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Prevalence of Glucose-6-phosphate Dehydrogenase Deficiency in Aligarh, India: A Pilot Study

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Abstract

Introduction: Deficiency of glucose-6-phosphate dehydrogenase (G6PD) enzyme in erythrocytes is among the prevalent X-linked recessive genetic disorders and affects approximately 400 million people across the globe. It causes neonatal hyperbilirubinemia, also hemolysis eventually leading to hemolytic anemia when exposed to oxidative stress. **Purpose:** The purpose of the study was to find out the prevalence of G6PD deficiency in Aligarh (Western Uttar Pradesh) India. **Materials and Methods:** The data and samples were collected from a total of 106 individuals. All the required information were recorded in a standardized questionnaire, blood was taken with their prior consent. Blood typing test and fluorescent spot test (FST) were performed, spots were arranged accordingly and observed. **Results:** Out of total 106 individuals surveyed, 10% of them were found to be deficient for G6PD enzyme, 5% were intermediate, and 85% were normal. Significant association was found for G6PD deficiency and gender ($P < 0.05$) of the individuals while non-significant association were reported for G6PD deficiency and consanguinity ($P < 0.8$) and for G6PD deficiency and blood groups ($P < 0.9$). **Conclusion:** FST is a good method for G6PD screening and detecting the deficiency in fields. This pilot study highlights the prevalence of G6PD enzymopathy in Aligarh and will help government programs of this area to prevent and control the genetic disorder.

Keywords: Blood typing, Fluorescent spot test, Glucose-6-phosphate dehydrogenase deficiency, Hemolytic anemia, X-chromosome

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Introduction

Glucose-6-phosphate dehydrogenase deficiency (G6PD) is one of the most common inherited enzymopathies in human subjects and lies in the category of X-linked recessive genetic disorders.[1] G6PD is a crucial enzyme of the pentose phosphate pathway, which provides protection to erythrocytes from oxidative stress by maintaining levels of reduced glutathione in cells.[2] Although, in most cases, the affected individuals appear asymptomatic, when they face induction of oxidative stress with foods (fava beans and legumes), drugs (primaquine and sulfa drugs) or infection with microorganisms, the condition elicits acute hemolysis.[3] The inherited deficiency also causes neonatal hyperbilirubinemia and chronic hemolytic anemia. The favorable effect of G6PD deficiency is reported to confer partial resistance to malaria in deficient males as well as in heterozygous females, explaining its higher prevalence in malaria-endemic regions.[2,4,5] G6PD deficiency was first identified in American blacks (African and Asian descent) in the course of studies of sensitivity to the hemolytic effect of primaquine.[6,7]

The telomeric region of the long arm of X chromosome (band Xq28) harbors the gene encoding G6PD and a span of about 18.5 kb.[8] Approximately 300-point mutations in the base sequence of the G6PD gene have been identified.[9] Almost 186 clinically relevant mutations have been identified for G6PD gene till date, encoding deficient variants of the physiologically normal enzyme.[8] Nearly 160 single nucleotide mutations at DNA level have been reported leading to reduction of enzyme activity.[3,10] G6PD deficiency is classified into moderate (<30% activity) and severe (<10% activity) depending on the percentage of enzyme activity.[11,12]

G6PD deficiency affects around 400 million people globally whereas in malaria endemic countries, it accounts for approximately 8%.[10,13] In India, it ranges from 2% to 27.9% in different communities[14,15] and is reported to be higher among tribals compared to caste population.[16] In general, the frequency of this defect is found to be higher in North India and West India as compared to the South India. The G6PD activity varies among males and females, males are more commonly affected than females.[2] In the present study, authors have focused on the screening of G6PD deficiency using fluorescent spot test (FST) and the blood typing test in the subjects and ascertaining its prevalence in the population from Aligarh, Western Uttar Pradesh, India. We have also evaluated the results for association with gender, consanguinity, and blood groups.

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Materials And Methods

Study Site and Data Collection

Aligarh is a city in the state of Uttar Pradesh in India and lies 307 km northwest of Kanpur and approximately 130 km southeast of the capital New Delhi. Aligarh is located at the coordinates 27.88°N and 78.08°E. It has an elevation of approximately 178 m (587 feet). The city is in the middle portion of the doab, the land between the Ganges and the Yamuna rivers. The survey was conducted for G6PD trait screening in the period between February and March 2019

on random basis. The study was confined to Aligarh city including different localities. More than 300 individuals were visited for the study but only 106 individuals have cooperated with the project fellow [Figure 1]. Data were collected on detailed interview with the respondent. Caste, consanguinity, religion, sect, age, sex, siblings live or dead, marital status, and family status (nuclear or joint) were recorded. Standing height of the subject was measured using measuring tape (Thermocare height measurement scale for kids and adults, India) to the nearest 0.1 cm and weight was measured using weighing scale (HealthSense Ultra-Lite personal scale, India) to the nearest 0.1 kg. Body mass index (BMI) was calculated using the values of height and weight for every individual in kg/m^2. All information was recorded in a standardized questionnaire.

Written Informed Consent and Ethical Approval

All the subjects gave written informed consent for the study. The study and protocol were approved by Institutional Ethical Committee, Aligarh Muslim University, Aligarh.

G6PD Deficiency Test (Beutler FST, 1966)

Preparation of stock solution

1. Glucose-6-phosphate sodium salt, 10 mmol/L. Dissolve 0.3 g of G6P in 10 ml of dH_2O. Stored at -20°C
2. β-NADP 7.5 mmol/L. Dissolve 0.0624 g in 10 ml dH_2O (-20°C for storage)
3. Saponin 10 g/L. Dissolve 0.1 g in 10 ml dH_2O. Stored at -20°C
4. Oxidized glutathione 8 mmol/L. Dissolve 0.049 g in 10 ml of H_2O (-20°C for storage)
5. Tris HCL 750 mmol/L, pH 7.8. Dissolve 9.085 g tris in 85 ml dH_2O and adjust to pH 7.8 with 6 mol/L HCL and make to 100 ml, stored at 4°C.

Preparation of working solution

In a closed tube, 0.2 ml of Glucose-6-phosphate, 0.1 ml of β-NADP, 0.2 ml of Saponin, 0.3 ml of Tris HCL buffer, 0.1 ml of oxidized glutathione, and 0.1 ml of dH_2O were added to make a volume of 1.0 ml of working solution and stored at 2°C.

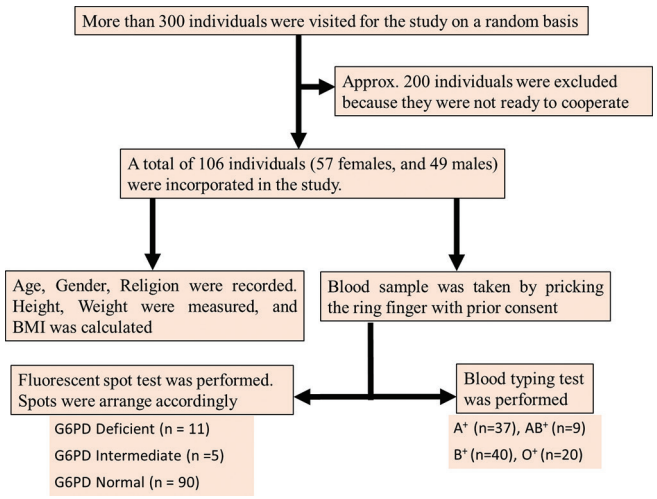

Figure 1: Study design: flowchart depicting the steps involved in recruitment process

Sample Collection, Blood Typing, and G6PD Deficiency Screening

These experiments were performed on the study site. Blood typing test of the subject was performed using blood typing kit (Tulip Diagnostics Pvt Ltd, Goa, India) provided with monoclonal antibodies (Anti- A, B and D). A clean glass slide was taken, and three circles were drawn on it. In the first circle Anti-A, in the second Anti-B, and in the third Anti-d were added using the dropper. Ring finger of the subject was wiped using alcohol swabs and blood sample was taken by pricking their fingertip with the help of needle. As blood started oozing out, it was allowed to fall on the three circles on the glass slide. Blood samples were mixed with the antibodies by using toothpick and result was observed.

For G6PD deficiency screening, 10 µl of blood was taken by micropipette and was added to 0.1 ml of working reagent in a small tube and kept at room temperature for 5 min. One drop of mixture was then put on Whatman No.1 filter paper and allowed to dry and then visually examined under long wave (340 nm) of UV light in the laboratory. A brightly fluorescing spot meant G6PD normal [Figure 2a] or positive condition, no fluorescence is G6PD deficient [Figure 2c] or negative condition. The intermediate one indicates heterozygotes [Figure 2b].

Statistical Analysis

Statistical analysis was conducted using Microsoft excel version 2019. Charts and graphs were drawn from the information given in the questionnaire. Frequency tables were made. A master chart was drawn containing all the data. Chi-square (χ^2) method was used to determine the significant difference for enzyme deficiency with consanguineous status of the subject, blood group, and gender. We took 106 samples from the survey by which normal, intermediate, and deficient subjects were identified and the results were presented.

Results

Gender, Age-group, and BMI Distribution among Surveyed Population

A total of 106 individuals were surveyed for the study in Aligarh city, out of which 57 (53.77%) were females and 49 (46%) were males [Table 1]. The age of individuals surveyed lies in the range 13–53 years. The largest age group of 16–30 years consist of 96 individuals which shares almost 90.56% of the total data and the smallest group consists of 0–15 years of age which consist only two individuals sharing 1.88% of total individuals [Table 1]. By calculating the BMI values, it was found that BMI between 18.5 and 24.9 kg/m^2 (normal BMI range) shows highest number of individuals and shares 77.36% of total data [Table 1], while individuals with over-weight BMI (25–29.9 kg/m^2) shares 10.37% and under-weight (<18.5%) shares 12.26% [Table 1] of total surveyed population.

Distribution of ABO Blood Group System

After performing blood typing test of the individuals surveyed, it was found that most prevalent blood group among them was B+, comprising approximately 38% of the total data size [Table 1 and Figures 3 and 4]. Smallest number of individuals belongs to O+ blood group (8.49%) followed by AB+ and A+ blood group. All the

Table 1: Master table showing all the parameters (numbers and percentage) recorded during survey, namely, gender (male and female), age-group (0–15, 16–30, 31–45 and 46–60 years), blood group (A+, B+, AB+, and O+), BMI: Body Mass Index, (<18.5= under-weight, 18.5–24.9= normal, and 25–29.9= over-weight), consanguinity (non-consanguineous and consanguineous), religion (Muslim, Hindu, and others)

Variables	*Normal*	*%*	*Intermediate*	*%*	*Deficient*	*%*	*Total*	*%*
Gender								
Male	40	81.63	0	0	9	18.36	49	46.22
Female	50	87.71	5	8.77	2	3.50	57	53.77
Age-group								
0–15	1	50	0	0	1	50	2	1.88
16–30	86	89.58	3	3.13	7	7.29	96	90.56
31–45	1	25	1	25	2	50	4	3.77
46–60	2	50	1	25	1	25	4	3.77
Blood group								
A+	32	86.48	1	2.70	4	10.81	37	34.91
B+	33	82.50	2	5	5	12.50	40	37.74
AB+	9	100	0	0	0	0	9	8.49
O+	16	80	2	10	2	10	20	18.86
BMI								
<18.5	12	92.31	0	0	1	7.69	13	12.26
18.5–24.9	71	86.58	4	4.87	7	8.54	82	77.36
25–29.9	7	63.64	1	9.09	3	27.27	11	10.37
Consanguinity								
Non-Cons.	86	85.14	4	3.96	11	10.89	101	95.28
Consanguineous	4	80	1	20	0	0	5	4.72
Religion								
Muslim	54	90	2	3.33	4	6.66	60	56.61
Hindu	35	77.77	3	6.66	7	15.55	45	42.45
Others	1	100	0	0	0	0	1	0.94

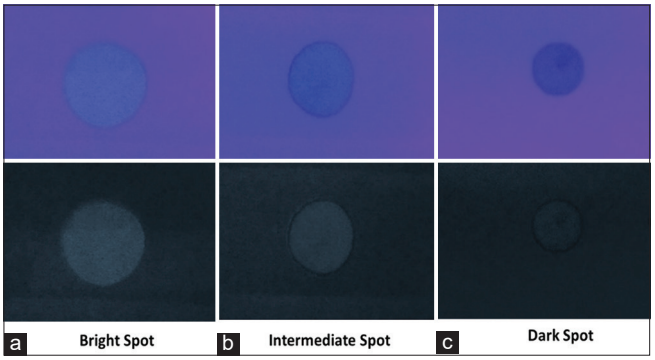

Figure 2: Depicting Whatman no.1 filter paper observed under UV light (wavelength of 340 nm), observation of a bright fluorescence under UV light means a normal condition for glucose-6-phosphate dehydrogenase (G6PD) enzyme (a), while observation of intermediate fluorescence in UV light denotes heterozygous condition for G6PD enzyme (b) and no fluorescence observed under UV light meant deficient condition for G6PD enzyme (c)

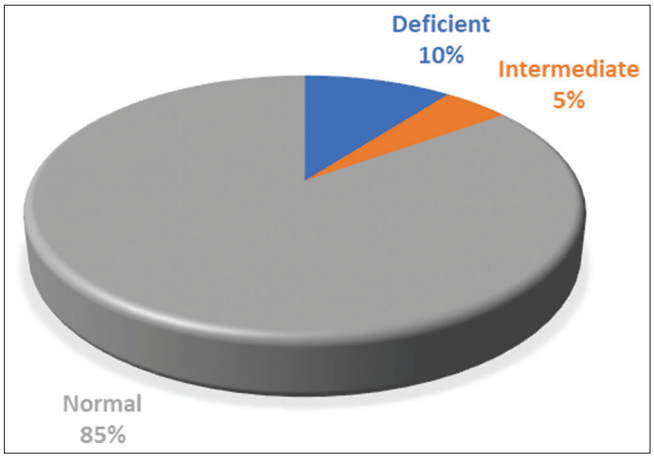

Figure 3: Pie chart depicting the distribution of G6PD deficiency in study population. Only 10% of the population identified as G6PD deficient, intermediate stands for heterozygous condition for the disease accounting 5%, rest are normal for G6PD enzyme

individuals surveyed for the study belongs to Rh+. No case for Rh^- was found in the surveyed population [Table 1 and Figures 3 and 4].

Distribution of Consanguinity and Ethnicity

From the data, it is clear that only a small percentage (approximately 5%) of the population is consanguineous. One third of the total surveyed population belongs to the Hindu and Muslim comprises 56.61% of the total surveyed population [Table 1 and Figures 3 and 4].

G6PD Deficiency

Data and samples were collected from a total of 106 individuals for G6PD deficiency screening in Aligarh city. Out of 106 individuals only 11 (10.37%) individuals were found deficient for G6PD and 5 of them were intermediate for the enzyme G6PD [Figure 5] and rest were found normal.

Gender-wise distribution of G6PD deficiency among the population of Aligarh shows that among males, G6PD deficiency accounts for 18.36% while in case of females, it is only 3.50% [Table 1]. Males being hemizygous and the allele frequency are the same as their phenotype frequencies. Among ABO blood group system, B+ blood group individuals show highest percentage (12.50%) of G6PD deficiency, while none of the subjects with AB+ blood group was found deficient for G6PD enzyme [Table 1 and Figures 3 and 4]. The categories of age-group consisting of largest percentage of G6PD deficient individuals are 0–15 years and 31–45 years which are 50% [Figure 4 and Table 1]. The individuals belonging to over-weight BMI (25–29.9 kg/m^2) accounts for 27.27% of deficient

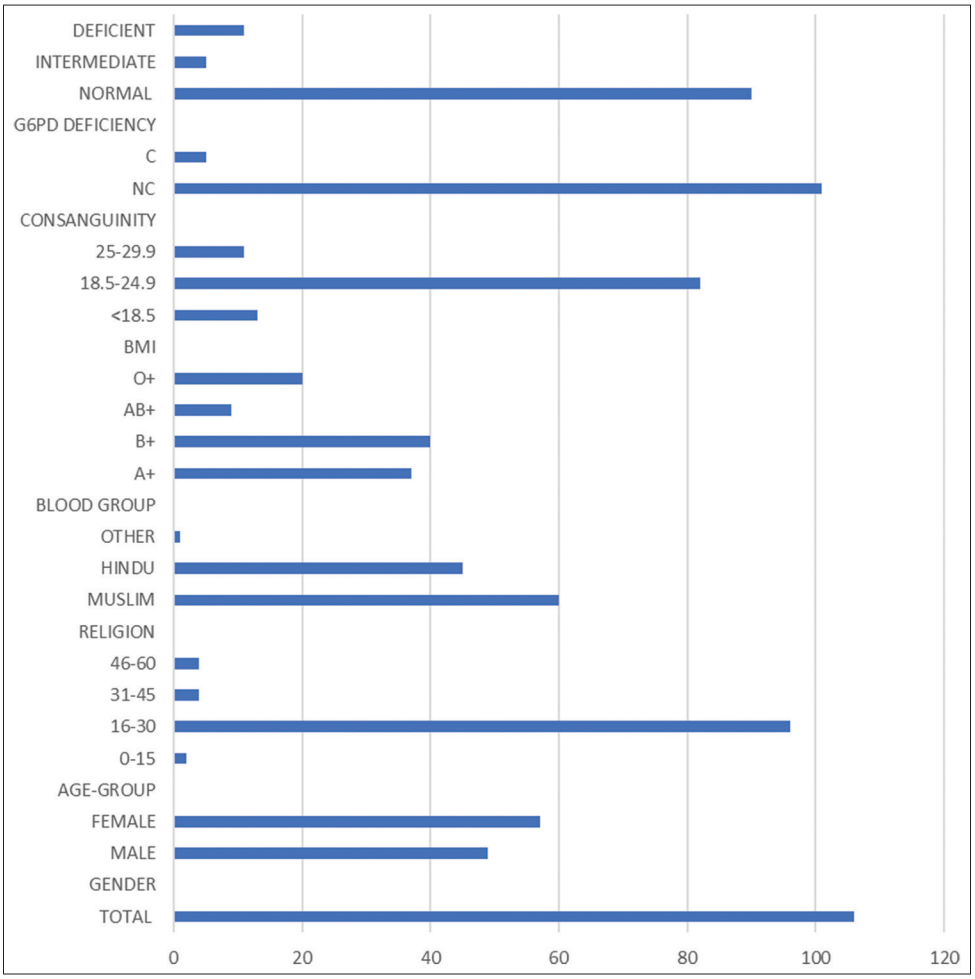

Figure 4: Bar diagram showing number of individuals for different parameters recorded during survey viz. Gender (male and female), Age-group (0-15, 16-30, 31-45 and 46-60 years), Religion (Muslim, Hindu and others), Blood group (A+, B+, AB+ and O+), BMI (Body Mass Index) (<18.5= under-weight, 18.5-24.9= normal, and 25-29.9= over-weight), consanguinity (non-consanguineous and consanguineous), G6PD deficiency (normal, intermediate and deficient)

individuals and 9.09% intermediate ones. Group of under-weight BMI (<18.5 kg/m^2) individuals accounts for 7.69% among deficient ones and normal ranging BMI (18.5–24.9kg/m^2) have 8.54% of G6PD deficient individuals [Table 1 and Figures 3 and 4]. In our data, no consanguineous individual was recorded to be deficient for G6PD screening test while 10.89% of non-consanguineous ones were found deficient. One third of the surveyed population belongs to Hindu religion, out of which 15.55% were deficient for G6PD and among Muslims, only 6.66% belongs to deficient category of G6PD screening test [Table 1 and Figures 3 and 4].

We also applied Chi-square statistics to determine the significant differences of G6PD deficiency to consanguinity, blood group, and gender. The population showing non-significant differences for consanguinity (χ^2yates = 0.351699, df = 2, *P*-value < 0.8) [Table 2] and blood group (χ^2yates = 1.1481, df = 6, *P*-value < 0.9) [Table 3] and for gender, the population showing a significant difference (χ^2yates = 8.3055, df = 2, *P*-value < 0.05) [Table 4].

Table 2: Cases of consanguinity among Normal, Intermediates, and deficient individuals with Chi-square values and Yate's correction

Consanguinity Phenotype	*NC*	*C*	*Total*
Normal	86	4	90
	e=85.75	e=4.25	0.01573
	χ^2=0.00073	χ^2_{yates}=0.015	
Intermediate	4	1	5
	e=4.76	e=0.236	0.3092
	χ^2_{yates}=0.0142	χ^2_{yates}=0.295	
Deficient	11	0	11
	e=10.48	e=0.52	0.026769
	χ^2=0.026	χ^2_{yates}=0.000769	
Total	101	5	106
	χ^2_{yates}=0.04093	χ^2_{yates}=0.310769	χ^2_{yates}=0.351699

NC: Non-consanguineous, C: Consanguineous, χ^2=0.351699, df=2, *P*<0.8

Discussion

Many methods have been reported to detect the G6PD-deficiency status in humans till date. FST has proved to be the best method in G6PD deficiency screening in fields and also it is the most widely used assay for diagnosis of G6PD in Asia.[17] Using this method, we screened a total of 106 subjects belonging to different age groups, blood groups, ethnicity etc., and identified approximately 10% of them as G6PD deficient [Figures 2 and 5] which lies close to 8.5% as

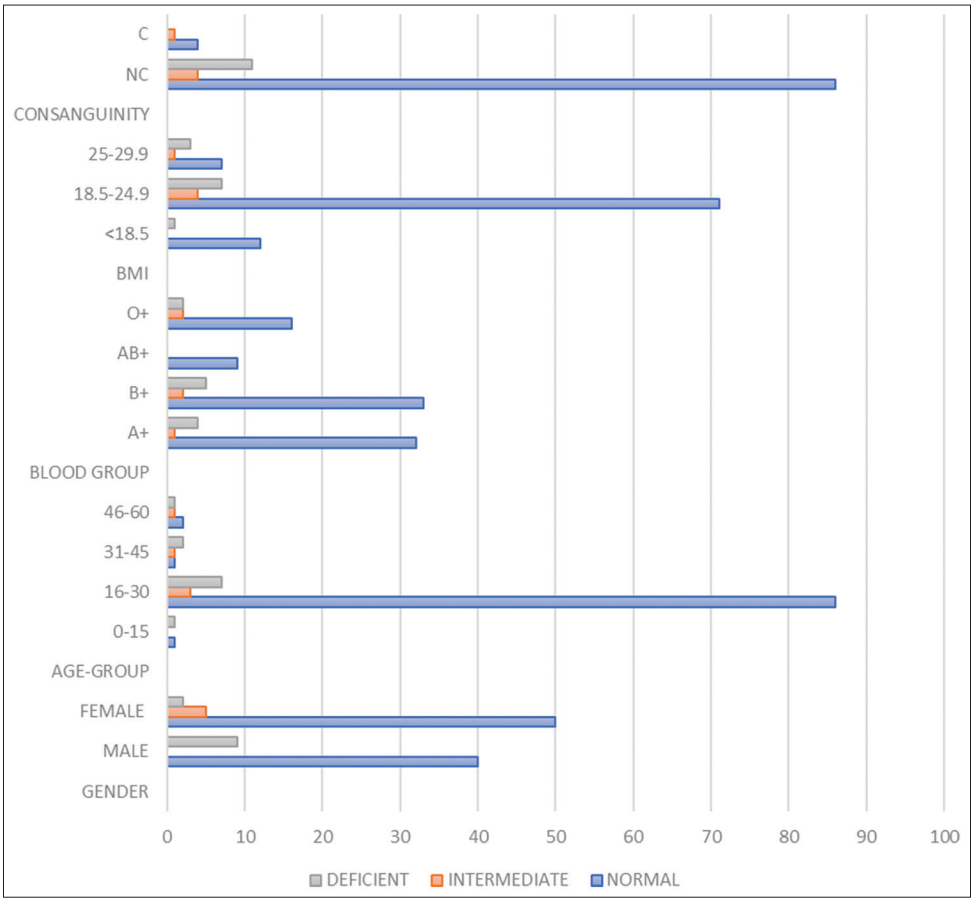

Figure 5: Bar diagram showing occurrence of different parameters with respect to G6PD deficiency (categorized into normal, intermediate and deficient). Parameters are Gender (male and female), Age-group (0-15, 16-30, 31-45 and 46-60 years), Blood group (A+, B+, AB+ and O+), BMI (Body Mass Index) (<18.5= under-weight, 18.5-24.9= normal, and 25-29.9= over-weight), consanguinity (non-consanguineous and consanguineous)

Table 3: Occurrence of Normal, Intermediates, and deficient individuals for G6PD deficiency for different blood groups with Chi-square value and Yate's correction

Blood groups Phenotypes	*A+*	*B+*	*AB+*	*O+*	*Total*
Normal	32	33	9	16	90
	e=31.41	e=33.96	e=7.64	e=16.98	0.3365
	χ^2=0.011	χ^2=0.027	χ^2=0.242	χ^2=0.0565	
Intermediate	1	2	0	2	5
	e=1.75	e=1.89	e=0.42	e=0.94	0.4701
	χ^2_{yates}=0.035	χ^2_{yates}=0.0869	χ^2_{yates}=0.0152	χ^2_{yates}=0.333	
Deficient	4	5	0	2	11
	e=3.83	e=4.15	e=0.93	e=2.08	0.3415
	χ^2_{yates}=0.0284	χ^2_{yates}=0.0295	χ^2_{yates}=0.1988	χ^2_{yates}=0.0848	
Total	37	40	9	20	106
	0.0744	0.1434	0.456	0.4743	χ^2_{yates}=1.1481

χ^2=1.1481, df=6, *P*<0.9

reported by Pradeep *et al*., (2016) in Indian population.[6] From the present study, we reported 5% individuals intermediate for G6PD enzyme and 85% as normal.

Bittles *et al*. (1991) have documented that in the societies where mother's sister's daughter and mother's brother's daughter unions are common, greater numbers of hemizygous, heterozygous, and homozygous children with G6PD mutation will be born.[18] According to our study, we did not find any significant relationship ($P < 0.8$) [Table 2] between consanguinity and G6PD deficiency;[19] this is may be due to the small sample size and also due to the ethnicity of the population. Approximately one third of the surveyed population belongs to Hindus, where marriage between close relatives or blood relatives is not generally preferred, that's why the population did not show a large proportion of consanguineous unions. Only five individuals that account 4.72% [Table 1] of the surveyed population are the offspring of consanguineous union which is actually a small number. Approximately 11% of non-consanguineous individuals

Table 4: Depicting phenotypic frequencies for both sex with Chi-square value and Yate's correction

Phenotype / *Sex*	*Normal*	*Intermediate*	*Deficient*	*Total*
Male	40	0	9	49
	e=41.60	e=2.31	e=5.084	5.049
	$\chi^2 = 0.615$	χ^2_{yates}=1.418	$\chi^2 = 3.016$	
Female	50	5	2	57
	e=48.39	e=2.68	e=5.91	
	χ^2=0.0535	χ^2_{yates}=1.236	χ^2_{yates}=1.967	3.2565
Total	90	5	11	106
	0.6685	2.654	4.983	χ^2_{yates}=8.3055

χ^2yates=8.3055, df=2, *P*<0.05

from the data are found deficient for G6PD and almost 4% belongs to intermediate category [Table 1], and no individual was found deficient for G6PD enzyme among consanguineous group.

As far as the relationship of ABO blood group and G6PD deficiency is concerned, our study suggests that there is no significant relationship ($P < 0.9$) [Table 3]. Our finding is supported by Saha *et al*., (1971). In 1971, Saha *et al*. have found that G6PD deficiency is independent of ABO blood group system.[20] In our data, we found maximum number of individuals belonging to B+ blood group, which accounts almost 38%, out of which 12.50% individuals were found to be deficient for G6PD enzyme [Table 1].

We also found that male individuals were more affected with the deficiency (18.36%) than females (3.50%) [Table 1 and Figure 4]. This genetic disorder occurs more frequently in males than in females because the red blood cell G6PD is synthesized by a gene on the X chromosome (location Xq28).[21] We found significant relationship between gender of the subject and G6PD deficiency ($P < 0.05$) [Table 4]. Unlike the males who are hemizygous for this gene and can be either normal or G6PD deficient, the females, in spite of having two G6PD genes, could be either normal or deficient (double heterozygotes or homozygous), or intermediate (heterozygous). Lyon hypothesis can explain some situations of lack of difference in prevalence between genders. Therefore, because of X chromosome inactivation, heterozygous females are mosaics and since X inactivation is non-random, there can be varying phenotypes in heterozygotes females, with normal, intermediate, or grossly deficient G6PD RBC activity.[22] On molecular basis, it is known that female subjects show mosaic phenotypes, showing normal, intermediate, or high degree G6PD deficiency depending on that lyonization of X chromosome. In fact, in females, only one X chromosome is active, the second one remains inactive as Barr bodies. This fact can explain that G6PD activity could be similar in male and in female although it was supposed to be its double.[23]

Since Hardy-Weinberg equation states that the amount of genetic variation in a population will remain constant from one generation to next in the absence of disturbing factors, our data suggest that the population is not in equilibrium, this may be due to mixed population or migration. Small size of sample also may be the cause of in-equilibrium in surveyed population.

Conclusion

The present study highlighted the prevalence of G6PD deficiency (10%) in Aligarh, Western Uttar Pradesh, and India. The FST used to detect the genetic disorder has been proved best for on-site screening of G6PD deficiency in subjects, also easy to perform and require small amount of blood sample. This is the first study to determine the prevalence of G6PD deficiency from Aligarh, India. The crucial plan of action for successful management and control of G6PD deficiency are early detection and prevention. The affected individuals are asymptomatic (until exposed to oxidative stress) and hence pass the mutation to the next generation. This pilot study will help in control and conduct of government programs for the successful management of G6PD deficiency in future.

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