recovered well. Recovery in most of them (91.1%), was within the first 3 days. This period was also found by Frank JE.(30), IbidS (33), and Hilmi FA. (37)

Conclusion:

Hemoglobin and blood morphology with hyperbilirubinemia were useful hematologic indicators of hemolytic process, but renal function was not affected, especially in patients with early presentation followed by early treatment. A significant number of patients showed an early reduction of G6PD enzyme within the course of the disease. Blood transfusion was the most used therapeutic measure, and recovery was expected within 2-3 days.

Author's contributions:

Muthanna Falah Athab and Anwer Sabeeh Al-Zubaidi: data collection and interpretation.

Hayder Hadi Al-Momen: data analysis and critical revision

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