Hematological Findings and Severity of G6PD Deficiency in Vataliya Prajapati Subjects

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Abstract
Objectives: The present study was undertaken to assess the clinical implication of G6PD deficiency in Vataliya Prajapati (VP) subjects in Surat.
Method: Blood samples of 954 children and 690 adults were collected in camps. Cord blood samples of 57 neonates born to VP mother were also collected. Medical history and other relevant information of all subjects were obtained. Samples were screened for G6PD deficiency by NBT test and the enzyme activity was estimated by WHO method. Hematological parameters were measured on hematology analyzer while reticulocyte count was measured using new methylene blue dye.
Results: The G6PD enzyme deficiency was detected in 27.5% males and 12.8% females. The enzyme levels in deficient subjects suggested class II variant. Hematological studies indicated mild anemia in G6PD deficient persons. Reticulocyte count was slightly raised (p <0.05). Out of eight G6PD deficient neonates one developed mild jaundice. Five deficient male adults gave the history of hemolytic crisis, three of them had typhoid, one tuberculosis and remaining one had fever of unknown origin.
Conclusion: G6PD deficiency in majority of Vataliya Prajapati subjects is of mild type. However it is essential to test every Vataliya Prajapati subject for G6PD deficiency as certain infections and drugs can cause crisis in deficient person.

INTRODUCTION
In glucose–6-phosphate dehydrogenase deficiency (G6PD) hemolysis could be triggered after ingestion of certain drugs or even after exposure to oxidant free radicals generated by leukocytes in the course of infection.1 The drugs implicated include anti-malarials, sulfonamides, nitrofurantoins etc.2 The infections, which are particularly associated with hemolytic crises in deficient patient are viral hepatitis, pneumonia and typhoid fever.1 G6PD deficiency can present even without exposure to oxidant stress in form of neonatal jaundice or chronic hemolytic anemia.1

In Surat City there are about 20,000 inhabitants of Vataliya Prajapati community who are migrated from Amreli and Bhavnagar districts of Gujarat. Our earlier study showed high prevalence of G6PD deficiency in them.3,4 No systematic study is available to know the clinical importance of deficiency in them. Hence the present study was undertaken.

MATERIAL AND METHODS
After having detail discussion with community leaders about the project and clearance from the Institutional Ethical Committee this project was initiated. Camps were arranged in school or community hall in the Amroli and Katargam regions of Surat city where majority of the Vataliya Prajapati community resides. The medical officer, technicians and volunteers managed the camp. Prior to camp, community leader requested them to bring their medical files with them if they had any illness in the past. The proforma consisting information like past and present illness, intake of medicine, history of drug induced hemolytic anemia or crisis, neonatal jaundice etc. were filled for all 1644 participants. Informed consent was obtained from the subjects before collecting their blood. Blood samples in EDTA vials were collected for G6PD enzyme and hematological studies. After completion of screening a special camp was organized for G6PD deficient subjects to confirm their G6PD status and to get their medical history again.

Cord blood samples were collected from the two maternity hospitals (Amroli and Katargam) where Vataliya Prajapati women registered for delivery. Doctors/nurses conducting the delivery collected the
cord blood in EDTA vials. They also filled the proforma. The infants were watched for neonatal jaundice. G6PD enzyme deficiency was detected by Nitroblue tetrazolium (NBT) test. G6PD activity was measured in samples showing deficiency or inconclusive results, by quantitative assay. Hematological parameters were measured on automatic hematological analyzer (Nihon Khoden) while reticulocyte count was done by new methylene blue supravital staining method. Blood smears were stained by Wright’s stain.

Mean and standard deviation (SD) were calculated on Excel software of MS Windows. χ² and “t” tests were employed for statistical evaluation of the results using standard methods.

**RESULTS**

The study includes 57 infants, 954 children and 690 adults, consisting 1020 males and 681 females. G6PD deficiency was detected in 280 (27.5%) males and 87 (12.8%) females. Among 57 neonates there were 35 males and 22 females of which five males and three females were G6PD deficient. Neonatal jaundice on 3rd day of life was observed in one deficient male infant. His indirect serum bilirubin level was 9 mg/dl. The results of quantitative assay showed the G6PD enzyme activity range in deficient males and females as 0 to 0.82 IU/10^10 RBC and 0 to 0.77 IU/10^10 RBC respectively. In normal individuals the range was 1.3 to 3.2 IU/10^10 RBC. About 82% of the G6PD deficient male and female individuals had the activity <10% of the normal, suggesting Class II G6PD variant.

Medical history was obtained from all 1644 subjects, but due to illiteracy or if children attended the camp without parents it was not possible to get reliable information. Majority of them (Normal as well as deficient) gave a history of fever. But they did not know whether it was due to malaria, typhoid or any other cause. However most of them were never hospitalized. Out of 367 G6PD deficient subjects reliable medical history could be obtained from 125 G6PD deficient children and adults. Similarly 450 normal subjects revealed reliable medical history. The pattern of illness was identical in both the groups. Table 1 presents analysis of 125 G6PD deficient cases with respect to history of recent infections. The history of malaria (*P. falciparum* or *P. vivax*) was noted in 19 (15.2%), typhoid 17 (13.6%) and jaundice in 8 (6.4%) subjects. In 18 (14.4%) persons there was a history of fever of unknown etiology, skin infection or diarrhea. Five individuals reported the hemolytic crisis, hematuria or drug-induced hemolytic anemia (DIHA). Three of them had received treatment for typhoid, one tuberculosis and one for fever of unknown etiology. They had received drugs like Ciprofloxacin, Quinine, Paracetamol, Sulphadoxine, Amoxicillin, Ceftriaxone, Etophylline, Theophylline etc. It was difficult to identify the drug responsible for hemolysis.

Table 2 and 3 give the mean ± SD values of different hematological parameters in G6PD deficient as well as normal males and females respectively. The results indicate that deficient subjects generally have mild anemia. Compared to normal subjects mean values of Hb were lower and the values of reticulocyte count and mean cell volume (MCV) were higher in G6PD deficient subjects. Comparison of mean ± SD values by “t” test showed significant difference in some categories (p<0.05).

The G6PD deficient and normal subjects who had Hb levels below 11 g/dl were further analyzed to rule out
the possibility of iron deficiency anemia and/or beta thalassemia trait. The numbers of subjects having MCV values ≤ 75 fl and mean cell hemoglobin (MCH) values ≤ 27 pg were comparable by $\chi^2$ test in normal and G6PD deficient groups. But those having low MCH were increased in G6PD deficiency category (p<0.05).

**DISCUSSION**

G6PD enzyme deficiency can cause jaundice at birth or chronic hemolytic anemia in the later life. In the present study majority of G6PD deficient subjects had enzyme activity below 10% of the normal value, thus suggesting WHO class II variant. Sukumar et al have observed that Vataliya Prajapati subjects have G6PD Mediterranean mutation and a C at 1311 nucleotide position. It is known that Mediterranean variant of G6PD due to C563 CT point mutation results in acute hemolysis triggered by oxidants. However according to Vulliamy et al in the middle east and India the clinical symptoms of G6PD Mediterranean mutation are mild in spite of severe enzyme deficiency. Our study suggests that most of the Vataliya Prajapati individuals live a normal life without being aware of their G6PD deficient status. The DIHA associated with hemoglobinuria and drop in Hb levels occurs in sporadic cases only.

Neonatal jaundice is the most common manifestation of G6PD deficiency. In the present study, out of eight G6PD deficient newborns, only one developed mild jaundice. Kothari et al have reported that approximately one out of 10 G6PD deficient infants develop hemolytic jaundice. Prematurity and ABO incompatibility can increase the risk of hemolytic jaundice in G6PD deficient infants.

Hematological investigation in G6PD deficient subjects revealed mild anemia. Anemic cases having MCV < 75 fl and MCH < 27 pg, which could be due to iron deficiency or β thalassemia trait, were identified in normal as well as in G6PD deficiency category. This observation suggests that Vataliya Prajapati community should be investigated for these two conditions. MCV values were raised in G6PD deficient subjects compared to normal, probably due to folate deficiency.

Sarkar et al have found hepatitis, malaria, bacterial sepsis and drug intake as the factors causing hemolytic crisis in G6PD deficient children. G6PD deficiency is known to protect the person from falciparum malaria infection as the essential metabolites necessary for survival of parasite are present in insufficient quantities. Hence the parasite cannot survive inside the RBC. However in our study some subjects had falciparum malaria in spite of having G6PD deficiency. None of the 19 subjects, who had suffered from malaria, had any problem after consumption of anti-malarials. However physicians in Surat have seen hemolytic crisis in deficient Vataliya Prajapati individuals, after intake of anti-malarials, particularly Primaquine (personal communication).

In the present study three out of 17 persons who had suffered from typhoid infection, had hemolytic crisis. It may be due to the use of Quinolones in enteric fever. An association between hepatitis A and hemolysis in G6PD deficiency has been reported in the literature.

<table>
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<tr>
<th>Parameters</th>
<th>G6PD Status</th>
<th>Cord Blood</th>
<th>≤ 12 Years</th>
<th>13 to 17 Years</th>
<th>≤ 18 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>Deficient</td>
<td>15 ± 1.9</td>
<td>10.8 ± 1.4*</td>
<td>11.8 ± 0.9</td>
<td>11.8 ± 1.5</td>
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<td></td>
<td>Normal</td>
<td>16.3 ± 2</td>
<td>11.4 ± 1.9</td>
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<td>11.8 ± 2</td>
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<tr>
<td>Reticulocyte Count</td>
<td>Deficient</td>
<td>2.15 ± 0.9</td>
<td>0.4 ± 0.09*</td>
<td>0.37 ± 0.11*</td>
<td>0.37 ± 0.11*</td>
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<tr>
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<td>Normal</td>
<td>2.15 ± 0.6</td>
<td>0.24 ± 0.13</td>
<td>0.18 ± 0.08</td>
<td>0.2 ± 0.11</td>
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<tr>
<td>Mean Cell Volume</td>
<td>Deficient</td>
<td>114 ± 10</td>
<td>84.9 ± 8.8*</td>
<td>92.1 ± 8.9*</td>
<td>93 ± 9.3*</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>102 ± 11</td>
<td>77.4 ± 9.7</td>
<td>83 ± 7.7</td>
<td>84.3 ± 9.8</td>
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<tr>
<td>Mean Cell Hemoglobin</td>
<td>Deficient</td>
<td>37 ± 2.6</td>
<td>26.4 ± 2.6</td>
<td>28.5 ± 2.8</td>
<td>29.2 ± 3</td>
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<tr>
<td></td>
<td>Normal</td>
<td>33.5 ± 3.5</td>
<td>25 ± 4</td>
<td>26.7 ± 3.2</td>
<td>27.4 ± 3.7</td>
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<tr>
<td>Mean Cell Hemoglobin Concentration</td>
<td>Deficient</td>
<td>32 ± 2</td>
<td>31 ± 2.8</td>
<td>30.3 ± 2.8</td>
<td>30.2 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>32 ± 3.2</td>
<td>32 ± 2.8</td>
<td>32.2 ± 2.9</td>
<td>32 ± 2.4</td>
</tr>
<tr>
<td>RDW</td>
<td>Deficient</td>
<td>NT</td>
<td>12 ± 1.6</td>
<td>13.5 ± 0.85</td>
<td>13.5 ± 1.1</td>
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<tr>
<td></td>
<td>Normal</td>
<td>NT</td>
<td>13.2 ± 1.6</td>
<td>13.1 ± 0.8</td>
<td>13.1 ± 0.64</td>
</tr>
</tbody>
</table>

* Statistically significant difference by “t” test (p <0.05), RDW = Red Blood Cell distribution width, NT = Not tested

<table>
<thead>
<tr>
<th>Sex</th>
<th>G6PD Status</th>
<th>No. Tested</th>
<th>MCV ≤ 75 fl n</th>
<th>MCV ≤ 75 fl %</th>
<th>MCH ≤ 27 pg n</th>
<th>MCH ≤ 27 pg %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Deficient</td>
<td>86</td>
<td>22 ± 25.6</td>
<td>55* ± 63.9</td>
<td>22 ± 25.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>172</td>
<td>49 ± 28.5</td>
<td>82 ± 47.7</td>
<td>49 ± 28.5</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>Deficient</td>
<td>40</td>
<td>4 ± 10</td>
<td>6 ± 15</td>
<td>4 ± 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>190</td>
<td>43 ± 22.6</td>
<td>73 ± 38.4</td>
<td>42 ± 22.1</td>
<td></td>
</tr>
</tbody>
</table>

* Significant increase in deficient male subjects having low MCH compared to normal subjects.
recommmended in deficient subjects. In the present study 17 G6PD deficient individuals had jaundice of unknown etiology, probably hepatitis A, but none of them showed signs of hemolysis.

Hemolytic crisis occurred in one G6PD deficient patient having tuberculosis. However to our knowledge there is no report of hemolytic crisis in tuberculosis patient.

As per the current list the drugs, which are unsafe for class II and III G6PD variants are Acetanilid, Furazolidine, Nalidixic acid, Nitrofurantoin, Primaquine, Sulphanilamide etc. Drugs, which were earlier considered unsafe for these classes but are now in the list of safe drugs, are Ascorbic acid, Aspirin, Chloramphenicol, Chloroquine, Quinine, Isoniazoid etc. It was difficult to pin point the drugs responsible for lysis in our cases. The five adult patients having hemolysis had mainly consumed Ciprofloxacin, Quinine, Paracetamol, Sulphadoxine, Amoxycillin, Ceftriaxone, Etophylline, Theophylline, Aspirine etc. Mehta et al have reported a case in which two G6PD deficient boys had severe hemolysis after drinking large quantity of soft drink containing ascorbate. It is necessary to find out if such drinks can precipitate hemolysis in this community.

Present study concludes that G6PD deficiency though highly prevalent in majority of Vataliya Prajapati subjects, it is of mild type. Hemolytic crisis can mainly occur in deficient patients having typhoid. It is recommended that every Vataliya Prajapati subject should get tested for G6PD deficiency and carry G6PD status card while visiting doctor.

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REFERENCES


Announcement

National Conference on Infectious Diseases on 17th and 18th December 2005 at Hotel Hyatt Regency, New Delhi.

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