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# G6PD DEFICIENCY IN ADULT JAUNDICED SUBJECTS: A CASE STUDY IN HOSPITAL ATTENDING POPULATION OF BHOPAL, INDIA

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## ABSTRACT

Glucose 6 Phosphate dehydrogenase is the key enzyme in pentose phosphate shunt (PPS) where it catalyzes the entry step of G6P that forms part of glycolysis. In erythrocytes, this alternate anaerobic pathway for glucose metabolism remains the only source for reduced NADP. Deficiency of enzyme fails to protect RBCs from hemolysis. During the destruction of RBCs is one of the causes of jaundice in humans. The association between G6PD and neonatal jaundice has been reported independently in many countries. The present study investigates the incident of G6PD and correlates it to hematological test and serum Bilirubin test, liver enzymes in adults in hospital attending heterogeneous population of Bhopal. In thhe study patients visiting tree hospitals of Bhopal were analyzed G6PD deficiency by DCIP test for the detection of deficiency and another test like serum bilirubin; liver enzymes were profiled for correlation. The study found a significant difference in the incidence of disease prevalence between male and female. Moreover, G6PD deficiency in patients also showed a low level of hemoglobin and a significant increase in the serum bilirubin. The result of the present study was well in agreement with similar studies throughout the world. The study concludes that G6PD deficiency is predictive of an increased risk for adverse health effects that involve hemolytic anemia resulting in jaundice.

**KEYWORDS:** Glucose 6 Phosphate Dehydrogenase, Jaundice, Bilirubin, Liver Enzymes, Pentose Phosphate shunt.

## INTRODUCTION

Jaundice is a yellowish pigmentation of the skin, sclera and other mucous membranes caused by hyperbilirubinemia.[1] Jaundice is a multifactorial disorder with many symptoms primarily due to dysfunction of liver.[2] Jaundice is often seen in liver diseases such as hepatitis or liver cancer.[3] Jaundice in adults may be caused by a variety inputs either harmless or life-threatening disorders. One of the causes of jaundice in adults is hemolytic anemia, a condition in which red blood cells are destroyed and removed from the bloodstream before their normal lifespan is concluded.[4] The causes of hemolytic anemia vary and may be inherited or acquired. Inherited causes of hemolytic anemia include hereditary disorders such as G6PD deficiency is the most common enzyme deficiency in humans.[5] In the case of G6PD deficiency, the ability to protect erythrocytes against oxidative stresses from certain drugs, infections, and ingestion of fava beans is impaired leading to premature lysis of erythrocytes.[6,7] The inheritance of G6PD deficiency shows a typical X-linked pattern with a higher incidence disease in males than in females.[8]

Certain ethnic groups have a high prevalence of G6PD deficiency than others. The severity of G6PD deficiency also differs among different populations. In India, the prevalence of G6PD deficiency varies between 0-27% in different caste, tribe and ethnic groups. Prevalence of G6PD deficiency in urban and hospital attending heterogeneous populations of selective regions in India have been reported.[9-11] The symptoms associated with G6PD deficiency are basically the same for both children and adults. Despite the similarity in symptoms between children and adults, the impact on children can be greater. The association between G6PD deficiency and neonatal jaundice has been reported independently in many countries and is now unquestioned.[12,13] However, studies are meager on a G6PD deficiency in adult jaundiced subjects**.** The present study was, therefore, undertaken with the objective to assess the G6PD deficiency in adult jaundiced subjects in hospital attending heterogeneous population of Bhopal, India in the background of the fact that G6PD deficiency is predictive of an increased risk of adverse health effects that involve hemolytic anemia resulting in jaundice.

## MATERIAL AND METHODS

### Subjects

Subjects and their families frequently attending hospitals of Bhopal city for remedies of their ailments were identified for the present study. The subjects attending three selective hospitals

of Bhopal were sampled for the present investigation. For the present investigation, 1020 hospital attending subjects were tested for G6PD deficiency, anemia, and jaundice. Further, 84 adult individuals out of the total who suffered from Jaundice as diagnosed by the physicians were recruited for follow-up diagnostic tests and analysis. Identification and other essential details of the patient were recorded in structured schedules.

**Collection of blood samples**

All the consumables and chemical used for the present study were molecular biology grade and procured from HiMedia Pvt Ltd Mumbai, India. The samples were collected from the different hospital after patient and hospital consent. The source of blood samples for the present study includes Carewell Hospital, Chirayu hospital and Medical Centre, Jeevandhara Hospital, ESI Hospital Bhopal Madhya Pradesh. From the selected subjects who were suspected for jaundice and anemia the blood samples (5ml and 10ml) were collected in Becton Dickinson (BD) Vacutainer Blood Collection Tubes containing anticoagulants (EDTA) using disposable syringes and needles under sterile conditions. From each subject, after obtaining informed/written consent in the presence of a doctor from the selective subjects for Jaundice, hemolytic anemia and G6PD deficiency tests using standard techniques.

**Detection of Jaundice**

Using standard hematological protocol initial tests were carried out in the hospitals to diagnose jaundice and its severity that involved urine test in measuring the level of urobilinogen, serum bilirubin and liver enzymes as liver function tests. The physiological parameters were also determined for jaundice in selective subjects by physical examination, initial test results and medical history of the patient.

**Detection of hemolytic anemia**

Tests carried out for ascertaining hemolytic anemia involved total erythrocyte count, reticulocyte count, Heinz bodies and hemoglobin estimation by following standard hematological procedures. Here, each sample was examined and evaluated for hemolytic anemia in triplicate.

**Detection of G6PD Deficiency**

The qualitative enzymatic activity of G6PD was assessed by DCIP decolorizing test following Bernstein.[14] In this test NADPH evolved through the action of G6PD reduces the

dye DCIP into a colorless state ($DCIPH_2$). The rate and the degree of this decolorization are proportional to the G6PD activity in the RBCs. Phenazone methosulphate (PMS) was used as an electron carrier between NADPH and 2,6 DCIP in this test. 20 µl of whole blood followed by 0.5 ml of dye solution were mixed in a test tube containing 1 ml of triple distilled water and immediately overlaid by liquid paraffin, allowed to stand at room temperature ($37^oC$). The color of the mixture changes from blue to red in the presence of G6PD within 20 minutes for normal while it takes 90-120 minutes for deficient samples. The dye solution was prepared from the stock solutions containing 2 ml G6P anhydrous (50 mM/L), 10 ml NADP+ (3mM/L), 8.7 mg 2,6 DCIP (0.5 mM/L), 60 ml Tris HCl buffer (750 mM/L) and 20 ml distilled water containing 0.4 mg PMS, freshly prepared, forming the whole 92 ml is stored in dark reagent bottle at $4^oC$.

## RESULTS

### Incidence of Jaundice

Here, table 1 depicts incidence of jaundice in adults in the present study in the selected population. A total of 84 (8.23%) out of 1020 (678 males and 342 females) hospital attending subjects had shown Jaundice that involved 29 (34.52%) with normal Hb level and 55 (65.48%) with anemia. All Jaundiced subjects involved 59 (70.24%) males and 25 (29.76%) females. The incidence of Jaundice in all subjects being 1020 was 8.2% whereas; male and female populations exhibited significant variation in incidence depicting 5.8% and 2.4% respectively. Jaundice with normal Hb level was more frequent among males as compared to females being 25(29.8%) and 4 (4.8%) respectively. On the other hand, Jaundice with anemia was also more frequent among males as compared to females being 34(40.4%) and 21 (25.0%) respectively. It is important to note that incidence of jaundice with anemia was considerably higher among females as compared to females who were Jaundiced with normal Hb level confirming the general trend of high anemia incidence in most female populations in India.

Subsequently, table 2 and figure 1 depicts incidence of jaundice in gender groups. The incidence of jaundiced male subjects in all males being 678 was 8.7% whereas; incidence of female jaundiced subjects in all females being 342 was 7.3% showing the insignificant difference. As depicted earlier, jaundiced males were predominant in their incidence among all jaundiced subjects; a similar trend was noted when incidence was calculated in all male and female subjects separately being 678 and 342 respectively. The incidence of jaundice

males with normal Hb level in all males was 3.63% whereas; this in the case of the female population was 1.17. On the other hand, the incidence of jaundice males with anemia in all males was 5.01% whereas; this in the case of the female population was 6.14 again exhibiting a trend of high anemia incidence in the female population.

**G6PD Deficiency in Jaundice subjects**

Here, table 3 and figure 2 depicts G6PD deficiency in Jaundice subjects. The incidence of G6PD deficiency in jaundiced subjects was noted to be 10.71% of which majority at 9.5% had anemia. Only 1 (1.19%) out of 9 G6PD deficient jaundiced subjects had normal Hb level. Table 4 depicts the distribution of G6PD deficient and normal subjects. The total subjects encountered having G6PD deficiency was 17 out of 1020 hence, the incidence of G6PD Deficiency in all the subjects tested involving normal and jaundice was 1.67%. Incidences in males and females were 1.37% and 0.30% respectively showing statistically significant difference in males and females (Table 5 and Figure 4). Low incidence among females was obvious due to X-linked transmission pattern of G6PD gene resulting in a low probability of homozygous females for gene. Table 6 and figure 5 depicts activity of the G6PD enzyme in G6PD deficient and normal subjects. The mean G6PD enzyme activity among G6PD deficient subjects was 1.6 $\pm$ 3.4 below the normal enzyme activity. The mean G6PD enzyme activity among normal subjects was noted to be 7.9 $\pm$ 3.7.

## DISCUSSION

Jaundice in adults is usually presented with the more severe disease with poor prognosis as benign causes of jaundice are less common as compared to serious and mostly malignant. An ample approach together with proper laboratory tests is essential to find out correct etiology of jaundice in adults.[15] There may be Pre-hepatic causes assumed extremely rare in elderly patients as a cause of jaundice and can be divided into cases with increased RBC turnover involving myeloproliferative disorders (MPDs), megaloblastic anemia and hemolysis-autoimmune hemolytic anemia.[16] G6PD deficiency is also associated with RBC turnover and hemolysis, and its incidence in the adult population is not very low. Our investigation revealed the considerably high frequency of G6PD deficiency among jaundiced adults presented in the majority with anemia in heterogeneous urban population. Jaundice will be evident if the total bilirubin is >35 µmol/L. In jaundice, the essential and rapid differentiation of the main causes (hepatitis, biliary stasis, and hemolysis, resolution of hematoma or congenital causes) can often be achieved by assessing urinary bilirubin and urobilinogen.

Normal or raised urinary bilirubin with elevated urobilinogen suggests hepatocellular or increased red cell breakdown known as hemolytic jaundice.[16]

The association between G6PD deficiency and neonatal jaundice has been reported independently in many countries and is now unquestioned.[17] However, the nature of the association is far from clear, since by no means all G6PD-deficient babies develop jaundice. The extent of the risk seems to vary considerably between populations, and in the same population in different environments, and even at different times in the same population. It is affected by many genetic, exogenous and cultural factors. In areas where G6PD deficiency is commonly incidental, there remains a higher incidence of severe neonatal jaundice.[18-20] This typically occurs on Day 4 to 7, which is later than the hemolytic jaundice of ABO or Rh incompatibility. In G6PD deficient subjects, severe hemolytic jaundice resulting in kernicterus can occur beyond the first week of birth, sometimes even as late as three to four weeks of life, a situation not reported in the Caucasian population.[21,22] While the additional stress of oxidant drugs or agents used in the newborn responsible for some of the severe cases.

The symptoms associated with G6PD deficiency are identical for both children and adults. Despite the similarity in symptoms between children and adults, the impact on children can be greater. Jaundice tends to affect children in the first few months of life. It is, therefore, important to take care of potential signs of G6PD deficiency in children, particularly in boys of African, Asian, or Middle Eastern descent or if there's a family history of the disorder.[23] The association between G6PD deficiency and neonatal jaundice has been reported independently in many countries and is now unquestioned.[24] The role of G6PD is highly versatile and important in understanding etiology of the disease. As enzyme are highly promiscuous may bind to multiple substrates.[25,26] It has been proposed and demonstrated that enzyme could catalyze multiple biochemical reactions with different activities.[27-30] The diverse role of G6PD was studied[31] hence large population studies are essential to conclude promiscuous nature of G6PD.[32] However, the nature of the association is far from clear, since by no means all G6PD-deficient babies develop jaundice. The extent of the risk seems to vary considerably between populations, and in the same population in different environments, and even at different times in the same population. It is affected by many genetic, exogenous and cultural factors.

## CONCLUSION

There is increasing evidence based on research findings suggesting G6PD deficiency remains the most common human enzyme defect being present in more than 400 million people worldwide. The frequency of G6PD deficiency and occurrence of the disease varies among population due to genetic and environmental factors. The hemolytic anemia and jaundice are interlinked with increasing association with G6PD deficiency. Research investigations have shown several facts as versatile nature of G6PD and unique pattern of its inheritance. The hemolytic anemia as a result of G6PD is largely due to missense mutations in the gene. As mentioned above, the frequency of G6PD deficiency and prevalence of hemolytic anemia is multifactorial and hence it obvious to vary from population to population. Here, in Bhopal diverse endogamous ethnic population resides and entire Madhya Pradesh remains habitat of selected tribal and tribes' population. However, it's very little known about these populations and needs a proper screening to study disease etiology. Bhopal remains a historical city with industrial disaster methyl isocyanate (MIC) at December 03, 1984 and hence such study will be quite useful to understand the present scenario of G6PD in city and nearby population.

**Conflict of Interest:** The author declares no conflict of interest.

## ACKNOWLEDGMENT

I would like to thank Department of Biochemistry and Genetics, Barkatullah University, Bhopal, Madhya Pradesh, India for kind support providing resources and infrastructure for the study.

**Abbreviations;** Glucose 6 Phosphate dehydrogenase (G6PD), Methyl Isocynate (MIC), Pentose phosphate shunt (PPS), RBC Red Blood Cells (RBC), Becton Dickinson (BD), 2,6-Dichlorophenolindophenol (DCIP), NADP Nicotinamide adenine dinucleotide phosphate (NADP), Ethylenediaminetetraacetic acid (EDTA)

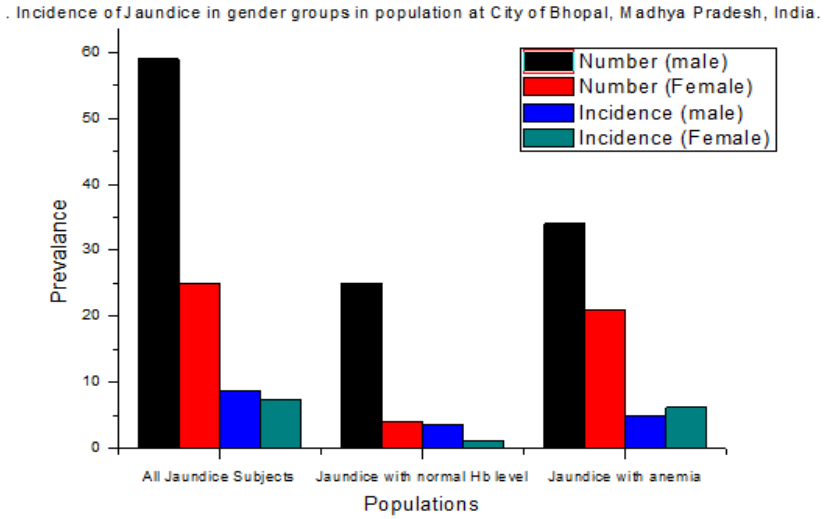



**Figure 1: Incidence of Jaundice in gender groups in population at City of Bhopal, Madhya Pradesh, India.**

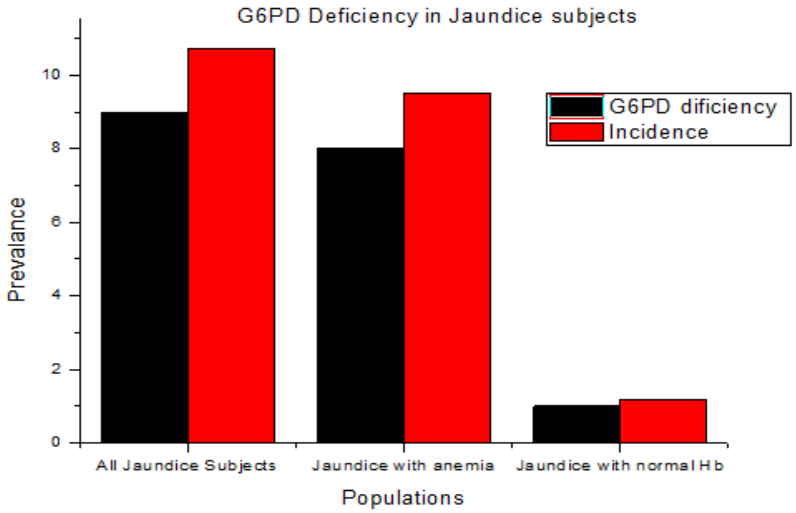
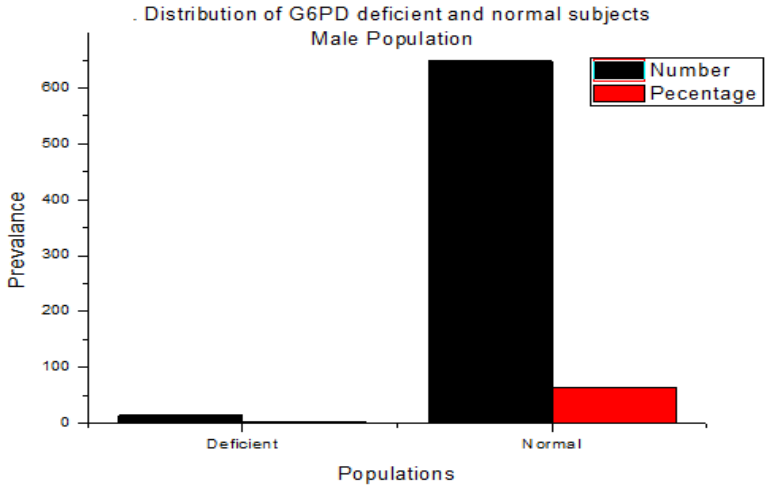



**Figure 2: G6PD Deficiency in Jaundice subjects.**



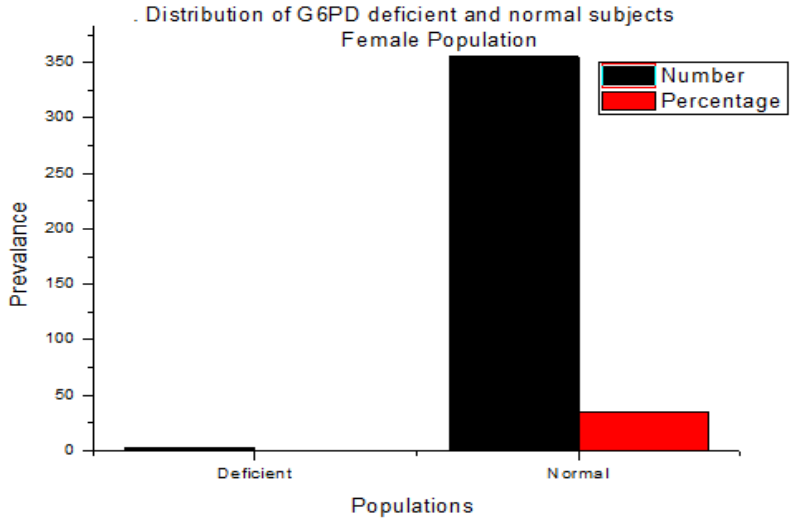



**Figure 3: Distribution of G6PD deficient and normal subjects.**

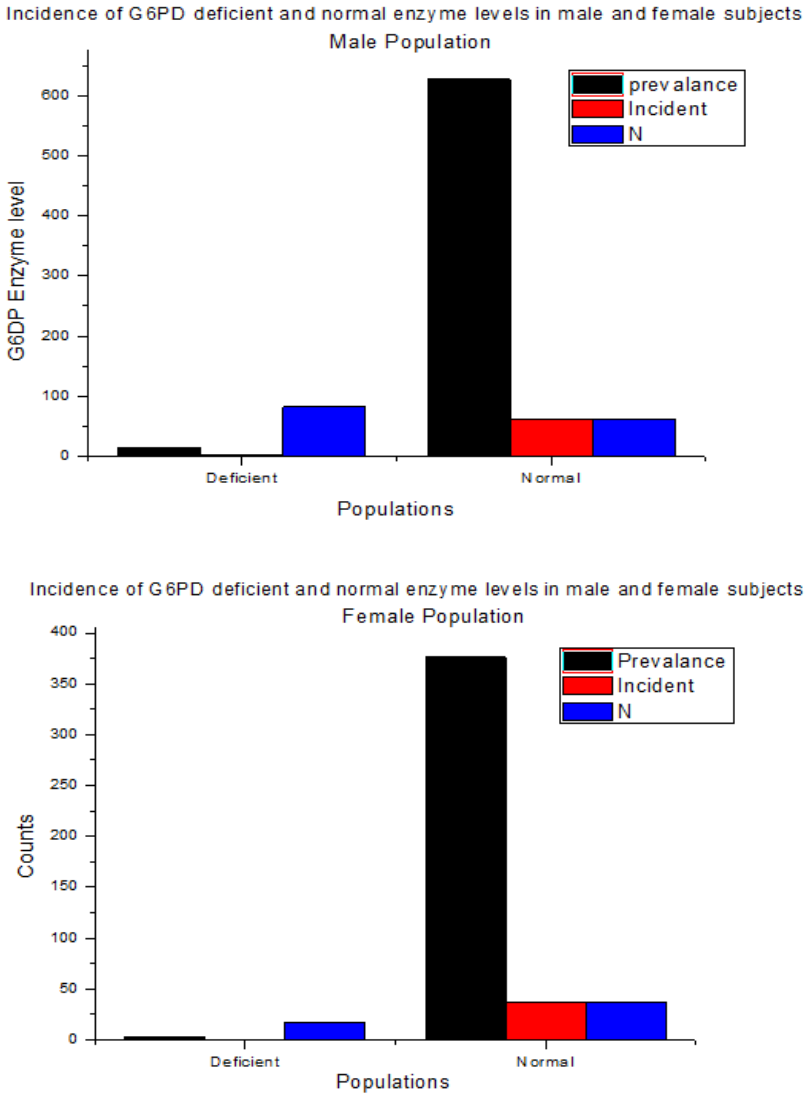



**Figure 4: Incidence of G6PD deficient and normal enzyme levels in male and female subjects.**

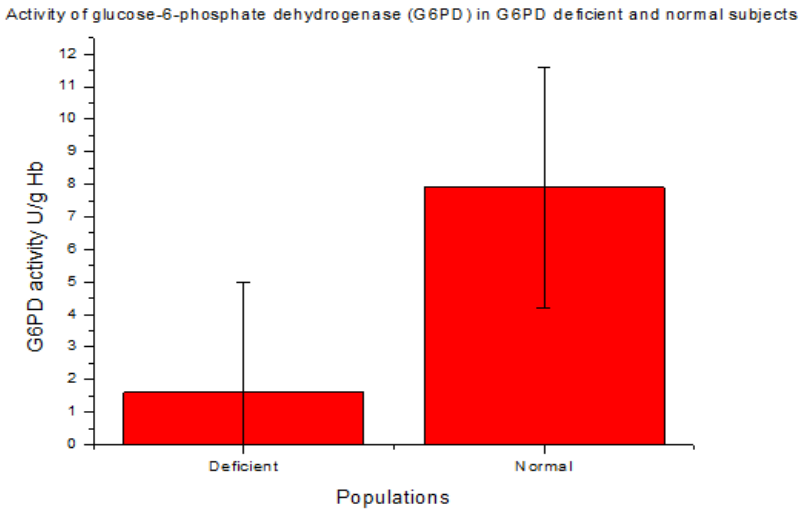



**Figure 5: Activity of glucose-6-phosphate dehydrogenase (G6PD) in G6PD deficient patients and normal subjects.**

**Table 1: Incidence of jaundice in adults population in City of Bhopal, Madhya Pradesh, India.**

| All Jaundice subjects | | | | | | Jaundice with normal Hb level | | | | | | Jaundice with anemia | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Count (n) | | | Incidence in all subjects (%) | | | Count (n) | | | Incidence in all Jaundice subjects (%) | | | Count (n) | | | Incidence in all Jaundice subjects (%) | | |
| All | M | F | All | M | F | All | M | F | All | M | F | All | M | F | All | M | F |
| 84 | 59 | 25 | 8.2 | 5.8 | 2.4 | 29 | 25 | 4 | 34.5 | 29.8 | 4.8 | 55 | 34 | 21 | 65.4 | 40.4 | 25.0 |

**Table 2: Incidence of Jaundice in gender groups in population at City of Bhopal, Madhya Pradesh, India.**

| All Jaundice subjects | | | | Jaundice with normal Hb level | | | | Jaundice with anemia | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Count (n) | | Incidence in all male subjects (%) | Incidence in all female subjects (%) | Count (n) | | Incidence in all male subjects (%) | Incidence in all female subjects (%) | Count (n) | | Incidence in all male subjects (%) | Incidence in all female subjects (%) |
| M | F | | | M | F | | | M | F | | |
| 59 | 25 | 8.70 | 7.30 | 25 | 4 | 3.63 | 1.17 | 34 | 21 | 5.01 | 6.14 |

**Table 3: G6PD Deficiency in Jaundice subjects.**

| Jaundice with normal Hb level | | Jaundice with anemia | | All Jaundice subjects | |
|---|---|---|---|---|---|
| G6PD deficient (n) | Incidence (%) | G6PD deficient (n) | Incidence (%) | G6PD deficient (n) | Incidence (%) |
| 1 | 1.19 | 8 | 9.5 | 9 | 10.71 |

**Table 4: Distribution of G6PD deficient and normal subjects.**

| Gender | G6PD normal | G6PD deficient |
|---|---|---|
| Male | 648 (63.52) | 14 (1.37) |
| Female | 355 (34.80) | 3 (0.30) |
| N (%) | 1003 (98.33) | 17 (1.67) |

**Table 5: Incidence of G6PD deficient and normal enzyme levels in male and female subjects.**

| G6PD Enzyme level | Gender* | | | | | |
|---|---|---|---|---|---|---|
| | Male | | | Female | | |
| | Count | Incidence (%) | Row N % | Count | Incidence (%) | Row N % |
| Deficient | 14 | 1.4 | 82.4 | 3 | 0.3 | 17.6 |
| Normal | 627 | 61.5 | 62.5 | 376 | 36.9 | 37.5 |

***Statistically significant difference (p<0.05).**

**Table 6: Activity of glucose-6-phosphate dehydrogenase (G6PD) in G6PD deficient and normal subjects.**

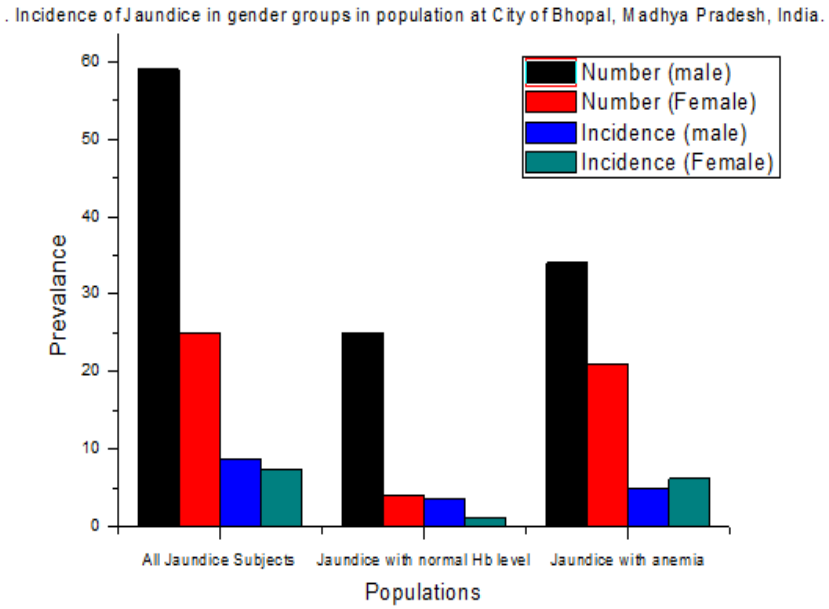
| G6PD enzyme level | G6PD activity, U/g Hb* | | | | |
|---|---|---|---|---|---|
| | Count (N) | Mean | Standard Deviation | Range | Variance |
| Deficient | 17 | 1.6 | 3.4 | 14.5 | 11.7 |
| Normal | 1003 | 7.9 | 3.7 | 16.2 | 13.9 |

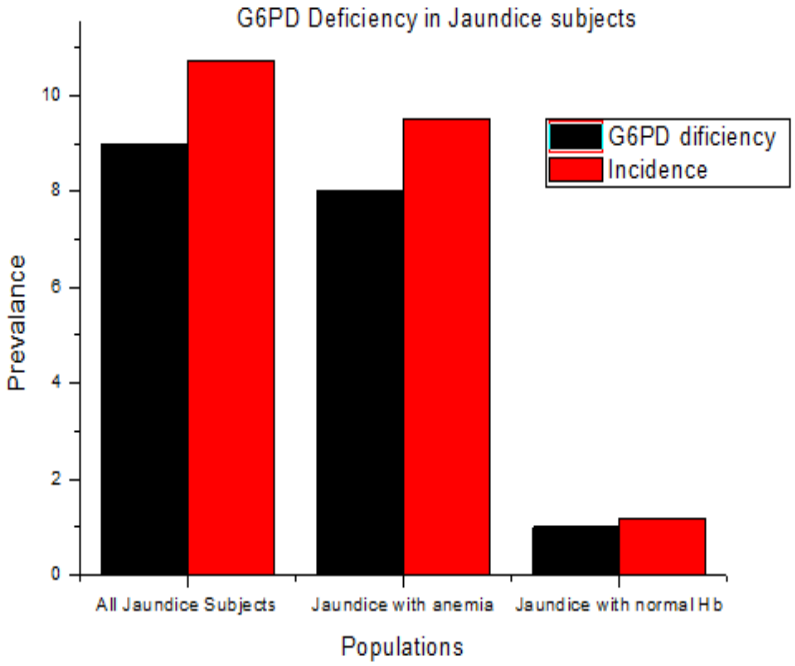
**Normal values: G6PDH activity (at 30° C): 4.6-13.5 U/g Hb**

**^Category based on G6PD qualitative DCIP decolourization test.**

***Statistically significant difference (p<0.05).**

**Figures**




G6PD Deficiency in Jaundice subjects
G6PD dificiency
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6
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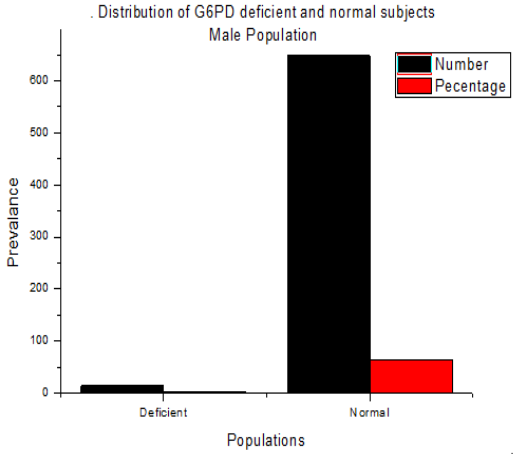
10
All Jaundice Subjects
Jaundice with anemia
Jaundice with normal Hb
Populations


. Distribution of G6PD deficient and normal subjects
Male Population
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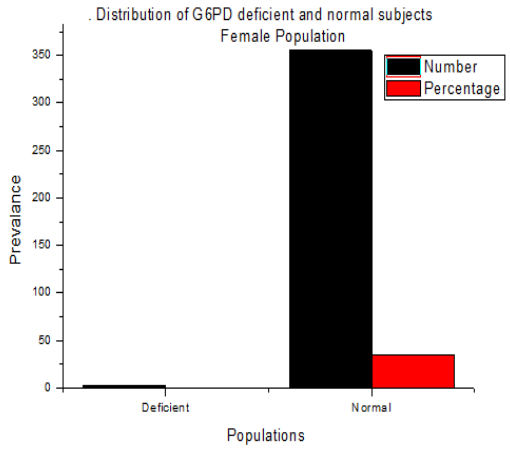
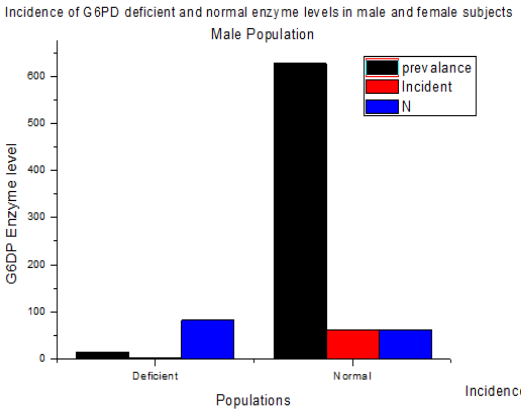
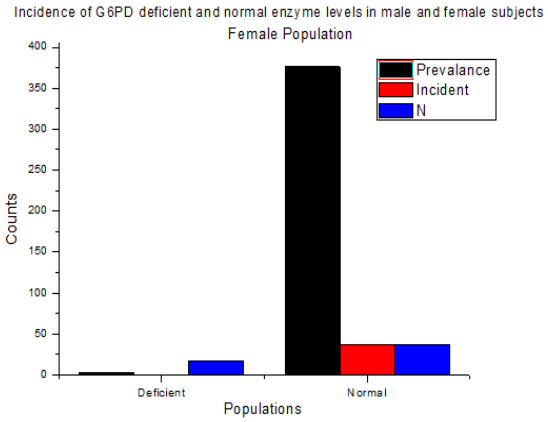
300
400
500
600
Deficient
Normal
Populations


. Distribution of G6PD deficient and normal subjects
Female Population
Number
Percentage
Prevalance
0
50
100
150
200
250
300
350
Deficient
Normal
Populations



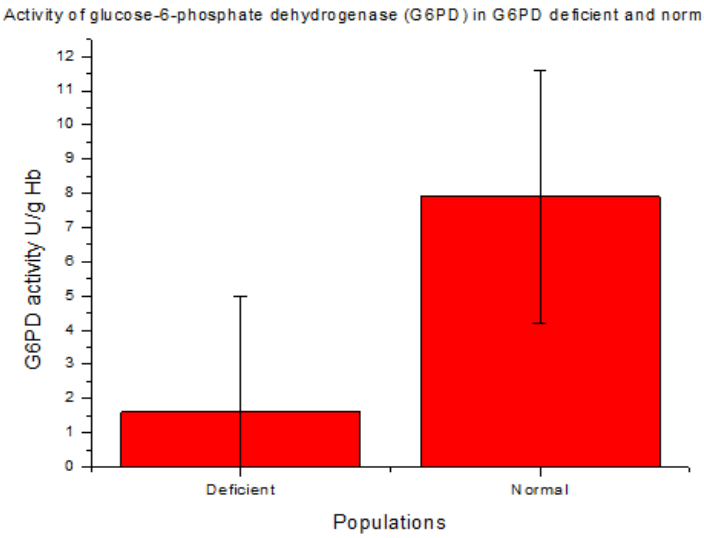




**Table 1: Incidence of jaundice in adults population in City of Bhopal, Madhya Pradesh, India.**

| All Jaundice subjects | | | | | | Jaundice with normal Hb level | | | | | | Jaundice with anemia | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Count (n) | | | Incidence in all subjects (%) | | | Count (n) | | | Incidence in all Jaundice subjects (%) | | | Count (n) | | | Incidence in all Jaundice subjects (%) | | |
| All | M | F | All | M | F | All | M | F | All | M | F | All | M | F | All | M | F |
| 84 | 59 | 25 | 8.2 | 5.8 | 2.4 | 29 | 25 | 4 | 34.5 | 29.8 | 4.8 | 55 | 34 | 21 | 65.4 | 40.4 | 25.0 |

**Table 2: Incidence of Jaundice in gender groups in population at City of Bhopal, Madhya Pradesh, India.**

| All Jaundice subjects | | | | Jaundice with normal Hb level | | | | Jaundice with anemia | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Count (n) | | Incidence in all male subjects (%) | Incidence in all female subjects (%) | Count (n) | | Incidence in all male subjects (%) | Incidence in all female subjects (%) | Count (n) | | Incidence in all male subjects (%) | Incidence in all female subjects (%) |
| M | F | | | M | F | | | M | F | | |
| 59 | 25 | 8.70 | 7.30 | 25 | 4 | 3.63 | 1.17 | 34 | 21 | 5.01 | 6.14 |

**Table 3: G6PD Deficiency in Jaundice subjects.**

| Jaundice with normal Hb level | | Jaundice with anemia | | All Jaundice subjects | |
|---|---|---|---|---|---|
| G6PD deficient (n) | Incidence (%) | G6PD deficient (n) | Incidence (%) | G6PD deficient (n) | Incidence (%) |
| 1 | 1.19 | 8 | 9.5 | 9 | 10.71 |

**Table 4: Distribution of G6PD deficient and normal subjects.**

| Gender | G6PD normal | G6PD deficient |
|---|---|---|
| Male | 648 (63.52) | 14 (1.37) |
| Female | 355 (34.80) | 3 (0.30) |
| N (%) | 1003 (98.33) | 17 (1.67) |

**Table 5: Incidence of G6PD deficient and normal enzyme levels in male and female subjects.**

| G6PD Enzyme level | Gender* | | | | | |
|---|---|---|---|---|---|---|
| | Male | | | Female | | |
| | Count | Incidence (%) | Row N % | Count | Incidence (%) | Row N % |
| Deficient | 14 | 1.4 | 82.4 | 3 | 0.3 | 17.6 |
| Normal | 627 | 61.5 | 62.5 | 376 | 36.9 | 37.5 |

***Statistically significant difference ($p<0.05$).**

**Table 6: Activity of glucose-6-phosphate dehydrogenase (G6PD) in G6PD deficient and normal subjects.**

| G6PD enzyme level | G6PD activity, U/g Hb* | | | | |
|---|---|---|---|---|---|
| | Count (N) | Mean | Standard Deviation | Range | Variance |
| Deficient | 17 | 1.6 | 3.4 | 14.5 | 11.7 |
| Normal | 1003 | 7.9 | 3.7 | 16.2 | 13.9 |

**Normal values: G6PDH activity (at 30° C): 4.6-13.5 U/g Hb**

**^Category based on G6PD qualitative DCIP decolourization test.**

***Statistically significant difference ($p<0.05$).**

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