

## Evaluation of liver and kidney function in favism patients

Akbar Dorgalaleh<sup>1</sup>, Muhammad Shahid Shahzad<sup>2</sup>, Mohammad Reza Younesi<sup>3</sup>,  
Esmaeil Sanei Moghaddam<sup>4</sup>, Mohammad Mahmoodi<sup>5</sup>, Bijan Varmaghani<sup>6</sup>,  
Zahra Kashani Khatib<sup>7</sup>, Shaban Alizadeh<sup>8</sup>

*Department of Hematology, Allied Medical School, Tehran University of Medical Sciences, Tehran, Iran.*

Received: 25 August 2012

Revised: 21 October 2012

Accepted: 23 October 2012

### Abstract

**Background:** G6PD deficiency is the most common enzymopathy of red blood cells. The clinical symptoms of favism are jaundice, hematuria and haemolytic anaemia that seem to affect liver and kidney in long term. Thus we evaluate kidney and liver function of favism patients in an endemic area of the disease with a high rate of fava beans cultivation.

**Methods:** This study was performed on favism patients and healthy controls referring to Iranshahr central hospital. Liver and kidney function tests were performed.

**Results:** The results showed a statistically significant difference between these two groups ( $p < 0.05$ ) for liver function tests, (AST, ALT and ALP), but not for renal tests (BUN and creatinine) ( $p > 0.05$ ).

**Conclusion:** Due to abnormalities were seen in the liver function tests of these patients, we suggest that these tests be regularly performed for favism patients who are constantly exposed to oxidant agents.

**Keywords:** G6PD, Favism, Liver, Kidney.

### Introduction

Glucose-6-phosphate dehydrogenase (G6PD) (E.C.1.1.1.49) is the key regulatory enzyme in the hexosemonophosphate shunt that catalyzes the oxidation of glucose 6-phosphate to 6-phosphogluconolactone (1,2). This first and rate-limiting step of shunt is associated with production of nicotinamide

adenine dinucleotide (NADPH) that is required for the generation of reduced glutathione, which is important for the protection against oxidative damages (3).

More than 442 variants of human G6PD are known (4); many of them are associated with anemia resulting from the impaired ability of the erythrocyte to respond to toxic stress (2, 5).

1. MSc of Hematology, Hematology Department, Allied Medical School, Tehran University of Medical Sciences, Tehran, Iran. dorgalaleh.1390@yahoo.com

2. MD, Iran Hospital, Nikshahr, Iran. onlyshahid@yahoo.com

3. MSc of Hematology, Hematology Department, Allied Medical School, Tehran University of Medical Sciences, Tehran, Iran. mohammadrezayounesi@yahoo.com

4. Manager of Blood Transfusion Center of Zahedan, High Institute for Research and Education in Transfusion Medicine and Zahedan Regional Educational Blood Transfusion Center, Iran. saso1334@yahoo.com

5. BSc of CLS Pars Pathobiology Laboratory, Golestan, Minoodasht, Iran. newassistant@yahoo.com

6. MSc of Hematology, Hematology Department, Allied Medical School, Tehran University of Medical Sciences, Tehran, Iran. bijan\_varmaghani@yahoo.com

7. BSc of CLS, Student of Scientific Research Center (SSRC), Tehran University of Medical Sciences, Tehran, Iran. zkashanikhatib@gmail.com

8. (Corresponding author), Assistant professor of hematology, PhD of Hematology, Hematology Department, Allied Medical School, Tehran University of Medical Sciences, Tehran, Iran. alizadehs@sina.tums.ac.ir

Precise molecular characterization of the G6PD gene showed that these 442 variants correspond to only 140 mutations. These mutations cause G6PD deficiency, a common enzymopathy affecting over 400 million people worldwide (6, 7). The disease can cause neonatal jaundice, drug- or infection-induced hemolytic crisis, favism, and nonspherocytic hemolytic anemia (4, 8). According to the level of enzyme activity, World Health Organization classified variants of G6PD to five groups (9).

Mediterranean variant with an estimated prevalence of 5% is the most common variant in Southern Europe, Middle East and India. Rate of incidence of Mediterranean mutation in Turkey, Iran, and India are 80%, 69% and 60.4% respectively; In Pakistan and Saudi Arabia the Mediterranean mutation is the prevalent variant (10, 11).

This variant of G6PD is often associated with favism, an acute hemolytic crisis induced by ingestion of fava beans (*Vicia faba*) by individuals deficient in glucose-6-phosphate dehydrogenase (12). Vicine and convicine as natural occurring toxic compounds in *Vicia faba* are involved in the formation of hemolytic anemia (13).

Clinical symptoms of favism are pallor, jaundice, hematuria, and acute hemolytic anemia that occurs suddenly 24 to 48 hours after consumption of fava beans. Depletion of reduced glutathione and impairment of some important enzymes induce oxidative stress in G6PD-deficient red blood cells and lead to acute hemolytic anemia (14-19).

Although the typical presentation of the disease is extravascular hemolysis, intravascular hemolysis also occurs (20-22). Intravascular hemolysis is the destruction of red blood cells in the circulation with the release of cell contents such as hemoglobin into plasma (23, 24). Then free plasma hemoglobin is filtered through the kidney and cause hemoglobinuria that is one of the most prominent clinical signs of excessive intravascular hemolysis and can cause renal failure (25-27).

In the other hand, extravascular hemolysis in favism occurs when altered RBCs during oxidative damage are phagocytized by macrophages in the spleen, liver and bone marrow; Therefore, free hemoglobin is not released into plasma (20, 22, 28).

Eventhough some believe that the breakdown of hemoglobin by the reticuloendothelial system causes increase in plasma bilirubin, others say that this increase, is largely the result of an impairment of liver function caused by G6PD deficiency in the liver (29, 30).

In spite of high prevalence of favism in many regions of Iran, fava is planted as a rich source of protein in these areas and since ingestion of fresh, dried or frozen forms of beans and even inhaling of its pollen can be associated with hemolytic events, favism patients can experience these episodes during the entire year.

It seems that frequent experience of these hemolytic episodes can have adverse effects on kidney and liver as the main organs involved in the hemolytic processes.

Thus the purpose of the present investigation is to evaluate the kidney and liver functions in favism patients by assessment of serum parameters implicate these organs status.

### Methods

This study was performed on 55 blood samples taken from individuals referring to Iranshahr central hospital between January 2012 and September 2012 and their G6PD deficiency tests were positive by fluorescent spot method (Kimia Pagouhan lot no 90607).

Ethics approval and patient consent statements were taken from all patients.

Short draw or hemolyzed samples and also sample of patients with history of liver and kidney disorders were excluded from the study and on remained samples, deficiency was subsequently confirmed by quantitative enzyme assays according to the manufacturer instructions (Biolabo-France).

Then alanine aminotransferase (ALT) (pars azmun lot no. 90002), aspartat amino transferase (AST)) (pars azmun lot no. 90005) and alkaline phosphatase (ALP) (pars azmun lot no. 90002) for assessment of liver function and creatinine (pars azmun lot no. 90009) and Blood urea nitrogen (BUN) (pars azmun lot no. 90009) for evaluation of kidney function were performed by Mindray chemistry analyzer (BS-200 China). Tests were also performed on an additional 60 random G6PD non- deficient individuals as control.

*Statistical analysis*

Obtained data of study was analyzed by

Statistical Package for Social Science (SPSS) software. Statistical independent t test was used to evaluate the significance of differences between two groups in mean of continuous variables.  $P < .05$  was considered statistically significant.

**Result**

In the study group of 55 patients, 44 were men and 11 were women, with an average age of 25 (SD=12) years (range 1-47). In the control group of 60 patients, 46 were men and 14 were women, with an average age of 28 (SD=11) years (range 3-51). Comparison among mean of some liver function tests in G6PD deficient and con-

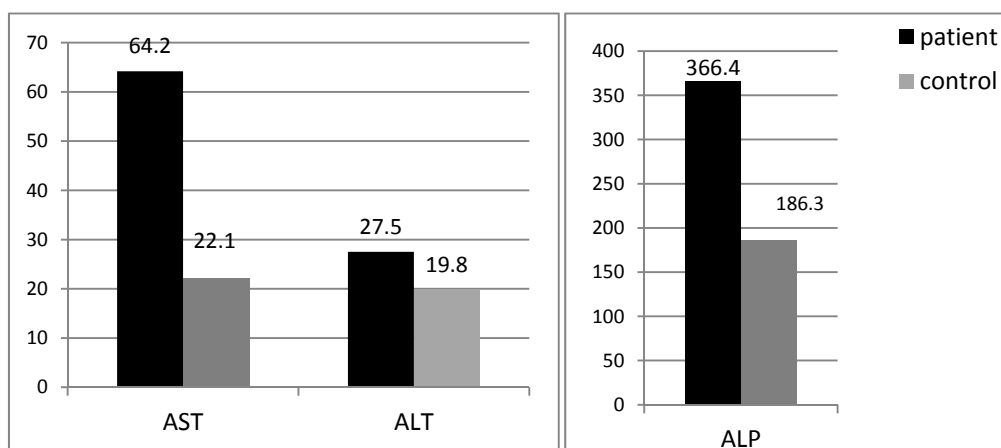


Fig1. Comparison among mean of some liver function tests in G6PD deficient and control groups, represents significance at the  $p=0.0001$  level for AST(U/L), significance at the  $p=0.001$  level for ALT (U/L) and significance at the  $p<0.05$  level for ALP (U/L)test.

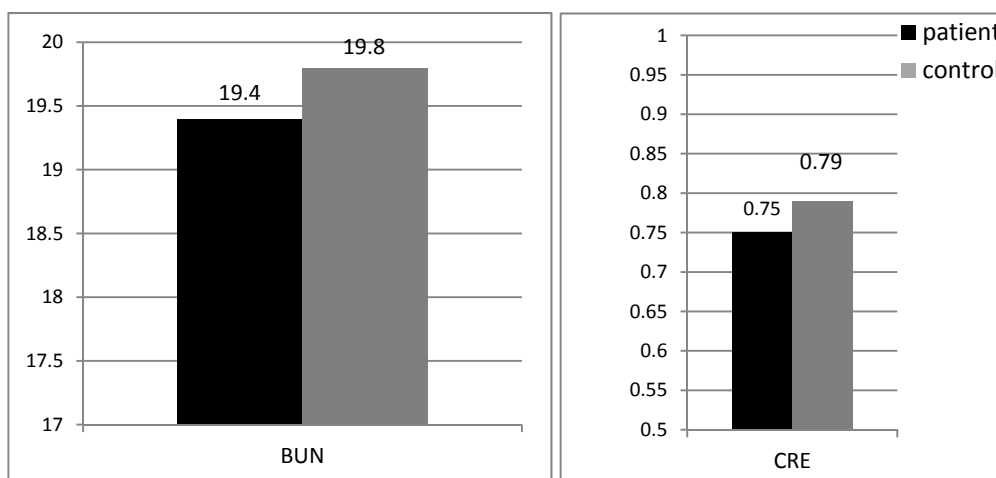


Fig 2. Comparison between mean of kidney tests in G6PD deficient and control groups represents no statistically significance different ( $p>0.05$ ). Numbers shown on unit of mg/dl.

control groups showed statistically significant difference ( $P < 0.05$ ). Serum AST, ALT and ALP were significantly elevated in patients than control group (Fig 1).

Comparison between mean of kidney function tests in these two groups did not show any statistically significant difference ( $p > 0.05$ ) (Fig 2).

### Discussion

Favism is a disease prevalent in Mediterranean countries and characterized by acute hemolytic anemia following ingestion of fava beans. The typical features of the disease are jaundice, hematuria and hemolytic anemia (14).

The fava beans are also common cultigens of Mediterranean area that can aggravate the disease in the G6PD deficient patients in this area. Since all types of fava beans can induce hemolytic episodes, favism patients can experience these episodes during the entire year (31, 32).

Occurrence of these frequent mild or severe episodes of hemolysis can, over time, lead to more serious complications, particularly in liver and kidney as the two main organs involved in the hemolytic process (25, 26, 33).

This study hypothesizes that in areas with endemic culture of fava beans, favism patients may be impaired in the performance

of these two organs. The results of this study partly confirmed this theory about liver, because means of liver function tests, AST, ALT and ALP were significantly higher in the case group than in the control group (64.2 IU/L vs 22.1 IU/L,  $p < 0.0001$ , 27.5 IU/L vs 19.8 IU/L,  $p < 0.001$  and 366.4 IU/L vs 186.3 IU/L,  $p < 0.002$  respectively). Although mean of ALT in patient group located in the normal range (Table 1). These elevated liver enzymes can be resulted from occurrence of recurrent hemolytic episodes that involve liver because a part of this hemolytic process can be extravascular. Moreover G6PD deficiency in liver can be a probable cause of liver complication in these patients but this claim require more investigations.

We did not observe any significant correlation between age and liver function enzyme levels ( $p > 0.05$ ).

On the other hand, BUN and creatinine as renal tests didn't show statistically significant difference between patient and control groups (19.4 vs 19.8,  $p > 0.05$  and 0.75 vs 0.79,  $p > 0.05$ ) (Table 1).

Our observations about liver function tests are in accordance with some other studies such as Alavis et al that reported elevated levels of AST in majority and ALT in some favism patients (34).

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Table 1. Statistical analysis of tests conducted on the patients and control groups. All liver function tests Showed statistically significant difference between these two groups ( $p < 0.05$ ), while kidney tests did not Show any significant difference ( $p > 0.05$ ).

		Number	Mean	SD	Min	Max	p value
AST (U/L)	patient	55	64.2	36.9	12	179	0.0001
	Control	60	22.1	6.7	4	40	
ALT (U/L)	patient	55	27.5	16.4	7	84	0.001
	Control	60	19.8	8.2	9	39	
ALP (U/L)	patient	55	366.4	130.6	100	770	<0.05
	Control	60	186.3	85.6	93	670	
BUN (mg/dL)	patient	55	19.4	9.2	10	52	>0.05
	Control	60	19.8	6.4	10	42	
CR (mg/dL)	patient	55	0.75	0.25	0.4	1.4	>0.05
	Control	60	0.79	0.19	0.4	1.2	

study showed high levels of AST and ALT in a G6PD deficient patient (35). But our results was incompatible with findings of those studies that claimed that tests are normal in these patients (36). It seems that this incompatibility originates from some features of their studies such as sampling time or study region.

Our study was performed in an endemic region with 24% frequency of G6PD deficiency and high cultivation of fava bean that can lead to induction of frequent hemolytic episodes in the patients (37). Moreover we found significant increase in serum ALP in case than control group that may implicate effects of hemolytic episodes on liver or gallbladder.

In this study we found non-significant disparity in kidney function tests between case and control group that is incompatible with some studies showed elevated levels of these elements (35). This is due to their samples that were collected during hemolytic crisis whereas our sampling was not in this situation.

### Conclusion

According to the results of this study and life style in these regions about consumption of beans as a traditional cheap and rich source of protein, we suggest that liver function tests be regularly performed for favism patients who constantly were exposed to fava bean or other oxidant agents and their hemolytic complications were recognized. With early diagnosis of liver complication and use of extensive and regular medical cares, development of liver dysfunction can be prevented.

Moreover Vicine and convicine can be eliminated from the fava bean substrate by microbial and Fungal enzymes or other available methods to facilitate use of this source of protein (38).

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