The in Vitro Effect of Chloramphenicol and Salicylate on Erythrocytes of Patients with Favism

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ABSTRACT:

BACKGROUND:

Glucose 6-phosphate dehydrogenase (G6PD) deficiency is the most common of all clinically significant enzyme defects. A long list of drugs thought to cause haemolysis in patients with this enzyme defect.

OBJECTIVE:

To determine whether chloramphenicol and salicylate can act as in vitro exogenous oxidizing agents and subsequently cause haemolysis of G6PD deficient erythrocytes and matching the result with the data obtained from the clinical observations which includes the intake of trimethoprim-sulfamethoxazole, salicylate or nalidixic acid by favic patient.

PATIENTS AND METHODS:

Sixty six patients admitted to the hospital (Karbala teaching hospital for Children, Karbala, Iraq) with history of sudden onset of pallor and dark urine after fava beans ingestion were studied. Each patient was fully examined and his parents were asked about the type of fava beans ingested and the past drug history.

Of the sixty six patients, ten were evaluated 1-3 months later and blood samples were taken from them along with blood samples from ten healthy volunteers. Blood samples from both groups were incubated in vitro with chloramphenicol and salicylate separately.

RESULTS:

Mean (SD) of methaemoglobin concentrations at baseline and after incubation with therapeutic and toxic concentrations of chloramphenicol (15 µg/ml and 25 µg/ml) and salicylate (150 µg/ml and 300 µg/ml) were calculated for both the control and the study groups. Paired t-test showed no significant differences (P> 0.05) in methaemoglobin concentrations at baseline and after incubation with therapeutic and toxic concentrations of these drugs. Mean percentage differences from baseline for G6PD deficient group were not significantly different from control group at both concentrations of these drugs as tested by student t-test.

CONCLUSION:

- Hemolysis in G_6PD deficient patients occurs mainly after fresh fava beans ingestion.
- chloramphenicol and acetylsalicylic acid do not cause significant hemolysis in G_6PD deficient erythrocytes in vitro .

KEY WORDS: G_6PD, methaemoglobin, haemolysis, favism, chloramphenicol , acetylsalicylic, salicylate.

INTRODUCTION:

Although many other red blood cell (RBC) enzyme deficiencies are now known[1,2]. Glucose 6-phosphate dehydrogenase (G_6PD) deficiency still reigns as the most common of all clinically significant enzyme defects, not only in hematology, but in human biology as a whole.

Biochemical characterization has led to the description of no less than 442 variants of (G_6PD) believed to be distinct. Two hundred ninety nine of these were characterized by methods agreed upon by World Health Organization (WHO) expert group[3].

The fact that primaquine was the only one of many drugs that precipitated haemolysis in (G_6PD) deficient individuals was recognized in many studies by in vivo challenge of ^{51}Cr-labelled erythrocytes[4].Therefore, in the 1950_s when a person with (G_6PD) deficiency developed hemolytic anemia, it was generally assumed that haemolysis has been precipitated by a drug, and whatever drug had been ingested was considered to be culpable.

As a result, a long list of drugs thought to cause haemolysis evolved. On more careful study, many of them have been proven to be quite innocent with

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respect to the cause of hemolytic anemia in (G_6PD) deficiency [5].

Favism, a clinical manifestation of (G_6PD) deficiency closely related to drug induced haemolysis, is the hemolytic anemia induced by ingestion of fava beans, vicia faba.

Patients with favism are always (G_6PD) deficient, but not all (G_6PD) deficient individuals developed haemolysis when they ingest fava beans. Thus, (G_6PD) deficiency is a necessary but not a sufficient cause of favism. Presumably some other factors, probably also genetic and very likely related to metabolism of the active ingredients in the beans is involved [6].

The most likely offenders in fava beans are vicine and convicine, β-glucoside of pyramidine compounds that are converted by β-glucosidases to their aglycones, vicine and isouramil, respectively. These compounds form reactive semiquinoid free radicals and can generate active oxygen species. This result in the formation of ferrihemoglobin, methemoglobin and inactivation of various enzymes [7,11].

New drugs continue to be introduced into medical practice and it would be extremely useful to be able to predict which of these cannot safely be given to favic patients; unfortunately those drugs that produce haemolysis have no clearly understood common denominator either in structure or chemical properties. Moreover, in some (perhaps in most) instances the injury to the enzyme deficient erythrocyte is not mediated by the chemical compound that is administered, but rather by a metabolic product.

In vitro systems have been advised in an attempt to mimic what occur in the body [11,12].

The aim of the present study is to evaluate the effect of certain drugs on the erythrocytes of favic patients in vitro and possibly in vivo.

PATIENTS AND METHODS:

The present study was conducted in Karbala teaching hospital for children from January 2007 to January 2008. Sixty six patients admitted to the hospital with a history of sudden onset of pallor and dark urine after fava beans ingestion were studied.

Each patient was fully examined and his parents were asked about the type of fava beans ingested (fresh, dried and frozen) and past history of intake of certain drugs (trimethoprim-sulfamethoxazole ,nalidixic acid and acetylsalicylic acid).

Of the sixty six patients, ten patients (study group) and 10 healthy children(control group)were evaluated 1-3 months after the initial attack.

Three milliliters of blood from each subject in the study and control groups were aspirated and collected in EDTA tubes. The blood samples were tested for G_6PD deficiency using the qualitative color reduction method (Kit number 506k, Sigma diagnostics, USA)[13]. Incubation and thorough mixing with drugs at 37^0C for 60 minutes was done for each sample as follows:

1. 0.5 ml of blood with chloramphenicol 7.5 µg at therapeutic concentration (15 µg/ml).
2. 0.5 ml of blood with chloramphenicol 12.5 µg at toxic concentration (25 µg/ml).
3. 0.5 ml of blood with salicylate 75 µg at therapeutic concentration (150 µg/ml).
4. 0.5 ml of blood with salicylate 150 µg at toxic concentration (300 µg/ml).

Mean (SD) values for methaemoglobin concentrations, which were measured spectrophotometrically at 630 nm before and after incubation of blood samples with drugs, were calculated. Methaemoglobin concentration values of more than 3% of the total haemoglobin were considered to be significantly indicating haemolysis[13]. Percentage differences between baseline values and those after incubation with drugs were determined by first calculating the percentage difference for each individual sample and then the mean (SD) of these individual percentages. Paired t-test was used to compare methaemoglobin at the baseline and after incubation with therapeutic and toxic concentrations of chloramphenicol and Salicylate mentioned above and Comparisons of the percentage differences between the study and control groups were performed using student's t-test. Statistically significant difference was defined as $P< 0.05$.

RESULTS:

Fifty eight patients were males and eight were females. Most of the patients 61(92.4%) developed favism during March and April.

Regarding the type of ingested fava beans, 62 patients (94%) ingested fresh cooked beans, 3 patients (4.5%) ingested cooked dried beans while one was a three months old breastfed infant developed haemolysis after ingestion of fresh cooked beans by his mother.

From detailed past history of each patient , it was found that: 52 patients (78.8%) received trimethoprim-sulfamethoxazole,48(72.7%) received salicylate and 42 patients(63.6%) ingested nalidixic acid once or more (most of the time) prior to the present attack without developing favism.

Red blood cells from 10 (G_6PD) deficient patients and 10 control healthy children were studied. Mean (SD) values for methaemoglobin at baseline, and

after incubation with chloramphenicol and Salicylate at concentration of (15 µg/ml, 25µg/ml) and (150 µg/ml, 300 µg/ml) which represent the therapeutic and toxic concentrations respectively were calculated. Paired t-test shows no significant difference ($p>0.05$) in methaemoglobin at the baseline and after incubation with therapeutic and toxic concentration of chloramphenicol and Salicylate mentioned above. Mean percentage changed from the baseline was calculated by first calculating the percentage of each individual specimens, and then the mean percentage change of these individual percentages, mean percentage difference from baseline for (G_6PD) deficient group was not significantly different from controls, at both concentration of chloramphenicol and Salicylaate as tested by student t-test.

These information are represented in table 1 and table 2. A schematic representation of the data is also.

Table 1 :Mean± SD metHb values before and after incubation with Chloramphenicol summarized in figure 1 to figure 3.

	Baseline	Incubation with 15µg/ml	% change from baseline	Incubation with 25µg/ml	% change from baseline
MetHb (% of total Hb)					
G-6-PD deficient (n=10)	1.95±0.04	1.96± 0.05	0.57± 3.69	1.96± 0.04	0.34 ± 2.53
G-6-PD normal (n=10)	1.92±0.06	.9±0.06	-1.16± 2.87	1.963± 0.06	2.04 ± 3.98
p value			p=0.23		p=0.24

Table 2: Mean± SD metHb values before and after incubation with Salicylate

	Baseline	Incubation with 150µg/ml	% change from baseline	Incubation with 300µg/ml	% change from baseline
MetHb (% of total Hb)					
G-6-PD deficient (n=10)	1.95±0.04	1.964±0.05	0.62±4.02	1.95±0.06	0.07±4.68
G-6-PD normal (n=10)	1.92±0.06	1.915±0.06	-0.51±2.02	1.95±0.04	1.78±3.08
P value			p=0.4		p=0.32

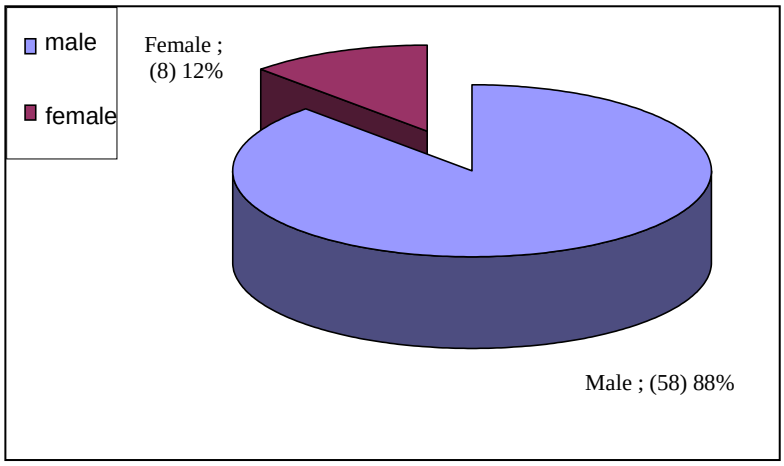

Figure 1 :Sex distribution of the patients

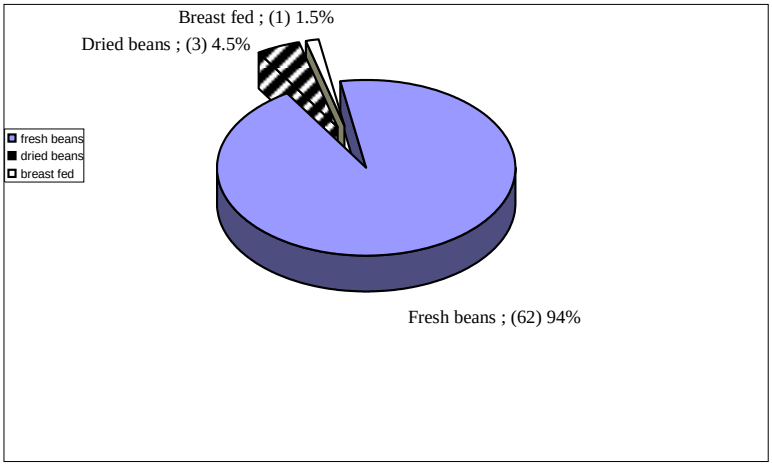

Figure 2: Types of fava beans ingested by the patients

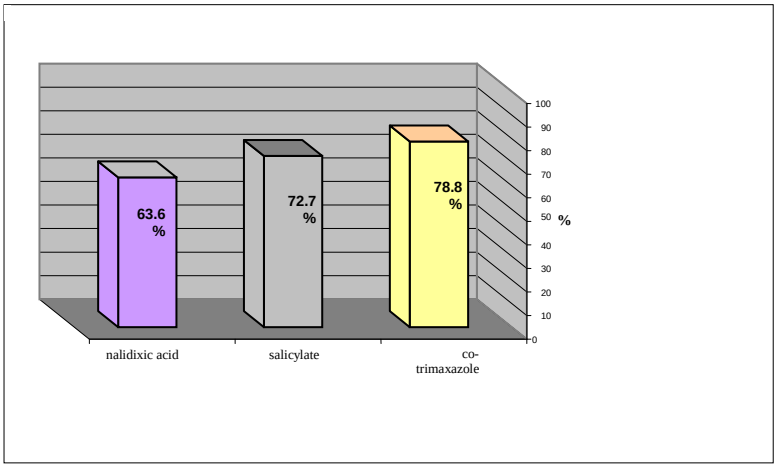

Figure 3:Past drug history of the patients

DISCUSSION:

Favism is a manifestation of G_6PD deficiency and is confined to relatively small geographical areas such as the Mediterranean region , the far East and Southern Asia , although sporadic cases have also been described elsewhere.[14,15] The G_6PD enzyme of subjects with favism has been characterized as the Mediterranean variant[16].

Synthesis of RBC G_6PD is determined by a gene on the X chromosome. Diseases involving the enzyme therefore occur more frequently in males than in females[17].

Eight (12.1%) of our patients were females which is a high figure compared to other x-linked recessive diseases due to high percent of consanguinity marriage in our society (affected male marrying relative heterozygous female) and random inactivation of the normal x-chromosomes in heterozygous female (Lyon hypothesis).

Sixty one patient (92.4%) developed favism during early spring months (March, April) when beans are ripening.

Sixty three patient (95.5%) developed favism after ingestion of fresh cooked fava beans (one infant through breast milk of his mother) while only 3 patients (4.5%) developed haemolysis after dried beans ingestion.

This study showed that favism occurs mostly during spring and mostly after fresh fava bean ingestion which is in line with other studies.[18,19,20]

From thorough details of past history of each patient , the following drugs were taken by the favic patients , once or more.

Fifty two patients (78.8%) received Trimethoprim-sulphamethoxazole ,48 patients (72.8%) received Acetylsalicylic acid while Nalidixic acid was taken by 42 patients (63.6%).

No single case of hemolytic anemia was observed after ingestion of these drugs by favic patients in the study group which in agreement with Markowitz N. who studied the effect of trimethoprim-sulphamethoxazole in a glucose 6 phosphate dehydrogenase deficient population[21] and AlMusawi ZM et al who studied the effect of fava beans and salicylate on G_6PD deficient patients[(22)].

Although data obtained from clinical observation are less reliable , over three decades of clinical practice in pediatrics, we and our colleagues , did not document any case of hemolytic anaemia after any drug ingestion in G_6PD deficient patients and we hear only about sporadic cases without any confirming evidence.

In the present study, we choose two drugs (acetylsalicylic acid and chloramphenicol) well known to cause hemolysis in G_6PD deficient patients[(17,23)]and studied their effect on erythrocyte of favic patient in vitro at therapeautic and toxic concentrations . These two drugs did not cause Significant oxidative damage (evidenced by an increased content of methemoglobin)when incubated with G_6PD deficient and normal erythrocyte.

Our study is in line with N.A.J Ali et al[24] and Beutler E[(25)]regarding acetylsalicylic acid where no significant effect was observed after incubation with G6PD deficient erythrocyte .

N.A.J. Ali et al observed little oxidizing effect (slight reduction in glutathione level of erythrocytes) after incubation with chloramphenicol and sulfonamides which is in line with our study [(24)].

The present study showed that chloramphenicol and acetylsalicylic acid did not cause significant hemolysis in G_6PD deficient erythrocytes in vitro. Drug metabolites may be the offending agents which need further studies for evaluation.

CONCLUSION:

- Hemolysis in G_6PD deficient patients occurs mainly after fresh fava beans ingestion.
- Chloramphenicol and acetylsalicylic acid do not cause significant hemolysis in G_6PD deficient erythrocytes in vitro.

Recommendations:

Further studies are needed to know the variants of G_6PD in different areas of Iraq and more drugs should be studied in vitro and if possible in vivo.

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