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Risk Factors Associated with Alteration of Hematological and Biochemical Parameters in G6PD Deficiency

by

Kamran Javed

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Dedicated to Almighty ALLAH and the Holy Prophet Muhammad (P.B.U.H)
and My Loving Family

CERTIFICATE OF APPROVAL

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Abstract

Glucose -6- phosphate dehydrogenase deficiency is a common enzyme disorder found almost in 400 million people worldwide. This enzyme is involved in the production of NADPH which protects red blood cells from oxidative damage. The current study investigates the relation of G6PD deficiency with different biochemical and hematological parameters. Regarding anthrophometry males were more deficient of G6PD as 91.8% (45) were male and 8.2% (4) were female, any how the age wise frequency distribution of G6PD deficient patients indicated that 51.0% (25) were of age less then ten years, 24.5% (12) were belonging to 11-30 years and 24.4% (12) were above 30 years. Over all cousin married individuals were more prone to the disease as it was calculated to be 77.5% (38). Six individuals 12.2% (6) with family history were studied while all the other cases 87.8% (43) were sporadic. The current study investigates the disease distribution among patients about 34.7% (17) were at neonatal stages, 42.9% (21) were at childhood stage and the remaining 22.4% (11) patients were diagnosed at adult stages, although these patients have G6PD deficiency before diagnosis but they remained asymptomatic. This indicate that G6PD patient can live normal life span, until they experience some kind of hemolysis after using some inducers. In G6PD deficient individuals certain drugs like sulphur and anti malarial drugs are identified as major stakeholder in triggering hemolysis. In current study two variables were selected like sulphonamides and anti malarial drugs. Out of studied patients 8.16% (4) were sensitive to sulphur drugs while 61.22% (30) were sensitive to anti malarial drugs, this indicates that high anti malarial frequency is due to lack of awareness in Pakistani population. Some antibiotics like ciprofloxacin 8.16% (4) patients were sensitive while 46.9% (23) were non sensitive. About 4.08% (2) out of 49 patients were sensitive to nalidixic acid. In present study the most important trigger for hemolysis is food and chemicals. Out of 49 patients, 34.6% (17), were sensitive to bean, 6.12% (3) were sensitive to banana, 4.0% (2) were sensitive to insecticidal spray while the remaining all were non sensitive. In current study hb level of patients i.e 20 (40.8%) were below 10g/dl, 24 (48.1%) patients were below 14 g/dl, while 6 (12.2%) patient hb level were above 14 g/dl. This variation indicates the

level of hemolysis. In the present study retics count for the patients was done. In the present study 65.3% (32) patients retic count were found between 2-4%, 12.2% (6) patients retic count was 4-6%, 6.1% (3) patients retic count was greater than 6% . The retics count of 16.3% (8) patients was normal it was due to slight anemia or mild hemolysis. Abnormal rbc's morphology is checked via microscopy and found 39 (79.6%) patients with abnormal morphology (macrocytosis, anicocytosis, poikilocytosis). Biochemical parameters include bilirubin, LDH, and AST which are directly linked with hemolysis show marked increase in their values. In present study their were about 65.3% (32) patient have neonatal jaundice, and about 34.7% (17) patients did not face jaundice in there early life.

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Abbreviations

A	Adenine
ARMS	Amplification-Refractory Mutation System
BLAST	Basic Length Alignment Sequence Tool
C	Cytosine
DNA	Deoxyribo Nucleic Acid
dNTPs	deoxyribo Nucleotide Triphosphates
EDTA	Ethylene Diamine Tetra Acetic Acid
G	Guanosine
G6PD	Glucose-6-Phospahate Dehydrogenase
Gd	Glucose-6-Phospahate Dehydrogenase Variant
IDT	Integrated DNA technologies
LDH	Lactate Dehydrogenase
MgCl2	Magnesium Chloride
NADP	Nicotineamide Adenine Dinucleotide Phosphate
NADPH	Nicotineamide Adenine Dinucleotide Phosphate Reduces
NCBI	National Centre for Biotechnology Informations
PCR	Polymerase Chain Reaction
PPP	Pentose Phosphate pathway
RFLP	Restriction Fragment Length Polymorphism
SPSS	Statistical Package for Social Sciences
T	Thymine

Chapter 1

Introduction

1.1 Background

Favism is a type of haemolytic reaction which usualy occur in some people after ingestion of food stuff which is composed of or contain a specific type of bean called Fava bean (Broad bean). Fava bean is the bean of a leguminous plant called Vicia faba. The haemolytic reaction starts after 6 to 24 hours after ingestion of bean. Favism is a type of acute haemolytic reaction which results from massive destruction of RBCs due to bean ingestion. This reaction is induced by bean because bean contain certain glucoside (Divicine & Convicine). These glucosides induce oxidative stress inside cells therefor favism usually occur in patients who are deficient an important enzyme called G6PD. Favism is more common and more fatal in children than adults (Isbir et al., 2016).

In a recent study out of 410 neonates, 24.63% (101) were G6PD-deficient and have no difference in gender (male, 11.95% (49) female, 12.68% (52). G6PD activity level was significantly (p¡0.001) decreased in G6PD-deficient male (2.06 ± 0.82 U/g Hb vs 16.63 ± 5.13 U/g Hb) and in female (5.42 ± 1.28 vs 19.73 ± 2.71 U/g Hb) compared to G6PD-normal controls. No variation reported in blood glucose level, but conjugated bilirubin (p =0.03) and total bilirubin (p =0.01) levels were significantly increased in G6PD-deficient than in G6PD-normal controls. Calcium

level was significantly (p =0.03) higher in G6PD-deficient while magnesium level did not vary. The level of serum AST (p=0.01) and ALT (p=0.03) levels were raised in G6PD-deficient neonates compared to G6PD-normal controls. AST and ALT levels in erythrocytes showed no changes (Fiogbe et al., 2018).

G6PD deficiency in patients in Trang Hospital, Trang province. The screening of G6PD deficiency was done by methemoglobin reduction test. The G6PD ratio were 0.033, 90 male G6PD patients and 0.87% G6PD deficiency where 12 of the females were obtained. In summary the data analysis would help G6PD deficient individuals and directs to a suitable treatment by drugs also the epidemiological material related G6PD deficiency in community in Trang Hospital, Trang Province (Nawanwat Chainuwong et al., 2018).

The exact mechanism of hemolysis in favism is still unclear but it is suggested that autooxidation of divicine which is an unstable aglycone present in fava bean results in production of anion free radicles that create oxidative stress inside the cells and ultimately cause acute haemolytic anemia (Favism) in G6PD deficient patients (Damonte et al., 1987).

In a study it was also suggested that autooxidation of divicine in G6PD deficient indivisual leads to late accumulation of intracellular calcium. Increased intracelluer calcium causes marked inactivation of calcium ATPase this increased intracellular calcium and marked inactivation of calcium pump are responsible for purtubation of rbcs membrane in favism (Manzo et al., 1992). G6PD enzyme deficiency is one of the most common disorder, and four hundred people are affected globally. It is predominant in African, Asian and mediterreandesecent. Its presentation may be as in the form of neonatal jaundace. A person with this deficiency have a high risk of episode of hemolysis after taking fava beans or being exposed by certain medication or drugs (Paul et al., 2016).

Enzyme G6PD is involved in catalyses of the initial step of pentose phosphate pathway, providing NADPH (reduced form of nicotinamide adenine dinucleotide phosphate) a reduced form to all cells. It permits cells to balance oxidative pressure that can be initiated by oxidative agents, to reserve the glutathione reduced form,

subsequently as red blood cells having no mitochondria, the pentose phosphate pathway is the source for NADPH; that provide defense against oxidative damage. (Cappellini and Fiorelli, 2008). Its deficiency is distributed more frequently in the eastern hemisphere tropical and subtropical zones. Deficiency of G6PD is infrequent amongst innate americans (Munir et al.,2014). Global estimatation indicates that 400 million people are carrying the defective gene of G6PD. The endemicity of malaria is parallels to the G6PD deficiency. Hence the prevalence of G6PD deficiency is seen typically high in Asia, Africa, Latin America and Mediterranean countries. Transmigration of the people led to its worldwide emergence. A current study showes that global G6PD deficiency prevalence is 4.5% and indicate the frequency of 1.8% in Pakistan. Though it is ignored in several of local published papers that reports the prevalence of G6PD deficiency is 2-4% in Pakistani males and high prevalence observed in Pathans 8% (Ronald et al., 1968).

People suffering from G6PD deficiency does not show any clear symptoms unless they are exposed to certain type of chemicals, foods, drugs and certain types of viral and bacterial infections. Common medical problem associated with G6PD deficiency is the haemolytic anaemia (Vulliamy et al., 1992). An insignificant phenotype considered through neonatal jaundice, favism and haemolytic anaemia has become shared with the world (Notaro et al., 2000) rising in areas of past-malaria threat such as Africa, India, the mediterranean and south east asia (Vulliamy et al., 1992). Glucose-6-phosphate-dehydrogense deficiency having one advantage is that it offers some resistant to malaria. Individuals with a reduced amount of activity of G6PD have a greater chance for numerous pathologies which can be risky if they are not treated timely. The cruelty of these circumstances are relevent with G6PD deficiency has motivated researchers to study this type of condition. G6PD deficiency in which red blood cells are destroyed easily due to which yellowing of skin, jaundice, dark urine, shortness of breath, tiredness and a rapid heart rate occurs. It is triggered by viral infections, bacterial or certain drugs, fava beans, inhaling pollen from fava plants (Genetic home refrence, 2016).

Screening and finding of G6PD deficiency are involved in decreasing haemolytic

crisis, over and done with suitable collection of treatment, patient psychotherapy, and moderation from disease-precipitating drugs such as antimalarial, bean andother agents. G6PD is a cytoplasmic enzyme and found in all the cells, participating to maintain the identity of the erythrocytes, inhibiting the oxidation of haemoglobin and other cellular proteins (Isaac et al.,2013).

In different periods of study to understand the G6PD pathogenesis in the rbc is inadequate. At the molecular and clinical level different allelic variation of G6PD is not clearly understands, how they occur. It requires a modern method which is suitable for understanding these variation (Shah et al., 2012). The G6PD gene loci is on the q arm of X-chromosome (Xq2.8). G6PD deficiency mode of inheritance is X-linked. The females, who are heterozygous for the G6PD, are considered as the carriers of the defected gene. These carriers may b normal, intermediate or severely affected depends upon the randomization of the X chromosome. Almost 400 variants of G6PD were reported so far (Moiz et al., 2011). G6PD gene is encoded by a human X linked gene. Mediterranean is the common variant that is linked with Favism. Molecular studies of several variants of G6PD deficiency revealed that G6PD deficiency is virtually caused by substitution of single amino acid. Some of the variants although do not associated with hemolysis so often identify by way of routine screening or may found in large population samples used as normal controle for research purposes. NADPH production and reduction of glutathione differ depending on the type of mutation in G6PD gene. Mutations associated with chronic hemolysis have a tendency to combine near the G6PD gene NADP-binding domain, while those associated with acute recurrent hemolysis or no hemolysis are dispersed in the gene remaining part (Angelo Minucci et al.,2007).

In Africans population the most common variant of G6PD is G6PD A-(202A>G) whereas in Southern Europe, Indian subcontinent and Middle East countries the most common variant is G6PD 563C>T (Saha et al., 1994). Two most common variants of G6PD are compared. According WHO the class II of G6PD is G6PD Mediterranean and class III is G6PD A-. Italian, Spanish, Jewish, Arabic and Grecian population is mostly affected by the class II G6PD deficiency, while African

population affected by class III deficiency. Neonatal hyperbilirubinemia is common in both classes but it may b more severe in class II. Favism is most common in class II G6PD deficiency but less common in class III G6PD deficiency. Hemolysis with oxidative drugs is common in both classes (Beutler, 1996; Omisakin et al.; Peters & Noorden, 2009).

The G6PD variations are categorized into following classes from class 1 to class 5. In class 1 lacking of enzyme activity is moderately high lead towards non-spherocytic and haemolytic anemia, although in second class lacking of enzyme is less than ten percent of normal and in third class slight lacking of enzyme, in fourth class no enzyme lacking and in the fifth class enzyme activity lead towards double (Tripaty and Reddy, 2007).

1.2 Aims

The most common enzyme deficiency in humans and about 400 million people globally are affected by G6PD deficiency. It is predominant in African, Asian and mediterrean desecent. By keeping in view the importance of G6PD, our study was designed with the aim to find out the variation in biochemical and haematological paramaters in G6PD deficiency.

1.3 Objectives

The major objectives of the study was:

1. To determine the risk factors responsible for Favism and hemolytic anemia.
2. To determine frequency distribution and variation in anthropometric, clinical presentation, biochemical and hematological paramaters in G6PD deficiency.

Chapter 2

Literature Review

2.1 Background Research

Leslie et al., (2013) reported 400 million people globally which were carrying the G6PD deficiency gene in which they characterized 140 G6PD variants genetically. About 4 hundred million people are effected with this enzyme deficiency and this figure matches to that of prevalence of malaria, furthermore the presence of malaria evolutionary effected the retention of G6PD mutation in human trait.

2.2 Prevalence of G6PD Deficiency

Aseefa et al., (2018) reported that the hypnozoite stage of Plasmodium vivax is the major hurdle as well as the opportunity to eradicate this specie. Therefore it is subclinical and couldnot identify untill starts a clinical attack with attendant morbidity, risk of mortality, and ways for further trasnsmission. The only authorised drug that destroy the plasmodium in hypnozoite stage is primaquine, attacks the hidden reservoir but creating serious obstacles in doing so-at hypnozoite-cidal doses, it induces a severe reaction in G6PD deficient individuals, a total of 8% population in the malaria prevalent areas are affected. That issue bypass a huge number of patients from the treatment of vivax malaria: G6pd deficient patients

include, young infants, lactating women. The following groups were comprises of population in a ratio of 14.3% globally about 95 countries. A very important hurdles regarding the killing of hibernate stage of P.vivax is its apparent metabolism to an active metabolite exclusively via cytochrome P-450 isozyme 2D6 (CYP2D6). A variety of genotypes express different enzymes in over 20% of people living in southeast Asia, about half of the plasmodium infections occur worldwide. The frequency of primaquine is impaired due to toxicity CYP2D6 activity composed over 35% of the populations at risk of vivax malaria. A lot of effort is to be done for the refinement of the data, probability errors, and improve their ethnographic granularity in order to inform control and elimination strategy and tactics.

Chu et al., (2018) reported that oxidatizing agents can induce hemolytic anemia in G6PD deficient individuals. The relationship between G6PD genotype and their phenotype is required to counter the acute haemolytic reactions. The hierarchy of oxidative hemolysis is decribed in G6PD deficient males and females; In heterozygous females hemolysis is common. It was observed that 8-aminoquinolines, especially primaquine and tafenoquine, triggers hemolysis in G6PD deficiency. To support wider use of primaquine in Plasmodium vivax elimination, a large data is required to analyze. 8-aminoquinolines in G6PD heterozygous females. Recently (in 2017) a study had given such precise data, provided precisely such data; and the main thing is to find the requirement for G6PD activity. Another priority is exploring alternative 8-aminoquinoline dosing regimens that are practical and improve safety in G6PD deficient individuals.

Sharma et al., (2018) found that mutation in the G6PD gene is an X-linked recessive genetic disorder which in actual is because of deficiency of G6PD . G6PD enzyme defects may causes pre mature breakdown of red blood cells (RBCs) resulting in haemolytic anemia. Hemolytic anemia is also a known hematological complication related with viral hepatitis, patients suffering with this condition may have hemolysis if there is any secondary injury to rbc in the form of membrane defect, oxidative stress, or lack of enzyme like in G6PD deficiency. Almutairi et al., (2018) reported that G6PD deficiency is a X chromosomal disorder expressed mostly in males. Globally, in enzyme deficiency is one of the most frequent. It

causes a variety of disease i.e, neonatal hyperbilirubinemia, acute and chronic hemolysis. The aim of the study was to find prevalence, risk factors of G6FD deficiency and its symptoms among general population of Dammam, Eastern Province of Saudi Arabia. A study was conducted during the period from 1 March 2016 to 30 July 2017. Data was collected from 182 individuals. Systematic random sampling technique was followed. Data was collected by using predesigned online questionnaire. The overall prevalence of Favism was 17%, of them, 67.7% were females and 32.3% were males. 15.2% of cases and 26.9% of the total sample had family history of the disease. Among the studied cases, 48.4% complained of pallor, 45.2% headache, 45.2% drowsiness, 12.9% nausea, 32.3% back pain, 64.5% tiredness, 25.8% abdominal pain, 38.7% discoloration of urine, 29% foot pain, 16.1% low grade fever and 19.4% complained of jaundice. Regarding risk factors of favism; 32.3% reported excess beans intake, 12.9% antibiotic intake and only 9.7% reported bacterial or viral infection. Conclusions: G6PDdeficiency is a common disorder as it affects 17% of the study population of Dammam, eastern province of Saudi Arabia. It was more common in females than males. Health education seminars and further epidemiological surveys are required because by early detection and diagnosis can lead to successful management and control of this genetic disease. Ahmed et al., (2018) reported that G6Pd ratio is elevated in the tropics because it covers heterozygous carrier females from severe malaria. G6PD deficient donors are asymptomatic unless they diagnosed. Nonetheless, G6PD-deficient donor red cells may show haemolysis when they encounter drugs or infections in recipient patients. Hence, WHO recommends prior screening of G6PD deficiency in deficient individuals prior donation in high prevalence area. Unluckily, in routine patients are not screened for G6PD deficiency in Nigeria. Hence, the ratio of G6PD deficient donors are not determined in North West Nigeria. Lee et al., (2018) reported that G6Pd deficiency is one the most common heriditory disorder, gene is located on the X chromosome. Primaquine is used to treat p.vivax and its relapse. Nowadays its use is to minimize the gametocyte and inturn by blocking transmission. However, Primaquine compounds oxidizes hemoglobin and produce certain reactive species which can induce hemolysis in

G6PD deficient persons. Moiz et al., (2011) reported that G6PD deficiency is an enzyme disorder and found at a prevalence rate of 2-4% in Pakistani population.

2.3 Prevalence of G6PD Deficiency on Different Ethnicities

Kawamoto et al., (2017) conducted a study in Veitnam in which 90 newborn deficient for G6PD from different kinds of ethnic groups including those belong to china, Kinh and Kho ethnic groups were selected. Eighty five out of 90 babies belong to Kinh ethnic group, 4 from chinese and one patient from Kho Kawamoto et al., (2017) conducted a study in Veitnam in which 90 newborn de- ficient for G6PD from different kinds of ethnic groups including those belong to china, Kinh and Kho ethnic groups were selected. Eighty five out of 90 babies belong to Kinh ethnic group, 4 from chinese and one patient from Kho ethnic group. These discoveries showed that the Kinh eth- nic gathering in southern Vietnam has 8 diverse G6PD variations, demonstrating that the individuals from this gathering have numerous precursors as far as G6PD variations from southeast Asia, China, and Oceania. In this study the frequency distribution of G6PD variations from Kinh population were compared with those of other southeast asian population and the distribution of G6PD Variants from Kinh population were found more likely to that found in the Thai population, however variable from it by the non appearance of G6PD Mahidol. group. In the patients from Kinh ethnic gathering, G6PD variations, for example, G6PD Viangchan (n=32), Canton(n=8), Kaiping (n=11), Chinese-5 (n=7), Union (n=5) and Quing Yuan (n=4) were identified. Silent mutation at 1311 C>T and IVS11 not 93 T>C was additionally distinguished in 17 cases. A novel Change (173 A>G) in exon 4 that leads to corresponding amino change in 58 Asp>Gly was likewise found in a Kinh infant young lady and her dad, and it was assigned as G6PD Ho Chi Minh. These discoveries showed that the Kinh ethnic gathering in southern Vietnam has 8 diverse G6PD variations, demonstrating that the individuals from this gathering have numerous precursors

as far as G6PD variations from southeast Asia, China, and Oceania. In this study the frequency distribution of G6PD variations from Kinh population were compared with those of other southeast asian population and the distribution of G6PD Variants from Kinh population were found more likely to that found in the Thai population, however variable from it by the non appearance of G6PD Mahidol.

According to Manzo et al., (2016) reported that any change in the G6PD gene may causes neonatal jaundice and intense hemolyticanemia. A total no of 186 changes were recognized in the recent review work. In this work, a review in the best in class in G6PD shortage, showing 217 mutations in the G6PD gene; they likewise integrated the data and found around 31 new changes, 16 of them were not characterized anyhow 15 have been accounted and characterized recently. With a specific end goal to improve the impacts of new depicted changes in G6PD gene, they found the point transformations in the understood three-dimensional structure of the human G6PD protein. It was that class I conversions have the most destructive impacts on the structure and stability of the protein.

Maiga et al., (2014) found that G6PD deficiency is in relation with protection of the phenotypes. They concluded that in sub Saharan Africa G6PD deficiency is due to 202A/376G G6PD A-allele, However, current studies unmask many discrepencies in G6PD202/376 linkage with severe malaria. There is proof that G6PD deficiency allele are present in the west Africa, and that allelic variation could explain these gaps.

2.4 Mutational Analysis of G6PD Gene

Haloui et al., (2016) described that G6PD deficiency is the main enzyme deficiency disorder. Above 200 transformations in the gene encode for G6PD have been depicted. In Tunisia, the A- African and the B- Mediterranean transformations exist the mutational range. The aim of their research was ARMS-PCR to the distinguish Gd A+, Gd A- and Gd B- variations in a companion of G6PD insufficient

people and to build up a phenotype/genotype association. About 90 patients were screened for enzymatic insufficiency by spectrophotometric technique. 50 unrelated patients were analyse by moleculer analysis. From the 54 altered chromosomes analyzed, it was found that 60% had the Gd A-transformation, in 18% Gd B-change were idntified and in 20% of cases, no transformations have been recognized. The ARMS-PCR indicated finish concordance with the endonuclease cleavage reference strategy and perfectly agreed with past Tunisian examinations where GdA and Gd B- were the most experienced. Additionally, likenesses in range transformations with North African and Mediterranean nations recommend quality movement from Africa to Europe through Spain. All in all, ARMS has been presented in this investigation for basic G6PD alleles distinguishing proof in Tunisia. It gives a few favorable circumstances compared with the conventional endonuclease assimilation strategy since it is more advantageous and time saving and furthermore offers the likelihood to be connected in mass screening studies.

Sarker et al., (2016) studied that G6PD deficiency is a X-chromosomal disease found in humans. The inherited deformity in G6PD deficiency is mainly due to sin- gle base missense mutation. It has been found globally that One hundred and sixty G6PD mutations which cause amino corrosive substitutions study was planned to discriminate mutations in the G6PD gene in the peoples of Dhaka (Bangladesh). Subjective fluorescent spot test and quantitative analysis of G6PD enzyme through Randox G6PD kit were performed for investigation of blood samples first to iden- tify G6PD-lacking or deficient patients, then the samples were processed by PCR followed by sequencing of the G6PD gene. These mutations either influence binding of NAADP coenzyme or upset protein structure. This study reveals that Ala44Gly and Gly163Ser are the most widely documented G6PD mutations in Dhaka, Bangladesh. It was the most important research of G6PD changes in Bangladesh.

Amini et al., (2011) designed a study in the Negrito tribe of the Malaysian Orang Asli with an objective to check the frequency and molecular basis of G6PD deficiency. Fours hundred and eighty seven individuals from Negrito community were

identified by using Flourescent spot test. G6PD deficient individuals DNA underwent PCR-RFLP analysis using thirteen known G6PD mutations. When the mutation could not be identified by PCR-RFLP, the entire coding region of the G6PD gene was subjected to DNA sequencing. It was found that only 44 individuals out of 486 individuals with 9% were G6PD-deficient. For PCR-RFLP and DNA sequencing only 25 samples were taken. Of these, three were found to carry Viangchan, one Coimbra and 16, a combination of C1311T in exon 11 and IVS11 T93C. Mutation for the five remaining samples were unknown. The mean G6PD enzyme activity ranged 5.7. IU/gHb in deficient individuals. Research results reveals that the occurrence of G6PD deficiency is higher among the Negrito Orang Asli than other Malaysian races. The presence of C1311T as well as IVS11 T93C in 64% of the deficient individuals (16/44) could well be a result of genetic drift within this isolated group.

EL-Menshay et al., (2016) reported that G6PD abcense is the most common metabolic disorder and red blood cell disorder, beside favism, drug-or infection induced hemolysis and chronic non spherocytic haemolytic anemia. Severe new born jaundice proved to be a common clinical condition and a worldwide important, most hazardous effect of G6PD deficiency. So the early depiction of G6PD activity provides a cause of analysis for neonatal jaundice (NJ), also to inform the family of new born with G6PD deficiency regarding hemolytic episodes prevention. The current study was formulated to find out the incidence of G6PD in Egyptian decent. The study comprises 53 infants presenting with neonatal jaundice 40 males (75.5%) and 13 females (24.5%) with a ratio 3:1. History of all the infants was taken, thorough physical examination and the following laboratory investigation, haematological estimation, total and direct serum bilirubin levels, direct coomb's test and we have done quantitative as well qualitative G6PD enzyme assay. The study exposed that 16 cases (30.2%) were G6PD deficient out of 53. In the G6PD deficient cases no evidence of other factors known to cause hyper bilirubineamia were detected. Out of the 16 G6PD deficient cases 12 cases (75%) were males and 4 cases (25%) were females with a male to female ratio 3:1 and 4 cases (25%) were markedly G6PD enzyme deficient and 12 cases (75%) were

moderately G6PD enzyme deficient. The occurrence of G6PD was significantly higher (66.7%) among the pre term infants compared to the frequency of (25.5%) among the full term infants (P=0.04). There was a significant difference between G6PD deficient cases and G6PD normal cases as regards total peak serum bilirubin (22.26+8.36, 18.14+3.82 respectively) (P=0.001). Hematological indices were unable to identify hemolysis as the hemoglobin levels, hematocrit values, reticulocytic count and percentage of anemia did not significantly differ between G6PD deficient and G6PD normal cases. Out of the 16 G6PD deficient cases 3 cases (18.8%) developed kernicterus compared to one case (2.7%) among G6PD normal cases which was statistically significant (P=0.04). On the other hand there was a note worthy difference between G6PD deficient and G6PD normal cases as regards frequency of using phototherapy, duration of its application (3.9+1.2, 2.85+0.56 respectively) and the need for exchange transfusion (P>0.05). Infants with marked G6PD deficiency were not significantly different from cases with moderate G6PD deficiency as regards sex distribution, hematological indices, time of appearance of jaundice, duration of phototherapy and the need for exchange transfusion. It was found that the incidence of G6PDdeficiency in jaundiced infants was high in this study this defines the role of G6PD deficiency in developing NJ among Egyptian infants. So early neonatal screening programme should be launched for the identification of G6PD deficiency where G6PD is prevalent.

G6PD shortage is a major enzyme that provides reduced energy to the cells by regenerating NADPH in the PPP and also occupied to maintain the intracellular redox potential which helps the cell to counter the oxidative pressure. Centrally with erythrocytes, these cells are at greater risk because of their oxygen carrying capability. Study suggested that if G6PD enzyme is completely absent it is not feasible for the cell to cope with its normal functioning (Luzzatto and Poggi, 2008).

2.5 G6PD Gene

Howes et al., (2012) suggested that G6PD is a abnormal enzymopathy and very identical to the other human red blood cells mutations,and mainly present in the previously malaria endemic areas. The extent of Plasmodium vivax malaria overlaps widely with that of G6PD deficiency; unluckily the drug used for the cure of malaria is primaquine which has side effects of being triggering hemolysis. The study conclude the recent and past data, which is very beneficial for malaria to eradicate this from the society inspite of controlling its clinical load. G6PD deficiency is a multi dimenshional disorder, in terms of spatial heterogeneity in frequency and molecular variants, as well as its connections with P. vivax and primaquine. Keeping in view the aspects of basic physiology, screenings, and risk factor of primaquine-induced haemolysis is required to highlight the advantages and dis advantages of prescribing primaquine in various geographic and demographic settings. It was suggested that haemolytically toxic antirelapse drugs will likely be the only remedial options for the upcoming decade.

Sukumar et al., (2004) reported that G6PD is present in india about thirty years ago and thirteen variants were identified. Research reveals a prevalence study about 14 different variants of population in India, out of the studied population 3166 males were screened, 332 (10.5%) were G6PD-deficient and the incidence ratio was varying from 5.7% to 27.9% in the various community groups. Molecular studies exposed that G6PD Mediterranean (563 C $\rightarrow$ T) was the most frequent (60.4%) deficient variant followed by G6PD Kerala–Kalyan (949 G $\rightarrow$ A; 24.5%) and G6PD Orissa (131 C $\rightarrow$ G; 13.3%). G6PD Mediterranean had a more widespread distribution as compared to G6PD Kerala–Kalyan and G6PD Orissa and was linked with both 1311 C and 1311 T polymorhism. G6PD Mediterranean have found to be less enzyme activity than the other two. G6PD Chatham (1003 G $\rightarrow$ A) with unknown red cell enzyme activity and G6PD Insuli (989 G $\rightarrow$ A) with normal G6PD activity were very rare in the Indian population. The large number of mutations causing G6PD deficiency points to the fact that the genetic diversity of these populations is considerably lowered than expected.

G6PD gene comprises of 13 exons and 12 introns over a 100 Kb region on the X chromosome. An identical locus G6PD gene, possibly a pseudo gene on chromosome 17 has been recognized. G6PD cDNAs from normal subjects and those with some mutations have been sequenced. 5′ end comprises non coding sequence. Certain cytidine goes on methylation at 3′ end having a regulatory role (Minucci et al., 2012).

They also found 140 mutations in G6PD gene. Vulliamy et al., (1992) critically analyzed the gene for 140 mutations. Reported mutations were further compared with other studies especially concerning the changes in the 3D structure of the enzyme.

2.6 Mechanism of Action of G6PD

G6PD is a cytoplasmic enzyme that is involved in the catalysis of initial step in PPP pathway, by producing a coenzyme, nicotinamide adenine dinucleotide phosphate (NADPH). In turn, NADPH regulates the quantity of glutathione which helps the red blood cells during any stress (Figure 2.1).

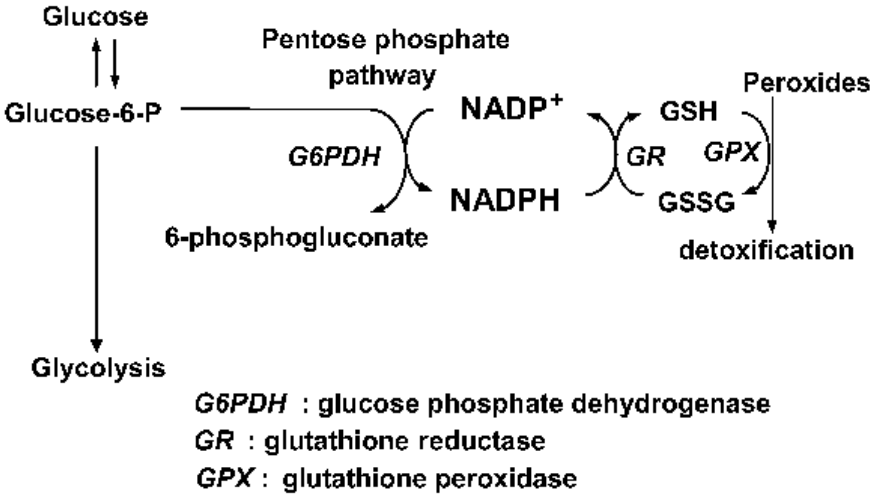

FIGURE 2.1: Pentose phosphate Pathway Triggering NADPH Production.

Gordon et al., (1995) reported that 17-20 ketosteroids are the major retarder of the NADP binding linked oxidation of glucose 6 phosphate by mammalian glucose 6 phosphate dehydrogenase. The inhibition is uncompetitive with respect to both NADP+ and glucose 6-phosphate, To determine the complete mode of action, steroidal effects were examined on human G6PD catalyzing the reverse reaction, i.e, the reduction of the gluconolactone by NADPH to avoid problems linked with the known instability of 6-phospho-δ-gluconolactone, the natural product of glucose 6-phosphate oxidation, the more stable 6-phospho-γ-gluconolactone was used.

Dehydroepiandrosterone, epiandrosterone, 16α-bromoepiandrosterone, and dehydroepiandrosteronesulfate all repressed the reverse reaction uncompetitively with respect to both NADPH and the γ-lactone. The Ki values for each of these steroids, determined by varying either coenzyme or substrate in forward and reverse reactions, are very similar, The study result shows that steroids avoid G6Pd attachment to the ternary enzyme-coenzyme-substrate ternary complex (es Arese et al., (1989) reported that haemolytic episodes in G6PD deficient patients by ingesting fava beans was lower than in the past. However, invivo oxidant damage naturally is by this process useful for the study of sensitive or damaged red blood cells (RBC) clearance from circulation. The following aspects have been considered:

- Pathophysiology of Favism, including incidence, salient features, and sequence of events.
- RBC variations during the haemolytic episodes: biochemical, rheological and morphological variations present in the rbc at any stage.
- Harmfull substances in Vicia faba and their mode of action: by treating with divivine and isouramil the G6PD deficient rbc's (substances in fava beans) initiates the same changes as observed during favism.
- Intravascular vs extravascular haemolysis: extravascular (i.e. phagocytic) in favism rbc removal of damaged cell is predominant.

- The rbc removal signal: in analogy with a recent model to recognize sensitive cells, we suggest removal of fava bean damaged rbc be mediated by a position of antiband 3 antibodies and complement C3 fragments, recognized as non-self recognition signal by monocytes and macrophages.

Studzinski et al., (1962) reported that Four TPN linked dehydrogenases – Isocitric Dehydrogenase, Glucose-6-phosphate Dehydrogenase, 6 Phosphogluconic Dehydrogenase and the Malic Enzyme – they were studied in adrenal cortex and found that they present in cell sap. On these enzymes the effect of corticotrophin wa studied and noted before and after the corticotrophin administration. G6PD ezyme showed increased elevation when corticotrophin is administered. The increase in adrenal gland was not directly related. G6pd enzyme is uniformely distributed. After corticotrophin administration however the marked elevation is present in the 3rd part of adrenal cortex, the current findings are in cotext to corticotrophin administration.

2.7 Symptoms Associated with G6PD Deficiency

In a G6PD deficient individual a number of clinical disorders are involved, i.e mycordial infarction, diabetes, sternous excercise. Mainly the exsisting infections or the oxidative drugs exposure are the basic cause of these infections. The exact mechanism by which the cell become sensitive and and leads towards the oxidative damge is still unknown. The clinical causes are fatigue, lower back pain, jaundice and anemia. The markers used to diagnose are LDH, increased level of unconjugated bilirubin, and reticulocytosis. Other symptoms include rapid heart rate, shortness of breath, urine that is dark or yellow, fatigue, dizziness, paleness, jaundice, or yellowing of the skin (Cappellini et al., 2008).

2.8 Treatment

Those individual having G6PD deficiency they do not need any special treatment. The reason for the Oxidative stress is removed by avoiding the causative agentse.g drugs, fava beans and other oxidants. The basic thing is to find out the causative agent is important which is responsible in the management of haemolysis in the individuals having G6PD deficiency. Heamolysis which is of mild type in the G6PD patients is usually cured in 8-14 days and the patient donot require any transfusion (Lawrence, et al., 2015).

Domingo et al., (2013) reported that by addressing both declared and undeclared malaria we could handle this. Recent available drugs are those belonging to 8-aminoquinolone group, such as primaquine. Unluckily people with G6PD deficiency when faces these drugs induce certain hemolytic reactions. G6PD deficiency is the most frequent enzyme disorder, affecting more than four hundred million people globally.

Scaling up the curing procedure for G6PD deficiency, at two levels: 1) on individual basis the safely case projection and management, 2) To guide the p.vivax treatment procedure. Technical and on ground discrepencies must be highlited, expand access to G6PD screening prior the administration of the drugs In this report from a stakeholder meeting held in Thailand on October 4 and 5, 2012, the G6PD redical cure testing is necessasary, the main emphasis is on the G6PD screening. G6PD testing in support of radical cure. The report also describes recommendations for evaluation of diagnostic tests for G6PD deficiency in support of radical cure.

Peters et al., (2009) reported that G6PD deficiency is a X chromosome disorder that include four hundred million people globally. To diagnose a heterozygous women with G6PD deficiency it is difficult, these womans having normal as well deficient red cell populations. To findout the difference between heterozygous females also homozygous females cytochemical test are the method of choice. G6PD deficiency is mainly present in those regions were malaria is more prevalent. In

these areas, malarial treatment is done with drugs which causes hemolysis. A most reliable test is done for screening of G6PD. In this review, it is concluded that both male and females are screened by two different methods so that G6PD deficiency is determined. The screeninig test that is fluorescent spot test is the ideal method for diffrentiating hemizygous G6PD-deficient men from non-deficient men. For women, the cytochemical assay is the method of chioce. However, this test is tougfh to perform and it is necessary to merge this test in a Kit (Frank et al., (2005).

G6PD deficiency, is the most frequent enzyme disorder, which is prevalent globally, causes a group of diseases including neonatal hyperbilirubinemia, acute hemolysis, and chronic hemolysis. Patient with these conditions may be asymptomatic. People of African, Asian, Mediterranean, or Middle-Eastern descent are more effected by this disorder. Approximately four hundred million people are affected globally. Homozygotes and heterozygotes can be symptomatic, The disease is more common in homozygous male. The change in NADPH formation in the erythrocytes results in the diagnosis. Usually this is done by methemoglobin reduction test. Variety of genetic changes cause a deviated levels of enzyme deficiency, classes assigned to each level of deficiency and its clinical causes,hemolysis due tony stimulant in the form of an infection, oxidative drug, or fava beans, cure is directly linked with the avoidance of these stressors. Acute hemolysis is self-limited, rarely it requires transfusion. Neonates with hyperbilirubinemia requires photo treatment and transfusion if necessary to avoid kernicterus. For the treatment of G6PD Antioxidants e.g vitamin E and selenium are not as much advantageous to reduce oxidative-induced haemolysis of G6PD-deficient. Research is going on to find out any medication.

2.9 Risk Factors Assocated with G6PD Deficiency

Major risk factors associated with G6PD deficiency are male sex, neonate, ethnicorigin of mediterranian, Africans or southeast asia, family history, recent exposure

to oxidative drugs, infections and recent exposure to fava beans. (Carvalho et al., 2011). Drugs which are the major risk factors and that induce hemolysis in Rbc's are listed in (Table 2.1) In haemolysis Folic acid and iron are used, while G6PD deficiency often shows no symptoms and the related haemolysis are generally brief.

TABLE 2.1: List of Common Drugs Causing Hemolysis in G6PD Deficient Patients. (Wandell et al., 1998)

S.No	Drugs
1.	Antimalarials: Primaquine, Pamaquine
2.	Sulfa drugs: Sulfonamide, Sulfamethoxazole
3.	Dapsone
4.	Nitrofurantoin
5.	Analgesics: Acetanilide
6.	Vitamin K
7.	Doxorubicin
8.	Methylene blue
9.	Nalidixic acid
10.	Furazolidine, Niridazole
11.	Phenazopyridine (Pyridium)
12.	Isobutyl nitrate
13.	Sulfapyridine
14.	Thiazosulfone
15.	Phenylhydrazine
16.	Toluidine blue

Huang et al., (2004) reported that the ratio of G6PD in whites as compared to Asian is lower. A case-control study was designed to identify the effects of eight deteremined risk factors [breast feeding, ABO incompatibility, premature birth, infection, cephalo hematoma, asphyxia, G6PD (G6PD) deficiency, and variant UDP-glucuronosyltransferase 1A1 (UGT1A1) gene] and a suspicious analog [organic anion transporter 2 (OATP 2) gene] on severe hyperbilirubinemia in Taiwanese neonates. The 100 control hospital and 72 studied subjects consisted of

neonates with marked serum bilirubin levels $\geq$ 342 μM and <256.5 μM, respectively. The RFLP-PCR– method was applied to detect the UGT1A1, OATP 2, and G6PD genes. The results of multivariate logistic regressions, adjusted for covariates, revealed odds ratios (ORs) of 4.64 [95% confidence interval (CI): 2.25–9.57; $p < 0.001$], 3.36 (95% CI: 1.54–7.35; $p = 0.002$), and 3.02 (95% CI: 1.30–6.99; $p = 0.010$) for neonates who were on breast feeding, and carry the variant UGT1A1 gene at nucleotide 211 and the variant OATP 2 gene at nucleotide 388, respectively, as well as feed with breast milk are at high risk to develop severe hyperbilirubinemia.

TABLE 2.2: List of Drugs that can be Safely given in Therapeutic Dosage in Patients of G6PD Deficiency with Nonspherocytic Hemolytic Anemia. (Wandell et al., 1998)

S.No	Drug
1	Acetaminophen
2	Acetophenetidine (Phenacetin)
3	Aminopyrine
4	Actazoline
5	Antipyrine
6	Ascorbic acid (vitamin C)
7	Benzhexol
8	Chloramphenicol
9	Chlorguanidine (Proguanil)
10	Colchicin
11	Diphenylhydramine
12	Isoniazide
13	Levo dopa
14	Menapathine
15	P-Aminobenzoic acid
16	Phenylbutazone
17	Phenytoin
18	Probenacid

19	Procainamide
20	Pyrimethamine
21	Quinine, Qunidine, Chloroquine, Mefloquine
22	Proguanil
23	Halofantrine
24	Streptomycin
25	Sulfacytine
26	Sulfadiazine, Sulfamerazine, Sulfisoxazole
27	Trimethoprim
28	Vitamin K

Chapter 3

Materials and Methods

3.1 Methodology and Techniques

3.1.1 Study Area

For present study, any person diagnosed with G6PD deficiency was included in any area of the Twin cities (Islamabad & Rawalpindi). Present sampling has representation from areas of Rawalapindi and Islamabad and samples were collected from different hospitals and private sector labs.

3.1.2 Ethical Approval

As per concern the involvement of human subject the current study was approved by the Ethical committee of Capital university of science and technology. Patient consent was also prescribed compulsory before sample collection.

3.1.3 Selection Criteria

A total of 150 individuals were screened for G6PD deficiency, out of them 49 positive cases were G6PD deficient which were selected for the current study.

Screening test for both sporadic as well familial cases were selected. For sporadic cases diagnosed patients of any age from any area of Pakistan were include for blood sample collection. While for Familial study, all members of the affected family were included for blood sample collection.

3.1.4 Sample Collection

In present study, familial as well as sporadic patients sampling was done. Patients from different area of Pakistan, suffering from G6PD deficiency and required criteria were selected for collection.

3.1.5 Data Collection

A Questionnaire was designed for current study, as reported earlier by (Al Sweedan et al., 2009). The questionnaire contain informations like Patient name, sample ID, age, gender, time of first diagnosis, time of onset of symptoms, martial status, triggers or inducers, clinical manifestation during haemolytic period, G6PD screening and other biochemical informations. The questionnaire also include history of neonatal jaundice, Favism and drug induced reactions. All questionnaire were properly filled before blood collection.

3.1.6 Blood Sample Collection

The venous blood was the sample of choice for this study. Venous blood was collected from the anti-cubital vein of forearm of the patient by using a fine 19 G sterile disposable Becton Dickinson (BD) syringe and following the good laboratory practices. A total 03 ml blood sample was collected in a special container having an anticoagulant K2/K3 EDTA (Di Potassium/Tri Potassium Ethylene Diamine Tetra Acetic Acid) to prevent the blood sample from clotting, a 02 ml blood sample was also collected from patients who do not have biochemical test information in heparin tubes for biochemical analysis. Blood collected in EDTA tubes were stored

at 4℃ while blood collected in heparin tubes were centrifuged at 3000 RPM for five minutes and the supernatant plasma were collected by using pastuerpipetts in a 1.5 ml eppendroff tube. The plasma were stored at -20 ℃for biochemical analysis.

3.1.7 G6PD Screening Test

All EDTA samples of patients were screened for G6PD deficiency by qualitative enzyme assay. The screening was done through a qualitative screening kit of Atlas medical UK. The principle of screening assay was that G6PD act on substrate Glucose 6 phosphate present in reagent vials, this results in the reduction of NADP to form NADPH which in turn reduces a colored substrate Dichlrophenol indophenol blue to a colourless DCPIP, the rate of decolorization of coloured substrate is proportional to concentation of enzyme in the sample. Screening was done according to following standard protocol.

1. First of all volume of blood required for screening was selected according to haemoglobin concentration of patient, as mentioned in (Table 3.1).
2. To the selected volume of blood 1 ml distilled water was added and allowed to stand for ten minutes to lyse the RBCs. The resulting solution now known as hemolysate.
3. Brought all reagents to room temperature.
4. Initially 0.5ml buffer (pH=8.0) was added to lypholized substrate powder (Glucose 6 phosphte & DCPIP) by gentle mixing, the solution was allowed to stand for ten minutes to completely dissoloved.
5. Then 1 ml of this prepared hemolyste was added to substrate containing Glucose 6 phosphate and colour substrate.
6. By Thoroughly mixing the mixture 1 ml of mineral oil was added.
7. Plugged the vial with cotton and placed in dark area for 1 to 2 hours.

8. After every 30 minutes the solution was checked for decolourization.

9. In the normal indivisual the decolourization time is 5 to 60 minutesin deficient indivisualsdecolourization time exceed 2 to 24 hours.

10. All samples were processed by assay and positive (Deficient) indivisuals samples were selected for haematological and biochemical analysis.

Table 3.1: Adjustment of Blood Volume According to Hb Level for G6PD Screening.

Hb Concentration (g/dl)	**Blood required (ml)**
7 – 9.5	0.04
9.6 – 11.5	0.03
11.6 – 13.5	0.025
13.5 – 15.0	0.02

3.1.8 Estimation of AST

The coupled enzymatic technique with UV monitoring of the NADH disappearance is the preferred, routine method.

3.1.9 Reagents

The commercial kit contains:

1. Buffer/ substrate containing Tris buffer and L-aspartate.

2. Enzyme/coenzyme/ α-oxoglutarate containing LD, MDH, NADH and α-oxoglutarate.

3. The working reagent was formed by adding an appropriate amount of buffer substrate into the bottle of enzyme reagent. It is stable for 24 hours at room temperature and 14 days on 2-8℃.

3.1.10 Procedure

1. By bringing all the reagents, including controls, to room temperature.
2. In a test tube 100ul of sample was added. Then 1.0ml of working reagent was added by gentle mixing.
3. Semi-automated analyser was set at kinetic mode, adjusted the instrument to zero with distilled water and noted the initial absorbance after 1 minute. Read again after 1, 2 and 3 minutes to determine $\triangle$A(rate of change of absorbance/min) at 340nm.
4. AST U/l = 1746 (factor) x $\triangle$A (340nm/min)

3.1.11 Reference Range

0-37U/l.

3.2 Serum Bilirubin

3.2.1 Principle

Total bilirubin was determined by Jendrassik and Grof methods. Bilirubin reaction with diazotised sulphanilic acid to form an azo dye in the presence of an accelator (caffeine),which releases albumin-bound bilirubin. Azodyegives a blue colour in an alkaline medium. Direct(conjugated) bilirubin reacts with diazotised sulphanilic acid without the addition of an accelerator to form a blue- colored complex .The indirect bilirubin(unconjugated) was calculatedfrom the difference between the total and the direct bilirubin.

3.2.2 Reagent

The commercial kit contains:

1. Sulphanilic acid with HCl
2. Sodium Nitrite
3. Caffeine with Sodium Benzoate
4. Tartrate with Sodium Hydroxide

3.2.3 Estimation of Total Bilirubin (TB)

1. By Bringing all the reagents, including controls, to room temperature.
2. The tubes was marked as sample Blank and Test.
3. 200μl of sulphanilic acid with HCl was added in both the tubes.
    - In both the test tubes 01 drop (5μl) of Sodium Nitrite was added.
    - In the test tube marked as 'test' 1.0ml of caffeine with sodium benzoate was added.
    - 200μl of serum sample was added in both of the tubes.
4. Sample are allowed to retain at room temperature for 5-30 minutes,and read the test against blank at 578nm (560-600nm).

3.2.4 Estimation of Direct Bilirubin (DB)

1. After serial 1&2 steps of TB estimation, added 200μl of sulphanilic acid with HCl in both of the test tubes marked as 'test' & 'sample blank' and then added 01 drop (5μl) of sodium nitrite only in tube marked as 'test'.
2. Normal saline i-e 2 ml (sodium chloride-9g/l) and 200μl of the serum sample was added in both of the test tubes. 3. By gentle mixing, tubes was allowed to stand for 5 minutes at room temperature and the absorbance of the Test against the Blank at 546 nm (530-560) wavelength was noted.

Chapter 4

Results and Discussion

In our study a total of 49 patients were selected both familial and sporadic cases of G6PD deficient were collected. There were three families found for G6PD Deficieny. For familial sampling, all normal and affected person were asked for sampling. They were properly labelled, clinically diagnosed with positive G6PD screening test. 44 sporadic cases were also collected. During blood collection all other required information in questionnaire included age, sex, drug inducers, HB level, serum total bilirubin, direct bilirubin and enzymes such as AST (aspartate amino transferase) and LDH (lactate dehydrogenase). This data was recorded in Microsoft excel sheets for further statistical analysis.

4.1 Frequency Distribution Based on Anthropometric Parameter

In present study out of 49 human subjects there were 45 males and 4 female patients. The percentage of screened individuals i.e, female was 8.2 % and male patients have a percentage of 91.8 % which clearly indicates that G6PD deficiency is X-linked disorder in which males were normally affected more as compared to females (Figure. 4.1).

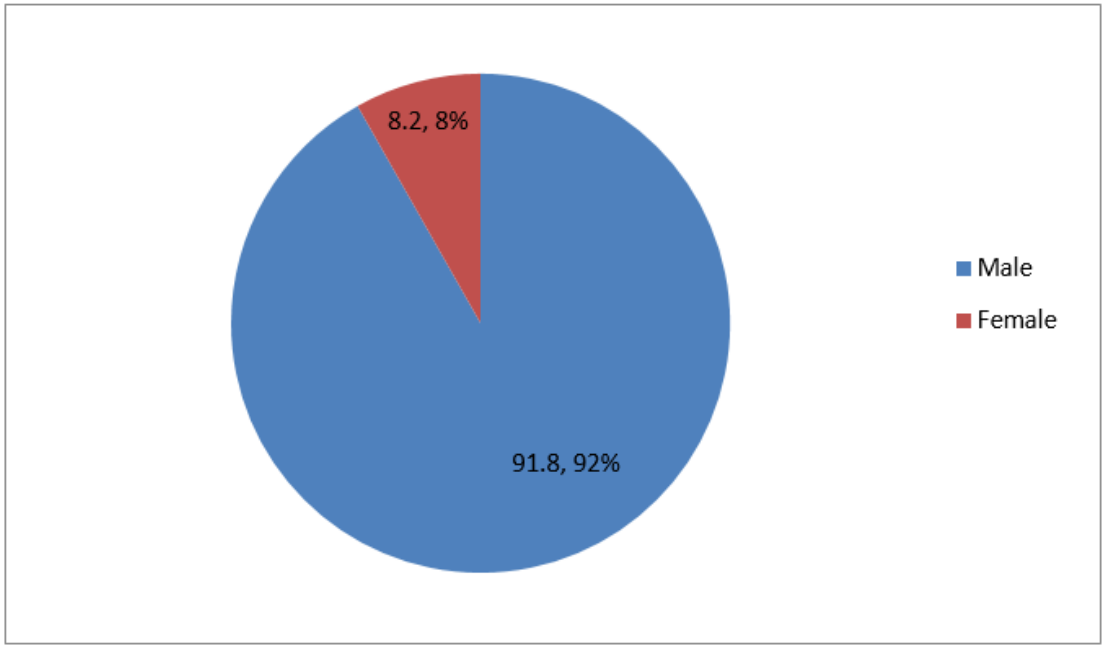

FIGURE 4.1: Frequency Distribution Based on Gender of G6DP Deficient Patients.

A study conducted by Guindo in 2007 reported that the responsible gene for G6PD deficiency is present on the X chromosome, as we know that males having one copy of X gene, so if male inherit only one mutant gene, it will be deficient but female require two genes to inherit the disorder and if chromosomal inactivation takes place in that case female would be deficient.

A Nawanwat at Trang hospital Thailand, reported that the occurance of G6PD deficiency were 90 male-G6PD deficiency patients and 12 female-G6PD deficiency patients and the prevalence rate of G6PD deficiency were 3.33%, of male and 0.87% of female.

Fu et al., 2018 discussed in his study that the gene for G6PD is present on the X chromosome, thus in males it occurs only as a normal or deficient hemizygous genotype; but females having two copies of X chromosome but inactivation in early in embryogenesis during the process of lyonization. In the current study, more patient were at the age of less then ten years including neonates. The number of patients below the age of ten years are with a percentile of about 51.0 % . Frequency distribution showed that about 24.5% patients were of the middle age

(11 to 30 years). The remaining 24.4% patients were above thirty years (Figure 4.2).

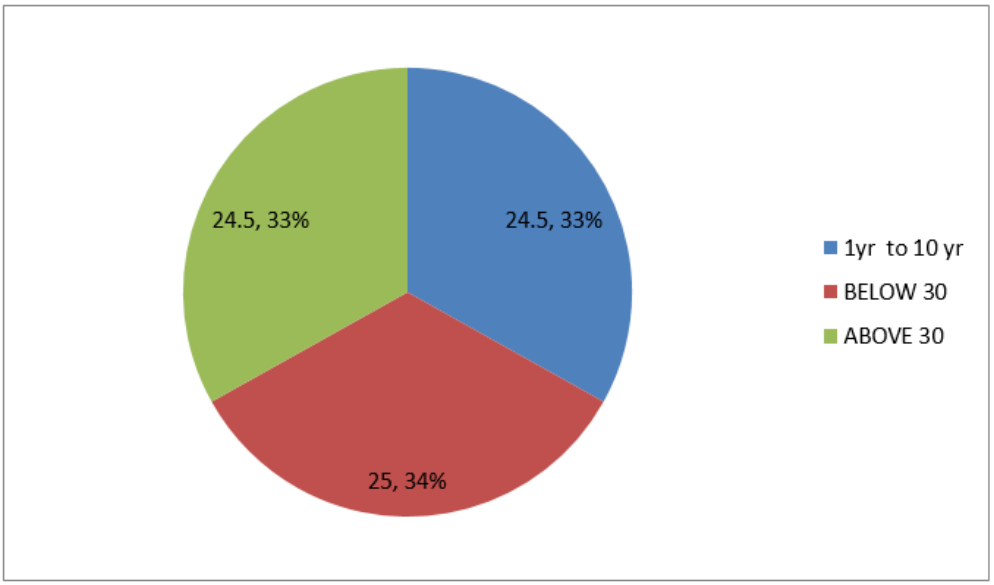

FIGURE 4.2: Frequency Distribution Based on Age in Years of G6DP Deficient Patients.

A study conducted by C.K. Firempong in 2016 according to his questionnaire, 312 subjects were included and had the same findings 56.13% were males and 43.87% were females with an overall average age of 10.4 ± 5.0 years.

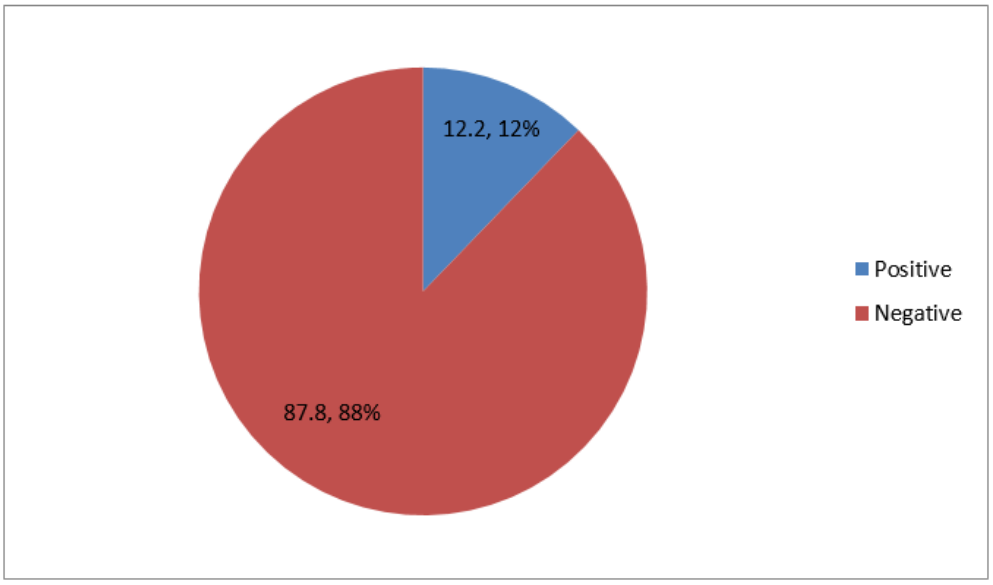

FIGURE 4.3: Frequency Distribution of G6DP Deficiency According to History of Subject Families.

In the current study, 3 families were selected for further investigation. About 6 patients were those who had a positive family history of G6PD deficiency. Such families had two or more patients in their families. The frequency distribution of familiel cases wasabout 12.2% . Patients whom family history about G6PD deficiency were negative with a percentile of 88.8%. The frequency of sporadic cases were about 88.8% . For familiel cases, the samples from whole family members like father, mother, brothers, sisters, grand father, grand mother etc were collected and screened for G6PD deficiency.

4.2 Frequency Distribution Based on Clinical Presentation

4.2.1 Frequency Distribution Based on Disease History

In the current study patients with a percentile of 34.7% were diagnosed first at neonate stage. This indicate that these patients have G6PD deficiency by birth. About 42.9%, were those who were diagnosed for the first at childhood stage. Although these patients have G6PD deficiency before diagnosis time but they were diagnosed when they experience some kind of haemolytic reaction after using some inducers of hemolysis. The remaining 22.4% patients remained asymptomatic before the adult stage. These patients were diagnosed at adult stage when they encountered jaundice or hemolysis after using triggering molecules. This indicate that G6PD deficient patient may live normal life span if they avoid the use of risk compounds. From the current study, it was observed that course of clinical manifestation was variable in such patients. The frequency distribution of clinical appearance for the first time was similer to first time diagnosis as discussed above. This indicate that patients did not screened their G6PD deficiency before they experience any haemolytic crisis.

S. Russ Richardson et al., 2018 reported that, in newborns, G6PD deficiency is referred as major threat for the progression of neonatal jaundice. Neonates with

G6PD deficiency are two times more likely to develop jaundice than the general population, and approximately 20% of kernicterus cases are associated with G6PD deficiency. Although rare, Neonates who encounter hyperbilirubinemia in first 24 hours should be considered for G6PD deficiency. In adults, common symptoms and exam findings of G6PD deficiency include those of hemolytic anemia or possibly red blood cell sequestration by the spleen. Some of these manifestations include pallor, jaundice, fatigue, splenomegaly, and dark urine.

The ANOVA for the disease history including first time diagnosis and onset of symptoms was also performed. The impact of stages of onset of disease was found significant with p¡0.05. Statistically significant values at p¡0.001 are represented by three stars (Table 4.1).

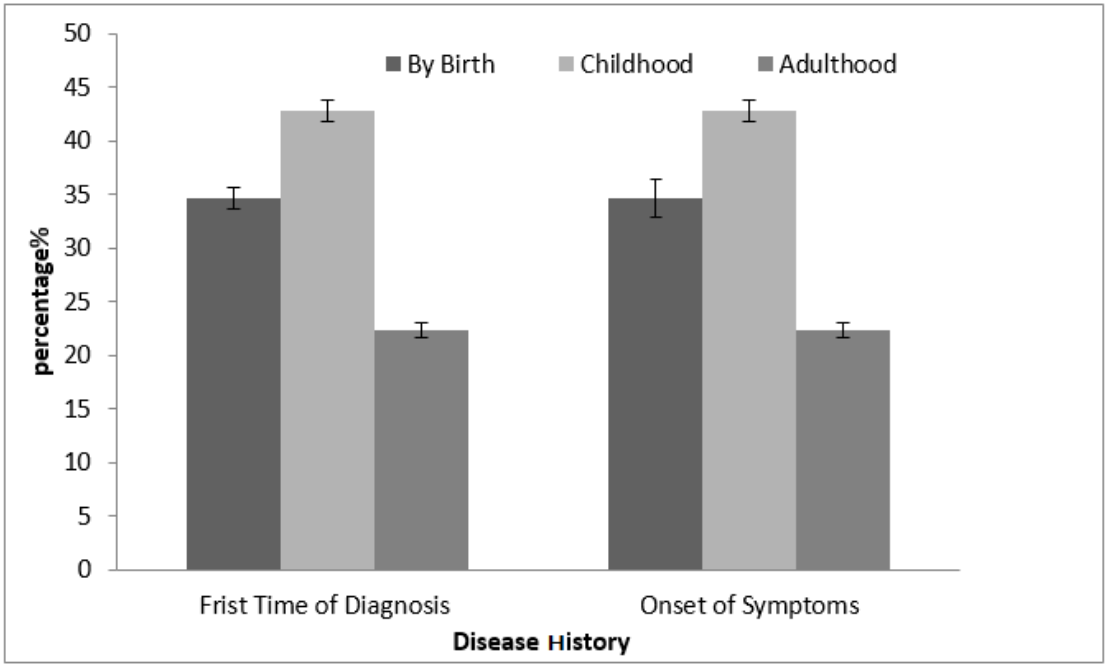

FIGURE 4.4: Frequency Distribution Among G6PD Patients Based on Disease History.

TABLE 4.1: Analysis of Variance for Frequency Distribution Based on Disease History

S. No	Source of Variation	P Value	P Value Summary	Significant
1	Interaction	1.0000	Ns	No
2	Stages of onset	0.0002	***	Yes
3	Disease history	1.0000	Ns	No

4.3 Frequency Distribution Based on Risk Factors

4.3.1 Drugs Sensitivity

There are certain drugs which causes complications in G6PD deficient indivisuals. The two most common medicines which acts as a trigger of complications in this disorder are sulphur drugs and anti malarial drugs. In the current study, these two variables were included to identify their sensitivity in such patients. Out of G6PD deficient patients, 8.16% were sensitive to sulphur medications like sulphones and sulphonamides. 61.22% patients were found sensitive to anti malarial drugs (Figure 6). The high frequency of sensitivity against anti malarial drugs in such G6PD deficient patients was because of high frequency of malaria, lack of awareness and common use of unprescribed medicines in Pakistani population.

Belfield et al., 2018 reprted in his recent study that primaquine is an anti malarial drug belonging to 8 aminoquinoline group, The use of this drug followed to the deficiency of G6PD during the Korean War in the 1950, when about 250000 soldiers were given that drug for a period of 14 days. The level of hemolysis induced is due to the variant involved. Variants other than African showed severe hemolysis even if the medication is not stopped. African variant patients have ability to cope G6PD deficiency somehow because the newly generated cells produce bit of G6PD. If the drug is not stopped the meditrerean variant showed severe life threatening hemolysis. There have been 14 documented primaquine-related deaths in the databases, with 12 occurring secondary to severe hemolysis. For G6PD deficient patients FDA approved a label for primaquine. It is common nowadays to check the G6PD status of the patient, until the prescription is made.

The two way ANOVA for drugs and its sensitivity was performed. The sensitivity of the patients towards drugs was also found statiscally significant ($p<0.05$). Interaction of drugs with their sensitivity in patients was also found is highly significant ($p<0.05$).Statistically significant values at $p<0.001$ (Table 4.2).

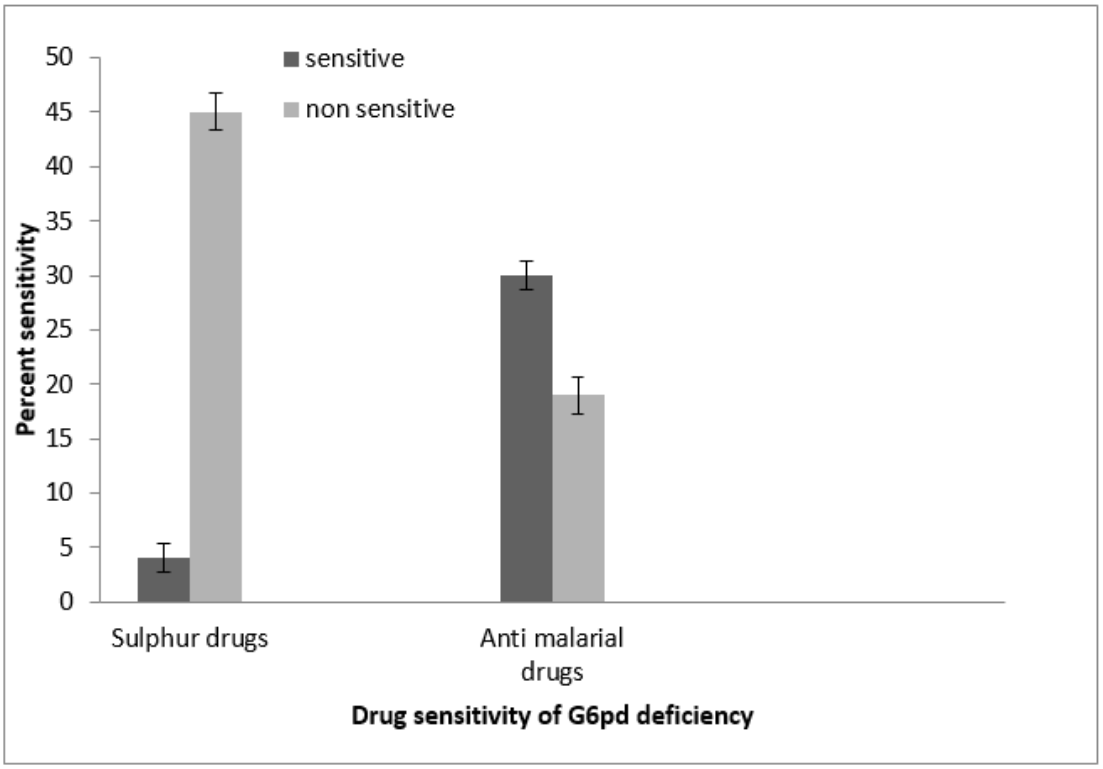

FIGURE 4.5: Frequency Distribution Based on Sensitivity of G6PD Deficient Patients for Sulphur & Anti Malarial Drugs.

TABLE 4.2: Two Way ANOVA for Frequency Distribution Based on Drugs Sensitivity.

S. NO	Source of Variation	P Value	P Value Summary	Significant
1	Interaction	<0.0001	***	Yes
2	Drugs	1	Ns	No
3	Sensitivity	<0.0001	***	Yes

4.3.2 Frequency Distribution Based in Antibiotics

Antibiotics was found another risk factor in such patients. In present study,2 antibiotics were found that causes hemolysis in these patients. Ciprofloxacin which belongs to Flouro Quinolones group of antibiotic was a common stimulator of complications in such patients. This antibiotic commonly used in UTI, respiratory infections and enteric fever. In the present study 8.16% patients were found to have sensitivity to ciprofloxacin. The patients non sensitive to this antibiotic were having 46.9% percentile (figure 4.7). Most of ciprofloxacin sensitive patient encounter hemolysis when they used these antibiotic for one of the above mentioned drug. Another important antibiotic which was found as inducer of hemolysis and

TABLE 4.3: Two Way ANOVA for Antibiotic Sensitivity.

S. NO	Source of Variation	P Value	P Value Summary	Significant
1	Interaction	<0.0001	***	Yes
2	Antibiotics	<0.0001	***	Yes
3	Sensitivity	<0.0001	***	Yes

other complication was Nalidixic acid. Nalidixic acid commonly used to treat UTI. This antibiotic belong to Quinolone group, in the present study it was observed that 4.08% patients have sensitivity to this antibiotic. (Figure 4.6)

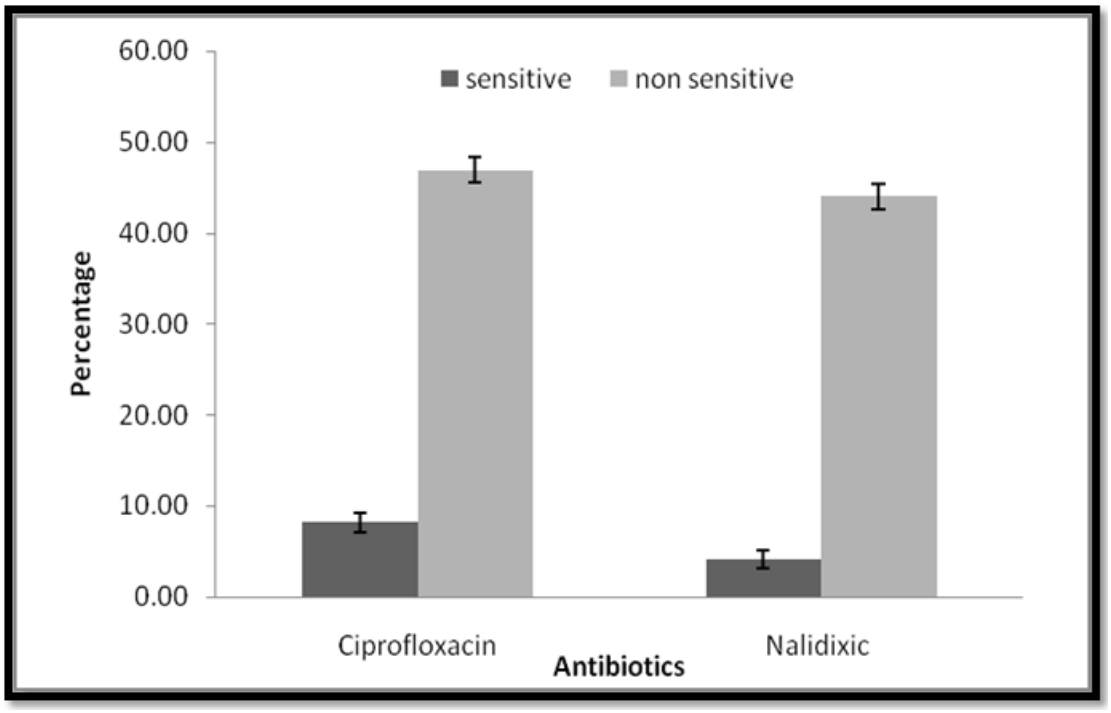

FIGURE 4.6: Frequency Distribution of Antibiotics Sensitivity of G6PD Deficient Patients. The Bars Represent Standard Deviation of Mean Values.

Chu et al., (2018) reported in a journal that most frequently administered drugs such as 8-aminoquinoine, Ciprofloxacin, Nalidixic acid, rasburicase, and other agents (methylene blue) may cause hemolysis in G6PD deficient persons, including heterozygotes. Two way ANOVA for frequency distribution based on antibiotics sensitivity was performed. The impact of antibiotics, sensitivity to these antibiotics and their interaction, all factors were found statistically significant ($p<0.05$) Statistically significant values at p¡0.001 are represented by three stars (Table 4.3).

4.3.3 Food and Chemical Sensitivity

In the present study, one of the most common trigger of hemolysis in G6PD deficiency was food. The variables belong to this category studied were bean, banana selected from different research papers. The most common food variable i.e. Bean causing acute haemolytic anemia also known as Favism. Some defiecient people were found that they when use bean they encounter haemolytic anemia, their percentage was 34.6%, while remaining 65.3% were non sensitive. The study also included new born babies who were not able to use bean so bean sensitivity might b more. It was concluded from this study that favism (bean sensitivity) was common in such patients.

Fruit like banana was also found in certain patient that causes hemolytic crisis. About 6.12% patients were found that when they use banana experience mild hemolysis, the remaining 93% were found non sensitive. Some chemical like insecticides may be the cause of hemolytic complications, in present study 4.0% patients were found that they face haemolytic crisis when they were exposed to insecticidal spray. The remaining 95.9% did not have such problem (Figure 4.7).

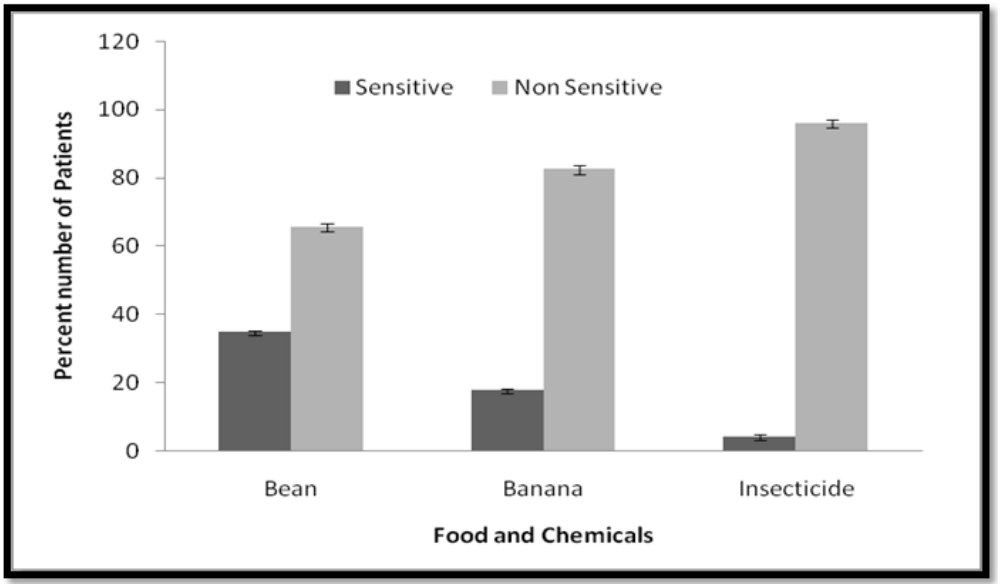

FIGURE 4.7: Frequency Distribution of Sensitivity of Foods and Chemicals in G6PD Deficient Patients. Bars Represent Standard Deviation from Mean Values.

Table 4.4: Two Way Anova for Frequency Distribution Based on Food and Chemicals.

S. NO	Source of Variation	P Value	P Value Summary	Significant
1	Interaction	<0.0001	***	Yes
2	Food and Chemicals	1	Ns	No
3	Sensitivity	<0.0001	***	Yes

Sebastien La Vieille et al., (2018) reported that fava beans can induce high haemolytic episodes with G6PD deficiency. In this review, the most suitable food i.e fava beans are conclusive because of oxidative nature.

Two way ANOVA for frequency distribution based on food and chemicals and its sensitivity was performed. The sensitivity of the patients towards food and chemicals was also found statistically significant ($p<0.05$) irrespective of type of food and chemicals studied, impact of food and chemicals with their sensitivity to them was also found statistically significant ($p<0.05$). Statistically significant values at $p<0.001$ are represented by three stars, (ns) represents non significance.

4.4 Frequency Distribution Based on Hematological Parameters

4.4.1 Frequency Distribution Based on Hemoglobin Level

In the current study, selected patients screened for G6PD enzyme deficiency by qualitative assay. Almost all patient gave positive (Deficient) result in the assay. Hemoglobin levels were estimated in the current study. Patients regarding hb level, 40.8% were below 10g/dl. This very low hb level was because of high hemolysis of rbc's and anemia due to use of anti malarial drugs. Judith Recht et al., (2018) discusssed in his study that the major harmfull effect of primaquine is that it is dose dependant in G6PD deficiency, the most frequent enzyme disorder. This indicate that anti malarial dugs are the major risk factor from drug groups leads to the alteration in haematological parameters. S.Russ Richardson et al.,(2018)

discussed in his study that erythrocytes are exposed to ROS as cells are involved in oxygen transport as well mature cells could not prepare their cellular proteins.

In our study the hb level of 48.2% patients were below 14 g/dl. In this category most patients were neonate so there hb level were low because of neonatal jaundice. The remaining 12.2% patient hb level were above 14 g/dl, there hb level were normal as these were asymptomatic or hb level recovered after hemolysis. In a study by Esmeralda Vizzi et al., (2016) reported in his findings that biochemical screening unmask that about 23.6% patients were enzyme deficient and the common G6PD enzyme activity 4.5 ± 1.2 U/g Hb. Two way ANOVA was performed on Hb levels (Table 4.5). The impact of concentration of Hb level of G6PD deficients patients was significant, ($p<0.05$). Statistically significant values at $p<0.001$ are represented by three stars.

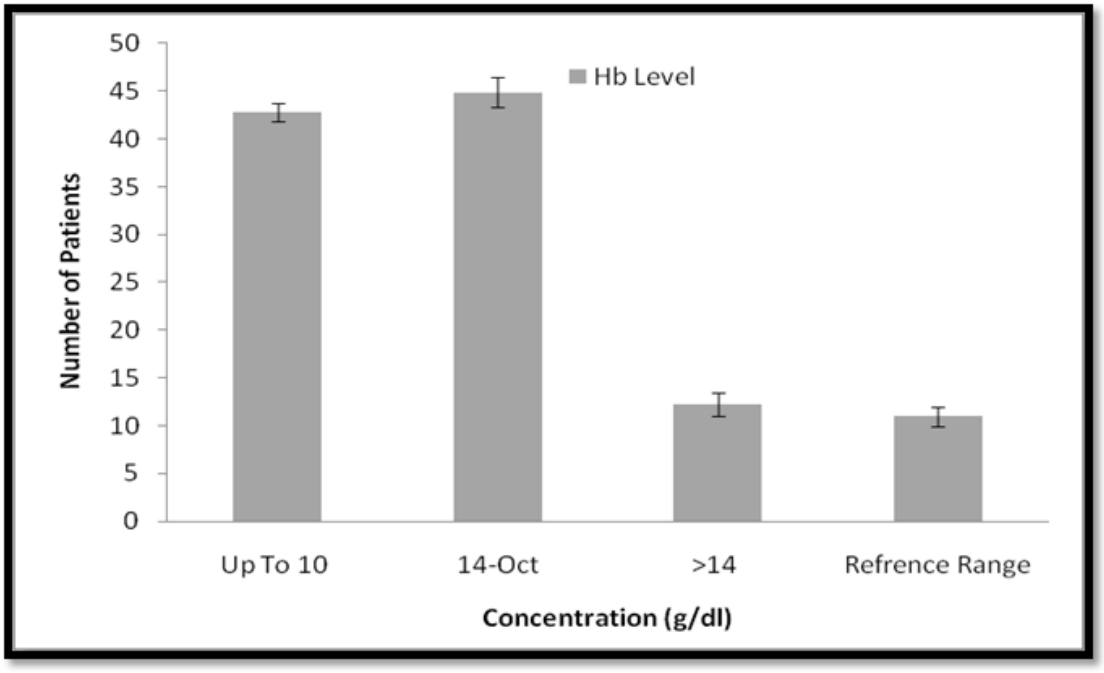

FIGURE 4.8: Frequency Distribution of Hemoglobin Level in G6PD Deficient Patients.

4.4.2 Frequency Distribution Based on Retics Count

As G6PD deficiency is associated with hemolysis and anemia.In haemolytic anemiareteiculocytes count usually increases.In the present study retics count was

TABLE 4.5: Two Way ANOVA for Frequency Distribution Based on Hemoglobin Level.

S. NO	Source of Variation	P Value	P Value Summary	Significant
1	Interaction	1	NS	NO
2	Concentration	<0.0001	***	Yes
3	Hb Level	1	NS	NO

TABLE 4.6: Two Way ANOVA for Retics Count in G6PD Patients.

S. NO	Source of Variation	P Value	P Value Summary	Significant
1	Interaction	1	Ns	No
2	Concentration	P<0.0001	***	Yes
3	Retic count	1	Ns	No

performed for all selected patients.Around 65.3% patients retics count were between 2% to 4% which was a high retics count, 12.2% patients retics count was between 4% to 6% which was more high, 6.1% patients retics count was greater then 6% which was a very high level. Retics count was found high in most selected patients because during hemolysis reticulocytes pull out from bone marrow to blood to compensate for deficiency of normal RBcs. Increased Reticulocytes were indication of ineffective erythrophoesis in such patients. The retics count of 16.3% patients was normal this was because these patients were asymptomatic or they have slight anemia or mild hemolysis. A study conducted by Nawanwat et al.,(2014) at Trang hospital and reported that in G6PD deficient individuals the retic count was 4.18±2.74%, which correlates with our findings.

Two way ANOVA for retics count was performed (Table 4.6). The impact of concentration of retics on G6PD deficiency was found statistically significant ($p < 0.05$). Statistically significant values at $p<0.001$ are represented by three stars.

4.4.3 Frequency Distribution Based on Morphology of Red Blood Cells

As G6PD deficiency is associated with haemolytic anemia. In haemolytic anemia rbc's morphology become change. Slide microscopy were performed to look and

observe rbc's morphology. In the current study 79.6% show abnormal rbc's morphology. This abnormality was of three types in all the patients, that was macrocytosis (Enlarge RBCS > 7.2 um in diameter), anisocytosis (variation in RBCs size) and poikilocytosis (variation in RBCs shape). Macrocytosis was because of abnormal rbc's coming from bone marrow to the blood circulation to compensate for loss of normal RBCs during hemolysis. It was also found that 25 to 50 percent cells show macrocytosis in sever haemolytic conditions. The haemolytic crisis also influence size and shape of rbc's. Cells were variable in size and shape like oval cell, elliptical cells and round cells etc. Again it was found that variation in size and shape was 25 to 50 percent in hemolysis period in selected patients. In a recent study Osime et al., (2018) reported that in G6PD deficient individuals the hematological parameters such as hemoglobin concentration, hematocrit, values, mean cell volume, mean cell haemoglobin and abnormal rbc morphology changes were observed to be in test participants when compared to the controls (P¡ 0.05, P = 0.001, P = 0.022, and P = 0.044, respectively).

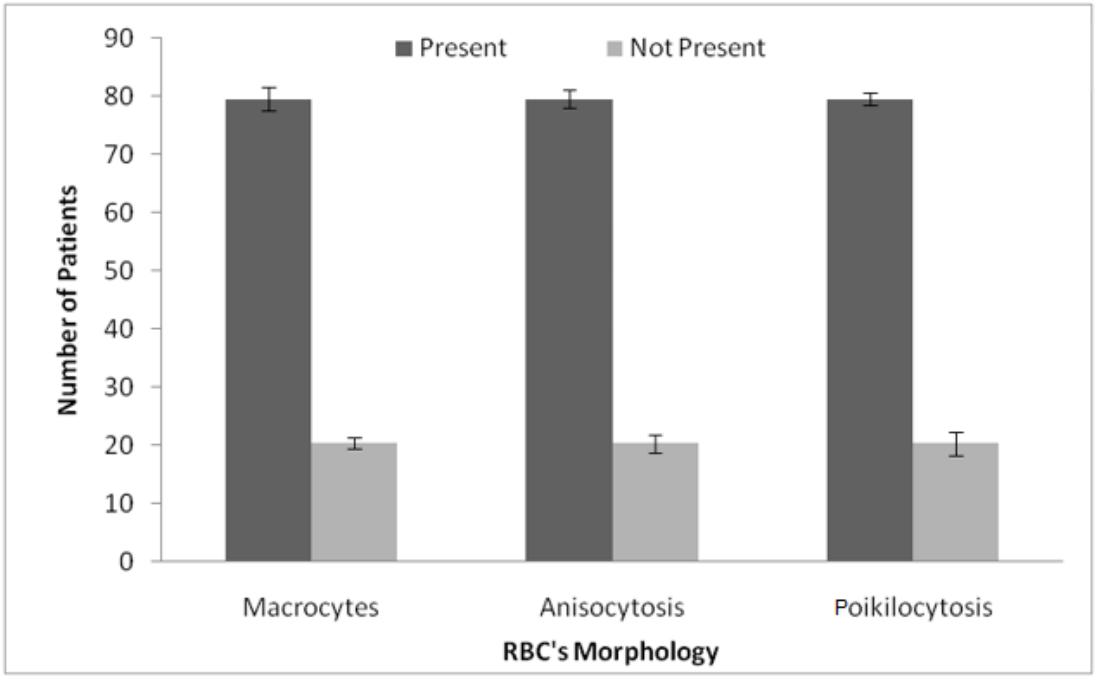

FIGURE 4.9: Frequency Distribution of RBC's Morphology in G6PD Patients.

Two way ANOVA for RBC's morphology was performed (Table 4.7). The presence of variation in size and shape of RBC's was found stasitically significant with

Table 4.7: Two Way ANOVA for RBC's Morphology in G6PD Patients

S.No	Source of Variation	P Value	P Value Summary	Significant
1	Interaction	0.178	Ns	No
2	Morphology	1	Ns	No
3	Type of variation (Size & shape)	<0.0001	***	Yes

impact (P<0.05). Statistically significant values at p<0.001 are represented by three stars.

4.5 Frequency Distribution Based on Biochemical Parameters

4.5.1 Serum Bilirubin Level

Serum bilirubin level was another biochemical variable that was estimated in the present study for selected patients. Total bilirubin level of 30.6% patients were between 1mg/dl to 6 mg/dl in this study. This was high level then normal range. Total bilirubin level in51.0% patients serum was between 6.1 mg/dl to 12.0 mg/dl which was more high level of bilirubin. Total bilirubin level of 18.4% patients was greter then 12.1 mg/dl and exceed to 30 mg/dl in some patients. Serum total bilirubin level was high in G6PD deficient patients because bilirubin is the end product of haemoglobin breakdown, so increase hemolysis as found in such patients always lead to hyerbelirubinaemia. The variation in hyperbilirubinemia was because of variability in haemolytic crisis in selected patients. Indirect bilirubin was another biochemical factor analysed for selected patients in the study. Serum indirect bilirubin level of 30.6% patients was between 1mg/dl to 6 mg/dl which was high level. Indirect bilirubin level of 46.9% patients was between 6.1mg/dl to 12 mg/dl which was more high. Indirect bilirubin level of 22.4% patients was more then 12.1 mg/dl which was very high level. Serum indirect bilirubin level was high in these patients as increased hemolysis always lead to high level of indirect bilirubin which is also called unconjugated bilirubin. The variability in indirect

Table 4.8: Two Way ANOVA for Serum Total Bilirubin Level in G6PD Patients.

S. NO	Source of Variation	P Value	P Value Summary	Significant
1	Interaction	1	Ns	No
2	Concentration	P<0.0001	***	Yes
3	STB	1	Ns	No

Table 4.9: Two Way ANOVA for Serum Direct Bilirubinlevel in G6PD Patients

S. NO	Source of Variation	P Value	P Value Summary	Significant
1	Interaction	0.1161	Ns	No
2	Concentration	P<0.0001	***	Yes
3	Direct bilirubin	1	Ns	No

Table 4.10: Two Way ANOVA for Serum Indirect Bilirubinlevel in G6PD Patients

S. NO	Source of Variation	P Value	P Value Summary	Significant
1	Interaction	0.5008	ns	No
2	Concentration	P<0.0001	***	Yes
3	Indirect bilirubin	0.7502	ns	No

bilirubin level in these patients was because of variability in haemolytic crisis in different patients.

In the study 63.2% patients have normal direct bilirubin level. Direct bilirubin usually have little significance in haemolytic condition as this type of bilirubin usually have high significance in liver diseses. Direct bilirubin level of 36.7% patients was slightly increased which has no or little significance. S. Russ Richardson in his recent publication stated that in newborns, G6PD deficiency is recognized as a serious threat for the development of neonatal Jundice002E Neonates with G6PD deficiency are two times more likely to develop hyperbilirubinemia than the general population, and approximately 20% of kernicterus cases are associated with G6PD deficiency.

Two way ANOVA for Serum bilirubin and was performed (Table 4.8). Impact of concentration of serum bilirubin including all i.e (direct, indirect and total) within G6PD patients was found statistically significant. Statistically significant values at $p<0.001$ are represented by three stars.

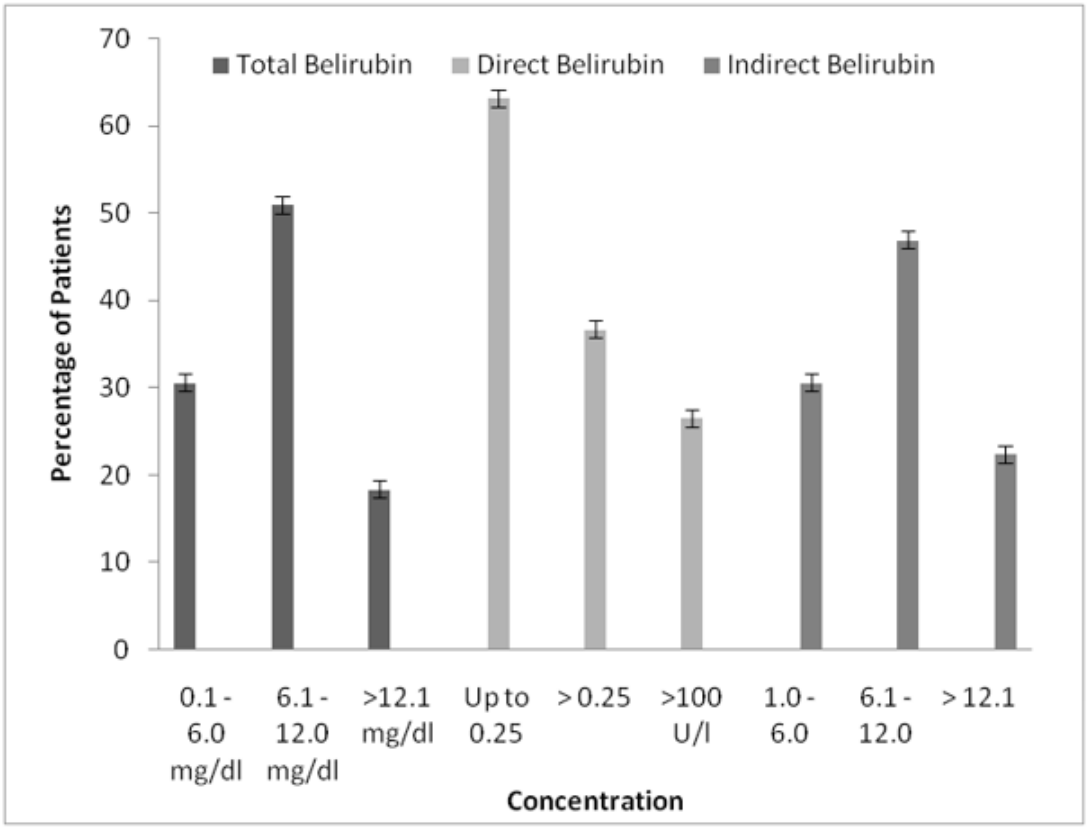

FIGURE 4.10: Frequency Distribution of Serum Bilirubin in G6PD Patients.

4.5.2 Serum Enzyme ELDH and AST

Incresed serum enzymes level, like LDH and AST were always associated with hemolysis. In the present study, these two chemical parameters were analysed. Serum LDH level of 28.6% patients was found between 500 U/l to 1000 U/l which was higher then normal value. Serum LDH level of 53.0% patients was greter then 1000 U/l which was very high level. As LDH is found in rbc, these high level was because of increased hemolysis of rbc's in such patients. Serum LDH level of18.4% patients was found normal. This was because these patients were asyptomatic or they experience mild hemolysis or anemia or they recovered from haemolytic crisis.

AST was another chemical parameter taken in this study. Serum AST level of 51.1% patients was between 40 U/l to 100 U/l which was higher then normal. Serum AST level of 26.5% patients was beyond 100 U/l which was very high. The higher values was because of increased destruction of rbc's in these patients as AST is also present in rbc's. Serum AST of 18.4% patients level were within normal

Table 4.11: Two Way ANOVA for LDH in G6PD Patients.

S. No	Source of Variation	P Value Summary	Significant
1	Interaction	Ns	No
2	Concentration	***	Yes
3	Enzyme	Ns	No

range as these patients were either asyptomatic or they face slight hemolysis or they were at recovery stage from anemia.

Kitchin et al., (2018) reported in his study that the hepatotoxicity of mammalian cells was assessed in dose results and structure activity studies in humans hep G 2 cells treated with 3 different nanoparticles, different CeO2, three SiO2, and one TiO2-based particles for 3 days. A number of biochemical parameters were raised, cellular growth, hepatic function, and oxidative stress, all but TiO2 showed a marked degree of cytotoxicity. Four nanomaterials (all three SiO2) seizes G6PD activity with marked decline in the enzyme activity obtained at 30 μg/ml. In this study, the more suitable and effective assays were G6PD, glutathione reductase, superoxide dismutase, LDH, and AST. The primary drug dose on which the level of rbc membrane damages itleads to the release of LDH (All $P < 0.01$ and more elevated than 2.4 fold) at 1000 μg/ml.

Compared to % LDH release, generally HepG2 cells showed the same or less degree of response with % AST. In two cases, nano CeO2 Y6 andTiO2 T8141, no significant % AST effects were observed even at 1000 μg/ml. Nano CeO2 Z7 again was the strongest CeO2 particle giving $P < 0.001$ responses at 100, 300, and 1000 μg/ml. The parameter % AST was increased by several doses of nano SiO2 J0 and nano SiO2 K1 (at 100, 300, and 1000 μg/ml).

Two way ANOVA for LDH and AST was performed (Table 4.11,4.12).The impact of concentration of AST and LDH on G6PD deficiency was found statistically significant. Statistically significant values at $p < 0.001$ are represented by three stars.

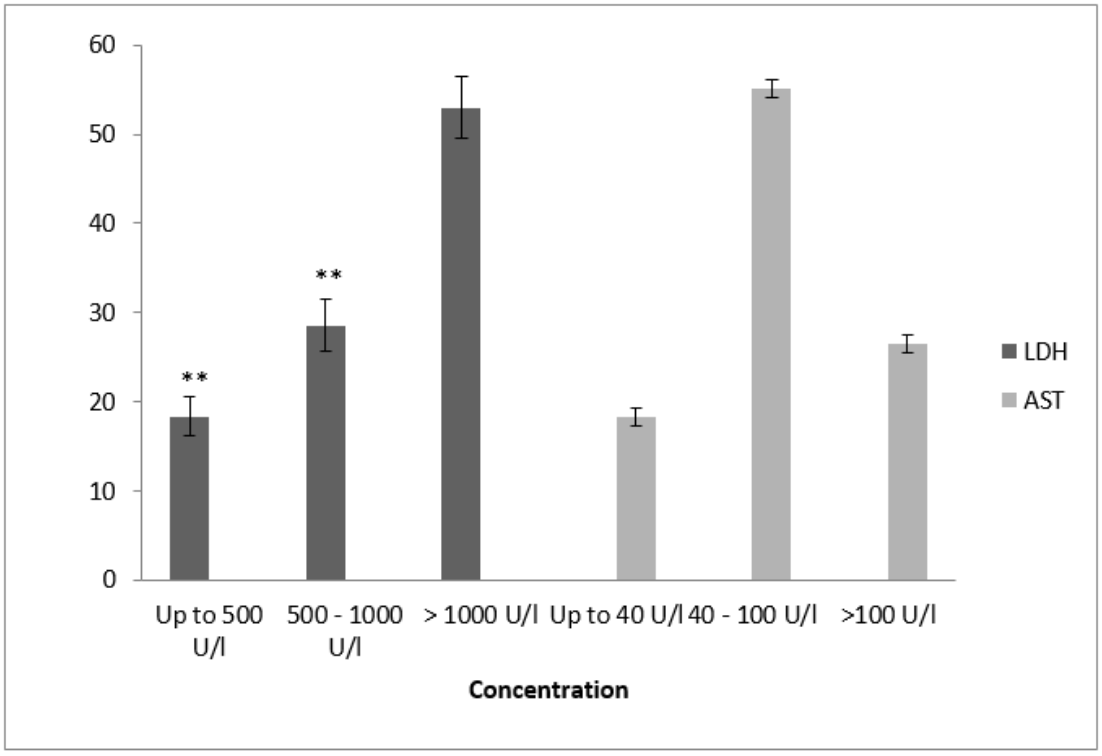

FIGURE 4.11: Frequency Distribution Based on LDH and AST.

TABLE 4.12: Two Way ANOVA for AST in G6PD Patients.

S. No	Source of Variation	P Value Summary	Significant
1	Interaction	Ns	No
2	Concentration	***	Yes
3	Enzyme	Ns	No

4.6 Frequency Distribution Based on Neonatal Jaundice and Favism

Neonatal jaundice is very common in G6PD deficient indivisuals.In the present study, different neonates were also selected. Neonates blood was screened for serum bilirubin to confirm jaundice. In case of adult, and children, history of neonatal jaundice were asked from them or their parents. About 65.3% patient have neonatal jaundice or history of neonatal jaundice although some of these patient were not diagnosed that time for G6PD deficiency. Neonatal jaundice occur in G6PD deficient persons either physiologically or because of certain drugs like vitamin K commonly given to newborn as there liver did not synthesizes

vitamin k in early stages of life.About 34.7%did not face jaundice in there early life.

Chunyunfu et al., (2108) reported in his study that the percentage of G6PD deficiency is 7.28% in new borns. The percentage is higher in Guizhou (1.94%), Guangzhou (3.7%), Chaozhou (2.68%) and Jiangxi (3.6%) in southern China, in Greek (7.7%) and Indian (7.8%). High prevalence is due to the patients selected from different areas of the populations. Although the cases are compound heterozygous, heterozygous, homozygous G6PD mutations were defined as G6PD deficient patients, considering female heterozygotes may also be deficient due to lionization.

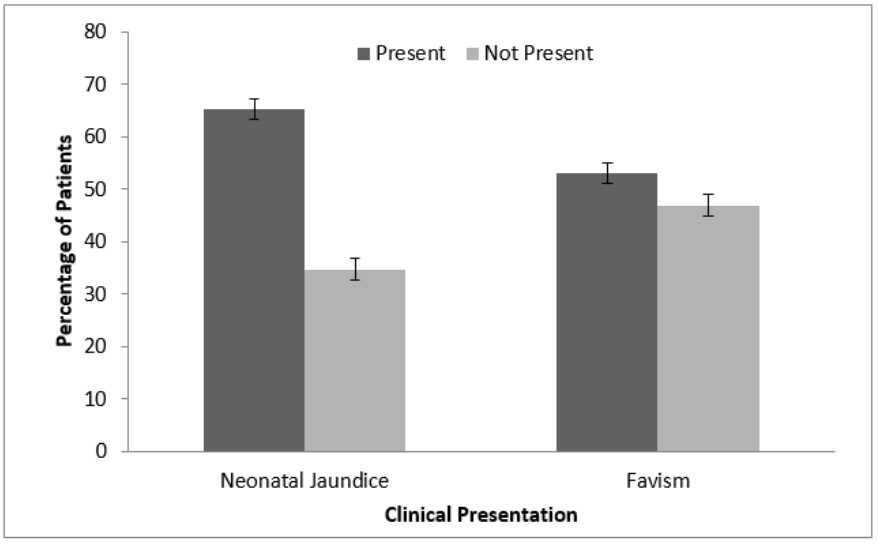

FIGURE 4.12: Frequency Distribution of Neonatal Jaundice and Favism in G6PD Patients.

In present study, Favism was specifically targeted as it is one on the most important complication in G6PD deficient person after eating bean. About 53.1% patients in the current study were found that they face acute haemolytic anemia when they eat bean. It was also observed in the study that these patients when ingest bean they encounterhemolysis after few hours of eating bean. This indicate that bean contain some kind of strong oxidative compound that causes severe and intense hemolysis.

TABLE 4.13: Two way ANOVA of Neonatal Jaundice and Favism in G6PD Patients.

S. No	Source of Variation	P Value Summary	Significant
1	Interaction	***	Yes
2	Neonatal Jaundice and Favism	Ns	No
3	Row Factor	***	Yes

Two way ANOVA for neonatal Jaundice and favism was performed (Table 4.13) The impact of occurance of neonatal jaundice and favism, with G6PD deficient patients is highly significant ($p<0.05$). Statistically significant values at $p<0.001$ are represented by three stars.

Chapter 5

Conclusions and Recommendations

In the current study, about 49 G6PD deficient patients were studied, different pa- rameters like anthrophometric, biochemical & haematological were checked. We observed that G6PD deficient patients could experienced hemolysis when they encountered by any oxidative agent, like different drugs, food and chemicals. In the current study, we studied the levels of aspartate amino transferase (AST), lactate dehydrogenase (LDH), hemoglobin (Hb), retics count as wells as rbc morphology in G6PD deficient people, and found that all these parameters were elevated which is an evidence for hemolysis as well G6PD deficiency. In the current study we found that fava beans, anti-malarial drugs and biochemical parameters were found to be major risk factors of G6PD deficiency patients. Rest of the parameters i.e banana, insecticides and sulphur drugs were found to be minor risk factors of G6PD deficient patients. From the current study we found that a G6PD deficient patient can live a normal life span until they are encountered by any triggering molecule i.e, bean, certain antibiotics and chemicals

This study will help physicians to counter G6PD deficient patient, by avoiding certain anti malalrial and antibiotics (Ciprofloxacin, Nalidixic acid), few chemicals like insecticides sprays and foods like banana and favabeans which are oxidative

and can trigger hemolysis. Furthermore, mass screening for G6PD deficiency in the population is necessary to identify G6PD deficient individuals, and to educate them at least for their medications and diet.

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Appendix A

QUESTIONNAIRE FOR RESEARCH PROJECT

Project Title: Mutational analysis of G6PD in origin of Islamabad and Rawalpindi

Investigator(s): Capital University of Science & Technology, Expressway, Kahuta Road, Zone-V, Islamabad.

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BIODATA: (This information provided by Patient will be confidential)

First Name: ——————————Mid Name:——————

Last Name:——————DOB:——————Age:——————

Gender:——————Contact (Office)——————Home:——————

Cell:——————Email:——————

Permanent Address:

Address: ——————————

City:————————————————Province:————————————

Temporary Address:

Address: ————————————————————————————————

City: ————————————————Province: ————————————

CLINICAL INFORMATION:

Class of Disease:

(a) Class 1

(b) Class 2

(c) Class 3

(d) Class 4

When was the disease first diagnosed:

Onset of symptoms:

(a) By Birth

(b) Childhood

(c) Later

Hemolytic reaction occur through the use of:

(a) Drugs

(b) Fava beans

(c) Herbal medicine

(d) Insecticides and Fungicides

(d) Food colouring

Time for hemolytic reaction after use of hemolytic inducers:

Clinical findings during hemolytic reaction:

(a) Pale Yellow skin colour

(b) Yellow colouration of sclera of eyes

(c) Weakness

(d) Fatigue

(e) Dark yellow colour urine

(f) Paler than usual stool

(g) Pruritis

(h) Weight loss

(i) Vomiting

(j) Fever

Names and type of medicine which induce hemolysis:

Other information:

BIOCHEMICAL INFORMATION:

G6PD screening test

During hemolytic reaction: Hemoglobin level: Reticulocyte count: RBC's morphology: Serum bilirubin level:

- Direct
- Indirect
- Total

Hemoglobinuria:

Serum LDH level:

Other information:

Thanks for your Patience and cooperation.