*Pak. J. Biotechnol. Vol. 21(1), 190-197, **2024,*** http://doi.org/10.34016/pjbt.2024.21.01.866

Research Article

OPEN ACCESS

Available on http://www.pjbt.org
Pakistan Journal of Biotechnology
(PJBT)
(P-ISSN: 1812-1837 and E-ISSN: 2312-7791)

TREATMENT FOR MALARIA PATIENTS IN PAKISTAN AND THE PREDOMINANCE OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY

Syed Waqad Ali[1], Marium Raziq[1], Muhammad Muzammil Khan[1], Sania Tanvir[1], Syed Jamal Hyder Zaidi[3], Sidra Abid Syed[1], Bullo Saifullah[2], Shahzad Nasim[2*]

[1]Sir Syed University of Engineering & Technology, Biomedical Engineering Department, Karachi
[2]The Begum Nusrat Bhutto Women University, Sukkur
[3]IQRA University, Karachi
*Corresponding Author: shahzadnasim@live.com

Article Received 28-11-2023, Article Revised 10-01-2024, Article Accepted 25-01-2024.

ABSTRACT

Even though it predisposes carriers to hemolysis, glucose-6-phosphate dehydrogenase (G6PD) deficiency is linked with malaria endemicity. This fact supports the malaria prevention theory. The objective of this paper to determine whether and how much there is a protective relationship between malaria and G6PD deficiency. Twelve databases were searched for studies describing any G6PD connection in malaria patients. 38 of the 50 included papers qualified for the review. Results indicated that there was no harmful association between G6PD deficiency and uncomplicated falciparum malaria in Even though it puts carriers at risk for hemolysis, glucose-6-phosphate dehydrogenase (G6PD) deficiency is widespread in areas of Pakistan where malaria is also prevalent. This data supports the malaria protection hypothesis. Pakistan's annual malaria burden is estimated to be 1.5 million cases. The government needs to execute a successful malaria control and eradication program, given the prevailing circumstances. Destroying Plasmodium falciparum gametocytes and eradicating Plasmodium vivax hypnozoite reservoirs are possible with primaquine. However, when using this medication, those who lack the enzyme glucose-6-phosphate (G6PD) experience hemolysis. The distribution of malaria and G6PD deficiency in Pakistan must be mapped to create an effective medication to suppress the disease. No significant reports of G6PD deficiency (G6PDd) in malaria patients have come from Pakistan. This review article seeks to establish the existence and magnitude of a protective connection between malaria and G6PD deficiency.

Keywords: G6PD deficiency, malaria control, *Plasmodium Falciparum,* Plasmodium vivax hypnozoite.

INTRODUCTION

Pakistan, one of the seven WHO Eastern Mediterranean Region nations that collectively bear 95% of the burden of the region's malaria, is predicted to have 1.5 million cases of the disease per year. Plasmodium vivax malaria is more prevalent (88%) in this nation than Plasmodium falciparum malaria, It is only prevalent during rainy seasons or immediately following rain and accounts for just 12% of the malaria load (Ali *et al*., 2005). Given the current situation, Pakistan needs an effective malaria control and eradication effort. Because it kills P. vivax hypnozoites and P. falciparum gametocytes, primaquine, an 8-aminoquinoline, is the sole medicine for treating malaria (Beutrler *et al*., 1994). Those who lack the enzyme glucose-6-phosphate dehydrogenase (G6PD) are at danger from primaquine. The pentose pathway enzyme G6PD (NADP) catalyzes the rate-limiting step in converting nicotine amide to adenine dinucleotide phosphate. Reduced NADP is essential for keeping cells alive by eliminating free radicals. As a result, otherwise, asymptomatic G6PD-deficient people are susceptible to acute hemolytic anaemia brought on by medications, foods, or infections (C. Alison *et al*., 1961). Males are more likely to have G6PD than females because the G6PD gene resides on the X chromosome. A hereditary advantage in terms of malaria risk is suggested by the global distribution of G6PD deficiency (G6PDd), which coincides with that of past and contemporary malaria (Bouma *et al*., 1995). For hemizygous males and, to a lesser extent, heterozygous females In Asia, G6PD A-type confers modest protection against falciparum malaria (Azad Bakht *et al*., 2011). G6PD Mediterranean (G6PD-Med), a variant widespread in Asian countries, is similar to G6PD because it confers protection against vivax malaria in hemizygous men. An enzyme called G6PD (glucose-6-phosphate dehydrogenase) aids human cells in keeping their equilibrium when under oxidative stress. Red blood cells have a streamlined metabolism, so there is no other way to keep things balanced. In people with G6PD deficiency, oxidative

stress results in hemolysis, or the rupture or destruction of red blood cells (Alving *et al*., 1960).G6PD insufficiency is the most prevalent enzymatic disease in humans and is widespread in populations exposed to malaria. G6PD deficiency may act as a malaria preventative, despite conflicting evidence about the precise nature of this action. People with G6PD deficits frequently do not develop symptoms unless they are subjected to oxidative stress caused by certain causes. In particular, 8-aminoquinoline and anti-malarial foods like fava beans (D. toncheva *et al*., 1985). Previous research has shown that G6PD deficiency (G6PDd) is common in Northern Pakistan, with recurrence rates ranging from 2 to 8% in diverse ethnic groups. The most well-known G6PD variant in Pakistan is G6PD-Prescription. Ashley *et al*. found that those delivering G6PD-Drug experienced hazardous hemolysis on primaquine organization. This proves that the primaquine organization for the eradication of malaria in Pakistan requires strict G6PD testing. Because this test is not widely available, the expense of malaria will continue to be a financial burden on the country (Hedrick *et al*., 2011). The current study employed repeated DNA testing to determine the allelic recurrence of G6PD c.563C>T in male patients who did not have malaria. This small-scale study is expected to spark broader studies on the real weight of G6PDd before mass primaquine therapy is scheduled. With a projected 405,000 fatalities and 228 million cases worldwide in 2018 (clark *et al*., 1989), malaria continues to be a problem on a global scale. It is still endemic in 91 countries, putting a significant chunk of the population in danger, particularly those who reside in low-wage countries. Target 3.3 of the Feasible Improvement Objectives is to eradicate jungle fever, Helps, TB, and other tropical illnesses by 2030. Nepal has shown that it is committed to the Asia-Pacific region's goal of eradicating local malaria by 2030. Less than half of the nations are on track to accomplish the global plan goal of a 40% case and death reduction by 2020, although the prevalence of intestinal disease reduced globally by 41% between 2000 and 2015 (cappelini *et al*., 2008). It is difficult to treat malaria when the population has a G6PD deficit since intensive therapy utilising 8-aminoquinolines is associated with a risk of hemolysis. Asymptomatic G6PD insufficiency is the most well-known defect in human protein (khattak *et al*., 2013). In any case, people with G6PD deficiencies may experience the detrimental effects of severe hemolytic fragility when they are subjected to a few oxidative triggers, such as fava beans, diseases, or antimalarial medications like primaquine (AHA). AHA is the most well-known symptom of this deficiency and is linked to intra- and extravascular hemolysis, jaundice, and hemoglobinuria, with profound renal failure being the most catastrophic outcome (Kaplan *et al*., 1999). Individual factors impacting hemolytic risk include hematologic profile, particularly red platelet age appropriateness, drug dosage and use (dependent on CYP2D6 in primaquine), and G6PD aggregate and genotype. Men are typically thought to be more susceptible to hemolysis at the population level since the inadequate aggregate is more common in men, but recent research has revealed that heterozygous women with moderate G6PD aggregates are also at risk for severe hemolysis (kakar *et al*., 2010). It is estimated that 400 million persons globally are G6PD deficient, with a geographic distribution similar to that of intestinal disease but also with significant heterogeneity across various nations and areas. In nations where malaria is endemic, 220 million men and 133 million women are thought to reside. Pakistan has the greatest typical assessed predominance (7.5%) (Howes *et al*., 2012). In its Public Malaria Treatment Convention released in 2015, the Study of Disease Transmission and Infectious Prevention Division (EDCD) endorsed the method used by the World Health Organization (WHO) to test for G6PD deficiency in malaria patients (Leslie et., 2010). Determining the significance of G6PD deficiency and identifying vulnerable groups are necessary for Pakistan's malaria control strategy to be successful. In the present study, we aim to assess the incidence of G6PD deficiency and to illustrate the hidden changes in regions with an endemic malaria transmission (Khattak *et al*., 1992)It is guessed that the review discoveries will illuminate masterful courses of action for the malaria end program.Glucose-6-phosphate dehydrogenase is a catalyst start in cytoplasm of by and large cells catalyzing essential reaction in pentose phosphate way, insofar as dunking capacity to out and out cells in strategy of NADPH.NADPH permits cells to adjust oxidative strain that might be actuated through various oxidant arbiters, and to hold dense technique for glutathione (L.Luzzatto *et al*., 2001). G6PD deficiency was linked by guard in Plasmodium falciparum logical inconsistency and remains one of the most prevalent human chemical unfortunate deficits in the ecosphere. It is prevalent among the general population to varying degrees. Adolescents' advise comprehension was obtained; its socio-segment facts and logical appearances were also included with the assistance of an organized overview. G6PD deficiency was discovered subjectively during the preliminary G6PD screening (Kruatrachue *et al*., 1962).

MATERIALS AND METHOD

Data source: Prevalence of Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency in Malaria Patients and Treatment in Pakistan PubMed, WHO NCBI, Springer, and Google Scholar resources, Clinical Related Data Set. National Institute of Health (NIH) I. Ministry of National Health Services, Regulations, and Coordination Field Epidemiology and Disease Surveillance Division (FEDSD).

REVIEW METHOD

The prevalence of Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency among Malaria

Patients and Treatment in Pakistan context was reviewed. A total of 38 research papers have been included for examination (Figure-1). Given the ever-changing nature of the research, the mentioned references give the most recent healthcare claims.

Identification of studies:

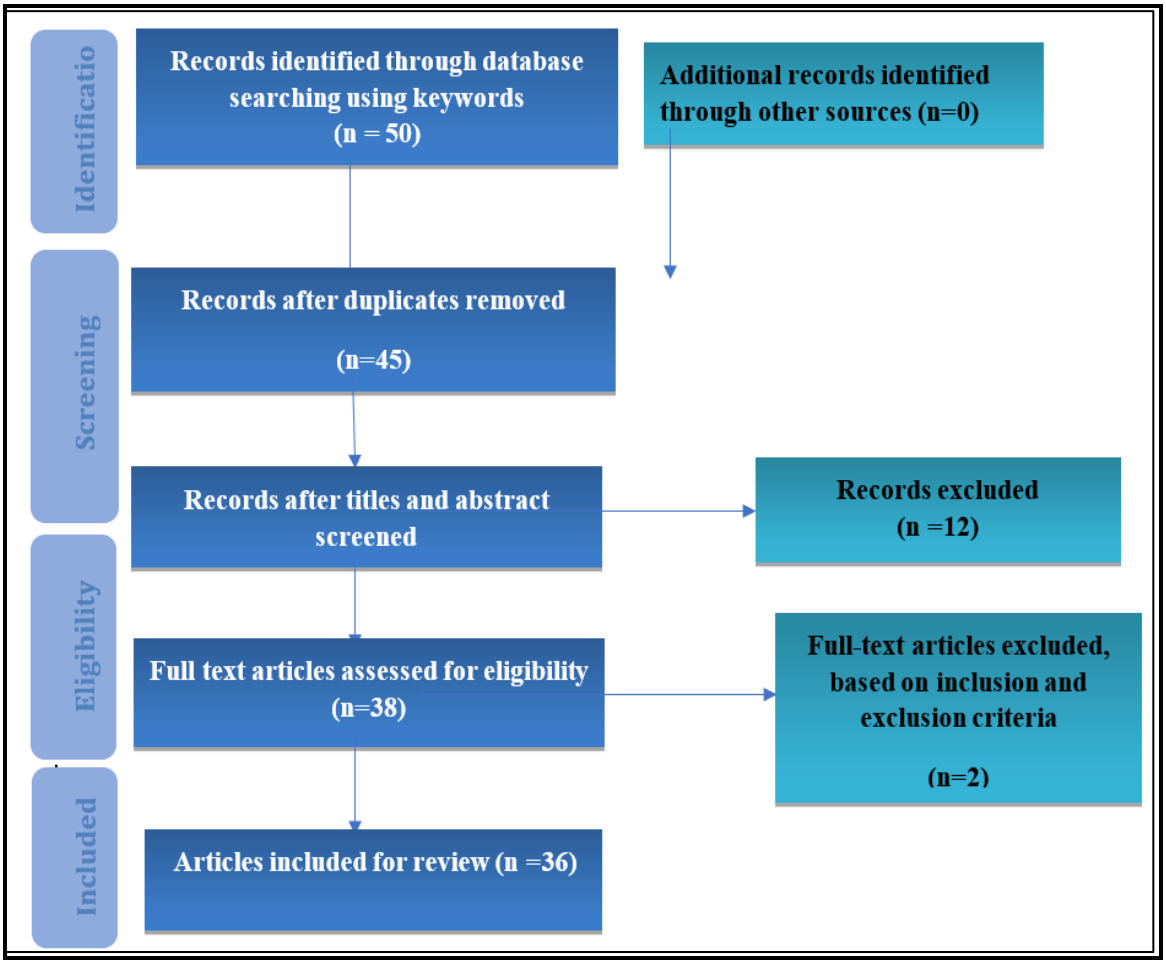

Figure 1. PRISMA flowchart for the search method of literature review

RESULT AND DISCUSSION

Around 98 million people with universal Glucose-6-phosphate dehydrogenase deficiency are recognized to be inherent in malaria-endemic nations, and the G6PDdeficiency was exposed to defend against malaria contagion. This illness frequently affects children under six (L.S. Greene *et al*., 1993). The current study was motivated by a lack of technical information on G6PD absence in malaria-affected children in Pakistan, and it was intended to control the occurrence of G6PD deficiency among children (aged 1-6 years) infested with Plasmodium falciparum in Lahore (Moiz *et al*., 2013) Punjab Province, Pakistan. From May 2017 to June 2018, 220 blood trials were composed of Plasmodium falciparum malaria offspring attending eight designated hospitals located diagonally in three senatorial regions of the country at Lahore General Hospital (R.E *et al*., 2012). The examples were prepared in EDTA tubes and transported directly to the General Hospital's Research Laboratory Subdivision in an ice-cooler box. The trial expedient consists of a monoclonal malaria antibody wrapped in a membrane (Ninokata *et al*., 2006). The Plasmodium falciparum Quick Trial Device (entire blood) is a quantitative membrane immunoassay for detecting Plasmodium falciparum antigen in whole blood. Plasmodium falciparum antibody keeps the membrane pre-covered. During testing, the entire blood sample retorts through a dye conjugate pre-covered in the test band (Moiz B *et al*., 2009). Previously, the combination travelled uphill on the membrane chromatographically via capillary exploit and responds via Plasmodium falciparum antibody on the membrane via trial line. If the sample contains Plasmodium falciparum antigen, a coloured line in the trial area indicates that it contains Plasmodium falciparum antigen. The development of the G6PD protein was evaluated abstractly in this paper using the fiscally open G6PD screening tests, as shown by the manufacturer's rules, who uses fresh blood tests, because pulse activity reduces cooling (Moiz *et al*., 2013). The glucose-6-phosphate dehydrogenase found

in red platelet hemolysate, which controls glucose-6-phosphate and lowers NADP+, reduces the quantity of 2, 6-dichlorophenolindophenol colored. Glucose-6-phosphate dehydrogenase (G6PD) insufficiency is the most common hereditary disease and one of the most common red cell enzymopathies in the world (Rehman *et al*., 1995). In 1956, Alving and his associates made the discovery while looking into the extraordinary primaquin sensitivity of Blacks' erythrocytes. Later it was found that other ethnic groups, besides Africans, were also prone to G6PD deficiency. Nearly a decade after its discovery, a number of related clinical disorders were identified. Around the world, 400 million people are thought to carry the defective gene. The regional distribution of malaria endemicity has previously been correlated with G6PD deficiency (Riskin *et al*., 2012). As a result, regions with high frequency frequently include those in Latin America, Africa, Asia, and the Mediterranean. Population migration was responsible for its international emergence. According to a recent meta-analysis, Pakistan has a 1.8% incidence of G6PD deficiency, compared to a global prevalence of 4.5%. It omitted a couple of our locally published non-indexed studies, which showed an 8% incidence among Pathans and a 2% to 4% prevalence in Pakistani men (Figure-2). 5-8 A newborn jaundice check was necessary in 26% and 30% of all hospital admissions for 1624 and 6454 babies, respectively, according to two major nationwide investigations. G6PD deficiency was found in 8% of infants with jaundice and malaria, according to research. Jaundice has also been linked to premature delivery, sepsis, ABO or Rh incompatibility, and premature birth (Ruwende *et al*., 1995).

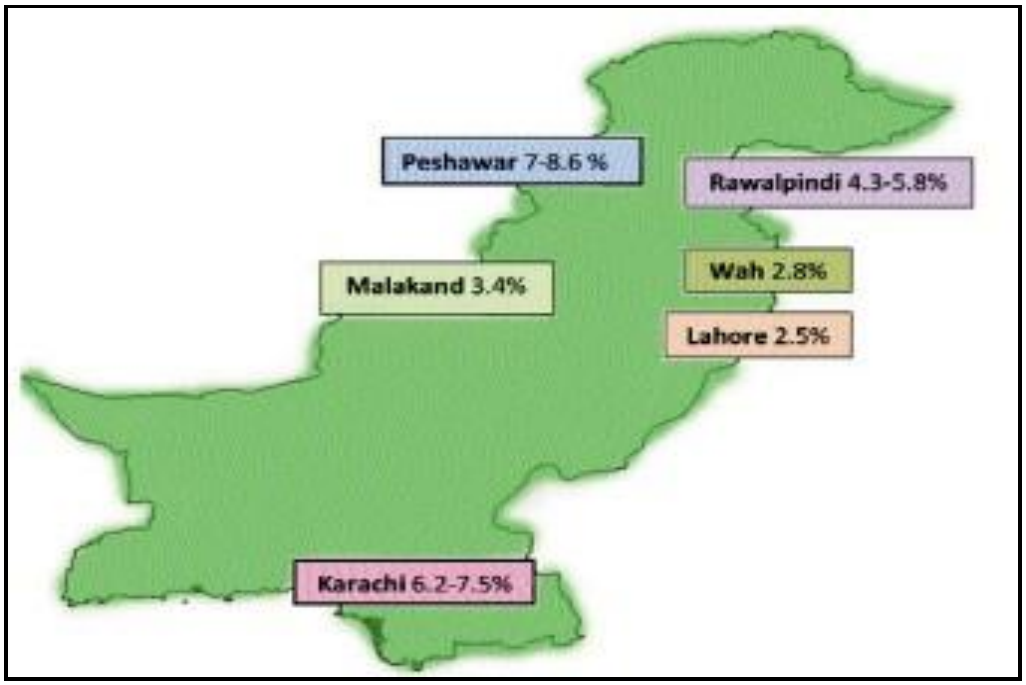

Figure 2. Prevalence of G6PD deficiency in Pakistan.

Structure and function of G6PD G6PD is a dimer having a molecular weight of 59 kDa and an amino acid sequence of 515. At neutral pH, each subunit of the tetrameric G6PD molecules has its own active site and contains NADP. (adenine dinucleotide phosphate with nicotine amide). As a result, NADP may be thought of as the G6PD molecule's structural and functional domains. The enzyme G6PD catalyzes the first step of the pentose phosphate pathway. Red blood cells are mostly involved in the latter kind of glycolysis (Sahan *et al*., 1994). The enzyme aids in the conversion of glucose 6 phosphate to 6-phosphonogluconolactone and is high in NADPH [reduced NADP]. Because it increases glutathione reduction, NADPH is necessary for red cell viability and catalase stabilisation, hence). negating their antioxidant capabilities (Figure-3). Erythrocytes are the body's primary generator of NADPH; nevertheless, they lack mitochondria and rely solely on PPP. Erythrocytes cannot withstand oxidative stress in the absence of G6PD and hence NADPH, and the accumulating free radicals cause them to lyse prematurely (shah *et al*., 2012). Passivity and Genetics Because G6PD is situated on the Xq2.8 long arm of the X-chromosome, the mode of inheritance for G6PD deficiency is sex. Women who are heterozygous have malfunctioning genes and, depending on X-chromosome randomization, may have normal, moderate, or severely diminished G6PD enzyme activity (Tikmani *et al*., 2010

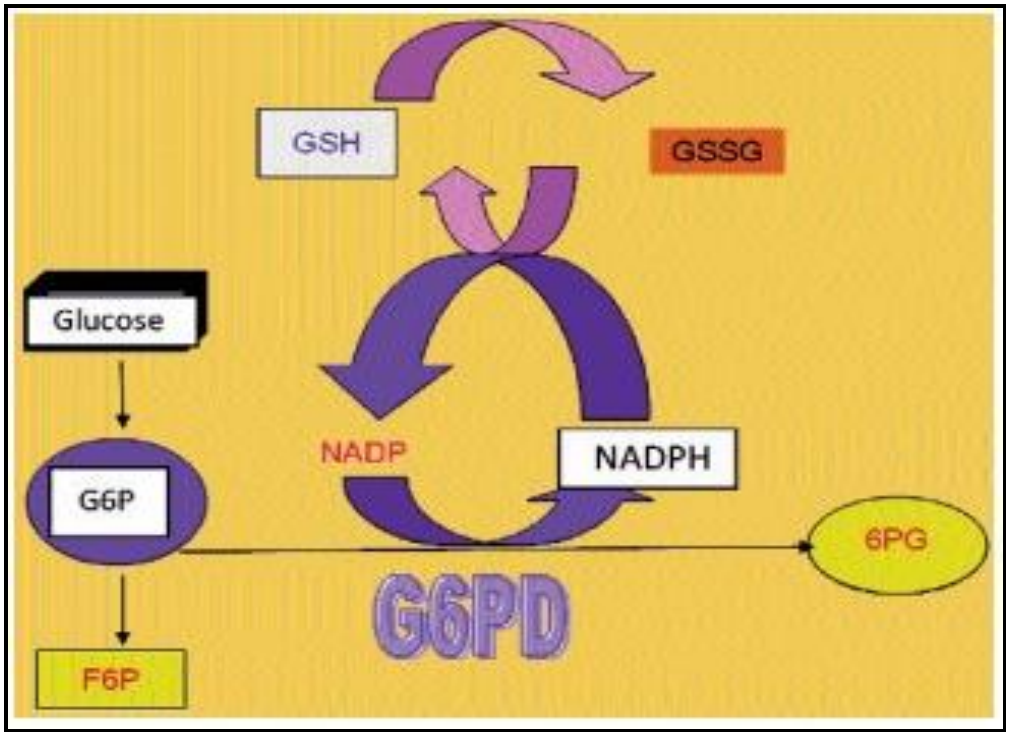

Figure 3. Biochemical role of G6PD enzyme in the cells [GHS: reduced glutathione; GSSG: oxidize glutathione; G6P: Glucose 6 phosphate; F6P: Fructose 6 phosphate; NADP: Nicotinamide adenine dinucleotide phosphate; NADPH: Nicotinamide adenine dinucleotide phosphate reduced].

78% of Pakistanis had lower rates of G6PD Chatham and Orissa, corroborating an earlier finding. A sizable proportion of individuals with G6PD 563C>T were also shown to have an associated 1311 C/T polymorphism (youngster *et al.,* 2010). Pathophysiology deficiency of G6PD..Under normal conditions, the small amounts of enzyme that G6PD-deficient red cells produce are sufficient for survival. However, when exposed to oxidative stress, these red cells lyse as a result of a lack of NADPH synthesis. Despite the ongoing stress, the hemolysis and icterus are self-limiting because the older erythrocytes with the lowest enzyme activity are haemolyzed first (stern MA *et al*., 1968) . Evidence suggests that inadequate hepatic processing of unconjugated bilirubin rather than enhanced hemolysis is the main contributor to newborn hyperbilirubinemia. The emphasis of interest in G6PD quality has also been on UGT1A1 change of the quality advertising or coding area, which results in a Gilbert-like situation in G6PD deficient newborns (Saleem *et al*., 1996). Therapeutic Highlights Except when exposed to oxidative loads caused by drug organisation, contaminations, or consumption of fava beans, G6PD-deficient patients are asymptomatic. Four factors are identified that cause severe illness or drug-induced hemolysis: Clinically, hemolysis is severe and intravascular, resulting in icterus, hemoglobinuria, and back pain. In the presence of common hepatic proteins, circuitous bilirubinaemia is typically associated with jaundice. Favism: Glycosides, divicine, and isouramil are examples of unidentified oxidants found in fava beans. Some G6PD-deficient people experienced significant hemolysis after consuming fava beans, although not all of them. Hyperbilirubinemia in the newborns (Ronald et al., 1968). The likelihood of severe encephalopathy, which can lead to irreversible brain damage, makes this the most catastrophic consequence of G6PD deficiency. G6PD insufficiency manifests as persistent non-spherocytic haemolytic paleness (CNSHA), an uncommon and severe condition (Rehman et al., 1995). is a rare condition. Subjects with CNSHA exhibit permanent hemolysis and splenomegaly, in contrast to the usual variation's rambling hemolysis on oxidative problems. Other clinical hypotheses have linked G6PD deficiency to helplessness to sepsis, albeit this has not always been proven. Complete G6PD deficiency is incompatible with life causing early foetal catastrophe. The CBC analysis of the CEOs' lab samples reveals normochromicnormocytic sickness (Nkhoma et al., 2009).Although platelets and white blood cells are typically normal, hemolysis may cause them to be slightly elevated. Sphercoytes, polychromasia, chomp, and rankle cells are typically visible on fringe film when reticulocytosis is suspected. As in any severe hemolysis, abnormal bilirubin and LDH levels are elevated. G6PD protein measurement is the demonstration test.Because recently created red cells are quickly replaced with catalyst and may give false average readings during hemolysis, this should be done after reticulocytosis has stopped. The formazan test, methaemoglobin reduction test, mono spot fluorescence test, and colour decolourization test are a few screening procedures that are accessible. Additionally, a brand-new methaemoglobin-based cytofluorometric test has recently been created. These

several tests assess NADPH production. The G6PD quantitative test is also offered in Pakistan. The reference scopes of the G6PD measure for Pakistani adults, kids, and teenagers are 6.7-14.3, 5.8-13.5, and 7.0-19 U/gHb, respectively. Because a heterozygote may shift G6PD in a manner that is usual or nearly typical, transporters can be challenging to identify with screening assays. Contraaction: A few drugs should be avoided since they have the potential to speed up hemolysis. When endorsing pharmaceuticals to G6PD deficient people, a list of these medications that should be avoided should be provided (Moiz et al., 2011)

The entire 230 offspring (125 men and 105 women, i.e. 4:3 man-to-woman relation) who are Plasmodium falciparum malaria positive were screened; 33 (12%) were found to have G6PD deficiency.

Figure 4 depicts the occurrence of G6PD deficiency in offspring studied using Plasmodium falciparum malaria. According to biochemical testing of 230 broods admitted otherwise offered to designated hospitals by Plasmodium falciparum malaria in the study, 36 offspring (17%) were found to be G6PD deficient, while the remaining 178 offspring (86%) are normal (M.J et al., 1981).

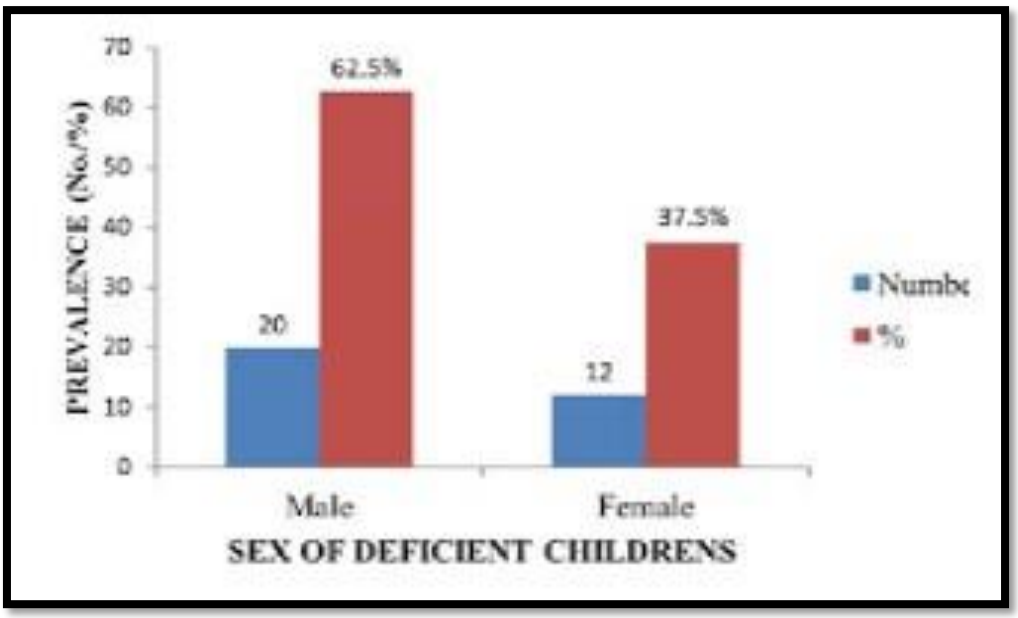

Figure.4 Occurrence of G6PD Shortage amongst Offspring Based on Gender

As a result, researchers discovered a statistically significant occurrence of 17% (36/230) among 230 Plasmodium falciparum positive offspring studied. This review show the result, the occurrence of G6PDlack in children (aged 1-6 years) infected with Plasmodium falciparum in Pakistan. The high prevalence of G6PD in the absence of numerous malaria-endemic republics explains the significant struggle in malaria (Mehmood *et al*., 2010).

CONCLUSION

In Lahore, Pakistan, G6PD deficiency in offspring brought on by Plasmodium falciparum malaria was discovered to be highly prevalent. The results revealed that G6PD insufficiency was more common in men (65%) than in women (35%). The bulk of the first child's progeny (76%) have G6PD deficiency (2 - 4 years). In the current circumstances, routine testing of offspring for G6PD deficiency is necessary to enable indication grounded organization of malaria in those offspring and to ensure escaping of food and things that may possibly predispose them to oxidative stress. Creating and directing educational responsiveness programs for G6PD deficiency, particularly among mothers, could also play a significant role. There is also a need to determine capacity amongst pediatricians in the current location to safeguard actual organization of children due to G6PD shortage else severe hemolysis or blood transfusions may be required.

REFERENCES

Ali, N., Anwar, M., Ayyub, M., Bhatti, F. A., Nadeem, M., & Nadeem, A. (2005). Frequency of glucose-6-phosphate dehydrogenase deficiency in some ethnic groups of Pakistan. *Journal of the College of Physicians and Surgeons--Pakistan: JCPSP*, **15**(3), 137–141.

Allison, C., & Clyde, D. F. (1961). Malaria in African children with deficient erythrocyte glucose-6-phosphatedehydrogenase. *British Medical Journal*, **5236**, 1346–1349.

Alving, A. S., Tarlov, A. R., Brewer, G., Carson, P. E., Keller-Meyer, R. W., & Long, W. K. (1960). Glucose-6-phosphate dehydrogenase deficiency: some biological implications. *Transactions of the Association of American Physicians*, ***73,*** 80–92.

Azadbakht, M., Hosseinimehr, S. J., Shokrzadeh, M., Habibi, E., & Ahmadi, A. (2011). Diospyros lotus L. fruit extract protects G6PD-deficient

erythrocytes from hemolytic injury in vitro and in vivo: prevention of favism disorder. *European Review for Medical and Pharmacological Sciences*, **15(**11), 1270–1281.

Beutler, E. (1994). G6PD deficiency. *Blood*, **84**(11).

Beutler, E. (1996). G6PD: population genetics and clinical manifestations. *Blood Reviews*, **10**(1), 45–52.

Bouma, M. J., Goris, M., Akhtar, T., Khan, N., Khan, N., & Kita, E. (1995). Prevalence and clinical presentation of glucose-6-phosphate dehydrogenase deficiency in Pakistani Pathan and Afghan refugee communities in Pakistan; implications for the use of primaquine in regional malaria control programmes Trans R Soc Trop Med Hyg. *Trans R Soc Trop Med Hyg*, **89,** 62–64.

Cappellini, M. D., & Fiorelli, G. (2008). Glucose-6-phosphate dehydrogenase deficiency. *Lancet*, **371**(9606), 64–74.

Clark, G., & Chaudhri, W. (1989). Some roles of freeradicals in malaria. *Free Radical Biology and Medicine*, **5**(3), 315–321.

Friedman, M. J., & Trager, W. (1981). The biochemistry ofresistance to malaria. *Science Americana*, **244**(3), 158–164.

Greene, L. S. (1993). G6PD deficiency as protection against falciparum malaria," An epidemiologic critique of populationand experimental studies. *American Journal of PhysicalAnthropology*, **36(**S17), 153–178.

Hedrick, P. W. (2011). Population genetics of malaria resistance in humans. *Heredity*, **107(**4), 283–304.

Howes, R. E., Piel, F. B., Patil, A. P., Nyangiri, O. A., Gething, P. W., Dewi, M., Hogg, M. M., Battle, K. E., Padilla, C. D., Baird, J. K., & Hay, S. I. (2012). G6PD deficiency prevalence and estimates of affected populations in malaria endemic countries: a geostatistical model-based map. *PLoS Medicine*, **9**(11),

Kakar, Q., Khan, M. A., & Bile, K. M. (2010). Malaria control in Pakistan: new tools at hand but challenging epidemiological realities. *La Revue de Sante de La Mediterranee Orientale [Eastern Mediterranean Health Journal]*, **16**(Supp.), 54–60.

Kaplan, M., Beutler, E., Vreman, H. J., Hammerman, C., Levy-Lahad, E., & Renbaum, P. (1999). Neonatal hyperbilirubinemia in glucose-6-phosphate dehydrogenase-deficient heterozygotes. *Pediatrics*, **104**, 68–74.

Khattak, A. A., Venkatesan, M., Nadeem, M. F., Satti, H. S., Yaqoob, A., Strauss, K., Khatoon, L., Malik, S. A., & Plowe, C. V. (2013). Prevalence and distribution of human Plasmodium infection in Pakistan. *Malaria Journal*, **12(**1), 297.

Khattak, M., Dawood, M., & Saleem, M. (1992). The prevalence of glucose-6-phosphate dehydrogenase deficiency in Northern Pakistan. *Pak Armed Forces Med J*, **42,** 35–38.

Kruatrachue, M., Charoenlarp, P., Chongsuphajaisiddhi, T., & Harinasuta, C. (1962). Erythrocyte glucose-6-phosphate dehydrogenase and malaria in Thailand. *Lancet*, **280**(7267), 1183–1186.

Leslie, T., Briceño, M., Mayan, I., Mohammed, N., Klinkenberg, E., Sibley, C. H., Whitty, C. J. M., & Rowland, M. (2010). The impact of phenotypic and genotypic G6PD deficiency on risk of plasmodium vivax infection: a case-control study amongst Afghan refugees in Pakistan. *PLoS Medicine*,**7**(5), e1000283.

Luzzatto, L., & Gordon-Smith, E. C. (2001). Inherited haemolyticanaemia. In *Postgraduate Haematology* (pp. 120–143). Arnold.

Mehmood, A., Akhtar, M., & Niazi, M. (2010). Frequency of glucose-6-phosphate dehydrogenase deficiency in asymptomatic Pakistani population. *Pak Armed Forces Med J*, **60**, 1–3.

Moiz, B., Nasir, A., Moatter, T., Naqvi, Z. A., & Khurshid, M. (2009). Population study of 1311 C/T polymorphism of Glucose 6 Phosphate Dehydrogenase gene in Pakistan - an analysis of 715 Xchromosomes. *BMC Genet*, *10*.

Moiz, B., Nasir, A., Moatter, T., Naqvi, Z. A., & Khurshid, M. (2011). Molecular characterization of glucose-6-phosphate dehydrogenase deficiency in Pakistani population: Molecular Characterization of Glucose-6-Phosphate Dehydrogenase Deficiency in Pakistan. *International Journal of Laboratory Hematology*, **33**(6), 570–578.

Moiz, Bushra. (2013). A review of G6PD deficiency in Pakistani perspective. *JPMA. The Journal of the Pakistan Medical Association*, **63(**4), 501–503.

Ninokata, R., Kimura, U., Samakkarn, W., & Settheetham-Ishida, T. (2006). Coexistence of five G6PD variants indicatesethnic complexity of Phuket islanders, Southern Thailand. *Journal Human Genetics*, **51(**5), 424–428.

Nkhoma, E. T., Poole, C., Vannappagari, V., Hall, S. A., & Beutler, E. (2009). The global prevalence of glucose-6-phosphate dehydrogenase deficiency: a systematic review and meta-analysis. *Blood Cells, Molecules & Diseases*, **42**(3), 267–278.

Rehman, H., Khan, M. A., Hameed, A., Roghani, M. T., & Ahmad, A. (1995). Erythrocyte glucose 6 phosphate dehydrogenase deficiency and neonatal jaundice. *JPMA. The Journal of the Pakistan Medical Association*, **45(**10), 259–260.

Riskin, A., Gery, N., Kugelman, A., Hemo, M., Spevak, I., & Bader, D. (2012). Glucose-6-phosphate dehydrogenase deficiency and borderline deficiency: association with neonatal hyperbilirubinemia. *The Journal of Pediatrics*, **161(**2), 191-6. e1.

Ronald, A. R., Underwood, B. A., & Woodward, T. E. (1968). Glucose-6-phosphate dehydrogenase deficiency in Pakistani males. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **62**(4), 531–533.

Ruwende, S. C., & Khoo, R. W. (1995). Natural selection ofhemi- and heterozygotes for G6PD deficiency in Africa byresistance to severe malaria. *Nature*, **376**, 246–249.

Saha, N., Ramzan, M., Tay, J. S., Low, P. S., Basair, J. B., & Khan, F. M. (1994). Molecular characterisation of red cell glucose-6-phosphate dehydrogenase deficiency in north-west Pakistan. *Human Heredity*, **44**(2),

Saleem A Glucose-6-phosphate dehydrogenase deficiency and hemolytic anemia. (1966). *J Pak Med Assoc*, **16**, 3–9.

Shah, S. S., Diakite, S. A. S., Traore, K., Diakite, M., Kwiatkowski, D. P., Rockett, K. A., Wellems, T. E., & Fairhurst, R. M. (2012). A novel cytofluorometric assay for the detection and quantification of glucose-6-phosphate dehydrogenase deficiency. *Scientific Reports*, *2*, 299.

Stern, M. A., Kynoch, P. A., & Lehmann, H. (1968). Beta-thalassaemia, glucose-6- phosphate-dehydrogenase deficiency, and haemoglobin DPunjab in Pathans. *Lancet*, **1,** 1284–1285.

Tikmani, S. S., Warraich, H. J., Abbasi, F., Rizvi, A., Darmstadt, G. L., & Zaidi, A. K. (2010). Incidence of neonatal hyperbilirubinemia: a populationbased prospective study in Pakistan. *Trop Med Int Health*, **15**, 502–507.

Toncheva, D., & Tzoneva, M. (1985). Prenatal selection and fetal development disturbances occurring in carriers of G6PDdeficiency. *Human Genetics*, **69**(1).

Youngster, I., Arcavi, L., Schechmaster, R., Akayzen, Y., Poplisti, H., & Shimonov, J. (2010). Medications and glucoses-6-phosphate dehydrogenase deficiency: an evidence-based review. *Drug Saf*, **33**, 713–726.