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# Title: Prevalence of 131 G to C Single Nucleotide Polymorphism of Glucose 6 Phosphate Dehydrogenase in The School Going Children of Kolkata.

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A B S T R A C T

**Background**: Glucose 6 phosphate dehydrogenase enzyme (G6PD) is an important enzyme for the shunt metabolism of glucose in humans. G6PD deficiency is an X linked recessive disorder and its partial deficiency is linked with the malaria endemicity. Severe free radical induced cellular membrane damage causing hemolysis occurs in cases of G6PD deficiency, particularly after Primaquin therapy used for treatment of Falciparum malaria. The overall deficiency of this deficiency is about 7.7 percent in our country. Among different genetic mutations, 131 G to C conversion has been reported in Eastern part of India.

**Methodology**: Prevalence of G6PD deficiency along with its 131 G to C variants were assessed in 251 school children. G6PD was assayed using the kinetic method and was expressed in U/g of hemoglobin. The 131 G to C variants were assessed using PCR amplification of the gene followed by restriction digestion.

**Results**: Only 7 children out of 251 were found to be deficient for G6PD and out of those 7 children only 1 child was found to have the mutant 131 C variety. The chi square and odds ratio for the mutant allele distribution were 0.7 and 0.12 to 9.4 (for 95% confidence interval) respectively. P value was insignificant for both.

**Conclusion**: The G6PD deficiency in the school going children in our study region was well within the normal reference range in the context of both national and regional scenario. The 131 G to C SNP was not significantly associated with this deficiency in this region.

Key words: G6PD deficiency, School going children, single nucleotide polymorphism, 131 G to C SNP.

## INTRODUCTION

**Abbreviations:** NCBI - National Center for Biotechnology Information; EMBL - European Molecular Biology Laboratory; HGP - Human Genome Project; CADD - Computer Aided Drug Designing.

According to David Lipman, the Director of the National Center for Biotechnology Information (NCBI), Margaret Oakley Dayhoff, an American chemist, is the "mother and father of bioinformatics"1. Paulien Hogeweg and Ben Hesper in 1970 put forward the the term “Bioinformatics” as the process of study of information processes in biological system 2-4. NCBI defined bioinformatics as a combination of biology, computer science, mathematics, physics and informational technology 5. The scope of bioinformatics includes computational resources along with organization and interpretation of a wide range of biological evidence gathered from study of genetics, proteomics, metabolomics, cell biology, molecular biology and similar information.

Due to its large inclusive zone bioinformatics has become one of the most important scientific informative resource tool in every field of science. Due to recent developments in the fields of genomics and proteomics, it has become one of the most informative tool in area of different branches of life science. At an increasingly integrative level, it examines and lists the biological pathways
and systems which are significant parts of system biology. In basic biological science, it helps in the recreation and displaying of DNA6, RNA6-7, proteins8 just as bimolecular interactions 9-10.

After the discovery of protein sequence of insulin by Sir Fedrik Sanger in the early 1950s, the computers became essential in the field of molecular biology. It became quite difficult to compare multiple sequences manually. We know that Margaret Oakley Dayhoff was a pioneer in this subject 11. She gathered first primary protein arrangement databases and published as books and she also discovered the pioneer methods of sequence alignment 12-13.

Bioinformatics, since multidisciplinary in approach, is considered as computational biology at present. Biological computation combines

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bioengineering, biology and info-tech to build biological computers, whereas bioinformatics helps understanding biology more vividly by using such computations. The important role of bioinformatics in the computational biology has enabled in generation of comprehensive output of information from several newly generated organic data inputs. Using the techniques of

**Introduction**

Glucose 6 phosphate dehydrogenase enzyme (G6PD) is an important enzyme for the shunt metabolism of glucose in humans as this enzyme plays a crucial role for maintaining a major metabolic pathway in the Red blood cells (RBCs)1. It generates the important cofactor NADPH which plays a pivotal role in neutralizing the hydrogen peroxide produced in the RBCs and protects the RBCs against oxidative stress induced damages e.g. conversion of hemoglobin to methemoglobin and severe free radical induced cellular membrane damage causing hemolysis. In spite of having significant high degree of free radical production, RBCs have no other source of NADPH other than the HMP shunt pathway and so, are exclusively dependent on HMP shunt for their protective machinery against the oxidative stress. Generally, under normal non oxidative stressful conditions, RBCs express about 2 percent of their total G6PD capacity2. G6PD deficiency is an X linked recessive disorder. Mutations causing G6PD deficiency are found in the distal long arm of X chromosome. Different mutations of single nucleotide polymorphisms have been attributed for this disorder. About 140 types of mutations have been reported for this deficiency, most of which are single nucleotide base changes or single nucleotide polymorphisms (SNPs)3.

Based on genetic patterns, different G6PD variants are found in Africa, Mediterranean region and India. In southern Ghana, 12.4% of school children aged between 5 and 12 years showed a G6PD deficiency with the SNP A376G/G202A of G6PD A allele4. Considerable variation in genetic polymporphism has been observed among Indian population varying from 2.3 to 27 percent with an overall prevalence of 7.7 percent with higher frequencies in Nagaland, West Bengal, Chattisgarh, and Gujrat5. In these regions G6PD deficiency is supposed to be 10 percent among the tribals. Overall, the prevalence of G6PD deficiency vary from 1.4 to 31.4 percent in Western India6-11, 3.4 to 21.5 percent in Central India6, 12, 13, 0 to 10.6 percent in Southern India6, 111.3 to 17.4 percent in Eastern India6, 11, 14 and 1.2 to 4.4 percent in the Northern India6. Commonest polymorphic mutations among the Indian tribal population have been reported to be 563 C to T i.e the G6PD Mediterranean type that was closely followed by the 949 G to A i.e the G6PD Kerala Kalyan type and 131 G to C i.e the G6PD Odisha type15. With all these prevalence data, it has been estimated that almost 4 million male population in India suffer from G6PD deficiency and are always prone to develop severe hemolytic reaction after primaquine therapy for eradicating sexual phase of P falciparum malaria. Hence, the need for screening for G6PD deficiency by both quantitative assay in blood and genetic screening by evaluation of common SNPs are most needed. It is most appropriate to diagnose this deficiency at the earliest which can be easily done by genetic screening among the school going children.

Lacunae in the present state of knowledge in the context of G6PD deficiency in West Bengal: Although, previous studies have reported the prevalence of G6PD deficiency among some tribal groups of West Bengal14, the researchers of the present study did not find any sufficient evidence of previous studies delineating the distribution of common SNPs linked to G6PD deficiency in the urban population of our state. Keeping these factors in mind, the hypothesis for the present study was constructed that one of the common genetic polymorphism 131 G to C i.e the G6PD Odisha variety reported in our nearby state Odisa may be related significantly with G6PD deficiency in our state also. Accordingly, the researchers planned to delineate the prevalence of this mutation among the school going children (5-14 years of age) in a metropolitan urban region.

Methodology:

Children attending schools in KolkataW were chosen in the study for an early diagnosis of G6PD deficiency. Written consents were obtained from the competent authority for collection of 2 ml of blood sample from the school children for this purpose. The study was initiated after getting the approval from the Institutional Ethics Committee.

a. Study Design/ Experiment Design- The present study was undertaken as a cross sectional observational study among the school going children in Kolkata. 2 ml of venous blood were collected from each student as per aseptic protocol for analysing the study parameters.

b. Rationality for choosing school going children: Children attending school were chosen in the study for screening at the earliest possible stage and hence for a better overall management and preventive outcome.

c. Study setting & timelines: The present study was planned to be conducted in various schools in Kolkata over a period of 1 (One) year from 2019 to 2020. However, due to outbreak of Covid 19 pandemic and closure of schools, the study had to be extended till 2021 for getting the adequate number cases and experimental reagents. Students were also selected from the adolescent clinic of CNMC Kolkata during their regular health check up programmes.

d. Sample size/ Sampling Design: Considering 95% Confidence level and about 1% confidence interval and prevalence of G6PD deficiency (p) to be 8 % and an absolute error of 5, the total sample size was calculated to be about 113 using the formula 4pq/d2 where p was the prevalence of disorder (0.08), q was 1-p and d was the degree of error (0.05). By applying Design effect, the sample size was 226. Considering 10% Drop-out rate, the ultimate sample size comes to around 251. Children from 5-14 years (i.e. from Class I- Class X) from a single school and considering 50 students from each class made the total number of study participants to be 500. We considered randomly any one section out of many in each class, and only considered either the odd or even roll numbers. In this manner the final sample size came to 260 after surveying 5 schools and getting students from the adolescent clinic of CNMC, Kolkata.

e. Inclusion/ Exclusion criteria: only healthy children were included for study. Those with Haemoglobinopathies or on to any medication were excluded.

f. Study variables & parameters: Demographic parameters like age, sex, education, G6PD activity and SNPs of the target genes.

g. Data collection and Interpretation: Data from the study were analysed for analysing the prevalence of G6PD deficiency and the SNPs of the target genes. We used the SPSS software version 21 for Window for our data analysis.

h. Laboratory Investigations, Parameters & Procedures: The quantitative assay for G6PD activity in the present study was done by enzyme kinetic method. Briefly, Glucose 6 Phosphate dehydrogenase present in the test blood sample oxidised Glucose 6 phosphate into 6 phosphogluconate. In this reaction NADP+ is proportionately reduced into NADPH and the rate of its production is measured kinetically per minute at 280 nm. The rate of change in absorbance of NADPH is calculated and calibrated using the given factor to find out the enzyme activity of G6PD. Furthermore, hemoglobin content in the sample is estimated using cyanmethemoglobin method and finally, the G6PD activity is calculated in the terms of U/g of Hb.

Analysis of genetic polymorphism was done by isolation of the DNA by chloroform phenol extraction followed by its amplification by PCR and noting the polymorphism by restriction fragment length polymorphism (RFLP) using an appropriate restriction enzyme.

k) Outcome definition & Parameters: Prevalence of the 131 G to C variants was estimated among the G6PD deficient and normal controls. Both prevalence were compared by odds ratio analysis for a 95% confidence interval.

**Results:**

Out of 251 school children, the prevalence was G6PD deficiency was noted to be 2.7%. (Table 1)

**Table 1:** Prevalence of G6PD deficiency in the school going children in an urban population

| G6PD levels in U/gHb | Number of students (n = 251) |
|---|---|
| >2.6 U/g Hb (Normal) | 244 (97.3%) |
| < 2.6 U/g Hb (Deficient) | 7 (2.7%) |

Distribution of G and C alleles of the 131 G to C SNP among the study population has been shown in the Table 2.

**Table 2:** Distribution of 131 G to C variants among school going children with normal G6PD level and deficient G6PD levels.

| | Wild allele (131 G) | Mutant allele (131C) |
|---|---|---|
| G6PD normal levels i.e >2.6 U/g Hb (n = 244) | 206 | 38 |
| G6PD deficient levels i.e < 2.6 U/g Hb (n = 7) | 6 | 1 |

**Chi square = 0.70, P = 1. Odds ratio = 1.1 with 95% confidence interval of 0.12 -9.4.**

As the interval has a lower range of 0.12 that is well below 1, so there is no associated risk of the mutant C allele of this SNP with G6PD deficiency in our region.

**Discussion**:

Prevalence of G6PD deficiency in the school going children of our study population was found to be 2.7 percent (Table 1) which is within the national prevalence range of 2.3 to 27 percent but well below the national average level of 7 percent5. It signifies that children in our region are not at higher risk of G6PD deficiency and its related complications in our region. In West Bengal only the Santhal tribe from the district of Midnapur showed a high prevalence of G6PD deficiency6.

Prevalence of the mutant allele 131 G to C was also not significantly higher among the G6PD deficient children in our study population. A Fisher' exact value of 0.7 with a P value of 1 and an odds ratio interval of 0.12 to 9.4 (Table 2) also signified that the mutant allele C of the 131 G to C SNP was not a risk factor for G6PD deficiency in our region.

As G6PD enzyme is a protein enzyme, genetic defects due to mutations or SNPs can lead to its deficient expression leading to a significantly reduced G6PD level in the blood. However, several mutations and SNPs have been found in the gene of this enzyme which varies significantly from region to region and ethnic variations. In our country among the three major SNPs studies so far the 131 G to C has been found in the Eastern part of the country mainly, particularly in Odissa. However, in the school going children of West Bengal the prevalence of this SNP is not significantly more in the G6PD deficient population as evident from the present study. In future, more extensive studies are needed involving more genetic polymorphisms to generate more conclusive data.

Limitations of the study: The present study involved only one SNP i.e 131 G to C. In future we plan to include more SNPs linked with G6PD deficiency for these types of studies.

Conclusion and impact:

After obtaining the prevalence rate of the concerned mutation and its association with the G6PD deficiency in children, better guidelines for monitoring including programming for ideal anti-malarial treatment regimen can be made. Moreover, as this mutation is associated with resistance to malaria, its prevalence can also put a major impact on the prevalence of malaria in children group of this region.

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