Original article

Comparison of prevalence of G6PD deficiency in General Population and admitted symptomatic children of Gandhi Memorial Hospital associated to Shyam Shah Medical College, Rewa,MP,India.

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ABSTRACT

Introduction: Malaria and infectious diseases both are highly prevalent in Rewa region. Anti-malarial drugs and oxidant antibiotics are the mainstay of treatment and these drugs sometime became disastrous when given to G6PD deficient individuals. In G6PD deficient individuals, sthese oxidant drugs cause hemolysis manifesting as moderate to severe morbidity; and in severe cases may lead to mortality. Therefore, knowledge of prevalence of G6PD deficiency in Rewa division is important, so that essential steps could be taken to avoid and combait complications related to G6PD deficiency.

Methods: The present study was conducted on 600 children; 300 from general population group (control group) and 300 from symptomatic group (admitted in hospital).G6PD quantitative test was used for the diagnosis of G6PD deficiency.

Observations and Results: Prevalence was found 1.7% in general population and 3.7% in admitted symptomatic children but no significant difference was found (P>0.05). The enzyme deficiency non-significantly involved male gender more than female gender (P>0.05) and the disease was found to be significantly more concentrated in the tribal community than the other communities (P<0.05).The disease was precipitated by both, drugs and infections.

Conclusion: From the study, we concluded that, all symptomatic children (anemic or jaundice) of Rewa division should be investigated for G6PD deficiency before giving them any oxidant drugs. Government should launch educational awareness program along with investigating facility at community health centers (CHC). There is need to train the pediatricians for cost effective management of G6PD deficient patients.

Keywords: G6PD deficiency, oxidant drugs, hemolysis, G6PD prevalence

INTRODUCTION

Glucose – 6 – phosphate dehydrogenase (G6PD) deficiency is the most common red cell enzymopathy in humans. A conservative estimate is that at least 400 million people have a G6PD deficiency gene (Nihoma et al, 2009) [1]. The disease has been reported in peoples from nearly all geographical locations; however it occurs most frequently in areas where plasmodium *falciparum* malaria had been endemic.[2]

In India, about 2 million confirmed malaria cases are reported annually.[3] Thus malaria imposes great socio-economic burden on humanity. When severe G6PD deficiency complicates malaria infection, treatment with Primiquine or Dapsone can lead to life threatening acute intravascular hemolysis and acute renal failure.[4]Viral hepatitis in the presence of G6PD deficiency associated with severe hemolysis, renal failure, hepatic encephalopathy and even death. To avoid there complications, screening for G6PD

deficiency should be instituted in early life and those found deficient. Should be vaccinated against hepatitis A and B.[5]

G6PD deficiency in the Indian community was first reported from the Parsi population of Mumbai in the year 1963 by Baxi et al[6]. Bhasin and Walter in 2006 reviewed the prevalence and distribution of G6PD deficiency in India by pooling data from 224 different studied based on geographical, occupational, ethnic and linguistic categories. Higher prevalence was reported from North and West than south India. Studies from the Eastern parts of India were few. In southern India only tribals of Tamil Nadu and Andhra Pradesh show high prevalence. The occupational groups did not show any difference in the prevalence of G6PD deficiency.[7]

In Rewa division both malaria and infectious diseases (including hepatitis) is highly prevalent; but lack of local evidence for prevalence of G6PD deficiency is a big obstacle towards health management of children in these regions. This study tries to bridge the gap of knowledge towards health care.

The present study was aimed :

1. To find incidence of G6PD deficiency in 'symptomatic children' admitted in Gandhi Memorial Hospital, Rewa M.P.
2. To find prevalence of G6PD deficiency in 'asymptomatic general population' of Rewa division.
3. To find which community has more prevalence of G6PD deficiency.
4. To find common precipitating factors for hemolysis in G6PD deficient subjects of Rewa division.

MATERIAL AND METHODS

Study Design:This prospective case study was done on 600 children having age between 1 to 12 years. Children were kept in two groups: control group and symptomatic group.

In control group, there were 300 children of which 168 (56%) were male and 132 (44%) were female. Children in this group were normal (asymptomatic) and selected randomly from various schools of the Rewa District. In symptomatic group, also 300 children were taken who were admitted to pediatric ward of Gandhi Memorial Hospital associated with Shyam Shah Medical College, Rewa, M.P, India. In this group subjects were either anemic (pallor) or jaundice (icterus) or having both symptoms and consisted of 174 (58%) males and 126 (42%) females. Mean age of children in control group was 5.72± 1.8 year and in symptomatic group was 4.9±1.9 year. Study was conducted in the department of pathology of Shyam Shah Medical College, Rewa, India.

SAMPLE COLLECTION AND METHODS

Three milliliters (3ml) of whole blood was collected from each children in vacutainer tubes (Pammvi Exports Private Limited, Mumbai India) containing ethylene-diamine-tetra-acetic acid (EDTA) as anticoagulant. The EDTA – anticoagulated blood was used for the screening of the subjects for G6PD deficiency using the Randox G6PD quantitative in vitro test (Randox laboratories, Crumlin, UK). Its principle is based on reduction of NADP+ by G6PD present in red blood cells. The NADPH generated fluoresces under ultraviolet light at a wave length of 340nm. Enzyme activity was determined by the rate of absorbance change. G6PD enzyme activity of ≥ 6.97 U/g Hb was regarded as normal, while value <6.97 U/g Hb were regarded as deficient at 37°C. Hemoglobin (Hb) was determined by using cell counter "ERMA PCE 210 (N)" (Diamond

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Diagnostic, Holliston, Massachusetts, USA). Hemoglobin <11g/dl were considered as anemic. Bilirubin in serum was determined by using fully automated "A 25 Random Access Analyzer" (Biosystems Diagnostics, Pvt. Ltd, Barcelona, Spain) and value >1.3 mg/dl were regarded as jaundice. Blood film was prepared using the push-wedge method and stained by Leishman stain to find out infectious cause (Bacterial or viral), if present.

Statistical Analysis:

Statistical analysis was done by using Minitab Software (Minitab Incorporated, Pennsylvania, USA, version 17.1.0). Parametric data and non-parametric data were compared by student's t-test and Chi-square test respectively. P value of <0.05 denoted a statistically significant difference in all statistical comparisons.

OBSERVATIONS AND RESULTS

Present study was conducted with the aim to find out difference in prevalence of G6PD deficiency among general population and children admitted to pediatric ward of Gandhi Memorial Hospital, Rewa ,MP,India. Table 1 shows that the prevalence of G6PD deficiency in "General Population" was 1.7% (5/300). Table 2 shows that the prevalence of G6PD deficiency in "Admitted Symptomatic Children" was more than two times (3.7%, 11/300) as compared to "General Population".

Table – 1: Prevalence of G6PD deficiency in control group.

	Overall Prevalence	Sex Distribution		Prevalence in different community		Association Pearson's Chi-square test	
		Male	Female	Non-tribal community	Tribal community	B/w male & female	B/w Tribal and Non-tribal
Total subjects	300	168	132	213	87	X^2 =1.19 p value =0.28 Not Significant	X^2 =6.42 p value =0.01 Significant
Deficient subjects	5	4	1	1	4		
Prevalence	1.67%	2.38%	0.76%	0.47%	4.6%		

Table – 2 : Prevalence of G6PD deficiency in admitted symptomatic children.

	Overall Prevalence	Sex Distribution		Prevalence in different community		Association Pearson's Chi-square test	
		Male	Female	Non-tribal community	Tribal community	B/w male & female	B/w Tribal and Non-tribal
Total subjects	300	174	126	211	89	X^2 =0.15 p value =0.70 Not Significant	X^2 =10.15 p value =0.001 Significant
Deficient subjects	11	7	4	3	8		
Prevalence	3.67%	4.02%	3.17%	1.42%	8.99%		

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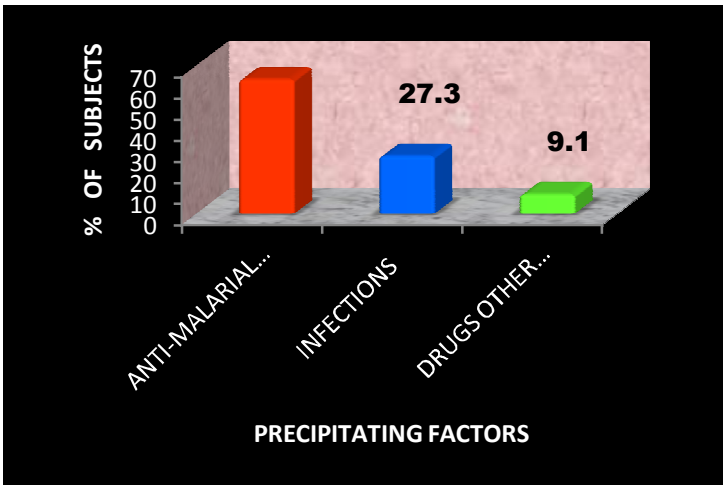
Both table shows that the deficiency was non-significantly more concentrated in males than females (P<0.05). Deficiency was significantly more prevalent in tribal community as compared to general population as evidenced by table 1 and table 2. Fig.1 shows that, among admitted G6PD deficient subjects, the most common precipitating cause of anemia or jaundice were anti-malarial drugs contributed 63.6%(7/11) of all factors and infections contributed 27.3% (3/11).

Fig. 1 Cause of precipitating hemolysis in G6PD deficient subjects.



DISCUSSION

Our country is endemic region for malaria. 1.5 to 2 million confirmed cases are reported annually[8]. Also, infectious diseases contribute about 30% of the disease burden in India.[9] In management of these diseases many drugs are used, which may cause severe hemolysis (Table 3 and 4) and leads various degree of morbidity and mortality in G6PD deficient individuals. Prevalence of G6PD deficiency in our country varies between 0-28%.[10] In area where prevalence of G6PD deficiency is high, before giving drugs (mentioned in table 3 and 4), it is necessary to investigate the patient for G6PD deficiency.

Table-3 Drugs to be avoided by persons with G6PD deficiency (Beutler Ernest, 2011)

Acetanilid
Dapsone
Diaminodiphenylsulfone
Cotrimoxazole
Furazolidone (Furoxone)
Glibenclamide
Isobutyl nitrite
Methylene Blue
Naphthalene

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Niridazole
Nitrofurantoin (Furadantin)
Nalidixic acid
Phenazopyridine (Pyridium)
Phenylphdrazine
Primaquine
Sulfacetamide
Sulfanilamide
Sulfapyridine
Sulfamithoxyzole
Thiazolesulfone
Trinitrotalurenbe (TNT)
Urate oxidase

Table -4 Drugs that carry possible risk of hemolysis in G6PD deficient individuals

Acetaminophen (paracetamol)
Acetylsalicytic acid (Aspirin)
Ascorbic acid (Vitamin C)
Aminopyrine
Chloramphenicol
Proguanil
Chloroquine
Ciprofloxacine
Colchicine
Isoniazid
L-Dopa
Norfloxacin
Phenytoin
Proguanil
Quinine
Streptomycine
Sulfadiazine
Vitamin K

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In our study, we found prevalence of 3.7% of G6PD deficiency in admitted symptomatic children. But, in general asymptomatic population prevalence was found 1.7%. Our finding was in accordance to Pao et al,[11] who show prevalence of 2.0% in Indian general population in his retrospective hospital based study (on 2479 males and females). Jain[12] found prevalence of 1.8% in Udaipur Rajasthan in general population (studied on 9433 individuals). We found more than two times prevalence in admitted children (3.7%) because they are selective in nature and chances becomes more positive for G6PD deficiency.Another finding of our study was that, the deficiency was more concentrated in males (2.4% males in general population; 4.0% in admitted children). Similarly, high predominance of disease in male was shown by Pao et al[11] (2.8% male, 1.1% female) in his retrospective hospital based study and by Joshi et al[12] (27.9% male, 9.7% females) in Vataliya Prajapati communities from Surat.

In G6PD deficiency disease males are predominantly involves, since disease is X-linked recessive.

But, Ramadevi et al[13] in her studies on neonates from South India found equal prevalence of disease in males and females. Wang et al in his study on children in Malaysia indicated that sex was not a significant predictor associated with actual G6PD enzyme levels.[14] This can be explained by the fact that hetrozygously deficient women have a mixed population of erythrocytes, owing to random inactivation of one of the two x-chromosomes, known as lionization (Lyon 1961; Davidson et al 1963). Due to lionization some of the heterozygote females are deficient and others are normal.[15]

Our study shows that the disease is significantly ($P<0.05$) more prevalent in tribal population of Rewa district (Table 1 & 2). Many authors from other regions of Indian subcontinent, also supported this facts.

Bhasin and Walter[10] in 2006 reviewed the prevalence and distribution of G6PD deficiency in India by posting data from 224 different studies based on geographical, occupational, ethnic and linguistic categories. Higher prevalence was reported from tribal groups. Saha et al[16] (Nine Mongoloid tribes of Eastern India). Rao et al[17] (tribes of Maharastra), Jain[12] (tribal area of Udaipur, Rajasthan), Devi et al[18] (tribes of Orissa and Kerala), Kaeda et al[19] (tribes of Orissa and M.P.) all shows higher prevalence in tribal population.

The variation can be explained in terms of the evolutionary history of the population and their endogamous nature and geographical spread of malaria.[20] Kar and Seth[21] in 1992 (in Nagas of Nagaland), Ruwende et al[22] in 1995 (in African Children) showed G6PD deficiency can reduce the risk of malarial infection by 46-58% in both, the heterozygous females and hemizygous males.

Malaria parasite breaks down hemoglobin for nutrition. The by-product of this process, oxidized iron is potentially toxic to parasite. In normal G6PD individual, reduced glutathione (G-SH) converts oxidized iron back to reduced iron state; but, in G6PD deficient individuals; oxidized iron checks the growth of parasite in RBC's by Tripathy and Reddy, 2007.[20]

In G6PD deficient individuals, due to lack of NADPH and reduced glutathione, reactive oxygen species cannot be reduced, leading to oxidation of hemoglobin to methemoglobin causing membrane damage and hemolysis (Ruwende and Hill 1998)[23] Reactive oxygen species are produced by activated

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leukocyte in infections (viral hepatitis, pneumonia, typhoid fever)[24] and by oxidant drugs (table 1 & 2). Apart from these; food like fava beans (contains divicine and isouramil glycosides responsible for hemolysis);[25] naphthalene (used for storing clothes), Zn-thiocarbamate (antifungal spray used in the fields); Aniline dyes (food- colouring agents) has been reported to cause hemolysis in G6PD deficient subjects.[26]

In our study (fig-1) both drugs and infection were the precipitating cause for anemia in G6PD deficient children. In 72.7% (63.6% + 9.1%) of cases hemolysis was precipitated due to using oxidant drugs which could be prevented, if screening was done for G6PD deficiency.Sukumar et al in 2002 reported that , most of drug induced hemolytic anemia in G6PD deficient individuals in India is due to administration of anti-malarial drugs.[27] But, the use of anti-malarial drugs under National malaria control program (NMCP) cannot be discontinued since cost-benefit analysis suggests that each rupee invested by the NMCP pays a rich divided of 19.7 reupees.[21]The majority of the G6PD deficient manifests within two days of ingestion of the offending agent, the patient will develop fever, dark "Coca-cola" urine, jaundice and anemia. Acute tubular necrosis may complicate hemolytic episode; maintenance of adequate renal blood flow, e.g. by forced alkaline diuresis, can prevent this complication. Exchange transfusion to remove the irreversibly damaged red cells that block the microcirculation, can also avert the renal complication.[22]

CONCLUSION

Due to high prevalence of disease in Rewa district, all suspected children from the region should be investigated for G6PD enzyme deficiency before administration of any oxidant, antimalarial or antibacterial drugs. To achieve this level, government should adopt some effective policies; like launch of educational awareness program to ensure complete avoidance of oxidant drugs and prevention of infections agents in previously diagnosed cases.

Government should include hepatitis A and B vaccination in National Immunization schedule. Government should establish nationwide program for screening of infants born in high prevalence area. For it, government should provide facility of investigating the disease at all community health centre (CHC) level; presently, even at our Medical College hospital, such facilities are not available. Lastly, there is need to build capacity among pediatricians to ensure cost effective management of G6PD deficient individuals.If, limited resources comes in way, direct above policies towards only male child of tribal community of Rewa division.

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