G6PD TESTING IN MYANMAR

Dr. Nwe Nwe Oo
Deputy Director and Head
Biochemistry Research Division
Department of Medical Research
(Lower Myanmar)
Introduction

- In Myanmar G6PD testing is not a routine test.
- In clinical practice this test is usually done when patient shows the signs and symptoms of haemolysis and jaundice in neonates.
- The whole country has more than 900 hospitals and out of these about 50 hospitals can do the G6PD test.
- Most of the tests are qualitative test (Methaemoglobin reduction test).
- In private sector quantitative assay kits are used.
- For the research purposes many researchers carried out the G6PD testing with different methods.
Glucose -6-Phosphate dehydrogenase deficiency in Burma

- **Authors:** Aung-Than-Batu and Hla Pe
- **Subject population:**
  All the subjects were unselected, unrelated, healthy adult males and they were born of parents both of whom came from the same race.
Methods

1. Glutathione instability test (Beutler 1957) for the presence of G-6-PD deficiency
   a reduced glutathione (G-SH) level of less than 30mg/100ml of RBC after incubation with acetylphenylhydrazine (APH) was considered deficient.

2. Nicotinamide adenine dinucleotide phosphate linked assay method (Bishop 1966)
   It determines the red cell G6PD enzyme activity.
### Results

<table>
<thead>
<tr>
<th>Ethnic Group</th>
<th>Total number of subjects</th>
<th>Deficient subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burmese</td>
<td>200</td>
<td>9% (n=18)</td>
</tr>
<tr>
<td>Mon</td>
<td>76</td>
<td>4% (n=3)</td>
</tr>
<tr>
<td>Karen</td>
<td>98</td>
<td>14% (n=14)</td>
</tr>
<tr>
<td>Kachin</td>
<td>125</td>
<td>8% (n=8)</td>
</tr>
<tr>
<td>All</td>
<td>499</td>
<td>9% (n=45)</td>
</tr>
</tbody>
</table>
Detection of Glucose 6 Phosphate Dehydrogenase (G6PD) enzyme deficiency in the field for the treatment of malaria

- Authors: Khin Lin, Aung Than, Mya Moe, Saw Lwin and Thein Tun
Method

- Hirono et.al., 1998
- 200μl each of DAEA (Diethyl Amino Ethyl-Amide) was mixed with Sophadex A 50 gel equilibrated with 0.1 Tris-HCl buffer pH 6.4 and MgCl mixture of G6P, 0.4nM NADP and 0.2% Saponin.
- 5μl of blood was taken from finger prick and mixed with 200 μl of above solution.
- The tube was placed in dark for 15 minutes.
- Orange ring, formazan colour was developed at the top of the solution in normal subjects.
## Results

<table>
<thead>
<tr>
<th>Ethnic Group</th>
<th>Total number of subjects</th>
<th>Deficient subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burmese</td>
<td>550</td>
<td>5.3% (n=29)</td>
</tr>
<tr>
<td>Mon</td>
<td>39</td>
<td>3.3% (n=3)</td>
</tr>
<tr>
<td>Shan</td>
<td>59</td>
<td>5.1% (n=3)</td>
</tr>
<tr>
<td>Karen</td>
<td>15</td>
<td>6.7% (n=1)</td>
</tr>
<tr>
<td>others</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>689</td>
<td>5.22% (n=36)</td>
</tr>
</tbody>
</table>
Glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency in Chin state

- Author: Nwe Nwe Oo, Myat Phone Kyaw, Ye Htut, Ni Ni Zaw and Maung Maung Mya
Method:

- In male subjects: Methaemoglobin reduction test (screening test)
- Agarose gel electrophoresis (for classification of G6PD)
- In female subjects: Cytochemical staining method (Cornelis et al., 1985)
Results:

<table>
<thead>
<tr>
<th>Male /Female</th>
<th>Total</th>
<th>Moderately deficient</th>
<th>Severe deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>147</td>
<td>4.08% (n=6)</td>
<td>0.68% (n=1)</td>
</tr>
<tr>
<td>Female</td>
<td>261</td>
<td>2.29% (n=6)</td>
<td>0.38% (n=1)</td>
</tr>
</tbody>
</table>
Glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency in Kayah and Rakhine State

- Author: Nwe Nwe Oo, Myat Phone Kyaw, Ye Htut, Ni Ni Zaw and Phyo Zaw Aung
Method:

- In male subjects: Methaemoglobin reduction test (Brewer's, 1962)
- Agarose gel electrophoresis (Myint –Oo, 1995)
- In male subjects G6PD deficient or normal was screened by methaemoglobin reduction test. In this test the reagents must be fresh and have good quality. If it is not, the false positive (false deficient) can occur.
- Agarose gel electrophoresis is a semiquantitative test. This test revealed three genotypes in the subjects, namely Gd$^B+$, Gd$^B-$ and Gd Myanmar. After staining with glucose 6 phosphate, MTT, Phenozone methosulfate, NADP and Tris –HCl buffer Gd$^B+$ gave a deep purplish blue colour (normal enzyme activity) and Gd$^B-$ type gave a faint blue coloration (moderately deficient) and Gd Myanmar (severe deficient) type showed no band.
In female subjects: Cytochemical staining method (Cornelis et al., 1985)

- For differentiation of homozygote and heterozygote in female by sensitive cytochemical staining method for G-6-PD activity in individual erythrocytes. Von Noorden and Vogels, 1985. In that method considerable formazan production occurs in most erythrocytes of a healthy person and only a small percentage of the cells appeared to be negative. Two cells populations of one completely negative and other with a variable amount of formazan per cell in almost equal size would be discerned in heterozygote for G-6-PD deficiency. Homozygous deficiency leads to a population of negative cells with a few positive ones after staining.
Results:

<table>
<thead>
<tr>
<th>State</th>
<th>Male/Female</th>
<th>Total</th>
<th>Moderately deficient</th>
<th>Severe deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kayah</td>
<td>Male</td>
<td>152</td>
<td>1.3% (n=2)</td>
<td>Not detected</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>252</td>
<td>0.4% (n=1)</td>
<td>1.98% (n=5)</td>
</tr>
<tr>
<td>Rakhine</td>
<td>Male</td>
<td>151</td>
<td>3.98% (n=6)</td>
<td>Not detected</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>250</td>
<td>0.8% (n=2)</td>
<td>Not detected</td>
</tr>
</tbody>
</table>
Early screening of G6PD deficiency among the healthy children living in malaria endemic area at Bogalay Township

- Authors: Moh Moh Htun, Kyaw Soe, Min Min Myint Thu, Myat Mon Oo, Hein Sithu Aung, Hnin Nu Htwe, Mya Thandar Win, Kay Thwe Win and Ohmar

- Poster presentation: Myanmar Health Research Congress (2009)
Method: Cogent test kit (Span Diagnostic Ltd.)

- Glucose-6-phosphate dehydrogenase, present in the red cell haemolysate, acts on glucose 6 phosphate and reduces NADP to NADPH which with the help of PMS, reduces blue coloured 2,6Dichlorophenol into a colourless form. The rate of decolourization is proportional to the enzyme activity.
Results:

<table>
<thead>
<tr>
<th>Male/Female</th>
<th>Total</th>
<th>Deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>53</td>
<td>15% (n=8)</td>
</tr>
<tr>
<td>Female</td>
<td>47</td>
<td>2.13% (n=1)</td>
</tr>
</tbody>
</table>
Malaria risk areas of Myanmar

High Risk

Moderate Risk

Low Risk

Risk Free
Malaria incidence in 2006-2010

- P. falciparum 73.73%
- P. vivax 24.349%
- P. malariae 0.19%
- P. ovale 0.004%
- Mix 1.723%
Usage of Primaquine in malaria treatment

- In *Plasmodium vivax* and *Plasmodium ovale*
- Chloroquine 10mg/kg/day 1\(^{st}\) and 2\(^{nd}\) day and 5mg/kg/day on 3\(^{rd}\) day
- Primaquine 0.25mg/kg/day for 14 days except pregnancy, Infants and G6PD deficient persons
- In G6PD deficient persons 0.75mg/Kg once a week for 8 weeks
The main bottle neck for G6PD testing

- Chemical are expensive
- Chemical must be fresh and qualified
- Tests are tedious and time consuming
- Need expertises
- Not available in remote areas
- Cannot diagnose genotype
Thank You For Kind Attention