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Research Article

PREVALENCE AND ALTERATION IN HAEMATOLOGICAL PARAMETERS OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENT PROTO-AUSTRALOID POPULATION OF MALARIA ENDEMIC HIMALAYAN BELT OF ASSAM, INDIA

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ABSTRACT: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common genetic disorder in malaria endemic regions and high among tribal population. To address the issue, the present study was framed to find out the prevalence of G6PD deficiency among the Proto-Australoid tribal population of malaria endemic Himalayan belt of Indo-Bhutan border areas of Assam and haematological changes in the target population. Screening for G6PD deficiency was done in 1436 normal individuals, out of which 6.62 percent (n=95) were found as deficient. Prevalence was higher in males (68.4%) compared to the females (31.6%). Complete Blood Count (CBC) was done in all samples. Further, analysis was performed to study the changes in the mean values of haematological parameters (both RBC and WBC indices) of G6PD normal and deficient subjects as well as between severe G6PD deficient and intermediate subjects. Subsequently association of haematological parameters with G6PD as well as between G6PD deficiency and gender was also studied. RBC indices *viz.*, Hb, RBC and MCHC showed significant positive correlation with G6PD. No significant correlation was seen with WBC parameters.

Key words: Glucose-6-Phosphate Dehydrogenase, Proto-Australoid population, Malaria, Haemolytic anemia, Hematology.

INTRODUCTION

The enzyme Glucose-6-Phosphate Dehydrogenase (G6PD) is a catalyst in the conversion of glucose-6-phosphate into 6-phosphogluconate, a rate limiting step of pentose phosphate pathway (Stanton 2012). The NADPH produced in this step controls the supply of reduced glutathione (GSH) to the Red Blood Corpuscles (RBC). This in turn saves the RBCs from oxidative stress (Au *et al.* 2000, Eferth *et al.* 2005). More than 400 million people of the world are affected by the deficiency of this enzyme (Nkhoma *et al.* 2009). G6PD deficiency causes premature breakdown of RBCs which results in haemolytic anemia. Generally, G6PD deficient individuals do not show any symptoms or suffer from any harmful effects, but exposure to certain factors like consumption of fava beans or certain anti-malarial drugs may trigger haemolytic anemia (Mehta *et al.* 2000). More than 400 mutants of G6PD have been reported on the basis of biochemical characterization and about 220 mutations are identified at DNA level (Gomez-Manzo *et al.* 2016). These mutations may result in changes in the protein structure thereby causing a decrease in its activity (Gomez-Manzo *et al.* 2014). The World Health Organization (WHO) has classified these mutations as Class I, II, III, IV and V based on the severity of the deficiency. Both class I and class II mutations show less than 10% enzyme activity causing chronic haemolytic anemia and periodic haemolysis respectively, and class III mutations exhibits 10-60% enzyme activity. The other two classes *i.e.*, IV and V show mild effect on the enzyme activity (WHO 1989).

The World Health Organization has established a population specific prevalence of G6PD deficiency in India ranging between 0-10%, with a higher prevalence among the tribal population (Tripathy and Reddy 2007). It has been estimated that a minimum of 3,90,000 children

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are born with this disorder every year in India (Verma and Bijarnia 2002). Higher prevalence of genetic diseases in tribal population is attributed to endogamy and consanguineous marriages. The Proto-Australoid population in Assam works in the tea gardens and they are known as tea tribe (Baishya 2016). They constitute about 17% of the total population of the state. Occurrence of G6PD deficiency has been studied in different tribal groups of India and the prevalence varies from 2.3 to 27.0 percent. Few studies have been conducted on the prevalence of G6PD in malaria endemic Eastern Himalayan state of Assam (Bhatia and Rao 1987, Islam *et al*. 2016). Recent study has reported 5.4% prevalence of the deficiency among the Mongoloid population of Tripura, Mizoram, Meghalaya and Arunachal Pradesh (Bharti *et al*. 2019) which is yet to be reported among the Proto-Australoid population of Assam. Thus, the present work is an addition towards existing knowledge pool through study on the prevalence of G6PD deficiency hither-to an understanding on alterations in the haematological parameters of the G6PD deficient Proto-Australoids of Assam, India in their usual steady state.

MATERIALS AND METHODS

Ethical approval and sample collection

The present study was approved by Institutional Ethics Committee, Bodoland University, Kokrajhar, Assam vide letter no. IEC/BU/ICMR/2019-2. Consent was obtained from the participants and parents/guardians of minors prior to the blood sample collection. A total of 1436 individuals including both male and female from four tea estates of Udalguri district were screened for G6PD deficiency using STANDARD G6PD Analyzer (SD Biosensor, Republic of Korea). All the individuals were asymptomatic to G6PD deficiency. Briefly, 10µl of whole blood was added to the extraction buffer and mixed thoroughly. From the mixture, 10µl was taken and applied to the test device for quantitative evaluation of G6PD in U/g of Hb as well as Hb level in g/dL. Blood samples (2 ml each) were collected from the deficient subjects in EDTA vials for further analysis. Complete Blood Count (CBC) was performed for all samples using Haematology Analyzer (Sysmex XP-100, Japan).

Prevalence and comparison of mean values of haematological parameters

The prevalence rate of G6PD deficiency and assessment of the mean values of haematological parameters i.e., RBC and WBC indices as well as platelet counts were calculated using SPSS (IBM Corp. released 2019, IBM SPSS for Windows, Version 26.0. Armonk, NY: IBM Corp.). RBC indices comprised of Haemoglobin (Hb), Red Blood Corpuscles (RBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Packed Cell Volume (PCV) and Red cell Distribution Width (RDW). WBC parameters include Total Leucocyte Count, Lymphocytes, Monocytes, Eosinophils and Basophils.

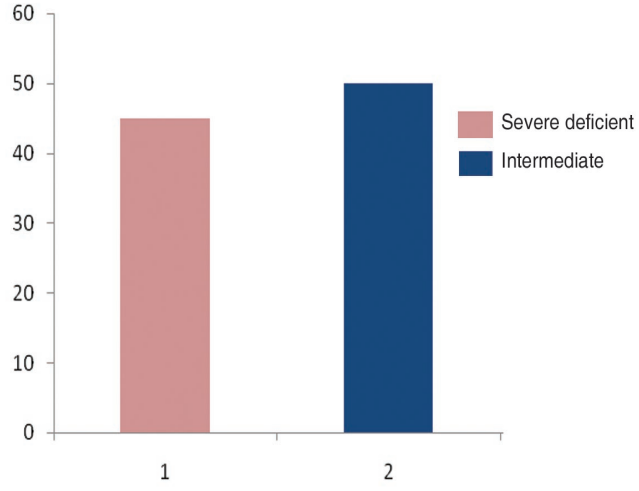

Fig. 1. G6PD status of the studied subjects.

Association between haematological parameters and G6PD

Karl Pearson's correlation coefficient was used to find out relationship between the haematological parameters and G6PD. Statistical significance between the aforesaid variables was also tested by using SPSS (IBM Corp. released 2019, IBM SPSS for Windows, Version 26.0. Armonk, NY: IBM Corp.).

Association between G6PD deficiency and gender

In the present study, we performed Chi-square test statistic to study the association between gender of the patient and G6PD deficiency.

RESULTS AND DISCUSSION

Occurrence of G6PD deficiency

A total of 1436 individuals were screened using STANDARD G6PD Analyzer. The device gives the quantitative value of Hb in g/dL and G6PD in U/g of Hb. Among these, 95 samples (6.62%) were found as G6PD deficient. Among the deficient individuals, 47.4% (45) were detected as severe deficient while 52.6% (50) were detected as intermediate (Fig. 1). This has been depicted in the recent past, wherein prevalence of G6PD deficiency in India was reported to be 8.5% (Kumar *et al.* 2016).

Previous study from North-Eastern states of India revealed that, prevalence of deficient individuals was 5.4% (Bharti *et al*. 2019). In two separate studies from Odisha indicated that, prevalence of deficiency syndrome ranged between 0.3-30.7% (Balgir 2010, Mukherjee *et al*. 2015, Kumar *et al.* 2020). The mean ± SD of RBC indices of G6PD normal and deficient subjects are presented in Table 1. Slightly lower mean values of RBC indices i.e., Hb, RBC, MCV, MCHC were seen in G6PD deficient subjects compared to the normal ones, whereas, PCV and RDW levels were increased in the deficient subjects. Table 2 represents the changes in WBC parameters of G6PD deficient subjects compared to the normal ones. The values were slightly higher in the deficient subjects. Further, comparison between severe deficiency and intermediate level of G6PD were also done. In Table 3, we have presented the mean ± SD values of RBC indices which showed lower Hb, MCV, MCH, PCV and RDW with higher MCHC and RBC count in severe deficient cases. Table 4 showed higher levels of WBC parameters in severe deficient subjects. No changes were seen in the Basophil levels in any of the cases. Platelet counts were lower in the deficient subjects compared to the normal subjects. Again, severe deficient subjects showed lower platelet count when compared with the intermediate ones (Table 5).

Correlation between haematological parameters and G6PD

The strength of association between haematological parameters and G6PD, measured by the Pearson Correlation coefficient has been tabulated in Table 6 and Table 7. The P-values for testing the significance of correlation coefficient between haematological parameters and G6PD have also been shown in the respective tables. Statistically significant positive correlations of G6PD have been observed with the RBC indices, namely, Hb, RBC and MCHC. Other RBC indices did not show significant correlation with G6PD. WBC indices such as Lymphocytes, Monocytes and Eosinophils showed negative correlation with G6PD but statistically insignificant. These observations indicate that G6PD deficient subjects were anaemic. Again, RBC, MCV, MCH levels were also low in the deficient subjects. These findings were in contrast to the previous observation of Ajlaan *et al.* (2000). They reported that G6PD deficient subjects had higher Hb, RBC, PCV, MCV and MCH level. Significant positive correlation was also reported with WBC count. Another study revealed mild anaemia and increased MCV levels in G6PD deficient subjects of Vataliya Prajapati population (Gupte *et al.* 2005). These

Table 1. Values of RBC indices of the G6PD normal and deficient subjects (Mean ± SD).

Parameter	Normal	G6PD deficient
Hb (gm/dl)	10.81 ± 1.56	10.59 ± 1.69
RBC (Million/Cumm)	4.27 ± 0.82	4.16 ± 1.04
MCV (pg)	84.45 ± 12.07	84.44 ± 11.99
MCH (fl)	25.38 ± 3.96	25.09 ± 3.89
MCHC (gm/dl)	29.90 ± 1.41	29.62 ± 1.63
PCV (%)	35.8 ± 4.73	35.43 ± 5.17
RDW (%)	15.56 ± 2.69	15.98 ± 3.07

Table 2. Values of WBC indices of the G6PD normal and deficient subjects (Mean ± SD).

Parameter	Normal	G6PD deficient
Leucocyte (Cells/Cumm)	8143.81 ± 2450.50	8161.05 ± 2441.52
Lymphocytes (%)	37.3 ± 9.09	38.68 ± 10.148
Monocytes (%)	6.31 ± 1.33	6.31 ± 1.46
Eosinophils (%)	5.70 ± 3.28	5.96 ± 3.94
Basophils (%)	0	0

Table 3. Values of RBC indices of the severe G6PD deficient and intermediate subjects (Mean ± SD).

Parameter	Severe deficient	Intermediate
Hb (gm/dl)	10.51 ± 1.71	10.66 ± 1.70
RBC (Million/Cumm)	4.33 ± 0.68	4.02 ± 1.26
MCV (pg)	82.91 ± 12.54	85.76 ± 11.46
MCH (fl)	24.711 ± 4.11	25.42 ± 3.69
MCHC (gm/dl)	29.711 ± 1.63	29.54 ± 1.64
PCV (%)	35.14 ± 5.01	35.69 ± 5.33
RDW (%)	15.85 ± 3.1	16.08 ± 3.07

Table 4. Values of WBC indices of the severe G6PD deficient and intermediate subjects (Mean ± SD).

Parameter	Severe deficient	Intermediate
Leucocyte (Cells/Cumm)	8356.82 ± 2085.54	7992.16 ± 2720.94
Lymphocytes (%)	38.84 ± 9.31	38.55 ± 10.89
Monocytes (%)	6.34 ± 1.58	6.27 ± 1.36
Eosinophils (%)	6.77 ± 5.13	5.25 ± 2.34
Basophils (%)	0	0

Table 5. Platelet count (in 10^3 µl).

Case	Mean ± SD
Normal	205.82 ± 66.64
G6PD deficient	199.27 ± 70.06
Severe G6PD deficient	192.7 ± 63.42
Intermediate G6PD	204.80 ± 75.39

Table 6. Pearson correlation between RBC indices and Platelets of G6PD deficient subjects with G6PD along with p-values.

Parameter	Pearson Correlation Coefficient with G6PD	p-value
Hb (gm/dl)	0.081	<0.05
RBC (Million/Cumm)	0.067	<0.05
MCV (pg)	-0.010	>0.05
MCH (fl)	0.012	>0.05
MCHC (gm/dl)	0.069	<0.05
PCV (%)	0.065	>0.05
RDW (%)	-0.046	>0.05
Platelets (10^3 µl)	0.059	>0.05

Table 7. Pearson correlation between WBC indices of G6PD deficient subjects with G6PD along with p-values.

Parameter	Pearson Correlation Coefficient with G6PD	p-value
Leucocyte (%)	0.047	>0.05
Lymphocytes (%)	-0.050	>0.05
Monocytes (%)	-0.007	>0.05
Eosinophils (%)	-0.012	>0.05

Table 8. Cross tabulation between G6PD deficiency and gender.

G6PD status	Gender		Total
	Male	Female	
Deficient	65 (68.4%)	30 (31.6%)	95 (100%)
Normal	208 (29.6%)	495 (70.4%)	703 (100%)
Total	273 (34.2%)	525 (65.7%)	798 (100%)

differences in observation of haematological parameters might not be solely due to G6PD deficiency since the role of G6PD deficiency on haematological parameters is not yet clearly understood (Tsegaye *et al*. 2014). Some earlier studies have reported a significant decrease in the haematological indices of G6PD deficiency, however, the deficient subjects had haemoglobinopathies too (Pengon *et al.* 2018, Balgir 2008).

Chi-square statistic for testing association between G6PD deficiency and gender

Statistical analysis was performed in order to study the association between G6PD deficiency and gender in our study subjects. The contingency table showing cross tabulation between G6PD deficiency and gender has been shown in Table 8. It shows that 31.6% (30) of the deficient cases were females with intermediate form of deficiency. This observation was similar to a previous observation that the prevalence of intermediate G6PD activity in females was 33.3% (May *et al.* 2000). The remaining deficient cases were males with either severe or intermediate form of deficiency. The value of Chi-square was = 54.66 with p-value <0.01, which indicated highly significant association between the two attributes, G6PD deficiency and gender. It has been observed from earlier studies that the prevalence of the deficiency in males was more than 60% (Goyal *et al.* 2015, Lauden *et al.* 2019, Sharma and Sharma 2019). We found that 68.1% of the deficient individuals were males which confirmed that the prevalence of G6PD deficiency was higher in males compared to females. This is in concordance with the X-linked inheritance pattern of G6PD (Kumar *et al*. 2016, Sanephonasa *et al*. 2021)).

CONCLUSION

Finally, it may be concluded that prevalence of G6PD deficiency in the tea tribe population was found to be 6.62%. Although the G6PD deficient individuals were not affected during their normal steady state, changes in the haematological parameters were seen. RBC indices like Hb, RBC and MCHC were positively correlated in the deficient subjects. No significant correlation was seen between other RBC indices as well as with WBC indices. Availability of population specific data on haematological parameters may help early detection of the enzymopathy as well as clinical advice in cases for malaria endemic regions and thus helpful for avoidance of future haemolytic crisis.

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