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:

Molecular detection of some mutations of G6PD in a sample of Iraqi patients

A Thesis

Submitted to the Council of Science College / Al-Nahrain University, In partial Fulfillment of the Requirement for the Degree of Master of Science in Biotechnology

By

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Hadeel mohammed.

AHA	Acute Haemolytic Anaemia
bp	base pair
CNSHA	Chronic Nonspherocytic Hemolytic Anemia
DF	Degree of freedom
DGGE	Denaturing Gradient Gel Electrophoresis
DNA	Deoxyribonucleic acid
DsDNA	Double strand Deoxyribonucleic acid
EDTA	Ethylene diaminetetra acetic acid
G6PD	Glucose-6-phosphate dehydrogenase

G6PD HF	Glucose-6-phosphate dehydrogenase high-frequency
GSH	Glutathione
GSSG	Oxidized Glutathione
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NCBI	National Center for Biotechnology Information
NNJ	Neonatal Jaundice
PCR	Polymerase Chain Reaction
PPP	Pentose Phoshate Pathway
RNA	Ribonucleic acid

RBC	Red blood cell
Rpm	round per minute
SNP	Single nucleotide polymorphism
UV	Ultra violate
V	Volt

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Summary

This study was constructed to discuss an issue regarding Glucose 6 phosphate dehydrogenase deficiency enzyme, and the genetic disorder that leading to heamolysis anemia.

A total of 50 blood samples were collected from different hospitals (Yarmook Hospital, Center Child hospital , AL Alweyaa , Medical City) beside to 20 sample as a control (healthy).

These samples were vary in the deficiency of the enzyme from mild to chronic according to the test of G6PD. The period time of collection samples took about three months from March to June 2014. According to the G6PD test, it was found that the percentage of mild was 42% , chronic was 40% and carrier G6PD was 18% as stated on statistics analysis of Chi square.

DNA was extracted from obtainable samples and subjected to PCR amplification using 3 specific primers designed for purpose of this study, the first primer (Frag I) with product length 115 bp, while second primer (FragII) with product length 1500 bp and third primer (Frag III) with product length 2000 bp That positive result obtained from respectively G6PD, as compared with those with chronic state, result of DNA sequencing reveled that percentage of substitution was 70% and insertion was 30%.

The other side of the study, concerned with the genetic inheritance of the disease by taking Iraqi families and Note inherited disease in these families where found that the lack of this enzyme found in the X chromosome and the father inherited disease to females and mother inherited disease to male and female and that the ratio of male will be more.

1. Introduction and Literature Review.

1.1. Introduction.

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common human enzyme defect, being present in more than 400 million people worldwide (Cappellini and Fiorelli, 2008). During G6PD deficiency, the red cells are unable to regenerate reduced nicotinamide adenine dinucleotide phosphate (NADPH), a reaction that is normally catalyzed by the G6PD enzyme (Ademowo and Falusi, 2002). Since the X chromosome carries the gene for G6PD enzyme, this deficiency mostly affects males. The two major conditions associated with G6PD deficiency are hemolytic anemias and neonatal jaundice, which may result in neurological complications and death (Luzzatto and Gordon-Smith, 2001). Screening and detection of G6PD deficiency helps in reducing such episodes, through appropriate selection of treatment, patient counseling, and abstinence from disease-precipitating drugs such as antimalarials and other agents.

G6PD is an enzyme present in the cytoplasm of all cells, acting specifically in the maintenance of the integrity of the erythrocytes, preventing the oxidation of hemoglobin and other cellular proteins so the deficiency of G6PD will cause hemolytic anemia and jaundice induced by ingestion of oxidative drugs and/or broad beans , there are different kinds of hemolysis from mild to severe that are seen to differences in variants of the disease (Au *et al*. 2006)

Molecular of studies have shown that the G6PD deficiencies are nearly always caused by single amino acid substitutions (Poggi *et al*, 1990). In addition, it is very important to underline the fact that some of these variants may be asymptomatic in adult subjects (male and females), since they do not

present hemolysis. These asymptomatic carriers are often discovered by means of routine screening or found in large population samples collected, as normal controls, for research purposes (Prchal and Gregg, 2005). This study was suggested to fulfill the aims of:

- Identification the SNP that cause the disease in Iraqi patients.
- Determination the severity of the disease in the relation the type of nucleotide change.

This achieved by:

- Collecting blood samples from G6PD patients.
- Isolation of DNA.
- Construction of new specific primers of G6PD gene.
- Amplification of blood samples by PCR.
- Sequencing of PCR product and performing alignment .
- Identification of the change at molecular level.

1.2. Literature Review.

1.2.1. Glucose 6 phosphate dehydrogenase deficiency.

The glucose 6 phosphate dehydrogenase enzyme plays a critical role in maintaining RBC integrity through catalysing a key step in the cell's metabolic production of reducing equivalents that maintain reduction–oxidation (redox) equilibrium of the cytoplasm. This protects the cell from oxidative attack by radicals derived from oxygen and organic compounds such as drugs and their metabolites(WHO Working Group, 1989). In spite of its vital function, the G6PD enzyme is highly variable, both biochemically and genetically. Detailed reviews of G6PD genetics, biochemistry and clinical characteristics have been previously published (Beutler, 1994, 1996; Mehta *et al*., 2000; Luzzatto, 2006; Mason *et al*., 2007 ; Cappellini and Fiorelli, 2008; ;Luzzatto ,2009, 2010).

1.2.1.1. G6PD Genetics and Inheritance.

The advent of molecular diagnostics following the successful mapping of the G6PD gene's 13 exons (Martini *et al*., 1986) which span 18.5 kb, and the gene's cloning and sequencing in 1986 (Persico *et al*., 1986; Takizawa *et al*. 1986) started to uncover the genetic basis to the enzyme's great variability (Vulliamy *et al*. 1988) . This Mendelian X-linked gene is one of the most highly polymorphic of the human genome with at least 186 mutations having been described (Minucci *et al*., 2012). That said, not all mutations are polymorphic and of public health significance, but many instead appear only sporadically within populations: almost half (66 of 140 mutations reviewed

in 2005 by Mason and Vulliamy) are associated with the most severe clinical phenotypes and are very rare (Mason and Vulliamy, 2005).

Most mutations are single point substitutions (121 of 140). Show figure (1-1) ,(Beutler and Vulliamy, 2002) leading to amino acid substitutions. The absence of more severe mutations reflects the enzyme's housekeeping function which requires some residual activity for cell survival. Knockout studies in mice found G6PD-null mutations to be lethal (Longo *et al*. 2002) and a high degree of evolutionary conservation of certain regions of the gene was identified by comparing the position of mutations across 42 different organisms, pinpointing certain regions of the gene as highly conserved, and hence essential for enzyme function and cell survival (Notaro *et al*. 2000). All known mutations have been found to affect the coding regions of the gene and none described in the regulatory regions, suggesting that reduced enzyme activity levels are associated with enzyme instability, rather than deficiencies in gene expression (Beutler and Vulliamy, 2002).

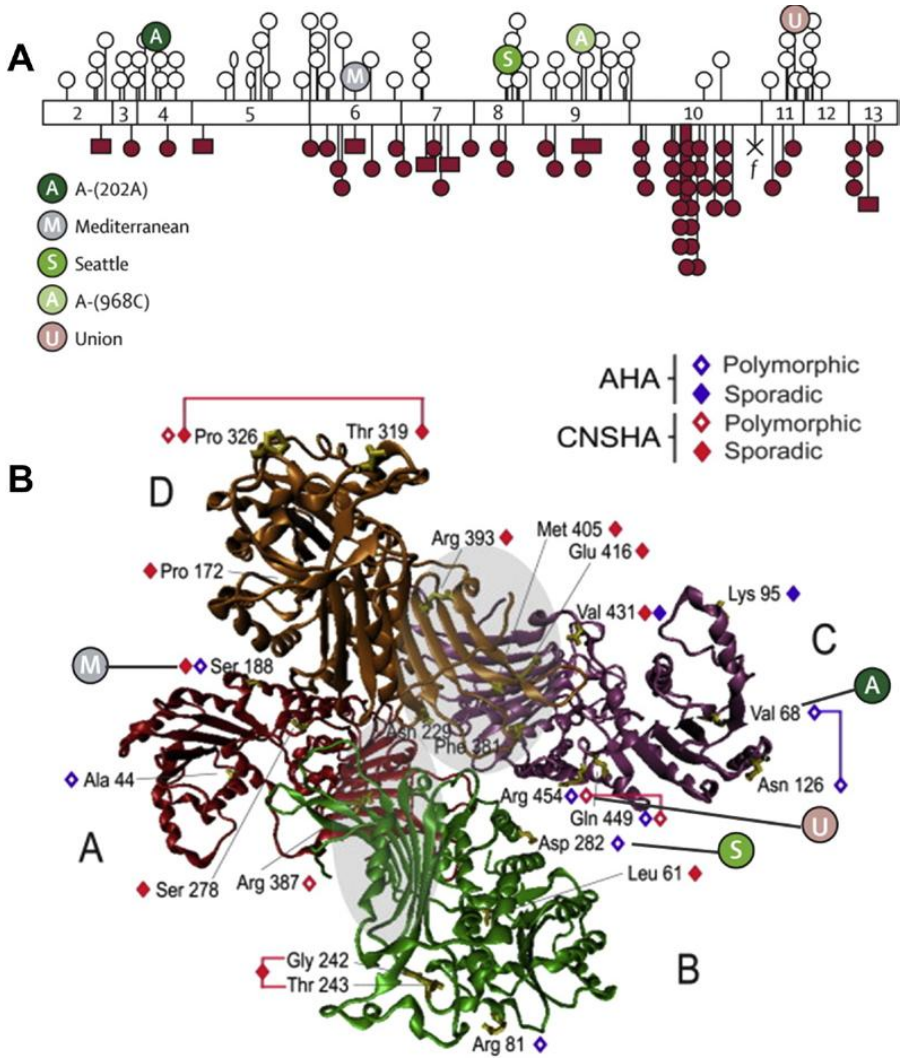
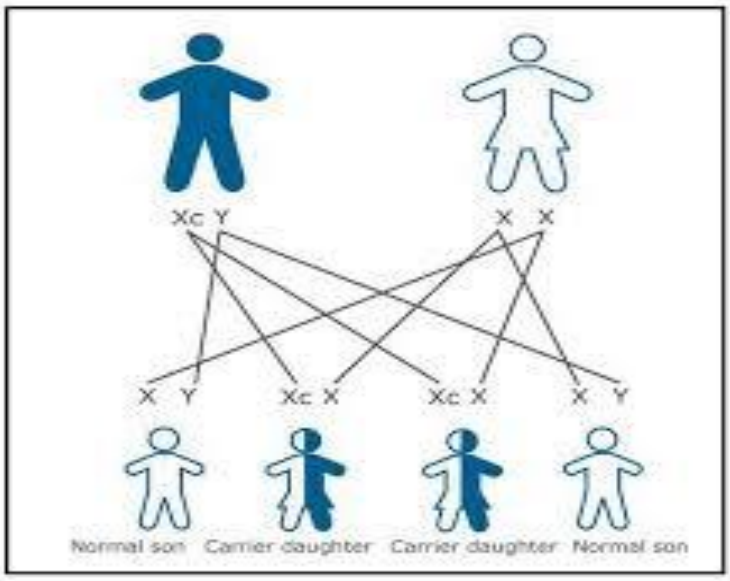


Figure: (1-1): Diversity of mutations in the G6PD gene and enzyme. Panel A shows the distribution of common mutations along the G6PD gene coding sequence. Exons are shown as open numbered boxes.

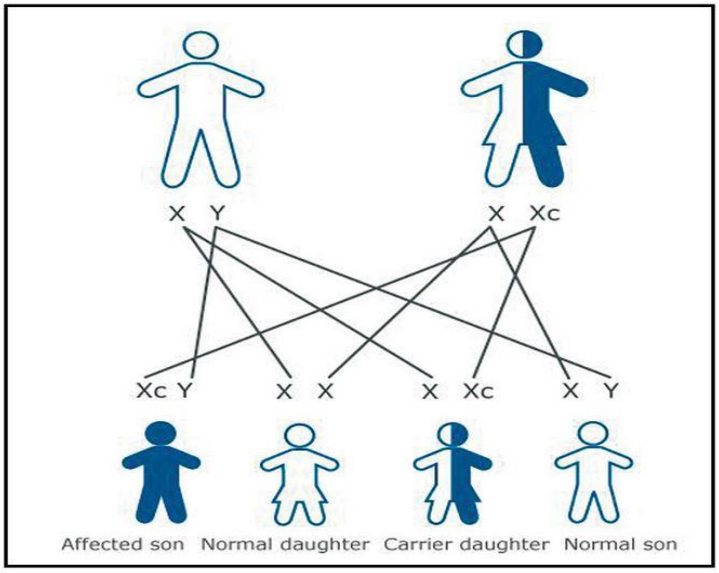
- **Open circles are mutations causing Class II and III variants**
- **filled circles are Class I variants**
- **filled squares are small deletions**
- **the cross represents a nonsense mutation; "f" shows a splice site mutation. (Cappellini and Fiorelli, 2008).**

The G6PD gene's position on the X chromosome has important implications for its population genetics. Unlike in males, for whom the G6PD phenotype was early-on observed to be binary with individuals being either deficient or nondeficient depending upon which allele was inherited (Beutler *et al*. 1955), the gene's X-linked inheritance means that deficiency in females is more complex. Females inherit two copies of the X chromosome and therefore have two populations of RBCs, each expressing one of the two G6PD alleles they carry. If females inherit two identical alleles (both either normal or deficient), their phenotype and clinical symptoms will be identical to those of homozygous males.

Heterozygous females, however, inherit one wild-type and one deficient allele but display a mosaic effect of expression as only one X chromosome is expressed in each cell. One population of cells will express the normal allele and the other population the deficiency (Beutler *et al*., 1962). The ratio of normal to deficient cells is variable, due to the phenomenon of Lyonization (Lyon, 1961). Lyonization is a random process and the resulting proportions of normal and deficient cells may deviate significantly from the expected 50:50 ratio, leading some heterozygotes to have virtually normal expression, and others with expression levels comparable with female homozygotes (i.e. entirely deficient). Heterozygotes may therefore express a spectrum of phenotypes; making appropriate diagnoses with standard binary methods much harder than for deficient males, as many heterozygotes will be phenotypically normal. At the population level, G6PD deficiency is more commonly expressed in males, though in populations with high frequencies of deficiency, homozygotic inheritance can be common, and the prevalence of affected heterozygotes may also be of public health concern show figure (1-2) (Beutler, 1994).



A. If the father has the abnormal gene, all of his daughters will get the abnormal genes as compared with sons.



B. Sons who inherit the abnormal gene from their mother will have the illness

Figure: (1-2): X – Linked recessive Inheritance.(Frank ., 2005).

1.2.1.2 The G6PD Enzyme.

The G6PD enzyme consists of either dimer or tetramer forms of a protein subunit consisting of 514 amino acids. Each subunit binds to an NADP+ molecule for its structural stability, which are positioned close to the interface where the two subunits of each dimer bind (Au *et al*., 2000). The majority of mutations disrupt the enzyme structural stability and thus reduce its overall activity. The effect of each mutation on enzyme structure and function depends on the location of the substituted amino acid. For example, many of the most severe mutations map to exon 10 (Mehta *et al*., 2000) which encodes the binding interface of the subunits and therefore disrupt its quaternary structure and stability. These mutations cause the most severe clinical symptoms and as such do not reach polymorphic frequencies; instead they usually result from independent spontaneous mutations. Mutations which do not cause such severe reductions in enzyme activity are widely distributed across the gene's coding region and throughout the enzyme structure (Figure 1-1), and have been found to reduce the efficacy of protein folding. The residual enzyme activity of G6PD variants ranges from <1% to 100% (Fiorelli *et al*. 2000).

As with all enzymes, G6PD activity decreases with cell age: it is estimated that in normal blood, reticulocytes have about five times higher activity levels than the oldest 10% of RBCs (Luzzatto, 2006). The oldest cells are therefore most vulnerable to oxidative stress. In individuals with intrinsically reduced G6PD enzyme activity due to genetic mutations, the ageing process is effectively sped up, with larger proportions of cells having lower enzyme levels and being at increased risk of oxidative damage. This has implications for the clinical severity of the mutations,. The properties of these enzyme variants correspond to a broad spectrum of enzyme biochemical phenotypes, i.e. electrophoretic properties, heat stability and enzyme kinetics. WHO guidelines (WHO Working Group, 1989) for standardised biochemical characterisation of the enzyme led to 387 variants of G6PD being described, though many of these would later prove to be genetic duplicates. (Beutler, 1990).

All the earliest evidence about the haemolytic risk of G6PD deficiency pertained to the African A- variant (G202A/A376G), due to the racial background of the "*primaquine sensitive*. Although rare as a genetic variant for having a double-point mutation (Domingo *et al.*2012), this type of deficiency is very common among individuals of sub-Saharan African origin. The A-variant characteristically expresses residual enzyme activity about 10% of normal levels (Beutler, 1991). It was studies with this variant which led to the discovery of G6PD deficiency (Carson *et al*., 1956). The Mahidol variant (G487A) is the predominant allele among many G6PD deficient populations of Myanmar and is also common among Thais. Enzyme activity is reduced to 5–32% of normal levels (Louicharoen *et al*., 2009). Finally, the Mediterranean variant (C563T) was originally known for its association with the clinical pathology of favism, and causes some of the most severely deficient phenotypes (Beutler and Duparc, 2007). This variant usually expresses <1% enzyme activity (table 1-1), with undetectable enzyme levels in older erythrocytes (Piomelli *et al*. 1968). Despite expressing such low levels of enzyme activity, carriers of this mutation are nevertheless asymptomatic until exposed to haemolytic triggers (Beutler, 1991).

Table (1-1): G6PD Variant Types and Their Key Characteristics (Luzzatto, 2009).

TYPE	Residual Enzyme Activity	Prevalence	Clinical significance

1.	**<10%**	**Sporadic, never polymorphic**	Severe and chronic: CNSHA
2.	**10-50%**	**Polymorphic**	Asymptomatic until triggered: risk of NNJ, AHA, favism
3.	**Normal (>50%)**	**Polymorphic(wild-type)**	**None**

1.2.1.3.Pentose phosphate pathway as an anti oxidative defence.

G6PD enzyme activity is necessary for RBC survival as it catalyses the only metabolic pathway capable of generating reducing power to these cells lacking mitochondria (Pandolfi *et al*. 1995). Reducing power, supplied in the form of NADPH, is necessary as an electron donor, i.e. chemical reduction, for detoxifying oxidative challenges to cells. The metabolic reactions concerned are part of the pentose phosphate pathway (PPP, also called the hexose monophosphate shunt), the first and rate-limiting step of which is catalysed by the G6PD enzyme: the oxidation of glucose-6-phosphate into 6-phosphoglucono-δ-lactone, which simultaneously reduces NADP+ to NADPH. The electron of NADPH passes to abundant glutathione dimers (GSSG) via another enzyme, glutathione reductase. Reduced glutathione monomers (GSH) represent the primary defence against hydrogen peroxides, organic peroxidaes, and free radicals, (Figure 1-3) .When G6PD functions normally, the drain of electrons from the NADPH pool caused by oxidative

challenge within the cell prompts the PPP to accelerate according to need, i.e. maintaining an NADP–NADPH equilibrium that strongly favours NADPH. This in turn maintains the oxidised–reduced glutathione (GSSG–2GSH) equilibrium strongly in the direction of the reduced state, i.e. 1:500 at steady state (Greene, 1993).

However, in cells that have a mutant and defective G6PD gene, the PPP may, depending upon the extent of the enzyme activity defect, function at near-maximum rate even at steady-state redox equilibrium. When oxidative challenge occurs and the equilibrium of NADP+ to NADPH shifts to the oxidised direction, the PPP is intrinsically unable to accelerate rapidly enough to force the equilibrium in favour of NADPH. This effectively stymies the flow of electrons to GSH, and that equilibrium shifts in favour of GSSG. The oxidants consuming these reducing equivalents, in turn, over-whelm the ability of the cell to provide them and damage may then occur. Visible evidence of such occurs in the form of Heinz bodies in the RBC membrane that attend acute primaquine-induced haemolytic anaemia. Heinz bodies cause the membrane to become rigid, and thus decrease the cells' lifespans. (Greene, 1993).

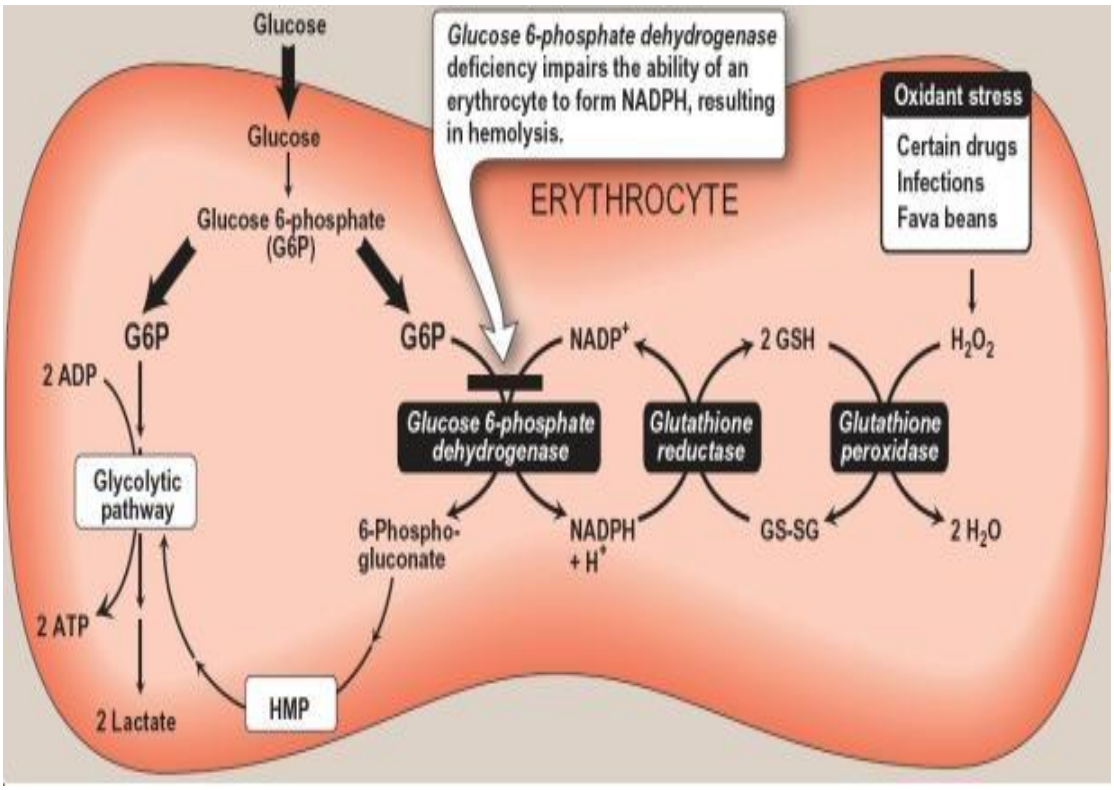


Figure (1-3): The Pentose Phosphate Pathway as an Anti-Oxidative (Golan, 2007).

1.2.2. The diagnosis of glucose 6 dehydrodenase deficiency .

The diagnosis of G6PD deficiency is made by a quantitative spectrophotometric analysis or, more commonly, by a rapid fluorescent spot test detecting the generation of NADPH from NADP+ (Gregg and Prchal, 2000).The test is positive if the blood spot fails to fluoresce under ultraviolet light (Glucose-6-phosphate dehyrdogenase deficiency (von Seidlein *et al.*2013). In field research, where quick screening of a large number of patients is needed, other tests have been used; however, they require definitive testing to confirm an abnormal result (Jalloh *et al.*, 2004 ; Lwai *et al.*, 2003). Tests based on polymerase chain reaction detect specific

mutations and are used for population screening, family studies, or prenatal diagnosis.

In patients with acute hemolysis, testing for G6PD deficiency may be falsely negative because older erythrocytes with a higher enzyme deficiency have been hemolyzed. Young erythrocytes and reticulocytes have normal or near-normal enzyme activity. Female heterozygotes may be hard to diagnose because of X-chromosome mosaicism leading to a partial deficiency that will not be detected reliably with screening tests (Gregg and Prchal, 2000; Reclos *et al.*, 2000 ; Ainooh *et al.*, 2003).

G6PD deficiency is one of a group of congenital hemolytic anemias, and its diagnosis should be considered in children with a family history of jaundice, anemia, splenomegaly, or cholelithiasis, especially in those of Mediterranean or African ancestry (Hermiston and Mentzer, 2002).

Testing should be considered in children and adults (especially males of African, Mediterranean, or Asian descent) with an acute hemolytic reaction caused by infection, exposure to a known oxidative drug, or ingestion of fava beans.

- Using complete blood count and reticulocyte counting; in active G6PD deficiency, Heinz bodies its can be seen in red blood cells on a blood film;
- The liver enzymes (to exclude other causes of jaundice).
- Lactate dehydrogenase enzyme (its elevated in hemolysis and meaning as a marker of hemolytic severity)
- The haptoglobin (decreased in hemolysis);

- A "direct antiglobulin test" (Coombs' test) – this should be negative, as hemolysis in G6PD is not immune-mediated

1.2.3. Molecular Diagnosis.

The molecular analysis may be useful for population screening, family studies, or prenatal diagnosis. Molecular tests are particularly important for the analysis of G6PD HF. Several molecular methods have been developed: amplification refractory mutation system (Du *et al* ,1999), gradient gel electrophoresis (DGGE) (Lam *etc al* ,1996), probe melting curve (Zhang *et al* ,2005), microarray (Bang *et al* ,2004), denaturing high-performance liquid chromatography (Tseng *et al* ,2005), matrix-assisted laser desorption/ionization-time of flight mass spectrometry (Zhao *et al.*,2004), reverse dot blot assay (Li *et al*, 2008), the single base extension assay (Farez *et al.*,2008), and finally restriction fragment length analysis performed by microcapillary chip electrophoresis (Minacci *et al.*, 2008). The identification of the specific G6PD mutations can better describe the clinical phenotype and provide additional epidemiological information regarding the different geographical distribution of the genetic variants. For a correct laboratory practice, the molecular diagnosis of G6PD deficiency should employ two analytical steps:

1) a first screening level, to research the most frequent mutations belonging to a specific geographical area. In this case, a PCR coupled to RFLP

represents a rapid, valid, and reliable molecular screening approach (Minacci *et al.,2008*);

2) a second level, based on the whole gene sequencing, finalized to the identification of the less frequent, or novel, mutations. DNA-based test for the screening of the most frequent mutations in a specific geographical area can be used as an alternative tool to the biochemical assay. The costs for chemicals dedicated to molecular test are comparable to those used for the enzyme assay. In the future, more advances systems should be utilized to improve the efficiency of the molecular assay (Vulliamy *et al* 1998).

1.2.4. The clinical feature of glucose -6- phosphate dehydrogenase deficiency.

Most G6PD deficient persons never suffer any clinical manifestations from this common genetic trait. The major clinical consequence of G6PD deficiency is hemolytic anemia (Cappelini and Fiorelli, 2008). Usually the anemia is episodic, but some of the unusual variants of G6PD may cause nonspherocytic congenital hemolytic disease. In general hemolysis is associated with stress, most notably drug administration, infection, the newborn period, and, in certain individuals, exposure to fava beans (Howes *et al* 2012)

1.2.4.1. Drug induce hemolytic anemia.

A large number of drugs and other chemicals that may have the capacity to precipitate hemolytic reactions in G6PD-deficient individuals are listed in Table (1-2). Some drugs, such as chloramphenicol, may induce mild hemolysis in people with severe, Mediterranean-type G6PD deficiency but not in those with the milder A– or Canton type of deficiency (Mc caffry *et al* .,1971). Furthermore, it appears that different individuals with the same G6PD variant experience a difference in the severity of their reaction to the same drug. For example, red cells from a single G-6-PD-deficient individual were hemolyzed in the circulation of some recipients who were given thiazolsulfone but their survival was normal in the circulation of others,

(Table 1-2). Sulfamethoxazole, which was clearly hemolytic in experimental studies, does not appear to be a common cause of hemolysis in a clinical setting (Chan and Mcfadzean, 1974). Undoubtedly, individual differences in the metabolism and excretion of drugs influence the extent to which G6PD deficient red cells are destroyed.

Table (1-2): Drugs to avoid in G6PD deficiency (Solé,2000).

Drug Name	*Use*
Dapsone	Antimicrobial for treatment of leprosy.
Flutmide(Eulexin)	Antiandrogen for treatment prostate cancer.
Mafenide cream(sulfamylon)	Topical antimicrobial.
Methylene blue(Urolene blue)	Antidote for druginduced methemoglobinemia.
Nalidixic acid(NegGram)	Antibiotic used primarily for urinary tract infections.
Nitrofurantion(Macrodantin)	Antibiotic used primarily for urinary tract infections.
Phenazopyridine(Pyridium)	Analgesic for treatment of dysuria
Primaquine	Antimalaria agent
Rasburicase(Elitek)	Adjunct to antineoplastic agents
Sulfacetamide(Klaron)	Antibiotic (ophthalmic and topic preparations)
Sulfamethaxazole(Gentanol)	Antibiotic used compination preparations(i.e.,trimethoprim-sulfamethoxazole)
Sulfanilamide	Antifungal agent for treatment of vulvovaginal *Candida albicans* infection

Chloramphenicol	Antimicrobial for treatment

Typically, an episode of drug-induced hemolysis in glucose 6 phosphate dehydrogenase -deficient individuals begins 1 to 3 days after drug administration is initiated. Heinz bodies appear in the red cells, and the hemoglobin concentration begins to decline rapidly (Beutler, 1969). As hemolysis progresses, Heinz bodies disappear from the circulation, presumably as they or the erythrocytes that contain them are removed by the spleen. In severe cases abdominal or back pain may occur (http;/www.Anemia in Children American Family Physician). The urine may turn dark – even black. Within 4 to 6 days, there is generally an increase in the reticulocyte count, except in instances in which the patient has received the drug in treatment of an active infection. Because of the tendency of infections and certain other stressful situations to precipitate hemolysis in G6PD deficient individuals, many drugs have been incorrectly implicated as a cause (Dausset and Contu, 1969). Other drugs, such as aspirin, have appeared on many lists of proscribed medications because very large doses had the capability of slightly reducing the red cell life-span (Stochman *et al* 1978).

In the A– type of G6PD deficiency, the hemolytic anemia is self-limited because the young red cells produced in response to hemolysis have near-normal G-6-PD levels and are relatively resistant to hemolysis. The hemoglobin level may return to normal even while the same dose of drug that initially precipitated hemolysis is administered. In contrast, hemolysis is not self-limited in the more severe Mediterranean type of deficiency(Beutler, 1969).

1.2.4.2. Hemolytic anemia occurring during infection.

Anemia has often developed rather suddenly in G6PD deficient individuals within a few days of onset of a febrile illness. The anemia is usually relatively mild, with a decline in the hemoglobin concentration of 3 or 4 g/dl. Hemolysis has been noted particularly in patients suffering from pneumonia and in those with typhoid fever (Shoenfield , 2008) . The fulminating form of the disease occurs particularly frequently among G6PD deficient patients who are infected with Rocky Mountain spotted fever (Rocky Mountain Spotted Fever). Jaundice is not a prominent part of the clinical picture, except where hemolysis occurs in association with infectious hepatitis. In that case it can be quite intense. Presumably because of the effect of the infection, reticulocytosis is usually absent, and recovery from the anemia is generally delayed until after the active infection has abated. Diabetic ketoacidosis has usually been considered a cause of hemolysis in G6PD deficiency, but a review of 36 episodes of diabetic ketoacidosis in G6PD deficient subjects yielded only 10 in whom hemolysis occurred, and these were all associated with infection or drug ingestion(Kitabchi,*et al* 2009). It has been suggested that hypoglycemia may precipitate hemolysis.

1.2.4.3. Favism.

Favism is potentially one of the gravest clinical consequences of G6PD deficiency. It occurs much more commonly in children than in adults(Beutler, 2008). The onset of hemolysis may be quite sudden, having been reported to occur within the first hours after exposure to fava beans. More commonly the onset is gradual, hemolysis being noticed 1 to 2 days after ingestion of the beans. Occasional hemolysis has been reported to occur after ingestion of other foodstuffs such as unripe peaches (Simoons, 1998). The urine becomes red or quite dark, and in severe cases shock may develop within a short time (Luzzatto 2009).

1.2.4.4. Neonatal icterus.

Icterus neonatorum with no evidence of immunologic incompatibility occurs in some infants with G6PD deficiency. The jaundice may be quite

severe and, if untreated, may result in kernicterus (Luzzatto ,2010). Thus G-6-PD deficiency is a preventable cause of mental retardation, and this aspect of the disorder has considerable public health significance. An increased incidence of neonatal icterus has been observed in Mediterranean infants with G6PD deficiency, and among the Chinese(Beutler,2008). It seems to occur quite rarely among neonates with the A– type Of enzyme deficiency in the United States, but some cases have been reported in G6PD-deficient infants in Africa. The cause of the difference is unknown, but it may be related to some environmental factor such as vitamin E intake. As noted above, anemia is mild or absent in these infants, and hepatic dysfunction may play a major role in the pathogenesis of the jaundice(Doxiadis and Valaes,1994 ;Luzzatto,2006).

1.2.4.5. Hereditary nonspherocytic hemolytic anemia.

Some of the rare types of G6PD deficiency are associated with hereditary nonspherocytic hemolytic anemia. Occasionally, patients with the common, Mediterranean type of defect have also been found to have this disorder (Rahman and Jamal 2002). The reason some individuals with the Mediterranean type of enzyme deficiency have chronic hemolysis while the majority have hemolysis only under conditions of stress is not clear; it is possible that hemolysis is due to some as yet undefined associated abnormality (Luzzatto,2010).

Hereditary nonspherocytic hemolytic anemia due to G6PD deficiency is usually first noted during infancy or childhood. In some instances, neonatal jaundice has been present. Hemolysis is often exacerbated by febrile illnesses or by the administration of drugs. Splenomegaly is commonly present (Shah, 2004).

1.2.5. The geographic distribution of G6PD deficiency.

It has been estimated that more than 400 million people worldwide are affected by G6PD deficiency(Howes *et al*. 2012). Individuals with G6PD deficiency have an evolutionary survival advantage in malaria-endemic

regions as G6PD deficiency confers a degree of protection against severe malaria
(Ruwende *et al*, 1995 ; Ruwende and Hill, 1998). The selective advantage of G6PD deficiency under malaria pressure has left its mark in the human genome as a "selective sweep" surrounding the G6PD gene. Not surprisingly G6PD deficiency is most prevalent in regions where malaria is or was prevalent Figure (1-4) (Sabeti *et al.* 2002).

Howes and co-workers have identified 1,734 community G6PD surveys globally of which 1,289 (74%) were conducted in malaria-endemic countries and used this evidence-base to model a continuous prevalence map of the deficiency (Howes *et al.* 2009). The prevalence of G6PD deficiency was lowest or absent in the Americas, and highest in sub-Saharan Africa. The region with the single highest predicted prevalence was in the eastern province of Saudi Arabia. The prevalence of G6PD deficiency tended to be lower, but heterogeneous across central and Southeast Asia, with "hotspots" identified in eastern India, at the Thai-Lao border and in the Solomon Islands. Despite the very large number of studies under review there remains uncertainty regarding the geographic distribution of G6PD deficiency prevalence in many areas. Uncertainty metrics accompanying the modelled map allow prioritization of areas where additional community surveys are needed. Furthermore, the prevalence of the deficiency is far from homogeneous, with considerable variations even within countries further emphasizing the need for additional data. An acknowledged limitation of the currently available information is the variability in G6PD deficiency tests used. It is difficult to extrapolate from the reported prevalence of G6PD deficiency to the risk of severe primaquine-induced haemolysis. The modelled prevalence maps are supplemented with data on genetic variants, which allows a better prediction of the risk of haemolysis. The maps will hopefully help policymakers to optimize safer strategies to deploy primaquine (Louchroen *et al.* 2009) .

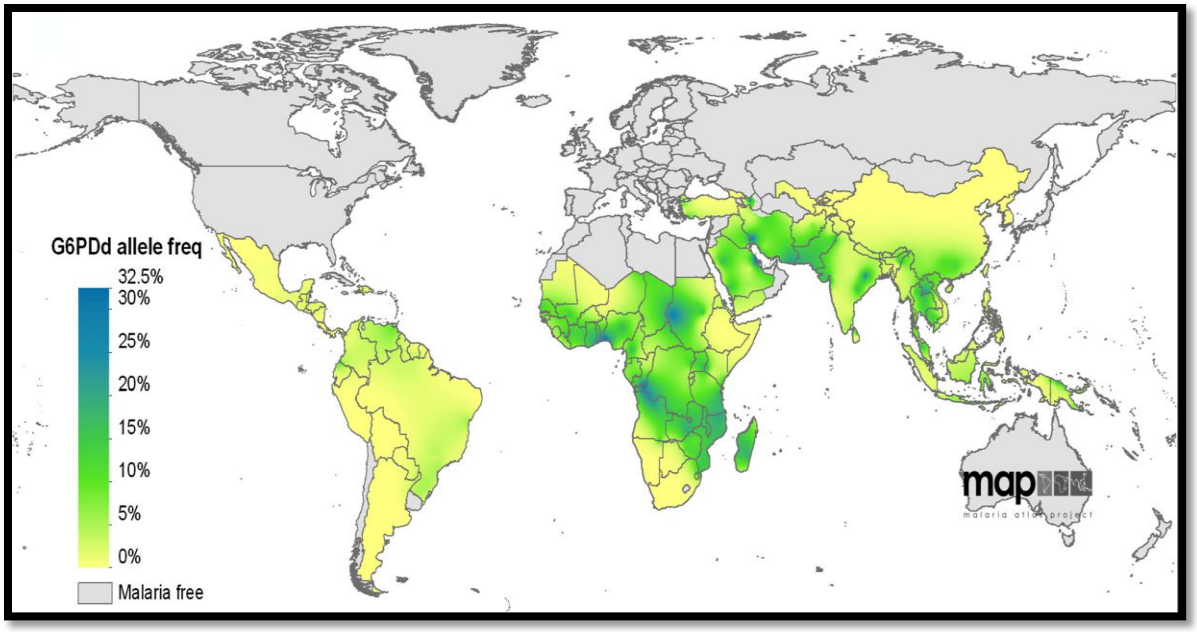


Figure (1-4): The geographic distribution of G6PD deficiency (Louchroen *et al*. 2009).

2. Materials and Methods.

2.1. Materials.

2.1.1. Apparatus.

Various apparatuses used in this study ae listed in table (2-1)

Table (2-1): Apparatuses used in this study

Apparatus	Company / country
Analytical balance	FC-400 / China
Autoclave	HIRAYAMA
Bench centrifuge	Uni-Media / Korea
Electrophoresis equipment	JUNY1-JY200C
Gel –documentation	Bio-Red / U.S.A
Heater – magnetic stirrer	Stuart / England
Microcentrifuge	MIKRO120 – Hettich
Microwave	LG / Korea
Nanodrop spectrophotometer	Techne / U.K.
Oven	Sanyo / Japan
Gradient PCR thermal cycler	Techne / U.K.
Conversional PCR thermal cycler	Techne / U.K.
ph meter	Martini / Japan
Sensitive balance	Mettlev / Switzerland

U.V. Transilliuminator with camera	Flowgen / U.K.
Vortex	Scientific industries / U.S.A.
Water bath	Memmert / Germany

2.1.2. Chemicals.

various chemicals material used in the study listed in table (2-2).

Table (2-2): List of chemicals.

Chemicals	Company / country
Absolute ethanol	Phamacia / sweeden
Agaros	Biobasic / Canada
DNA ladder	Promega / USA
Deionized water	Bioneer / korea
Ethedium bromid	Biobasic / Canada
Loading dye	Biobasic / Canada
Green master mix	Promega /USA
Primers	Bioneer / Korea
Tris Borate EDTA (TBE)	Biobasic / Canada
Methylene blue	Fluka / Germeny
Sodium nitrate	Fluka / Germeny
Cell Lysis (FABG buffer)	Taiwan
Proteinase K Solution (PK)	Taiwan
Elution	Taiwan
Wash buffer and W1 solution	Taiwan

Binding (ethanol)	Taiwan

2.1.3. Kits.

Table (2-3) shows the kite used in this study.

Kits	Company	**Country**
DNA Extraction from blood	Favorgen	Taiwan

2.1.4. Primers.

Sequences of the primers used.

Name	Oligonucleotides	Tm	GC%	Product length	Sequence (5'-3')	Referances.
Frag l	Forward primer	53.83	55.00	115 bp	CTGAAATCTGGCCTCTGTCC	Saunders *et al*, 2002
Frag l	Reverse primer	53.83	55.00	_	GTTCAGCCCCATCTTAGCAG	Saunders *et al*, 2002
Frag ll	Forward	55.88	60.00	1600 bp	ACCACAAGGTGGC	Saunders *et*

	primer				AGCGTTG	*al*, 2002
Frag ll	Reverse primer	60.21	66.67	_	TGCCTTGCTGGGC CTCGAAGG	Saunders *et al*, 2002
Frag lll	Forward primer	55.88	60.80	2000 bp	CCAGGGACGTGAT GCAGAAC3	Saunders *et al*, 2002
Frag lll	Reverse primer	60.80	58.33	_	GGGCAGGGACATG GACAGTAAGAG	Saunders *et al*, 2002

2.2. Study subjects.

This study included collecting blood samples from 50 patients suffering from Glucose 6 phosphate dehydrogenase deficiency during the period from March 2014 to June 2014. These patients were submitted to from the hospital child protects (hospital in a medical city) Child hospital AL yarmook hospilal and Alalwyia hospital. The apparently healthy 20 person individuals from college of science / Al Nahrain University were selected to represented the control group. The mean ages of patients were represented 7 days to 20 years and the main ages of control were 25 to 35 years. Informed consents from patient as well as control were taken which included age first relative family history of anemia or G6PD deficiency.

2.3. Blood samples collection.

Venous blood 5 ml was obtained from both of patients and the control group then was collected kept in EDTA tube and preserved in – 20 C° until been used.

2 ml was obtained from patient his age 7 days.

2.4. Green Master Mix

Go Taq® Green Master Mix is a premixed, ready to use solution containing bacterially derived Taq DNA polymerase, dNTPs, MgCl 2 and reaction buffers at optimal concentration for effective amplification of DNA templates by PCR.

2.5. DNA ladder (100 bp).

- DNA ladder 100- 3000 bp containing 13 fragments supplied in storage buffer 10mM Tris-HCL pH (7.5), 0.1 mM EDTA.
- DNA ladder 100- 2000 bp containing 13 fragments supplied in storage buffer 10mM Tris-HCL pH (7.5), 0.1 mM EDTA.
- DNA ladder 100- 1000 bp containing 13 fragments supplied in storage buffer 10mM Tris-HCL pH (7.5), 0.1 mM EDTA.

2.6. Methods.

2.6.1. Sterilization methods.

- Autoclaving: Buffers and solutions were sterilized by the pressure vessel (autoclave) at 121 ^{0}C and 15 bar for 15 minutes.
- Dry heat: A laboratory oven was used for glassware sterilization. Glassware was placed in the oven at 160-180 ^{0}C for 2 hours.

2.6.2. Laboratory Detection of Glucose 6 phosphate dehydrogenase .

Material:	Tube A	Tube B
Sodium nitrite	0.05 ml	0.05 ml
Methylene blue	0.05 ml	_
Blood sample	2 ml	2 ml
Tube A and B were placed in water bath (37^{0}C) for 3 hours *Tube B should be always brown.		

It depends on the color of tube A

- Red color means no G6PD deficiency

- Brown color (like tube B) deficiency of G6PD
- Between red and brown color means intermittent expression of G6PD deficiency.

2.6.3. DNA Extraction from blood samples.

2.6.3.1. Procedure:

The Extraction was briefly carried out as follow:

- The blood Sample Was mixed thoroughly for at least 10 minutes in a rotisserie at room temperature.
- A liquate of 30 µl of the Proteinase K Solution was added into Microcentrifuge tube.
- A liquate of 200 µl of blood was added to the Proteinase K Solution and mixed briefly.
- The solution was put in the water bath at 60 C° for 15 min
- A liquate of (FABG) buffer (200 µl) to the solution
- The solution was put in the water bath at 70 C° for 15 min (at this period we invert the solution every 3 min).
- A liquate 200 µl ethanol to the tube and vortex for 10 seconds.
- FABG column was placed in a 2ml collection tube.
- The sample mixture was transfer to FABG column carefully, centrifuged for 5 min at a full speed 14000 rpm, the supernatant was discarded and replaced by a fresh collection tube.

- FABG column was washed with 400 µl of W1 buffers, centrifuged for 1 min, the supernatant was discarded and replaced by a fresh collection tube.
- FABG column was washed with 600 µl of wash buffer, centrifuge for 1 min, the supernatant was discarded and replaced by a fresh collection tube.
- For additional 3 min was centrifuged to dry the column
- FABG column was placed to new 1.5 ml microcentrifuge tube, a liquate 50 ml of preheated elution buffer stand for 3-5 min or until the solution absorbed by the membrane.
- For 1 min was centrifuged , after 1 h, stored at -20 C°

2.6.3.2. Measurment of extracting DNA concentration and purity.

Ananodrop spectrophotometer (Techne / U.K) was used to estimate the concentration and purity of the extracted DNA (from blood of patient and control) according to the following procedure:

2.6.4. PCR Protocols.

Extracted DNA from blood samples and healthy was used in PCR for amplification of FragI, Frag II, Frag III

2.6.4.1. PCR amplification.

For PCR amplification of G6PD gene the following PCR protocols was followed

1. The Go Taq® Master (Promega / USA) Mix was thawed at room temperature. The master mix was mixed by vortexing then it was spine briefly in a micro centrifuge.

Component of Taq® Master mix are illustrated in table (2-5)

Table (2-5) component of Taq® Master mix.

NO.	Component	Quality / concentration
1-	Taq polymerase	2.5µ
2-	dNTP (dATP, dCTP, dGTP, dTTP)	250 µM
3-	Tris – HCL (PH 9.0)	10 mM
4-	KCL	30mM
5-	MgCl	1.5mM

2. The reaction mix was prepared by combining the table:

Table (2-6): The optimal values of various steps in the amplification.

Components	Volume	Concentration
Go Taq® Green Master Mix	12.5 µl	1x
Forward primer	0.1 µl	0.2 µM

Reverse primer	0.1 µl	0.2 µM
DNA template	3 µl	< 250 ng
Nuclease free water	9.3 µl	-
Final volume	25 µl	

3. The mixture then put in microcentrifuge and spinner for better mixing.
4. After mixing the master mix tubes were transferred to the thermocycler (Techne 32 thermal block / UK) which was previously programmed with the above protocol according to the G6PD gene.

2.6.4.2. Optimal protocol of PCR amplification

For PCR amplification of G6PD gene (FragI, Frag II, Frag III) the following PCR protocols was followed :

Thermal cycler protocol	No. of cycle	Temperature –time
Initial Denaturation	1 cycle	94^0C for 5 minutes
Denaturation	35cycle	94^0C for 1 min
Annealing		61^0C for 1 min
Extension		72^0C for 1 min
Final Extension	1 cycle	72^0 C for 10 min.

PCR Amplification using frag ll primer

Thermal cycler protocol	No. of cycle	Temperature –time
Initial Denaturation	1 cycle	94^0C for 5 minutes
Denaturation	30cycle	94^0C for 1 min
Annealing		60^0C for 1 min
Extension		72^0C for 1.30 min
Final Extension	1 cycle	72^0 C for 10 min.

PCR Amplification using fraglII primer

Thermal cycler protocol	No. of cycle	Temperature –time
Initial Denaturation	1 cycle	94^0C for 5 minute
Denaturation	30cycle	94^0C for 1 min
Annealing		59^0C for 1 min

Extension		72^0C for 2 min
Final Extension	1 cycle	72^0 C for 10 min.

2.7. Agarose gel electrophoresis (Maniatis *et al*., 1982).

- Two percentage agarose gel was prepared by mixing: 100ml of 1X TBE buffer and 1.5 gm in a glass bottle. A glass bottle was heated in a magnetic stirrer with heater until the affairs was dissolved.
- This solution was cooled to 70^oC, 2 µl ethidium bromide was added from stock solution and mixed thoroughly.
- The clean glass mold (17X12X4 cm) was set on a horizontal section of the bench. The comb was set in position 0.5-1.0 mm above the surface of the mold so that a complete well was formed when agarose was added.
- The warm agarose – solution was poured into the mold.
- After the gel was completely (20-30 min. At room temperature), the comp was carefully removed and the gel mounted in the electrophoresis tank which contain previously small amounts of 1X TBE buffer.
- A volume of 600ml of 1X TBE was added to cover the gel in depth about 1mm.
- A volume of 10 µl of the sample of DNA (PCR product) was added slowly into the slots of the submerged gel using micropipette.
- A volume of 5 µl of DNA marker was mixed with 1 µl of loading buffer. The mixture was set slowly into the slots of the submerged gel using an automatic micropipette.

- The gel was subjected to electrophoresis at 70 volts until the bromophenol blue tracking dye, migrated at least two-thirds of the way down the gel.
- The gel was examined by ultraviolet light using a UV transilluminator them photographed.

2.8. Measurement of DNA concentration before sequencing.

Before sequencing DNA concentration of the G6PD gene (Frag I, II, III) PCR products from 50 blood samples and 20 healthy was measure using nanodrop (techne UK) as in section 2.7.3.2. All products gave concentration more than 100n g/µl which is the least concentration required for DNA to be sequenced.

2.9. DNA sequencing .

Polymerase chain reaction products of G6PD gene Frag I, II and III 30 blood samples and 3 healthy from control were sent for DNA sequencing. The obtained sequences were aligned using NCBI software with normal sequence from NCBI GenBank and examined of the presence of SNPs.

2.10.Statistical Analysis.

The statistical Rackage for the social science (SPSS, version 14) was used for statistical analysis Chi- sequre was used for testing the deviation from Hardy Weinberg equilibrium distribution of different groups between patients and control, p value < 0.01 was considered statistically significant.

3. Result and discussion.

3.1. The distribution of the studied group.

Glucose -6-phosphate dehydrogenase (G6PD) deficiency is the common set disease-producing enzyme disorder of human beings. More than 300 variants of G6PD characterized by standard method are now known and recent isolation of G6PD gene that promises important fundamental advances in the understanding of enzyme structure and function (Farhud and yazdanpanah, 2008).

In this study, fifty sample were collected from patient dignosed with G6PD (males 37, females 13) as show in table (3-1) and figure (3-1).

Table :(3-1). Description of sample study according to gender.

Gender	Number	Percentage (%)
Male	37	74.00
Female	13	26.00
Total	50	100%
Chi-square value	---	11.826 **
** (P<0.01).		

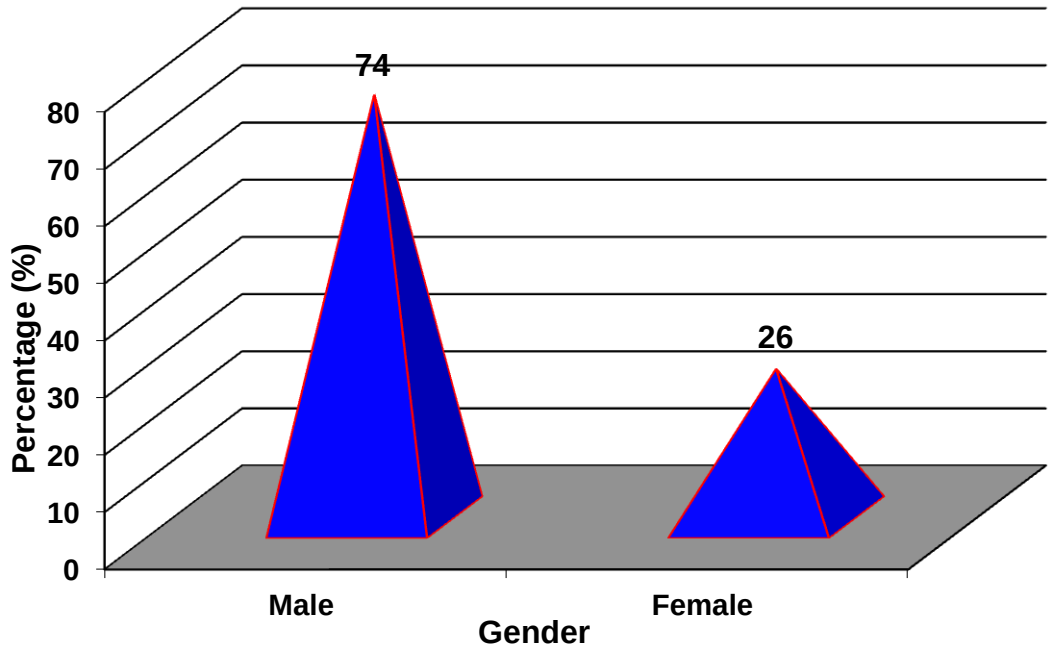


Figure (3-1): Description of sample study according to gender

50 samples divided into three groups depending on the severity of the disease as show in table (3-2) and Figure (3-2)

Table: (3-2). Description of sample study according to severity diseases

Severity diseases	Number	Percentage (%)
Carrier	9	18.00
Mild	21	42.00
Acute	20	40.00
Total	50	100%
Chi-square value	---	9.072 **
** (P<0.01).		

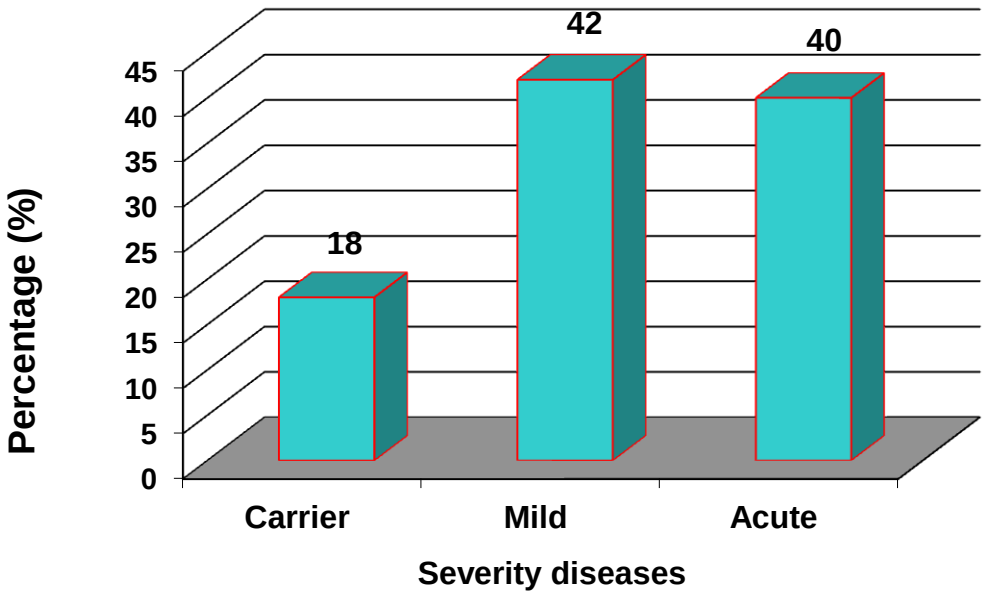


Figure (3-2): The distribution of patient depending on the severity disease

.

In figure (3-2) show that carrier and affected G6PD deficiency. However patients classified mild and acute G6PD disease.In chi square test the carrier group were significant on the other group and the P value was (P = 0.018), degree of freedom was (DF=2).G6PD deficiency in the most common enzymopathy in man and it is estimted to affected 400 million people world wild. The frequency being reported from same regional contries. For instance, it has been found at frequency of 65% in Saudia Arbia population affected (Nabeel, *et al* ,2004).

3.2. Molecular detection of G6PD deficiency by PCR technique.

3.2.1. G6PD gene:

In this study three primer sets were designed using the NCBI Primer-Design online tool in order to amplify a specific region in the G6PD gene by using a routine PCR technique.

The first primer set used in this PCR technique (Frag I) specific for the intron (6) of G6PD gene from NCBI primer design with product length (115 bp) which is shown in the figure (3-3)

Figure (3-3): PCR product for frag I primer, Agarose gel electrophoresis (2% agarose gel, 10 minutes at 100 voltage and then lowered to 70 Volts, 50 minutes). visualized under U.V light after staining ethidium bromide Line M: 100bp marker,line: 1-10 from patients,line: C from control (healthy),line: N from negative control.

Primer (Frag I) used in this study were previously designed to amplify the G6PD gene, show the samples 4,6 and 10 did not give any bands in the PCR

amplification because the patients lose all the G6PD gene and the samples 1,2,3,5,7,8 and 9give band in PCR product (115 bp) because those patients carried the disease

The second primer set used in this PCR technique (Frag II) specific for the exon (7) of G6PD gene from NCBI primer design which product length 1500 bp which was shown in figure (3-4).

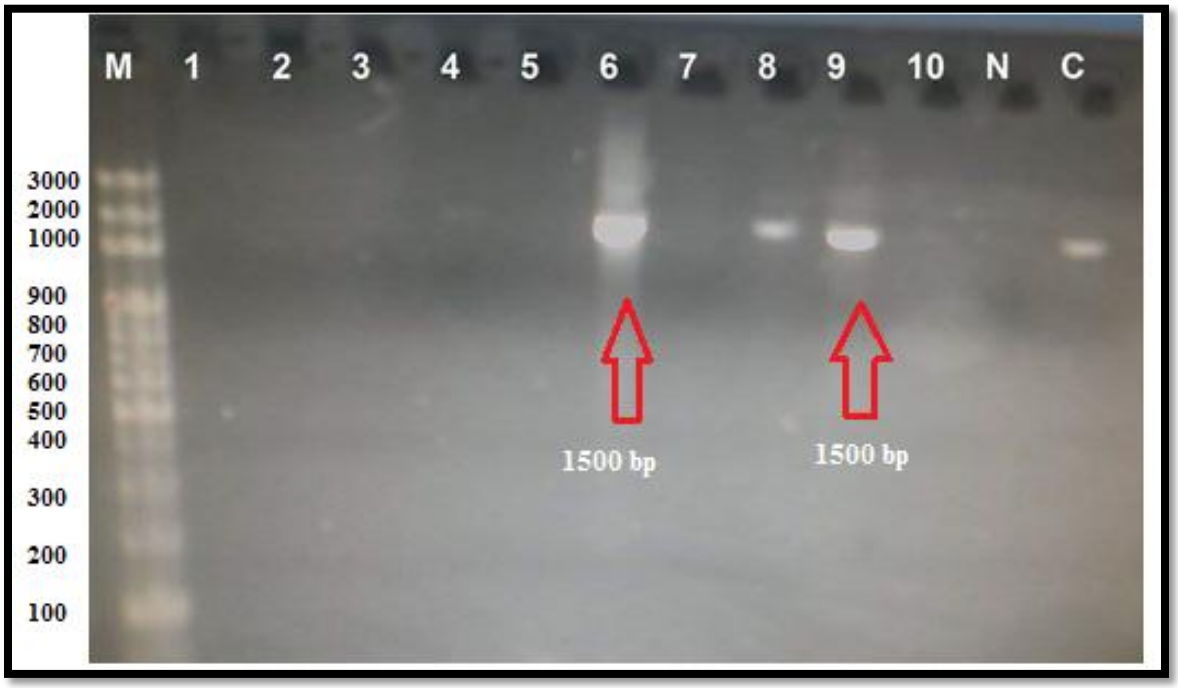


Figure (3-4): PCR product for frag II primer, Agarose gel electrophoresis (2% agarose gel, 10 minutes at 100 voltage and then lowered to 70 volts, 1 hour). visualized under U.V light after staining ethidium bromide, Line M 100bp marker: 1-10 from patients; Line C: from control (healthy) and line N :from negative control.

Primer (Frag II) used in this study were previously design to amplify the G6PD gene, show the samples 1, 2, 3, 4, 5, 7 and 10 did not give any band in PCR amplified because the patients lose the G6PD gene and samples 6, 8

and 9 give the band in PCR product (1500 bp) because this patients carried of the diseases

The third primer set used in PCR technique (Frag III) specific for the exon (9) from NCBI primer design which product length (2000 bp) which shown in figure (3-5).

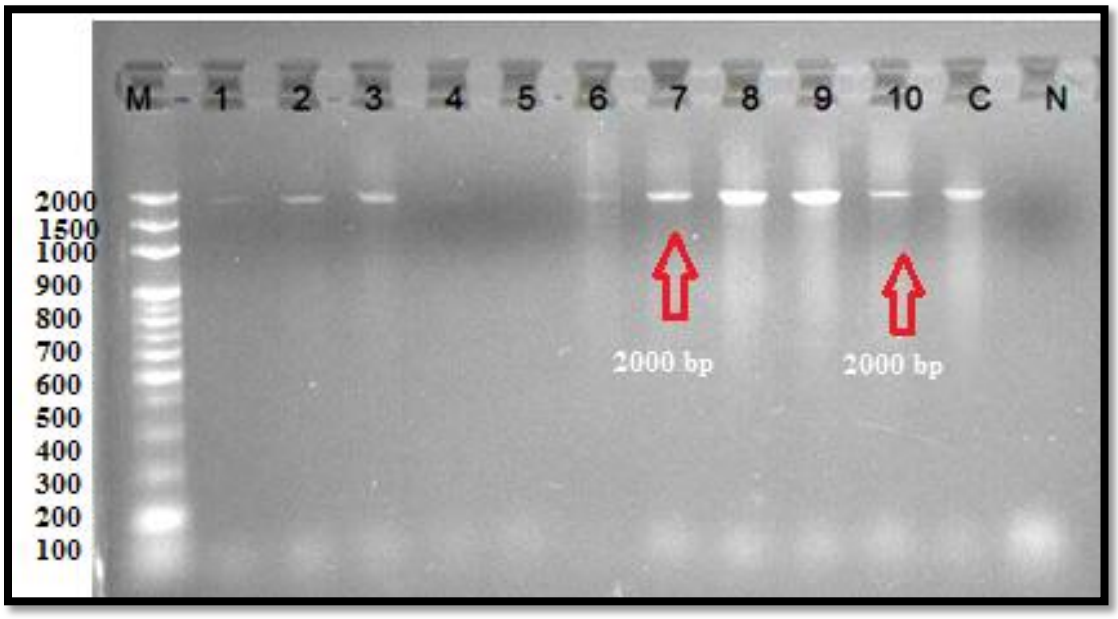


Figure (3-5): PCR product for frag III primer, Agarose gel electrophoresis (2% agarose gel, 10 minutes at 100 voltage and then lowered to 70 volts, 1 hour and 30 minutes) visualized under U.V light after staining ethidium bromide, Line M:100bp marker; line 1-10: from patients; line C: from control (healthy) and line N: from negative control.

Primer frag III used in this study were previously design to amplified the G6PD gene show the sample (5) did not give any band by PCR amplified because this patient lose all the G6PD gene and samples 1,2,3,4,6,7,7,8,9 and 10 give the band in PCR product (2000 bp) because this patients carried the disease.

The G6PD enzyme exits in its active form, as a dimer (or tetramer) each of which consist of 515 amino acid polypeptide subunits (indeed 514 amino acid since first N-terminal Met is not present in the mature protein) (Mason *et al* , 2006). Each dimer contain tightly bound NADP that plays both structural and functional roles. The enzyme is encoded by G6PD gene, is located on the long arm of the X-chromosome (Xq28) and spans over 18 kilobases (Kb) consisting of 13 exons (encoding sequences) and 12 intron (non- coding sequances) (Chen *et al* ,2009).

3.3.Molecular analysis of G6PD gene.

Direct sequncing of the G6PD gene from the all patients in Iraqi populations using primer Frag I , II and III located in intron 6, exon 7 and exon 9

3.3.1. Type of mutations.

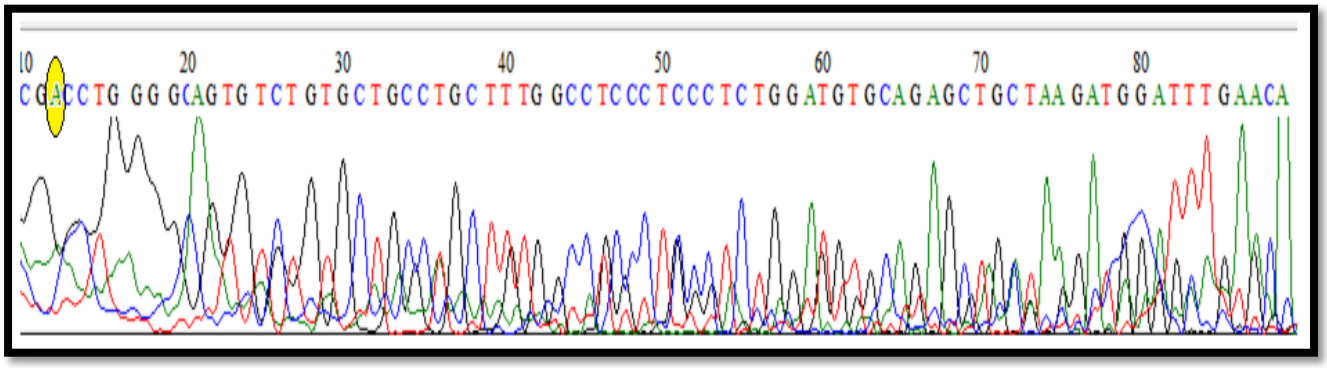
Point mutation in G6PD patients were undetectable during diagnosis by conventional PCR. The PCR products of G6PD patients were screened by sequencing. The result was directly compared with Iraqi healthy control (by using Mega 6 program) and with the NCBI nucleotide blast.

- **Control (Iraqi healthy)**

```
Query  13     CCTGGGGCAGTGTCTGTGCTGCCTGCTTTGGCCTCCCTCCCTCTGGATGTGCAGAGCTGC  72
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  16966  CCTGGGGCAGTGTCTGTGCTGCCTGCTTTGGCCTCCCTCCCTCTGGATGTGCAGAGCTGC  17025

Query  73     TAAGATGG  80
              ||||||||
Sbjct  17026  TAAGATGG  17033
```

Figure (3-6A): The automated sequencing of the (G6PD) gene Control (Iraqi healthy) gives %100 match with NCBI nucleotide blast.



CCCGTCGCGCGACCTGGGGCAGTGTCTGTGCTGCCTGCTTTGGCC

TCCCTCCCTCTGGATGTGCAGAGCTGCTAAGATGGATTTGAACA

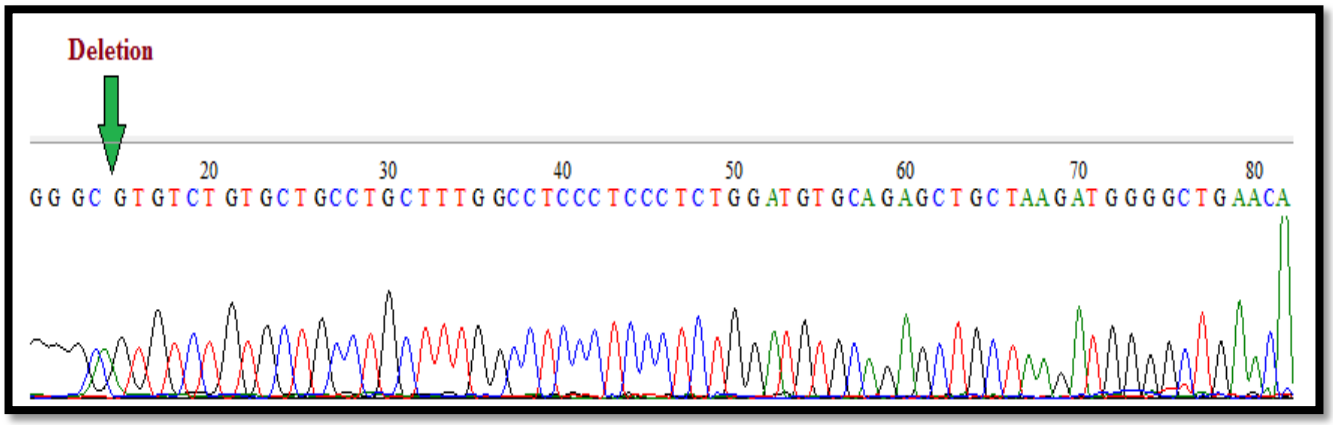
Figure (3-6B): A chromatogram for control (Iraqi healthy).

- **Sample:**

```
                    Deletion
                       ↓
Query  6      GCCTGGGGC-GTGTCTGTGCTGCCTGCTTTGGCCTCCCTCCCTCTGGATGTGCAGAGCTG  64
              ||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  16965  GCCTGGGGCAGTGTCTGTGCTGCCTGCTTTGGCCTCCCTCCCTCTGGATGTGCAGAGCTG  17024

Query  65     CTAAGATGGGGCTGAAC  81
              |||||||||||||||||
Sbjct  17025  CTAAGATGGGGCTGAAC  17041
```

Figure (3-6A): The automated sequencing of G6PD gene display a deletion in (16974/ A) base pair in intron (6) of the G6PD gene when comparing in the NCBI nucleotide blast.



GGGTCGCCTGGGGC_GTGTCTGTGCTGCCTGCTTTGGCCTCCCTC

CCTCTGGATGTGCAGAGCTGCTAAGATGGGGCTGAACA

Figure (3-6B): A chromatogram for sample display a sequence and the deletion region.

Figure (3-7) comparing between control (normal Iraqi) and G6PD patient in the MEGA 6 program.

The current utilized forward primer for sequencing the G6PD gene in 30 patients compared with healthy control and NCBI nucleotide blast.

The second DNA sequence of the G6PD gene located exon 7 composed from patients, control (Iraqi healthy) and NCBI nucleotide blast, show the figure (3-8A and B), (3-9 A and B) and (3-10A and B).

- **Control :**

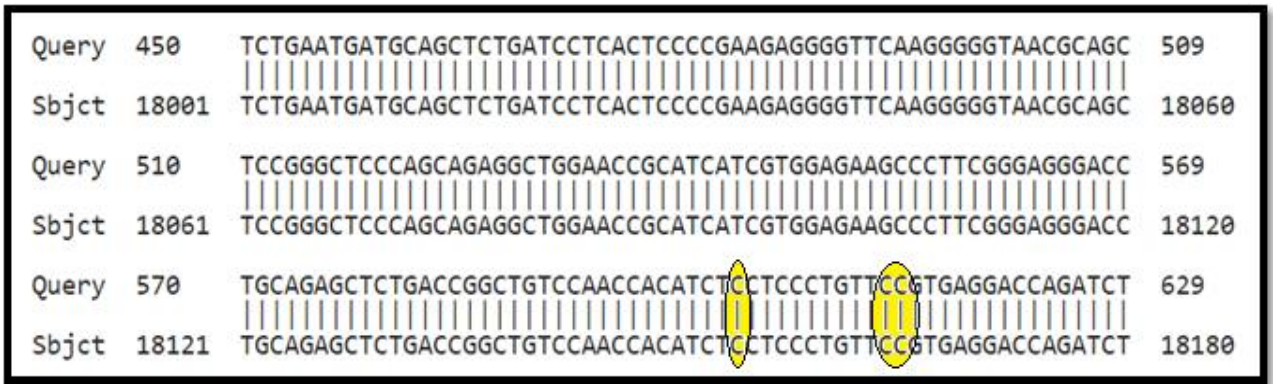
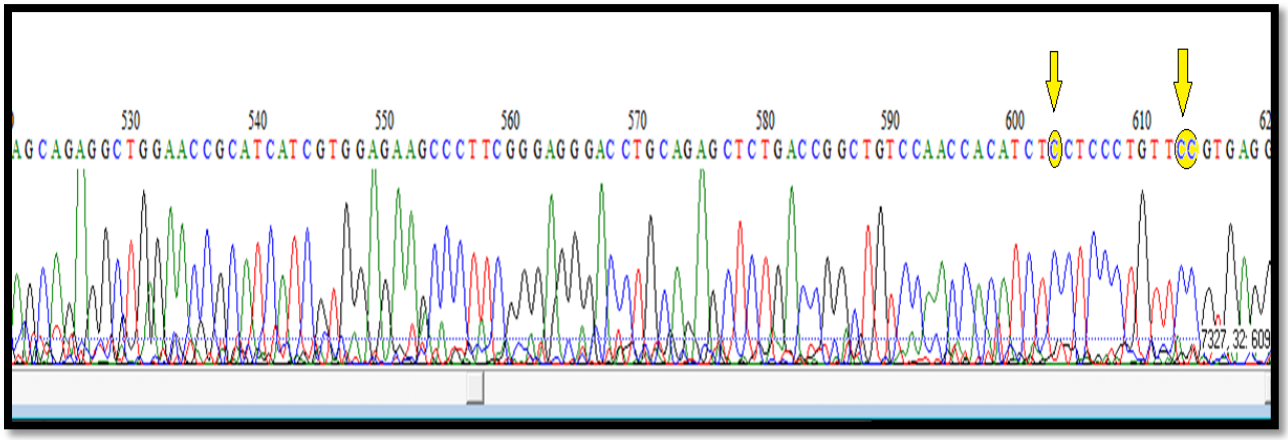


Figure (3-8A): display Iraqi healthy control comparing in NCBI nucleotide blast .



CAGCAGAGGCTGGAACCGCATCATCGTGGAGAAGCCCTTCGGG
AGGGACCTGCAGAGCTCTGACCGGCTGTCCAACCACATCTCCTC
CCTGTTCCGTGAGGACCAGATC**Figure (3-8B): A chromatogram for control (Iraqi healthy).**

- **Sample I:**

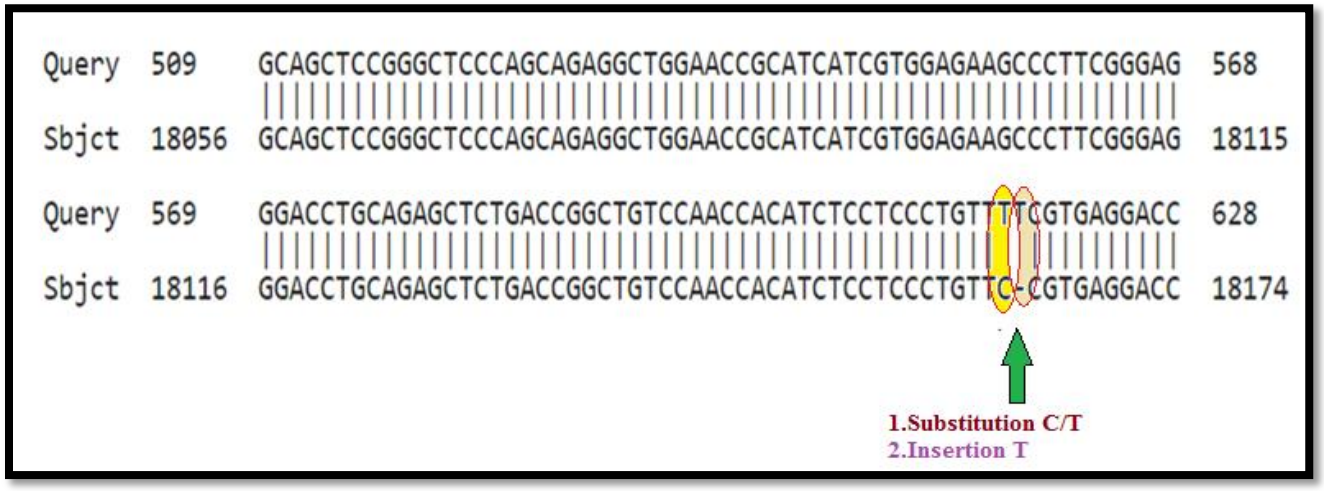
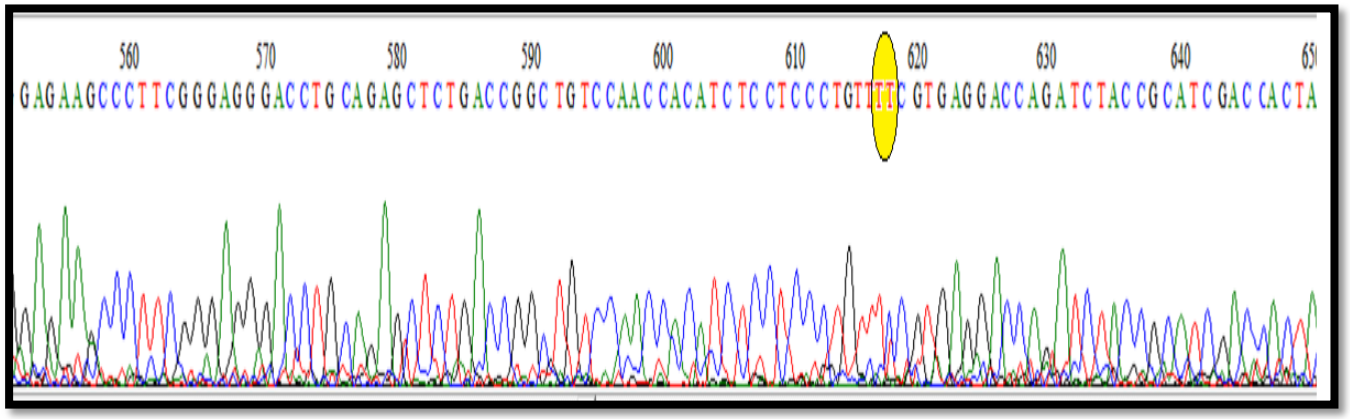


Figure (3-9A): display a sample of G6PD comparing in the NCBI nucleotide blast and show the sequence of exon (7).



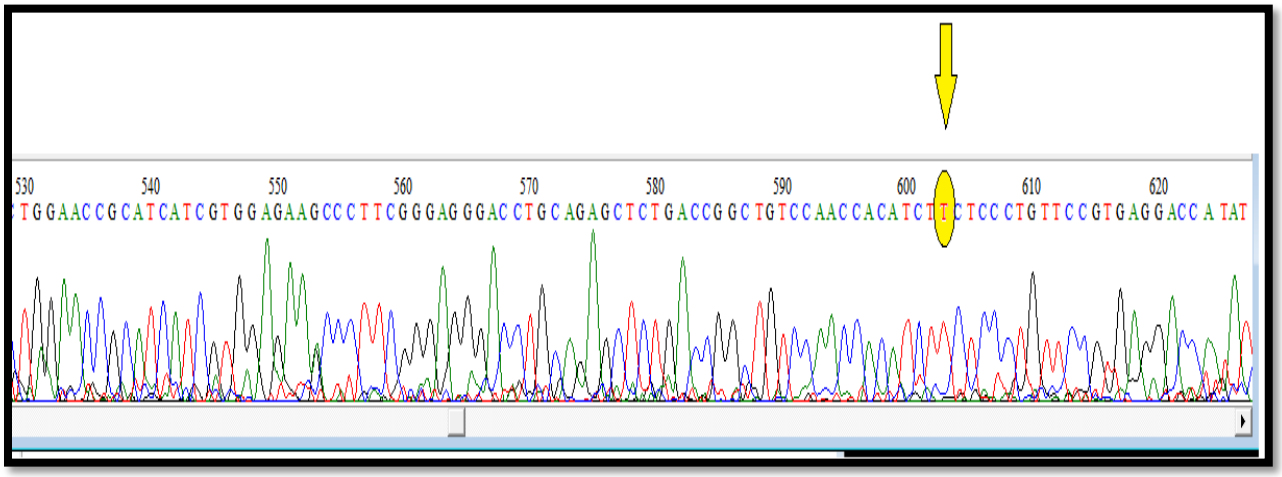
ACATCTCCTCCCTGTTTTCGTGAGGACCAGATCTACCGCATCGAC
CACTACCTGGGCAAGGAGATGGTG

Figure (3-9B): A chromotogram for a sample of G6PD.

In the sample I of the second primer showed a SNP at position C / T ,this replacement cause a silent mutation because its coded the same amino acid phe / phe, (Matsuoka ,*et al* .2007) found in G6PD gene silent mutation C/T that give the same amino acid Tyr / Tyr.

- **Sample II :**

Figure (3-10A): display a sample of G6PD comparing in NCBI nucleotide blast and show the sequence of exon (7) .



CTTCTCCCTGTTCCGTGAGGACCATATTTTACCGCATCGACCACTA
CCTGGGCAAGGAG

Figure (3-10B): A chromotogram for a sample of G6PD.

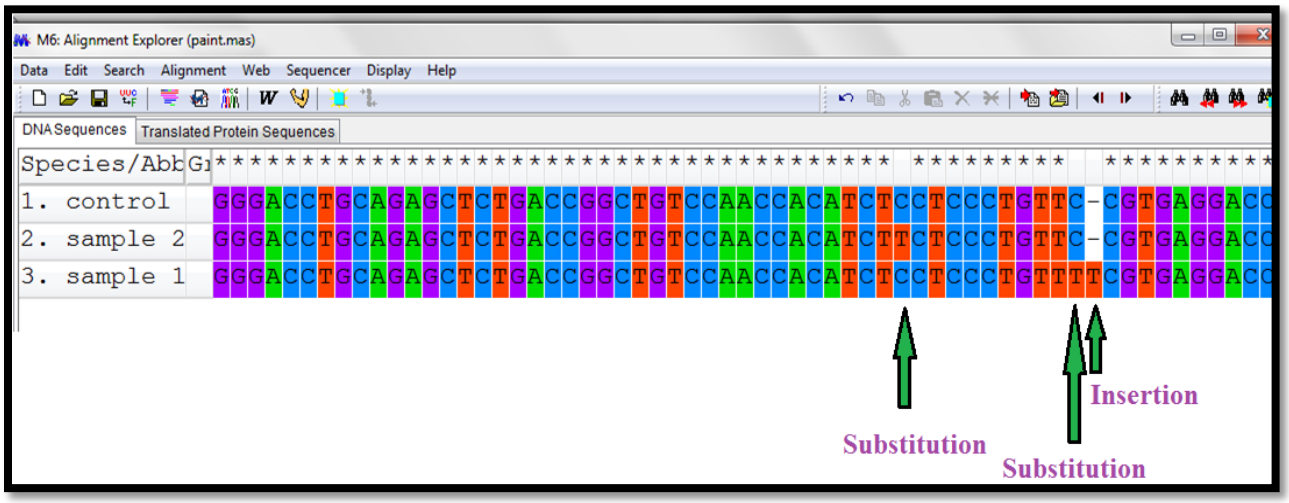


Figure (3-11): Comparing between control (Iraqi healthy) and samples of patients

In the sample (II) of the second primer was show a SNP as substitution C / T , was observed this replacement cause a missense mutation because its coded for other amino acid Ser /phe , (Taki, *et al* ,2001). The same substitution mutation C / T in exon 8 of the G6PD gene that chang amino acid pro to ser ,(Vulliamy,*et al*,1988) C / T change at position 563 in exon 6, resulting in an amino acid change Ser / Phe at position 188,this variant called the Mediterranean variant , this variant has higher affinity for G6PD and reduced thermo stability and associated with acute hemolytic anemia in response to therapeutic drugs and fava beans (Cappellini , *et al*, 2008).

The third DNA sequence of the G6PD gene located in exon 9 composed from patients and NCBI nucleotide was shown in figure (3-12A and B)

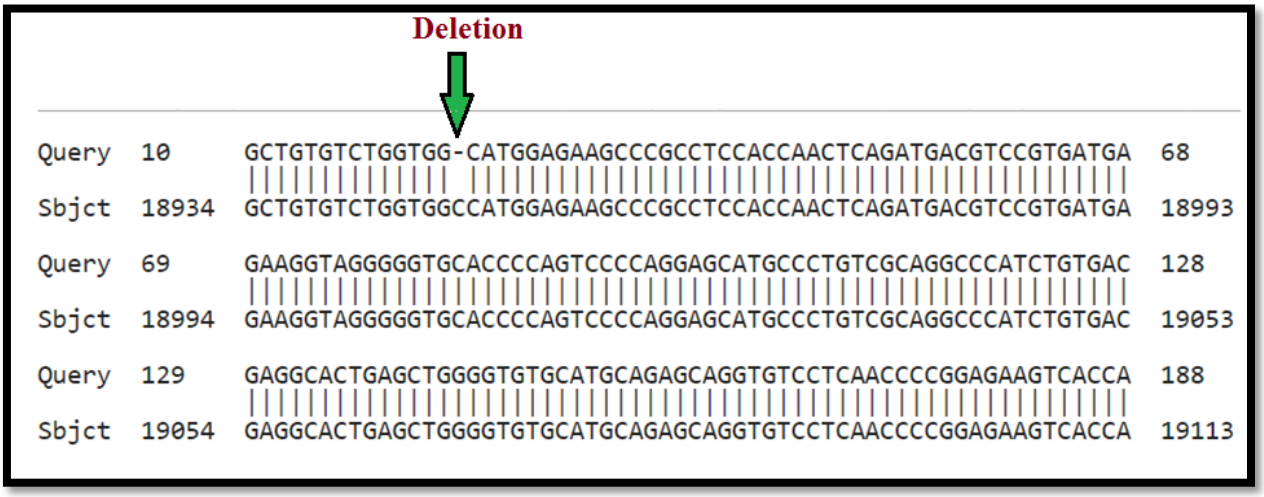
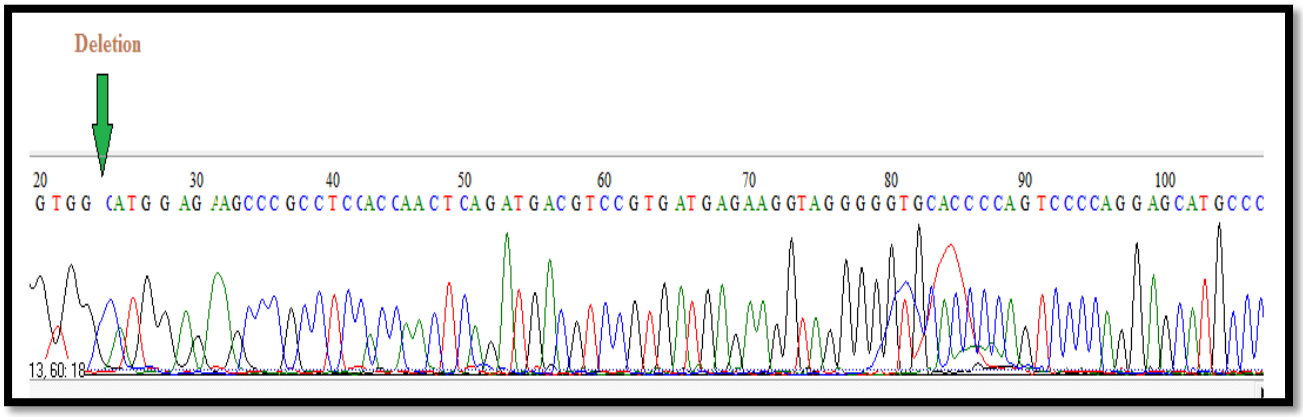


Figure (3-12A): display a sample of G6PD comparing in the NCBI nucleotide blast and show the sequence of exon (9).



ACCCGGACAGCTGTGTCTGGTGG_CATGGAGAAGCCCGCCTCCAC

CAACTCAGATGACGTCCGTGATGAGAAGGTAGGGGG

Figure (3-12 B): A chromatogram for a sample of G6PD.

A sample of the third primer showed a deletion in nitrogen base 18947-18949/GCC,in exon (9) that caused deletion in amino acid Gly/del , (Moradkhani ,*et al* ,2010) found point mutation in exon (9) of G6PD gene ACC that caused Thr /del

There are more than 130 mutation in the G6PD gene have been identification most of the muttion deleted so far are point mutation causing single amino acid substitution (Mehta *et al* ,2000). While show the table (3-3), differnted mutation of one or more than located gene region. However point mutation , substitution , deletion and insertion affected the G6PD gene in Iraqi patients.

Table (3-3) point mutations detect in patient samples:

No. of patient samples	Wild type	Mutant type	Change in amino acids	Site Of N.A.	Type of Mutation	Effect on translation
Patient 1 (fragII)	TTC	TTT	Phe_Phe	191	Substitution	Silent
	CGT	TCG	Arg _Ser	192	Insertion	Frame shift
Patient 2 (frag II)	TCC	TTC	Ser_Phe	188	Substitution	Missense
Patient 1(frag III)	GGC	_CA	Gly _ deletion	8	Deletion	Frame shift

This result agree with those of (Monika *et al*,2007; Hesham *et al* , 2011) who reported the direct sequencing of the G6PD gene located intron 6, exon 7 and exon 9 change the position 191, 188 and 8 these different type mutation caused the G6PD deficiency.

3.3.2. Percentage of mutations.

Analysis of G6PD gene by sequencing for Iraqi patients exhibited the existence of many genetic alteration. Three types of mutations namely substitution, deletion and insertion were present. Table (3-4) shows the percentage of mutation types that showed 50% for substitution 25% for deletion and 25% for insertion.

Table (3-4): percentage of mutation type

Type of Mutation	Percentage%
Substitution	50%
Deletion	25%
Insertion	25%

3.3.3. Effect of mutations.

Mutation in G6PD gene affects the regulation of apoptosis and proliferation. Table (3-5) shows that there was a missense mutation (25%) causing impact on phenotype that leads to replacement in amino acid. The deletion mutation lead to fram shift which represented 50% in this study. These mutations result in a completely different translation detect in G6PD enzyme.

Table (3-5) : percentage of effect of mutation .

Effect of mutation	*Percenage%*
Missense	**25%**

Silent	**25%**
Frame shift	**50%**

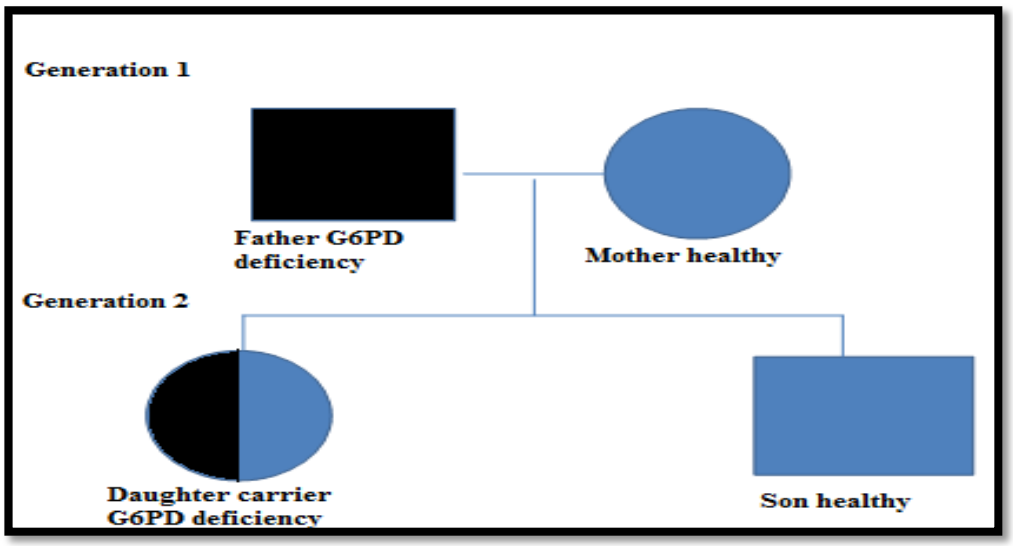
Molecular analysis of G6PD gene relarted more than 100 different mutations or combination of mutations that cause G6PD diffeciency, and the vast majority of these variant are sporadic, rather than polymorphic .

However each population has a characteristic profile of polymorphic G6PD varients (Beutler *et al* 2006). The G6PD Mediterranean variant (nt 563 C-T ; S188 F) predominant in the Mediterranean, Middle East and parts of India (Al Ali *et al* 2002).

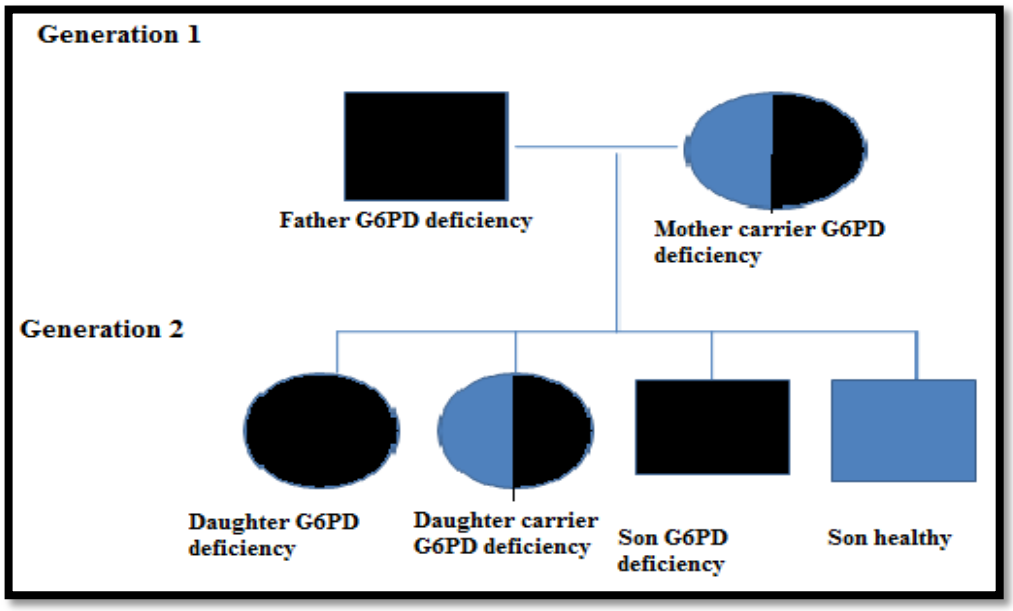
3.4. Pedigree analysis of families showing G6PD deficiency.

Pedigree analysis is an elassical test to reveal relationship among families and detect the transfer of genetic trait in generation.This type of analysis is employed to detect the transfer of G6PD deficiency in families that were selected for this study pedigree analysis of a families with G6PD deficiency is showed in figures (3-13 a,b,c,d,e)

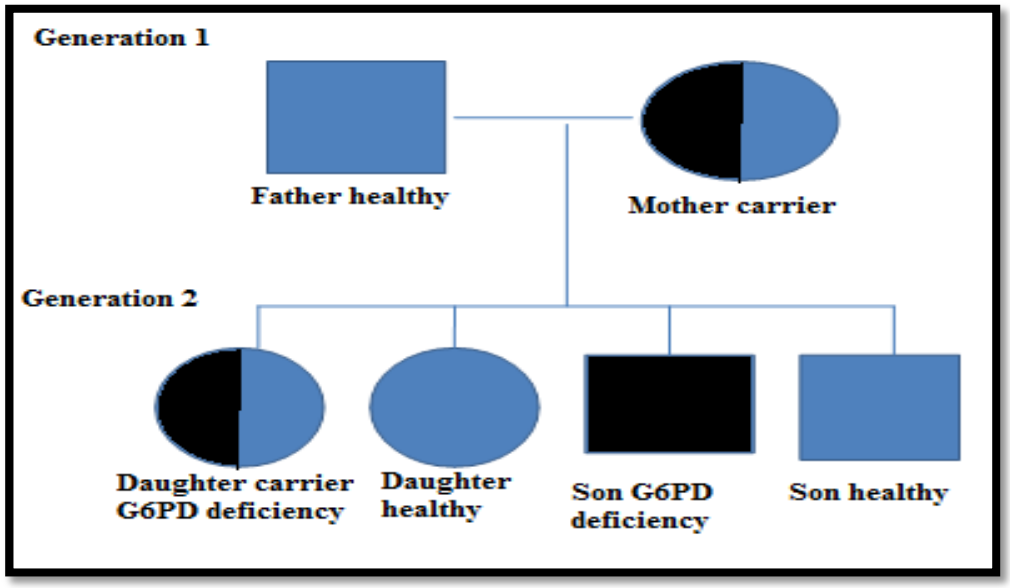
(a):



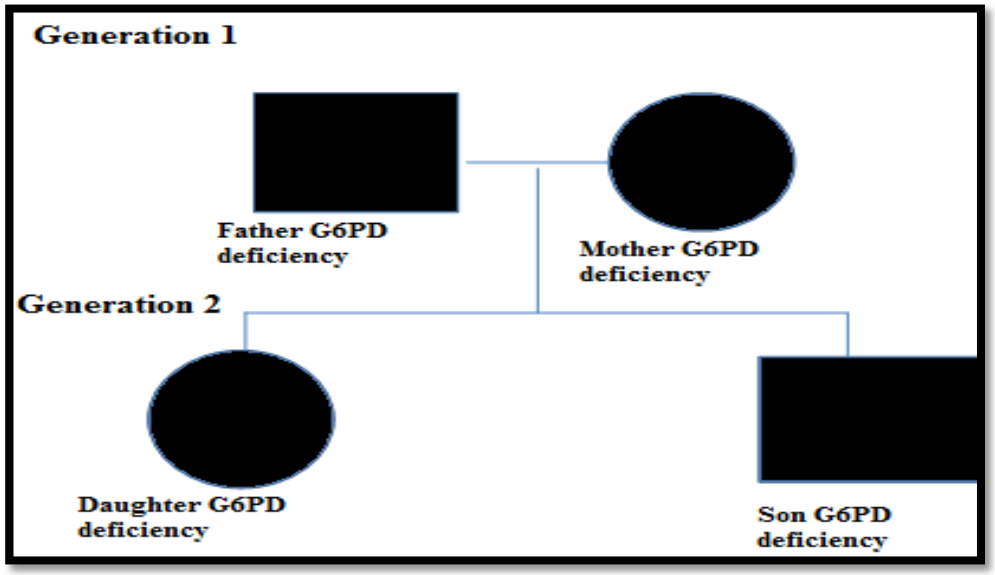
(b):


Generation 1
Father G6PD deficiency
Mother carrier G6PD deficiency
Generation 2
Daughter G6PD deficiency
Daughter carrier G6PD deficiency
Son G6PD deficiency
Son healthy

(c):


Generation 1
Father healthy
Mother carrier
Generation 2
Daughter carrier G6PD deficiency
Daughter healthy
Son G6PD deficiency
Son healthy

(d):


Generation 1
Father G6PD deficiency
Mother G6PD deficiency
Generation 2
Daughter G6PD deficiency
Son G6PD deficiency

(e):

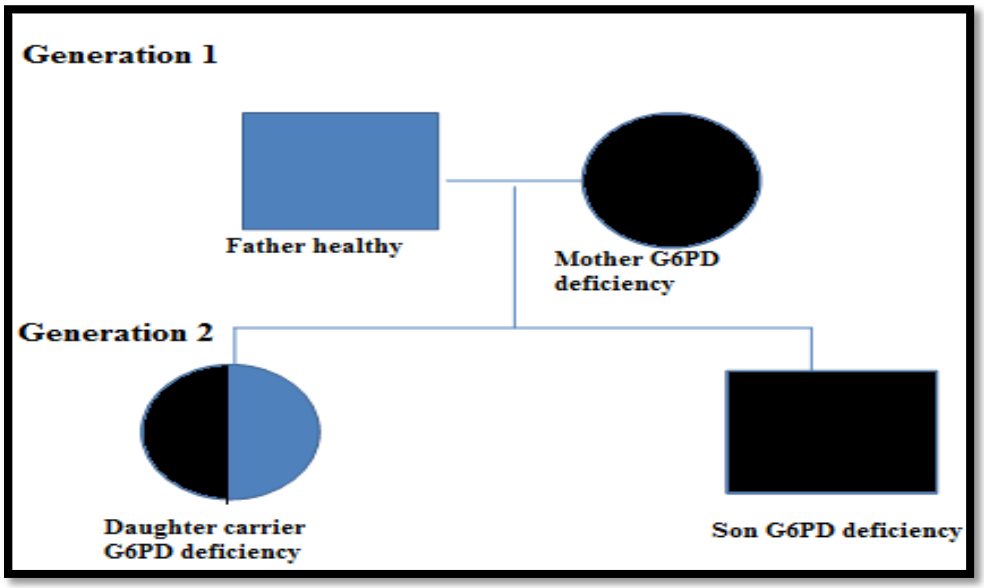

Generation 1
Father healthy
Mother G6PD deficiency
Generation 2
Daughter carrier G6PD deficiency
Son G6PD deficiency

Figure (3-13): Pedgree analysis.

The light square represents normal male square with dark represent a male with G6PD deficiency the circle light represent normal female, circle dark represent a female with G6PD deficiency and the circle with dark and light represent female with carrier G6PD deficiency.

Depending on the history of the families, the father was G6PD deficiency he was married to the mother who was normal ; thus the frequency of the arrive G6PD deficiency to increase in female show figure (3-13 a) when the mother carried G6PD deficiency married father G6PD deficiency the frequency of the son 25% affected, 25% healthy and daughter 25% affected and 25% carried show the figure (3-13b)

When the father healthy married carrier the frequency of G6PD deficiency increased in male show figure (3-13 c) when the father and mother affected the G6PD deficiency all sons and daughter affected the G6PD deficiency show figure (3-13d), and when the father healthy married mother G6PD deficient the frequency of the G6PD deficiency to increased in made show figure (3-13e).

This condition is inherited in an X-linked recessive pattern. The gene associated with this condition is located on the X chromosome, which is one of the two sex chromosomes. In males (who have only one X chromosome), one altered copy of the gene in each cell is sufficient to cause the condition. In females (who have two X chromosomes), a mutation would have to occur in both copies of the gene to cause the disorder. Because it is unlikely that females will have two altered copies of this gene, males are affected by X-linked recessive disorders much more frequently than females. A striking

characteristic of X-linked inheritance is that fathers cannot pass X-linked traits to their sons (Glader,2009 ; Mason, *et al*,2007 ; Westman, 2005).

4. Conclusions and Recommendation.

4.1. Conclusions..

1- Findings will help design a focused population screening approach and provide better management for G6PD deficiency patients.

2. The ratio of G6PD disease in all samples was about 82% and 18% were a carrier of disease.

3. males were more deficient than females, with 74% and 26% percentage respectivly.

4.Molecular analysis of this study compared with NCBI showed that the percentage of insertion was 30% , while substituted was 70%.

4.2. Recommendations.

1. Recording Iraqi Date in NCBI.

2. Study the expression of the gene using RT PCR.

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Appendix-1-

Case profile :

Name				
Gender	Male		female	
Age				
Address				
Occupation				
Education				
Time of disease				
History of disease				
Symptoms				
Nutrition				
Medication				

Appendix-2-

History of family

Name:

Gender	Male Female
Age	
Time of disease diagnosis	
History of disease	
No. of children	
No. of children affected	
Medication	

Appendix-3-

Sequences.

```
>DQ832766 Homo sapiens glucose-6-phosphate dehydrogenase (G6PD) gene, exons
hrough 9 and partial cds

Length = 3102

E-value = 0, Score = 821, Bitscore = 1517.22, Identities = 997/1077 (92%),
Positives = 997/1077 (92%), Gaps = 31/1077 (2%)
Frame = +1

Query        30 CGCTCTGCTT-CCCAAAGGCCCGGCCAGGCCGCAGGGTGGCAGCCTTGCTCTGCGAATG
                CGCTCTGCTT CCCAAAGGCCCGGCCAGGCCGCAGGGTGGCAGCCTTGCTCTGCGAATG
DQ832766   1047 CGCTCTGCTTCCCCAAAGGCCCGGCCAGGCCGCAGGGTGGCAGCCTTGCTCTGCGAATG

Query        89 AGCATGGTCCGCGCTGGGTGGTTTCCCAACCCAGCCAGAGGCTCTTGTCCTCTGGCTGG
                AGCATGG CCGCGCTGGGTGGTTTCCCAACCCAGCCAGAGGCTCTTGTCCTCTGGCTGG
DQ832766   1107 AGCATGGCCCGCGCTGGGTGGTTTCCCAACCCAGCCAGAGGCTCTTGTCCTCTGGCTGG

Query       149 TTTGAATGCGGGGGTAGTAAAGCAAAGGTCCTCTTCTCATTTTCAAAACCAATGAGGAA
                TTTGAATGCGGGGGTAGTAAAGCAAAGGTCCTCTTCTCATTTTCAAAACCAATGAGGAA
DQ832766   1167 TTTGAATGCGGGGGTAGTAAAGCAAAGGTCCTCTTCTCATTTTCAAAACCAATGAGGAA

Query       209 CCATGGCTTGGATGCCTCCTCCCCCTGCTCCCCTACAGGCCTTCAGGCCACTCAGACCC
                CCATGGCTTGGATGCCTCCTCCCCCTGCTCCCCTACAGGCCTTCAGGCCACTCAGACCC
DQ832766   1227 CCATGGCTTGGATGCCTCCTCCCCCTGCTCCCCTACAGGCCTTCAGGCCACTCAGACCC

Query       269 CCGGGGACCCAGCATGAGGCAGGAGGGGAACGGGCCCCCGGCAGCATGCCAGCAATGCC
                CCGGGGACCCAGCATGAGGCAGGAGGGGAACGGGCCCCCGGCAGCATGCCAGCAATGCC
DQ832766   1287 CCGGGGACCCAGCATGAGGCAGGAGGGGAACGGGCCCCCGGCAGCATGCCAGCAATGCC

Query       329 CCCTGGCACCCAGGGTGGGAAGGCTTCCCGGAAGGTGTTGAGCCAGAGGGTCATCTGGG
                CCCTGGCACCCAGGGTGGGAAGGCTTCCCGGAAGGTGTTGAGCCAGAGGGTCATCTGGG
DQ832766   1347 CCCTGGCACCCAGGGTGGGAAGGCTTCCCGGAAGGTGTTGAGCCAGAGGGTCATCTGGG

Query       389 ACACAAGGCACGGGAGGTGGCCACGGGGGCGAGGAGGTTCTGGCCTCTACTCCCCTGGG
                ACACAAGGCACGGGAGGTGGCCACGGGGGCGAGGAGGTTCTGGCCTCTACTCCCCTGGG
DQ832766   1407 ACACAAGGCACGGGAGGTGGCCACGGGGGCGAGGAGGTTCTGGCCTCTACTCCCCTGGG

Query       449 GGGCGTCTGAATGATGCAGCTCTGATCCTCACTCCCCGAAGAGGGGTTCAAGGGGGTAA
                GGGCGTCTGAATGATGCAGCT TGATCCTCACTCCCCGAAGAGGGGTTCAAGGGGGTAA
DQ832766   1467 GGGCGTCTGAATGATGCAGCTGTGATCCTCACTCCCCGAAGAGGGGTTCAAGGGGGTAA

Query       509 GCAGCTCCGGGCTCCCAGCAGAGGCTGGAACCGCATCATCGTGGAGAAGCCCTTCGGGA
                GCAGCTCCGGGCTCCCAGCAGAGGCTGGAACCGCATCATCGTGGAGAAGCCCTTCGGGA
DQ832766   1527 GCAGCTCCGGGCTCCCAGCAGAGGCTGGAACCGCATCATCGTGGAGAAGCCCTTCGGGA

Query       569 GGACCTGCAGAGCTCTGACCGGCTGTCCAACCACATCTCCTCCCTGTTTTCGTGAGGAC
                GGACCTGCAGAGCTCTGACCGGCTGTCCAACCACATCTCCTCCCTGTT  CGTGAGGAC
DQ832766   1587 GGACCTGCAGAGCTCTGACCGGCTGTCCAACCACATCTCCTCCCTGTTC-CGTGAGGAC

Query       629 AGATCTACCGCATCGACCACTACCTGGGCAAGGAGATGGTGCACAACCTCATGGTGCTG
                AGATCTACCGCATCGACCACTACCTGGGCAAGGAGATGGTGCA AACCTCATGGTGCTG
DQ832766   1646 AGATCTACCGCATCGACCACTACCTGGGCAAGGAGATGGTGCAGAACCTCATGGTGCTG

Query       689 GGTGGGGCCAATCCTGGGCCGGGGGGAACTTGGCGGGGGTGGCACTCAGTGAGCTCTCA
                GGTGGGGCCAA CCTGGGCC GGGGGA C  GG GGGGGTGG ACTCAG GAGC CTCA
DQ832766   1706 GGTGGGGCCAAGCCTGGGCC-GGGGGACCAGGGTGGGGGTGGTACTCAG-GAGC-CTCA

Query       749 CTGGGCCCACTGCCTCCCCCGAGGACGAATTCTTCCAAAACTCAGACAAGGGGTGACCC
                CT GGCCCACTGCCT CCCCGAGGACGAATTC TCCA AACTCAGACAAGGG TGACCC
DQ832766   1763 CT-GGCCCACTGCCT-CCCCGAGGACGAATTCCTCCAGAACTCAGACAAGGG-TGACCC

Query       809 TTCACATTGTGGCCCCTTGCACCACAGAGGCCCAAGGTCAGTTCCTTCCCCTTTGCCCC
                T CACAT GTGGCCCC TGCACCACAGAGGCCCAAGGTCAGTTCCT C CCTT GCCCC
DQ832766   1820 T-CACAT-GTGGCCCC-TGCACCACAGAGGCCCAAGGTCAGTTCCTCCACCTT-GCCCC

Query       869 TCCCCTGCAAACTTTGCCAATCAG-ATCTTCCGGCCCCATCCTGGAACCGGGGACAATC
                TCCC TGCA A TTTGCCAA CAG ATCTTC GGCCCCATC TGGAACCGGG ACAA C
DQ832766   1875 TCCC-TGCAGA-TTTGCCAA-CAGGATCTTC-GGCCCCATC-TGGAACCGGG-ACAA-C

Query       928 TCTCCTGGCGTTACCCTCTCTTTCAATGGAGCCCCTTGGGCTCTGAGGGTCCGGGGGGC
                TC CCT GCGTTA CCTC C TTCAA GGAGCCC TTGG C CTGAGGGTC  GGGGGC
DQ832766   1928 TCGCCT-GCGTTATCCTCACCTTCAA-GGAGCCCTTTGG-CACTGAGGGTCGCGGGGGC

Query       988 ATTTTCATAAATTTGGGAACATCCCTGTTACAGTT-TTCCTCCTCTCTGGGGAAGTTTG
                ATTT  AT AATTTGGGA CATCC  GT A AG T TTCCTC TCTC  GGGA G T G
DQ832766   1985 ATTTCGATGAATTTGGGATCATCCG-GTGAGAGCTCTTCCTC-TCTCCTGGGAGGCTGG

Query      1047 AAAAGGGTGGCAGAACCCTTTCACCCCGCCGTGGTCTTATCCTTCCTATATTGGGGG 1
                A  AGGGTGGCAGA CC  T CACCC GC G GG CT  TC T CCTAT TTGGGGG
DQ832766   2043 AC-AGGGTGGCAGAGCCAGT-CACCCTGCAG-GG-CTACTCTTCCCTATCTTGGGGG 2
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LOCUS       DQ832766               3102 bp ds-DNA     linear   PRI 06-AUG-
DEFINITION  Homo sapiens glucose-6-phosphate dehydrogenase (G6PD) gene, exo
            through 9 and partial cds.
ACCESSION   DQ832766
VERSION     DQ832766.1  GI:111052658
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostom
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 3102)
  AUTHORS   Yan,T., Cai,R., Mo,Q., Zhu,D. and Xu,X.
  TITLE     Incidence and complete molecular characterization of
            glucose-6-phosphate dehydrogenase (G6PD) deficiency in Guangxi
            Zhuang Autonomous Region of southern China: description of four
            novel mutations
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 3102)
  AUTHORS   Yan,T., Cai,R., Mo,Q., Zhu,D. and Xu,X.
  TITLE     Direct Submission
  JOURNAL   Submitted (27-JUN-2006) Department of Medical Genetics, Souther
            Medical University, Tongbo, Guangzhou, Guangdong 510515, China
```

```
>NG_009015 Homo sapiens glucose-6-phosphate dehydrogenase (G6PD), RefSeqGene on
 chromosome X

Length = 23182

E-value = 0, Score = 899, Bitscore = 1661.26, Identities = 1021/1075 (94%),
Positives = 1021/1075 (94%), Gaps = 28/1075 (2%)
Frame = +1

Query          20 CGCCTTT-CGCTCTGCTT-CCC-AAGGCCCGGCCAGGCCGCAGGGTGGCAGCCTTGCTCT    76
                  CGCCTTT CGCTCTGCTT CCC AAGGCCCGGCCAGGCCGCAGGGTGGCAGCCTTGCTCT
NG_009015   17568 CGCCTTTCCGCTCTGCTTCCCCAAAGGCCCGGCCAGGCCGCAGGGTGGCAGCCTTGCTCT 17627

Query          77 GCGAATGCAGCATGGCCCGCGCTGGGTGGTTTCCCAACCCAGCCAGAGGCTCTTGTCCTC   136
                  GCGAATGCAGCATGGCCCGCGCTGGGTGGTTTCCCAACCCAGCCAGAGGCTCTTGTCCTC
NG_009015   17628 GCGAATGCAGCATGGCCCGCGCTGGGTGGTTTCCCAACCCAGCCAGAGGCTCTTGTCCTC 17687

Query         137 TGGCTGGTTTTGAATGCGGGGGTAGTAAAGCAAAGGTCCTCTTCTCATTTTCAAAACCAA   196
                  TGGCTGGTTTTGAATGCGGGGGTAGTAAAGCAAAGGTCCTCTTCTCATTTTCAAAACCAA
NG_009015   17688 TGGCTGGTTTTGAATGCGGGGGTAGTAAAGCAAAGGTCCTCTTCTCATTTTCAAAACCAA 17747

Query         197 TGAGGAAGCCATGGCTTGGATGCCTCCTCCCCCTGCTCCCCTACAGGCCTTCAGGCCACT   256
                  TGAGGAAGCCATGGCTTGGATGCCTCCTCCCCCTGCTCCCCTACAGGCCTTCAGGCCACT
NG_009015   17748 TGAGGAAGCCATGGCTTGGATGCCTCCTCCCCCTGCTCCCCTACAGGCCTTCAGGCCACT 17807

Query         257 CAGACCCACCGGGGACCCAGCATGAGGCAGGAGGGGGAACGGGCCCCCGGCAGCATGCCAG   316
                  CAGACCCACCGGGGACCCAGCATGAGGCAGGAGGGGGAACGGGCCCCCGGCAGCATGCCAG
NG_009015   17808 CAGACCCACCGGGGACCCAGCATGAGGCAGGAGGGGGAACGGGCCCCCGGCAGCATGCCAG 17867

Query         317 CAATGCCACCCTGGCACCCAGGGTGGGAAGGCTTCCCGGAAGGTGTTGAGCCAGAGGGTC   376
                  CAATGCCACCCTGGCACCCAGGGTGGGAAGGCTTCCCGGAAGGTGTTGAGCCAGAGGGTC
NG_009015   17868 CAATGCCACCCTGGCACCCAGGGTGGGAAGGCTTCCCGGAAGGTGTTGAGCCAGAGGGTC 17927

Query         377 ATCTGGGAACACAAGGCACGGGAGGTGGCCACGGGGGCGAGGAGGTTCTGGCCTCTACTC   436
                  ATCTGGGAACACAAGGCACGGGAGGTGGCCACGGGGGCGAGGAGGTTCTGGCCTCTACTC
NG_009015   17928 ATCTGGGAACACAAGGCACGGGAGGTGGCCACGGGGGCGAGGAGGTTCTGGCCTCTACTC 17987

Query         437 CCCTGGGAGGGCGTCTGAATGATGCAGCTCTGGTCCTCACTCCCCGAAGAGGGGTTCAAG   496
                  CCCTGGGAGGGCGTCTGAATGATGCAGCTCTG TCCTCACTCCCCGAAGAGGGGTTCAAG
NG_009015   17988 CCCTGGGAGGGCGTCTGAATGATGCAGCTCTGATCCTCACTCCCCGAAGAGGGGTTCAAG 18047

Query         497 GGGGTAACGCAGCTCCGGGCTCCCAGCAGAGGCTGGAACCGCATCATCGTGGAGAAGCCC   556
                  GGGGTAACGCAGCTCCGGGCTCCCAGCAGAGGCTGGAACCGCATCATCGTGGAGAAGCCC
NG_009015   18048 GGGGTAACGCAGCTCCGGGCTCCCAGCAGAGGCTGGAACCGCATCATCGTGGAGAAGCCC 18107

Query         557 TTCGGGAGGGACCTGCAGAGCTCTGACCGGCTGTCCAACCACATCTTCTCCCTGTTCCGT   616
                  TTCGGGAGGGACCTGCAGAGCTCTGACCGGCTGTCCAACCACATCT CTCCCTGTTCCGT
NG_009015   18108 TTCGGGAGGGACCTGCAGAGCTCTGACCGGCTGTCCAACCACATCTCCCTCCCTGTTCCGT 18167

Query         617 GAGGACCATATTTTACCGCATCGACCACTACCTGGGCAAGGAGATGGTGCAGAACCTCAT   676
                  GAGGACCA AT  TACCGCATCGACCACTACCTGGGCAAGGAGATGGTGCAGAACCTCAT
NG_009015   18168 GAGGACCAGATC-TACCGCATCGACCACTACCTGGGCAAGGAGATGGTGCAGAACCTCAT 18226

Query         677 GGTGCTGAGGTGGGGCCAAGCCTGGGCCGGGGGACCAGGGTGGGGGTGGTACTCAGGAGC   736
                  GGTGCTGAGGTGGGGCCAAGCCTGGGCCGGGGGACCAGGGTGGGGGTGGTACTCAGGAGC
NG_009015   18227 GGTGCTGAGGTGGGGCCAAGCCTGGGCCGGGGGACCAGGGTGGGGGTGGTACTCAGGAGC 18286

Query         737 CTCACCTGGCCCACTGTCTCCCCGATGACCAACTCCTCCAGAACTCATACAAGGGTGACC   796
                  CTCACCTGGCCCACTG CTCCCCGA GAC AA TCCTCCAGAACTCA ACAAGGGTGACC
NG_009015   18287 CTCACCTGGCCCACTGCCTCCCCGAGGACGAATTCCTCCAGAACTCAGACAAGGGTGACC 18346

Query         797 TCTCAC-TGTGGCCCCTGCCCCCACAGATGTCCAAGGTCATTTCCTCCA-CTTGCCCCTC   854
                   CTCAC TGTGGCCCCTGC  CCACAGA G CCAAGGTCA TTCCTCCA CTTGCCCCTC
NG_009015   18347 CCTCACATGTGGCCCCTGCA-CCACAGAGGCCCAAGGTCAGTTCCTCCACCTTGCCCCTC 18405

Query         855 CCTGCAAATTTGCTCATCTGGATCCTTCGGTCCCTATTCTGGTACCGAGAC-ACATCGCC   913
                  CCTGCA ATTTGC CA C GGATC TTCGG CCC A TCTGG ACCG GAC ACATCGCC
NG_009015   18406 CCTGCAGATTTGC-CAACAGGATC-TTCGGCCCC-A-TCTGGAACCGGGACAACATCGCC 18461

Query         914 TGCGTTATCCTCACCTATCAATGGAGCCCTTCTGGCACTCGAGGGTCGCGGGAGGCTACT   973
                  TGCGTTATCCTCACCT TCAA GGAGCCCTT TGGCACT GAGGGTCGCGGG GGCTA T
NG_009015   18462 TGCGTTATCCTCACCT-TCAA-GGAGCCCTT-TGGCACT-GAGGGTCGCGGG-GGCTATT 18516

Query         974 TCGATGAATTTGGGATCATCCTGGTGAGTAGCTCTTCCTTCTCCTCCTGGGAGGCTGGCA  1033
                  TCGATGAATTTGGGATCATCC GGTGAG AGCTCTTCCT CT CTCCTGGGAGGCTGGCA
NG_009015   18517 TCGATGAATTTGGGATCATCC-GGTGAG-AGCTCTTCCT-CT-CTCCTGGGAGGCTGGCA 18572

Query        1034 ACGGGTTAC-GAG-CAGTCACCCTGCATG-CTACCTCTTTCCCTATTTCTTGGGG  1085
                  GGGT  C GAG CAGTCACCCTGCA G CTAC TCTT CCCTAT  CTTGGGG
NG_009015   18573 CAGGGTGGCAGAGCCAGTCACCCTGCAGGGCTAC-TCTT-CCCTAT--CTTGGGG 18623
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LOCUS       NG_009015              23182 bp ds-DNA     linear   PRI 18-SEP-2014
DEFINITION  Homo sapiens glucose-6-phosphate dehydrogenase (G6PD), RefSeqGene
            on chromosome X.
ACCESSION   NG_009015
VERSION     NG_009015.2  GI:575006674
KEYWORDS    RefSeq; RefSeqGene.
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
COMMENT     REVIEWED REFSEQ: This record has been curated by NCBI staff in
            collaboration with Sujatha Mohan. The reference sequence was
            derived from AC244090.3.
            This sequence is a reference standard in the RefSeqGene project.
            On Jan 17, 2014 this sequence version replaced gi:213385264.
            ~Summary: This gene encodes glucose-6-phosphate dehydrogenase. This
            protein is a cytosolic enzyme encoded by a housekeeping X-linked
            gene whose main function is to produce NADPH, a key electron donor
            in the defense against oxidizing agents and in reductive
            biosynthetic reactions. G6PD is remarkable for its genetic
            diversity. Many variants of G6PD, mostly produced from missense
            mutations, have been described with wide ranging levels of enzyme
            activity and associated clinical symptoms. G6PD deficiency may
            cause neonatal jaundice, acute hemolysis, or severe chronic
            non-spherocytic hemolytic anemia. Two transcript variants encoding
            different isoforms have been found for this gene. [provided by
            RefSeq, Jul 2008].
//
This sequence is too large to show in the text view.  To see the entire sequence in GenBank flat format, export it.
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الخلاصة:

جائت هذه الدراسة للتحري عن نقص الانزيم المسبب لفقر الدم التحللي الناتج عن اثر الباقلاء و الاثر الوراثي المؤدي له.

جمعت 50 عينه من دم اشخاص مصابين من مستشفيات عدة (مستشفى اليرموك و مستشفى الطفل المركزي و مستشفى العلوية و مستشفى مدينه الطب) تم مقارنتها مع 20 عينه من اشخاص اصحاء.

لوحظ تباين من ناحية المرض فمنها ذات تاثير قليل او متوسط ومنها اخذت من اشخاص ذو مرض مزمن ، امتدت فترة جمع العينات ثلاثة اشهر ابتدات من شهر ايار الى حزيران سنة 2014 .

بعد اجراء الفحوصات المختبرية وجد ان نسبة 42% تعود الى اشخاص ذوي حالة متوسطة من المرض بينما كانت نسبة ذوي الحالة المزمنة 40% والحاملين للمرض كانت النسبة 18%.

تم استخلاص الدنا من عينات الدم تحت الاختبار ودرست المنطقة المشفرة للانزيم بواسطة 3 بادئات متخصصة وبأستخدام تقنية PCR .

البادئ الاول (Frag I) ذو منتج 115 ز.ق بالاضافة الى البادئ الثاني (Frag II) ذو منتج1500 ز.ق، والبادئ الثالث 2000 ز.ق، والتي استطاعت تضخيم الموقع المشفر للانزيم في الحاملين للمرض والمصابين به بدرجة متوسطة.

بعد مقارنة النتائج لسلسلة الدنا المستخلصة من الاشخاص الذين يعانون المرض وجد ان نسبة الاستبدال كانت 70% ونسبة الاضافة في موق الجين كانت 30%ز

الجزء الاخر من الدراسة اختصت بوراثة المرض ضمن العوائل والذي اوضح ان هذا المرض مرتبط بالكروموسوم الجنسي X وان الاب يستطيع ان يورث هذا المرض الى بناته دون ظهور الاعراض بينما يظهر هذا المرض المزمن في الذكور.

الكشف الجزيئي لبعض الطفرات لأنزيم G6PD في عينة من المرضى العراقيين

رسالة

مقدمة الى مجلس كلية العلوم- جامعة النهرين كجزء من متطلبات نيل

درجة الماجستير في كلية العلوم/ التقانة الاحيائية

من قبل

هديل محمد خلف

)2006(بكالوريوس تقانة احيائية

بإشراف

د. رحاب صبحي رمضان

استاذ مساعد

تشرين الاول 2015 ذي الحجه 1436