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Research Article

Estimation Of G6pd Status And Analysis Of Its Common Genetic Variants In The School Going Population Of Kolkata.

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A B S T R A C T

Background: Glucose-6-phosphate dehydrogenase (G6PD) plays a crucial role in the Hexose Monophosphate (HMP) shunt by generating NADPH, essential for protecting red blood cells from oxidative stress. Deficiency of this enzyme predisposes individuals to hemolysis, particularly following infections or exposure to oxidant drugs such as primaquine. Although several common G6PD mutations (131G→C, 563C→T, 949G→A) have been reported across India, molecular data from Eastern India—especially Kolkata—remain limited. This study aimed to determine the prevalence of G6PD deficiency and its common genetic variants among school-going children in Kolkata.

Methods: A cross-sectional study was conducted among 260 school children aged 5–14 years in Kolkata. G6PD enzyme activity was measured using standard quantitative assays. Genotyping for three major Indian G6PD single nucleotide polymorphisms (131G→C, 563C→T, 949G→A) was performed using PCR–RFLP. The prevalence of enzyme deficiency and the distribution of Single Nucleotide Polymorphisms were analysed to assess potential associations.

Results: The prevalence of G6PD deficiency in the study population was low (2.3%). None of the three common G6PD variants—131G→C (Odisha type), 563C→T (Mediterranean type), or 949G→A (Kerala–Kalyan type)—showed significant association with measured enzyme deficiency. Despite Kolkata being a region with ongoing malaria transmission, the expected correlation between high malaria burden and higher prevalence of G6PD-deficient alleles was not observed.

Conclusion: This study demonstrates that common Indian G6PD polymorphisms are not major contributors to G6PD deficiency among school-going children in Kolkata. The overall low prevalence of deficiency in an urban malaria-affected region suggests distinct regional genetic patterns and highlights the need for broader population-based studies. The findings reinforce the value of early, school-based screening and support the use of simple point-of-care testing methods for community-wide G6PD surveillance.

Keywords: G6PD deficiency; genetic variants; PCR–RFLP; school children; Kolkata; malaria.

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) is a key enzyme in the Hexose Monophosphate (HMP) shunt, the only metabolic pathway in red blood cells (RBCs) capable of generating nicotinamide adenine dinucleotide phosphate (NADPH)[1]. NADPH maintains reduced glutathione and other antioxidant systems, protecting RBCs from oxidative injury. As RBCs lack mitochondria, adequate G6PD activity is essential under oxidative stress, such as during infections or exposure to drugs including aspirin, sulphonamides, and antimalarial agents like primaquine[1],[6].

G6PD deficiency is the most common RBC enzymopathy worldwide and is inherited in an X-linked recessive manner[2],[3]. More than 180 genetic variants—predominantly single nucleotide polymorphisms (SNPs)—have been described, with marked ethnic and geographic variation.[7],[24] Approximately 400 million individuals are affected globally, with the highest prevalence in malaria-endemic regions.[2],[8] This distribution supports the hypothesis that G6PD deficiency provides partial protection against Plasmodium falciparum, contributing to the maintenance of these alleles through balanced polymorphism.[23]

India exhibits significant molecular and ethnic heterogeneity in G6PD deficiency, with reported prevalence ranging from 2.3% to 27% across states and communities.[3],[4],[11] Eastern India, including West Bengal, shows prevalence between 1.3% and 17.4%, with certain tribal groups displaying higher rates.[4,12] Three SNPs—131C→G (G6PD Odisha), 563C→T (G6PD Mediterranean), and 949G→A (G6PD Kerala-Kalyan)—represent the most common variants in Indian populations.[3],[37],[38] However, data on their distribution among children in Kolkata remain scarce.

Screening school-aged children offers a strategic public health opportunity, as this age group is a primary target of school health programmes and malaria control initiatives[6,10]. Furthermore, primaquine, essential for blocking malaria transmission, can induce severe hemolysis in G6PD-deficient individuals[6]. Baseline epidemiological and genetic data are therefore crucial in malaria-endemic areas.

Against this backdrop, the present study aimed to estimate the prevalence of G6PD deficiency and examine the distribution of the three common SNPs (131C→G, 563C→T, and 949G→A) among school-going children (5–14 years) in Kolkata. The overarching goal was to determine whether these variants are associated with G6PD deficiency in this population.

Materials And Methods

Study Design and Participants

This cross-sectional study included school-going children aged 5–14 years in Kolkata over five years. Participants were randomly selected from multiple schools and the adolescent clinic of Calcutta National Medical College (CNMC). Healthy children of both sexes were eligible; those with hemoglobinopathies or under medication were excluded.

Ethical Considerations

The study was approved by the Institutional Ethics Committee (IEC No. CNMC/BIO/DST Project-1) and conducted in accordance with the Helsinki Declaration and ICMR guidelines. Written informed consent was obtained from parents/caregivers, and assent was obtained from children.

Sample Size Determination

Using a 95% confidence level, expected prevalence of 8%, and 5% absolute error, the minimum sample size was calculated as 113[44]. Accounting for design effect and 10% dropout, 251 children were required; 260 were enrolled.

Data Collection and Variables

Demographic variables (age, sex) and laboratory parameters (G6PD enzyme activity and SNP genotypes) were collected Primary outcomes:

Prevalence of G6PD deficiency (U/g Hb)
Prevalence of three G6PD gene variants: 563C>T (Mediterranean), 949G>A (Kerala-Kalyan), 131C>G (Odisha)

Laboratory Procedures

Measurement of G6PD Activity

Venous blood was collected in EDTA vials. G6PD activity was measured using a kinetic enzymatic assay based on NADPH generation[1]. Hemoglobin concentration was determined by the cyanmethemoglobin method, and results expressed as U/g Hb[20].

DNA Extraction and Genotyping

Genomic DNA was extracted using phenol–chloroform. Target SNPs were amplified by PCR using NCBI Primer-BLAST-designed primers and analyzed by restriction fragment length polymorphism (RFLP) with MboII (563C>T), MnlI (949G>A), and HaeIII (131C>G). Digested fragments were visualized on 4% agarose gels. Twenty samples were validated by sequencing for quality control.

Statistical Analysis

Data were analyzed using SPSS v21. Prevalence was summarized descriptively. Associations between G6PD deficiency and SNPs were assessed using Chi-square or Fisher's exact test, with odds ratios and 95% confidence intervals.

Results

G6PD Enzyme Activity in the Study Population

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A total of 260 school-going children aged 5–14 years were evaluated for G6PD enzyme levels. Of these:

- 254 children (97.7%) exhibited normal enzyme activity (> 2.6 U/g Hb), and
- 6 children (2.3%) were classified as G6PD deficient (< 2.6 U/g Hb).

The distribution of enzyme activity is summarised in Table 1.

Detection of G6PD Gene Variants

Three common G6PD gene variants—131C>G, 563C>T, and 949G>A—were analysed by PCR–RFLP. Restriction digestion produced distinct fragment patterns enabling differentiation of wild-type, heterozygous, and mutant homozygous genotypes. DNA sequencing confirmed the nucleotide substitution in the 949G>A variant.

Representative digestion patterns and sequencing chromatograms are shown in Figures 1–4.

Distribution of G6PD Variants and Association with Enzyme Levels

Allelic frequencies for the three SNPs were compared between children with normal and deficient G6PD activity.

131C>G Variant

- Normal G6PD group: 84.2% G allele, 15.8% C allele
- Deficient group: 100% G allele, 0% C allele
- No association observed ($\chi^2 = 0.70$, P = 1.0; OR = 0.40, 95% CI: 0.02–7.37)

563C>T Variant

- Normal group: 80.5% C allele, 19.5% T allele
- Deficient group: 83.3% C allele, 16.7% T allele
- Not statistically significant ($\chi^2 = 0.129$, P = 0.719; OR = 0.742, 95% CI: 0.145–3.79)

949G>A Variant

- Normal group: 86.6% G allele, 13.4% A allele
- Deficient group: 100% G allele, 0% A allele
- No significant association ($\chi^2 = 0.27$, P = 0.601; OR = 0.52, 95% CI: 0.02–9.48)

Overall, none of the three SNPs showed a statistically meaningful relationship with G6PD deficiency in this study population.

Sex-Wise Distribution of G6PD Deficiency

Fisher's exact test found no significant difference in deficiency between males and females (P = 0.43), indicating no sex-based clustering in this cohort.

Table 1.Distribution of G6PD Enzyme Activity among the Study Population (n = 260)

G6PD Activity (U/g Hb)	Number of Students	Percentage (%)
> 2.6 (Normal)	254	97.7
< 2.6 (Deficient)	6	2.3

Table 2.Distribution of 131C>G Variant among Normal and Deficient G6PD Groups

Group (n)	Wild Allele G	Mutant Allele C	χ^2	P value	Odds Ratio (95% CI)
Normal G6PD (>2.6 U/g Hb, n=254)	214 (84.2%)	40 (15.8%)			
Deficient (<2.6 U/g Hb, n=6)	6 (100%)	0 (0%)	0.70	1.0	0.40 (0.02–7.37)

Table 3.Distribution of 563C>T Variant among Normal and Deficient G6PD Groups

Group (n)	Wild Allele C	Mutant Allele T	χ^2	P value	Odds Ratio (95% CI)
Normal G6PD (n=254)	202 (80.5%)	50 (19.5%)			
Deficient (n=6)	5 (83.3%)	1 (16.7%)	0.129	0.719	0.742 (0.145–3.79)

Table 4.Distribution of 949G>A Variant among Normal and Deficient G6PD Groups

Group (n)	Wild Allele G	Mutant Allele A	χ^2	P value	Odds Ratio (95% CI)
Normal G6PD (n=254)	220 (86.6%)	34 (13.4%)			
Deficient (n=6)	6 (100%)	0 (0%)	0.273	0.601	0.52 (0.02–9.48)

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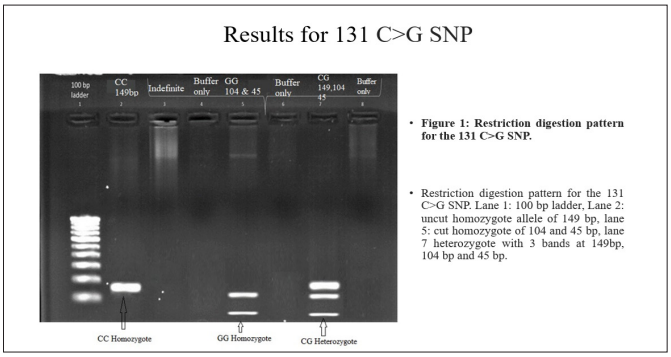

Figure 1.Restriction digestion pattern for the 131C>G SNP.
Lane 1: 100 bp ladder; Lane 2: uncut wild-type allele (149 bp); Lane 5: mutant homozygote (104 bp + 45 bp); Lane 7: heterozygote showing three bands (149 bp, 104 bp, 45 bp)

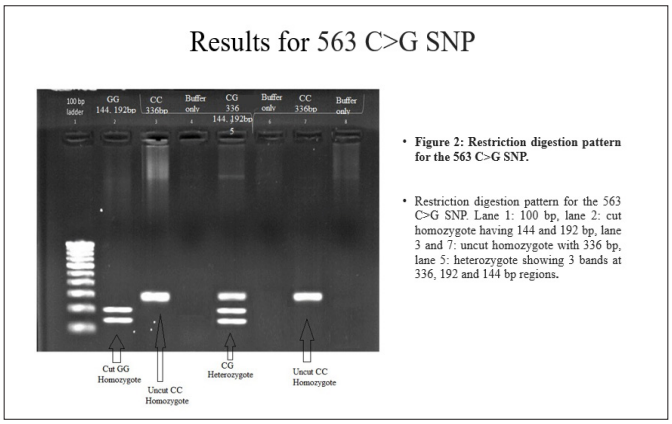

Figure 2.Restriction digestion pattern for the 563C>T SNP.
Lane 1: 100 bp ladder; Lane 2: mutant homozygote (144 bp + 192 bp); Lanes 3 and 7: wild-type (336 bp); Lane 5: heterozygote showing 336 bp, 192 bp, and 144 bp bands.

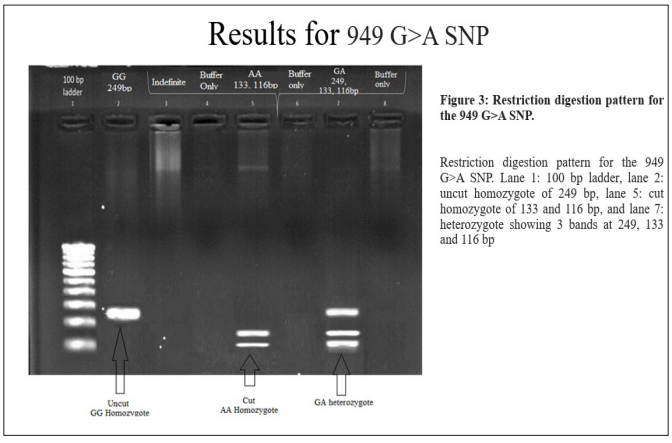

Figure 3.Restriction digestion pattern for the 949G>A SNP.
Lane 1: 100 bp ladder; Lane 2: uncut wild-type (249 bp); Lane 5: mutant homozygote (133 bp + 116 bp); Lane 7: heterozygote with three bands (249 bp, 133 bp, 116 bp).

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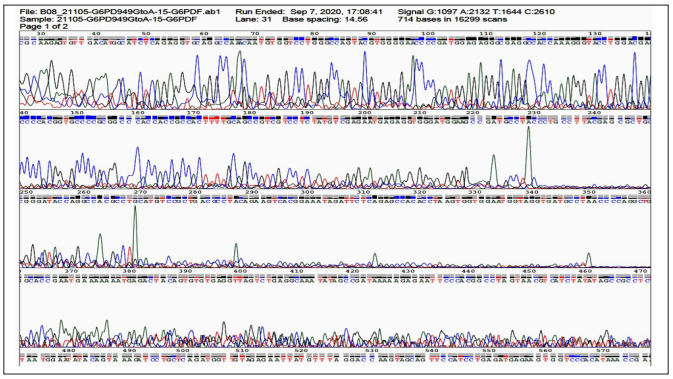

Figure 4.DNA sequencing chromatogram of the 949G>A locus confirming the A→G substitution at the 133 position of the amplicon.

Discussion

In this cohort of urban school children, G6PD deficiency prevalence was 2.3%, consistent with reports from Eastern India.[3,4,11,12] Despite Kolkata's malaria endemicity[42], the low prevalence suggests a balanced polymorphism, with protective effects against P. falciparum not necessarily leading to high deficiency prevalence[23].

Analysis of three common Indian SNPs—Mediterranean (563C>T), Kerala-Kalyan (949G>A), and Odisha (131C>G)—revealed no significant association with enzyme deficiency. Only one deficient child carried the Mediterranean mutation (16.7%), while the other two variants were absent among deficient individuals. These findings align with reports indicating substantial geographic heterogeneity and the contribution of other, less common variants to G6PD deficiency[7,37,38,42].

The primary limitation was the exclusive inclusion of school-going children, limiting generalizability to other age groups and subpopulations. Nonetheless, this group is critical for malaria control and primaquine administration[6,10]. The study highlights the need for broader community-based genotyping and validates the utility of point-of-care G6PD tests, such as fluorescent spot and chromatographic assays.[20,43,45]

Conclusion

G6PD deficiency prevalence is low among urban school children in Kolkata, and the deficiency is not significantly associated with three common Indian variants (563C>T, 949G>A, 131C>G). These results suggest the presence of other genetic determinants in this population. Further community-based studies across diverse age groups are warranted to inform targeted screening and malaria-control strategies.

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Conflict of Interest

The author declare that they have no conflict of interest related to this study.

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