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# DETECTION OF POLYMORPHISM AND MUTATION IN GILBERT SYNDROME GENE IN PATIENTS WITH SUSPECTED HEPATITIS

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**BY**

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DETECTION OF POLYMORPHISM AND MUTATION IN GILBERT SYNDROME GENE IN PATIENTS WITH SUSPECTED HEPATITIS

By Rasha Mahmood Assad ASAAD

January 2023

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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## ABSTRACT

# DETECTION OF POLYMORPHISM AND MUTATION IN GILBERT SYNDROME GENE IN PATIENTS WITH SUSPECTED HEPATITIS

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Master of Science in Biology

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January 2023

This study aims to estimate the levels of liver enzymes in patients with liver disorders or similar symptoms like yellowing skin and eyes, abdominal pain, lethargy, or who have a genetic history of Gilbert syndrome. 100 sample were collected, with age range 20 to 50 years, in addition to 100 healthy volunteers, with age range 22 to 48 years. There was a great difference in GPT, GOT and G6PD enzyme concentration and total bilirubin between the two groups. Genetic study results showed that the genotype (TC) had the highest frequency of 10 % in patients versus the control group of 1%. The genotype (TT) had a frequency of 90% in patients compared to 99 % in control group. The results also showed that the genotype (GC) had the highest rate of 6% in patients. The genotype (GG) had a frequency of 94 % in patients compared to 100 % in control group. As a conclusion from 200 sample, the blood groups O and A have the highest frequencies among patients, indicating that these blood groups may be more susceptible to elevation bilirubin in patients with liver disorders. The differences in serum level of G6PD between study groups referred that this enzyme may have an important role in hyperbilirubinemia condition among patients. High frequency of genotypes TC of G6PD Aures (143 T→C) and GC of UGT1A1 (position 179G>C) genes polymorphisms in patients in comparison with control, indicated that these genotypes may play an important role in variation in the level of G6PD, abnormality in liver enzymes as well as hyperbilirubinemia.



**2023, 64 pages**

**Keywords:** Hyperbilirubinemia, Gilbert syndrome, Liver enzymes, DNA extracted.

## ÖZET

# HEPATİT ŞÜPHELİ HASTALARDA GİLBERT SENDROMU GENİNDE POLİMORFİZM VE MUTASYON TESPİTİ

Rasha Mahmood Assad ASAAD

Biyoloji, Yüksek Lisans

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Ocak 2023

Bu tez, cilt ve gözlerde sararma, karın ağrısı, uyuşukluk açısından karaciğer bozuklukları veya benzer semptomlardan muzdarip veya Culbert sendromu ile genetik öyküsü olan kişilerde karaciğer enzim düzeylerini tahmin etmeyi amaçlamaktadır. Yaşları 20 ile 50 arasında, 100 sağlıklı gönüllünün yanı sıra 22 ile 48 arasında değişiyordu. Sağlıklı ve hasta olmak üzere iki grup arasında ortalama GPT, GOT ve G6PD enzim konsantrasyonu ve toplam bilirubin açısından güçlü bir fark vardı. Genetik çalışma hakkında Sağlıklı ve hasta. Genetik çalışma ile ilgili olarak, sonuçlar, genotipin (TC) hastalarda en yüksek sıklığa sahip olduğunu (yüzde on), kontrol grubu 1'e göre daha düşük bir sıklığa (yüzde on), genotipin (TT) ise bir frekansa sahip olduğunu gösterdi. 90 (yüzde doksan). sağlıklı kontrol grubunda en yüksek nüks oranı 99 (yüzde doksan) ile karşılaştırıldığında. Ayrıca her iki grupta da 0'da (yüzde sıfır) genotip (CC) yoktu. Sonuçlar ayrıca genotipin (GC), kontrol grubu 0'a (yüzde sıfır) kıyasla hastalarda en yüksek tekrarlama oranına 6 (yüzde altı) sahip olduğunu gösterdi. , sağlıklı kontrol grubundaki en yüksek frekans 100 (yüzde yüz) ile karşılaştırıldığında, genotip (GG) hastalarda 94 (yüzde doksan dört) sıklığa sahipti. Ayrıca her iki grupta da 0'da (yüzde sıfır) genotip (CC) yoktu.



**2023, 64 Sayfa**

**Anahtar Kelimeler:** Hiperbilirubinemi, Gilbert sendromu, Karaciğer enzimleri, DNA.

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**Rasha Mahmood Assad ASAAD**

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| A | Absorbance |
| ALP | Alkaline phosphatase |
| ALT | Alanine aminotransferase |
| AP-2 | Activator protein 2 |
| AST | Aspartate Aminotransferase |
| CccDNA | Covalently closed circular DNA |
| CHD | Coronary heart disease |
| G6P | Glucose-6-phosphate |
| G6PD | Glucose-6-phosphate dehydrogenase |
| GOT | Aspartate Aminotransferase |
| GPT | Alanine aminotransferase |
| GS | Gilbert syndrome |
| GSH | Glutathione |
| HBV | Hepatitis B virus |
| HCl | Hydrochloric acid |
| HCV | Hepatitis C virus |
| HMP | Hexose monophosphate pathway |
| MgCl2 | Magnesium chloride |
| NaCl | Sodium chloride |
| NADPH | Nicotinamide adenine dinucleotide phosphate |
| PPP | Pentose phosphate pathway |
| R5P | Ribose-5-phosphate |
| Sp1 | Stimulatory protein 1 |
| TB | Total bilirubin |
| UGT1A1 | UDPglucuronosyltransferase 1A1 |
| UGTs | Diphosphate glucuronosyltransferases |

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# 1. INTRODUCTION

Gilbert syndrome is characterized by non-hemolytic unconjugated hyperbilirubinemia, which is caused by a failure in the hepatic absorption of unconjugated bilirubin (GS), which Augustin Gilbert first diagnosed in 1901 (Fretzayas *et al.* 2012). GS, 5–10% of the general population suffers with icterus intermittent juvenilis, often known as simple family jaundice and more usually strikes males (Aiso *et al.* 2017).

The presence of Gilbert syndrome (GS) at birth but may not be identified until the person exhibits mild hyperbilirubinemia or changes to their overt jaundice in their second decade of their lives (Monaghan *et al.* 1999, Rossi *et al.* 2005). Gilbert syndrome has been connected to symptoms in people taking drugs like irinotecan for therapy (Sieg *et al.* 1987). Small-scale recurrent jaundice, tiredness, and stomach pain brought on by stress, an illness, or menstruation are some of the clinical signs of GS Due to a mutation withinside the UGT1A1 gene, the bilirubin uridine diphosphate glucuronyl transferase enzyme hobby is diminished in GS. The UGT1A1 gene has more than 100 variations linked to the GS phenotype, albeit phenobarbital may be administered in severe cases. Generally speaking, GS has no effective treatments (Ha *et al.* 2017).

In GS, hyperbilirubinemia has been related to a cardio protective advantage that can be attributed to bilirubin's antioxidant and vasodilator capabilities, according to a prior study by Maruhashi *et al.* (2012). The UGT1A1 variants UGT1A1*28, UGT1A1*60, and UGT1A1*93 have all been reported. The UGT1A1*28 mutation is typically found in Caucasians. UGT1A1 promoter TA repeat expansion, with an increase from 6 to 7 repetitions of the TA dinucleotide (Aono *et al.* 1995).

Gilbert's syndrome is characterized by a homozygous polymorphism (A (TA) 7TAA) in the promoter of the UDP glucuronosyl transferase 1A1 (UGT1A1) gene. This is the most prevalent symptom of Gilbert's syndrome (Lee *et al.* 2011). Gilbert syndrome is an underestimated clinical condition in many countries since so few studies have looked at its prevalence and clinico-genetic patterns (Fretzayas *et al.* 2012).

Globally, Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common form of inherited enzymopathy. NADPH is generated by the pentose phosphate pathway (PPP) to maintain constant levels of reduced glutathione, which is vital for protecting red blood cells from oxidative damage. G6PD is a major enzyme in this metabolic system (Tiwari *et al.* 2017, Grace *et al.* 2018).

The gene that codes for the G6PD enzyme has 13 exons and 12 introns, and it is situated close to the telomere region of the distal arm of the X chromosome (Gomez-Manzo *et al.* 2016). A single nucleotide mutation in an exon of the G6PD gene that leads in an amino acid change resulting in decreased enzyme activity and stability, is the primary cause of G6PD deficiency (Gómez-Manzo *et al.* 2014). Due to the fact that G6PD deficiency is an X-linked genetic disorder, it is projected that males are more likely than females to experience this condition, which manifests in both homozygous males and homozygous or heterozygous females (Domingo *et al.* 2019).

**Aim of study**

- Estimate the levels of liver enzymes (AST, ALT and ALP) in both groups of study.
- Estimate the levels of total bilirubin and G6PD in both groups of study.
- Study the mutations of some genes in both groups of study.

# 2. LITERATURE REVIEW

## 2.1 Gilbert Syndrome

Gilbert syndrome is a disorder that is passed down through families in an autosomal recessive manner that causes problems with how the liver processes bilirubin. Repeated episodes of jaundice and unconjugated hyperbilirubinemia are brought on by reduced bilirubin glucuronidation (Bosma 2003).

Normally, the proportion of unconjugated bilirubin is about 95%. Gilbert syndrome must be recognized from other disorders with unconjugated hyperbilirubinemia since it doesn't need to be treated (Fretzayas *et al.* 2012). Patients who have unconjugated hyperbilirubinemia should be examined for additional conditions that can impact bilirubin absorption, conjugation, or overproduction (Erlinger *et al.* 2014). Hepatic absorption process, storage, conjugation condition, and excretion issues can result in both hyperbilirubinemia both conjugated and unconjugated. Unconjugated hyperbilirubinemia is a prominent symptom of Crigler-Najjar syndrome (Strauss *et al.* 2020).

Unconjugated hyperbilirubinemia is brought on by resorbing hematomas, ineffective erythropoiesis, and excessive bilirubin production. Unconjugated bilirubin levels may be raised as a result of diversity of hemolytic reactions, including warm autoimmune hemolytic anemia, and cold agglutinin sickness. They also include infections, medicines, poisons, hemoglobin apathies, RBC membrane abnormalities, and genetic enzyme deficits (Muraca *et al.* 1983).

Most people with Gilbert's Syndrome don't show any symptoms of liver disease, although occasionally they do. Fasting, concomitant illness, menstruation, dehydration, and other conditions can all result in unconjugated hyperbilirubinemia of Gilbert syndrome (Fretzayas *et al.* 2012). Several acute and chronic liver diseases are characterized by unconjugated and conjugated hyperbilirubinemia (Kwo *et al.* 2017).

Patients with hepatobiliary disorders have higher levels of conjugated bilirubin in their systems. As a result, it is important to consider viral, metabolic, and autoimmune liver diseases when evaluating people who have hyperbilirubinemia and jaundice. After a thorough clinical examination, targeted laboratory testing Gilbert syndrome should be discovered after the elimination of other pathologies related to unconjugated hyperbilirubinemia and the assessment of different acute and continual liver diseases. Once Gilbert syndrome has been diagnosed, the only treatment option is observation (Fretzayas *et al.* 2012). Patients with Gilbert syndrome have a fairly good prognosis (Horsfall *et al.* 2013).

### 2.1.1 Etiology

Gilbert syndrome patients may experience unconjugated hyperbilirubinemia and jaundice for a number of reasons Common precipitants include fasting, hemolytic responses, febrile illnesses, menstruation, and physical exercise (Felsher *et al.* 1970). A decrease in daily calorie intake to 400 kcal might cause a 2–3-fold rise in bilirubin within 48 hours (Barrett *et al.* 1971). Additionally, a normocaloric diet without added fat may result in a comparable rise in bilirubin (Gollan *et al.* 1976).

With a regular diet, bilirubin levels often recover to normal within 12 to 24 hours. Numerous explanations have been proposed to explain how dietary modifications might result in unconjugated hyperbilirubinemia. Bilirubin is released from fat cells, enterohepatic cycling of bilirubin is accelerated, and conjugation is reduced because there was a reduction in the amount of UDP-glucuronic acid, which is a co-substrate during the glucuronidation process (Kotal *et al.* 1996, Brink *et al.* 1996).

### 2.1.2 Epidemiology

Gilbert syndrome can occur anywhere between 4 and 16 percent of the time (Borlak *et al.* 2000, Roy-Chowdhury *et al.* 2002). Boys are more prone to experience clinical symptoms in the early stages of adolescence. The causes of the increasing prevalence rates are most

likely to be variations in sex steroid levels and higher levels of bilirubin production in males. Because of the accelerated haemoglobin turnover and the suppression of bilirubin glucuronidation caused by endogenous steroid hormones during puberty, this condition is most prevalent then (Muraca *et al.* 1984).

Japanese individuals have a lower frequency of promoter mutations than Whites and Blacks, who have a variant promoter frequency of 30% (Monaghan *et al.* 1996). In persons Having Gilbert syndrome and a lack of glucose-6-phosphatase, long-lasting neonatal jaundice can happen (Auclair *et al.* 1976).

### 2.1.3 Diagnosis

Gilbert syndrome, in contrast to CN syndrome, is often benign and may not need treatment. However, because these people may be more susceptible to the negative effects of certain drugs UGT1A1-metabolized medicines, caution is advised screening for this illness is essential for therapeutic purposes. In the vast majority of cases of Gilbert syndrome, according to our research, the UGT1A1*28 mutation is homozygous. So, we first check for this mutation using real-time PCR detection, a speedy and precise tool (Borlak *et al.* 2001).

If the patient with Gilbert syndrome seemed to be homozygous for this mutation, then no further testing would be required. If only one allele has the UGT1A1*28 mutation, or if the TATA boxes of both alleles are normal, we test for the G71R and Y486D mutations, which have also been reported to be present in patients with UGT syndrome common Gilbert. In order to seek for structural alterations on the opposing allele, the whole coding area should be sequenced if this screening is inconclusive. If so, the person might suffer from CN syndrome. The potential that the UGT1A1*28 mutation has a selective advantage or disadvantage is raised by the variation in the prevalence of this mutation between groups. Another indication of a selection process is the longer TATA box in humans comparing to other primates (Hall *et al.* 1999).

Such a shift can result from a choice in favor of a longer TATAA box. This implies that Gilbert syndrome must benefit from the longer TATA box in some way. Bilirubin's antioxidant qualities could result in a favorable selection. A number of oxidative aspects that lead to illnesses like cancer and cardiovascular disease may be inhibited by elevated bilirubin levels in the blood (CHD). In fact, multiple studies discovered a protective association between Caucasians' blood bilirubin levels and their risk of CHD (Mayer 2000).

However, the biggest population study revealed a U-shaped connection, suggesting that the risk was increased for both those with low and those with high bilirubin levels, such as those with Gilbert syndrome (Breimer *et al.* 1995). The protective role of bilirubin does not appear to favorably select for the Gilbert syndrome mutation, and as a person reaches reproductive age, CHD becomes a prominent cause of mortality. Cancer, another significant cause of death that develops after reproductive age, follows the same rationale. In conclusion, it seems unlikely that the UGT1A1*28 mutation would be positively selected for as a result of bilirubin's anti-oxidant characteristics.

Negative selection may also occur when the Gilbert syndrome mutation interacts with other polymorphism sites. Negative selection may be brought on, in particular, by interactions with conditions that increase the burden of unconjugated bilirubin. The relationship to G6PD deficiency is one prominent illustration. Together, Gilbert syndrome gene mutation and G6PD deficiency cause severe neonatal jaundice. B-thalassemia carriers engage in similar interactions (Tzetis *et al.* 2001).

Both interactions have the potential to result in kernicterus in the newborn stage. As a result, the Gilbert syndrome mutation, as a consequence, G6DH and b-thalassemia are selected for will come under pressure in populations where malaria is endemic. Asians are less frequent than Caucasians, which is consistent with a negative selection. More research is required to verify this alleged negative selection mechanism and to ascertain the allele frequencies in various communities impacted by malaria. Hereditary spherocytosis is another instance of an interaction that increases bilirubin load.

The GS mutation and this disease work together to increase the risk of bilirubinate gallstones (del Giudice *et al.* 1999).

### 2.1.4 Causes and clinical assessment

In 1901, Gilbert and Lereboulette discovered GS as a persistent, benign kind of jaundice that was unrelated to hemolytic disease (Gilbert 1901). The broad Caucasian population is where this variation was initially found, and Arias later reported it in 1962 (Arias 1962).

Therefore, a circulating TB concentration of more than 17.1 lmol/L (1 mg/dL) was used to make the diagnosis of GS. Internationally, the range of tuberculosis concentrations deemed harmful varies. For example, the Royal College of Pathologists of Australasia defines concentrations above the standard range as >20 mmol/L. despite the fact that Gilbert *et al.* initially described the condition in people whose TB levels were >1 mg/dL (>17.1 mmol/L) at the time (RCPA 2018Concentrations above 22 mmol/L, It has been determined by Canadian Association of Physicians and Surgeons that these values fall outside of the reference range (Royal 2017).

To diagnose GS, a person's blood must be drawn after an overnight fast, they must have elevated bilirubin levels twice in the previous six months, Moreover, they need to have normal serum transaminases and usually levels of gamma-glutamyl transpeptidase and alkaline phosphatase, two indicators of biliary injury or obstruction (Powell 1967).

In addition to this, a complete blood count that includes the reticulocyte count as well as a blood smear should be performed to rule out the potential of abnormalities in the structure of RBCs as well as the likelihood of enhanced RBC production or destruction respectively. GS individuals occasionally experience jaundice while under stress, ill, or fasting (Powell 2002). When bilirubin levels are greater than 40–45 mmol/l, jaundice frequently manifests. However, a small percentage of GS patients may have levels as high as 85 mmol/l. IBIL has the potential to be neurotoxic at doses greater than 300

mmol/L.however GS TB concentrations are not high enough to cause neurological symptoms.

If TB levels are discovered to be greater than 85 mmol/L, It is necessary to conduct further tests to determine the underlying cause of hyperbilirubinemia in order to exclude the possibility of hemolytic illness and other uncommon abnormalities regarding the metabolism of bilirubin (i.e. Crigler-Najjar Syndrome type 2).

Patients who have a TB level that is less than 85 mmol/L and who wish to confirm the etiology of their GS have the option of undergoing genotyping for one of the several UGT1A gene variations. This is an option that is available to patients including the UGT1A128 variation, yet a diagnosis of GS can be made without it. In other words, this genotyping helps to determine what is causing the hyperbilirubinemia in the first place. The association between UGT1A1 activity, IBIL serum concentrations, and hyperbilirubinemia criteria is summarized in Figure 2.1.

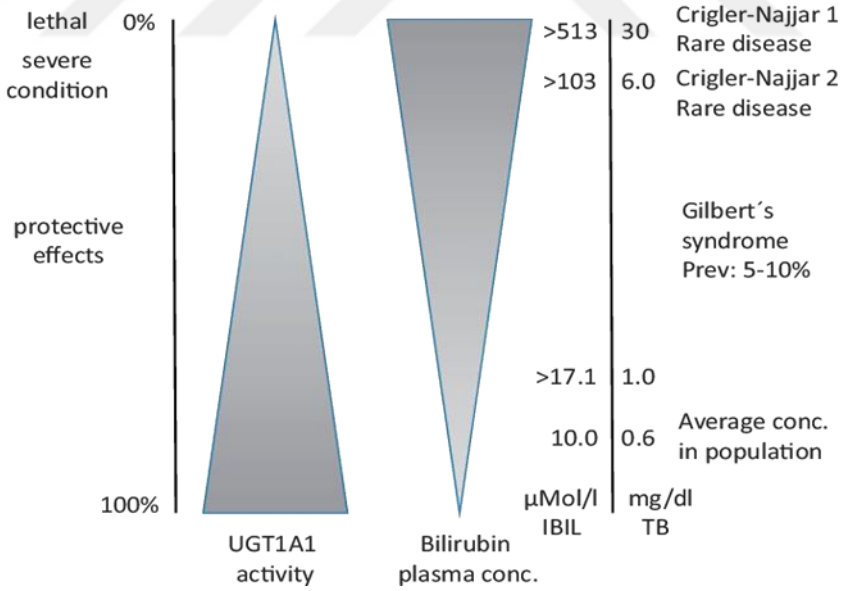


**Figure 2.1** Summary of the several hyperbilirubinemia syndromes (GS, CN-1 and CN-2, or kinds 1 and 2 of the Crigler-Najjar syndrome) based on varied UGT1A1 activities.

### 2.1.5 Pharmacological implications

Since the UDP-GT system is essential for the clearance of xenobiotics like drugs that undergo glucuronidation in addition to endogenous metabolites, patients with genetic hyperbilirubinemias may have impaired UDP-GT system metabolism. In addition to paracetamol, morphine, temazepam, ritodrine, diflunisal, zidovudine, and other medications, there are a great number of others that are direct substrates for the different isoforms of UDPGT.

Most of these medications also have phenol acceptor groups, which increases the possibility that they will be sulfated rather than glucuronidated. Other drugs like probenecid and Ibuprofen has carboxylic acid residues, which bind glucuronic acid more specifically than other acids. Some compounds have acceptor groups that are both phenolic and carboxylic, bile acids, and diflunisal, are two examples. Besides these substrates that bind directly, It is necessary for the production of acceptor groups for glucuronic acid that numerous additional medicines go through biotransformation using phase I activities. Given that GS affects a substantial portion of the overall population, it is likely that persons who have the condition will have a unique pharmacokinetic profile for glucuronidated drugs.

These folks might also be more susceptible to some drug side effects. However, Because of the large levels of inter- and intra-individual (age-related) variation, it might be challenging to accurately connect drug metabolism with UGT expression and activity. Numerous actions that occur throughout ontogeny are regulated in varying degrees by both genetic and environmental influences (Radu and Jacob 2001).

Hall *et al.* (1999)'S the investigation of the TA sequences that are located in the TATA box of the UGT1A1 gene revealed a considerable discrepancy in TA repeats across ethnic groups. There seem to be just two alleles among non-African groups. The same authors also emphasized the phylogenetic diversity that seems to have maintained throughout primate evolution. In nine distinct ape species, the number of TA repetitions has grown with time, with humans having the greatest. The situation is made worse by the existence

of alternative metabolic pathways and the ability organ-specific activity and/or ontogeny of other organs to partake in glucuronidation. As a result, it is not surprising that there is a lack of reliable data regarding the pharmacological effects of GS.

CPT-11 is the most well-known instance of medication toxicity associated with GS (irinotecan). CPT-11, a semisynthetic camptotecin derivative, is used to treat colon cancer with metastases. Tissue and serum carboxyl esterase convert it to SN-38, a metabolite with a high level of activity (7-ethyl-10-hydroxycamtothecin) before hepatic UGTs glucuronidate it.

It is hypothesized that SN-38 biliary excretion is associated to diarrhea, which is the primary dose-limiting hazard of irinotecan therapy. The degree of diarrhea is governed by SN-38 glucuronidation, and diarrhea is the predominant hazard (Iyer *et al.* 1998).

There was a considerable intersubject difference in the rates of SN-38 glucuronide synthesis in humans. Low UGT1A1 activity in GS patients makes them more susceptible to irinotecan toxicity. The data do not show that therapeutic paracetamol (acetaminophen) doses enhance GS patients' risk of hepatic or systemic toxicity. UGT1A6, not UGT1A1, is the major enzyme involved in paracetamol metabolism. (and to a lesser degree UGT1A9) (Esteban and Perez-Mateo 2004).

When cytochrome P4502D6 metabolism is compromised, glucuronidation is the main metabolic pathway for propafenon elimination. Researchers have looked into the possibility of increased medication toxicity in those with concomitant GS and poor metabolizers. It appears that propafenon toxicity is not increased in people with GS who also have slow metabolisms because the investigation did not support this assertion. Propafenone, however, may act as a substrate for UGT isoforms other than UGT1A1 (Dilger *et al.* 2000).

### 2.1.6 Clinical significance

It is unclear if mildly raised indirect bilirubin concentrations or asymptomatic jaundice with normal tests of liver function have therapeutic significance. Today, it is believed that GS is a benign, non-progressive condition that doesn't require therapy or ongoing medical care. It has been demonstrated that very low dosages of phenobarbital (0.05-0.15 g per day) can bring bilirubin levels back to normal. The risk that GS exacerbates other existing diseases or conditions must be considered, though.

### 2.1.7 GS and liver transplant

It is not against the law to use liver transplants from Gilbert syndrome donors (Miyake *et al.* 1998It has occasionally been seen that patients who have had liver transplants and who otherwise have normal liver function tests also have persistent unconjugated hyperbilirubinemia. According to research on the UGT1Agene TATAA-box in organ donor DNA, several transplant patients with chronic unconjugated hyperbilirubinemia had livers from donors with aberrant TATAA boxes in the bilirubinUGT1A-gene promotor region (Jansen and colleagues 1997).

### 2.1.8 Spherocytosis

Hereditary spherocytosis and GS may coexist, according to certain writers. A preventative splenectomy can be required if bilirubinate gallstones develop prematurely, which is a common side effect of hereditary spherocytosis. Co-inheritance of GS was shown to increase the probability of gallstone development in individuals with inherited spherocytosis.

Therefore, When thinking about prophylactic splenectomy in those people, it's important to evaluate whether they have GS (del Giudice and others 1999).

## 2.2 Crigler-Najjar Syndrome

In this case report, an adult with severe hyperbilirubinemia was homozygous for the Gilbert-type genetic abnormality and heterozygous for the Crigler-Najjar syndrome. This case demonstrates how a confluence of seemingly little genetic defects can result in a serious clinical condition (Chalasani *et al.* 1997).

## 2.3 Thalassemia

It appears that GS and hyperbilirubinemia are related in heterozygous beta-thalassemia. The genetic configuration of GS is homozygozity for the promoter (TA) 7 motif, is a risk factor for hyperbilirubinemia in heterozygous beta-thalassemia. (Galanello, Sampietro, 1997).Additionally, GS and alpha-thalassemia may coexist. Evidently, in this case, hyperbilirubinemia is caused by decreased bilirubin clearance rather than increased erythrocyte oxidation (Koiso and others 1998).

## 2.4 G6PD Enzymology

Glucose -6- phosphate dehydrogenase, often known as HMP, is the first and slowest enzyme in the pentose phosphate route (PPP). Glucose-6-phosphate (G6P) is transformed to ribose-5-phosphate through this pathway (R5P). R5P is required for glycolysis as well as the synthesis of DNA and RNA. G6PD produces nicotinamide adenine dinucleotide phosphate (NADPH) numerous reductive metabolic processes, including the production of cholesterol and fatty acids, requiring the transformation of G6P into 6-phosphogluconolactone (Figure 2.2).

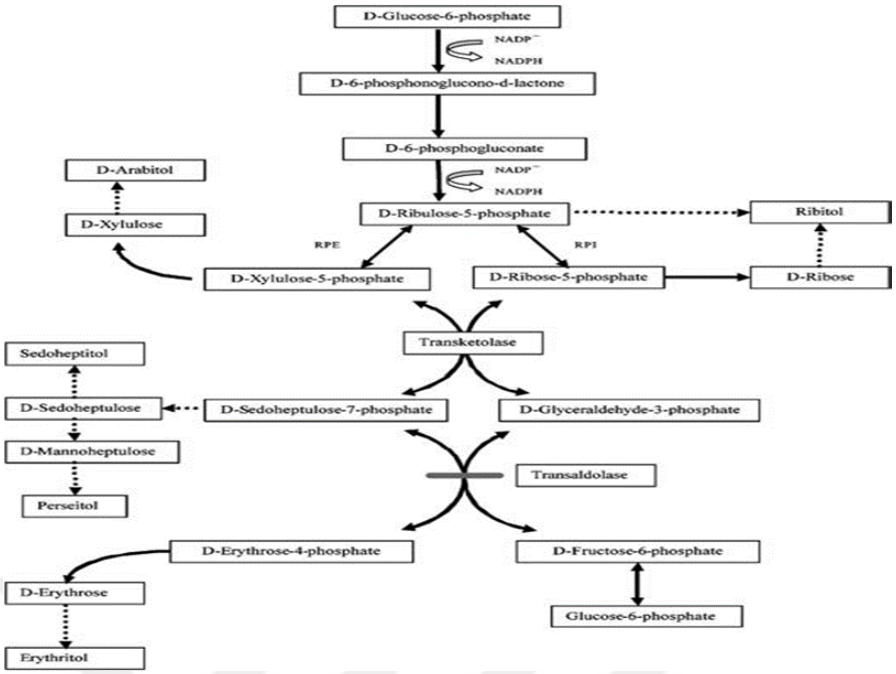


**Figure 2.2** Pentose Phosphate Pathway.

NADPH is essential for the regeneration of reduced glutathione (GSH), which is carried out by NADPH-dependent glutathione reductase under conditions of cellular oxidative stress. GSH is required for GSH peroxidase to effectively detoxify reactive free radicals and lipid hydroperoxides. Maintaining catalase's catalytic activity, which is necessary for the detoxification of hydrogen peroxide, is another crucial function of NADPH (Kirkman *et al.* 1987, Kirkman *et al.* 1999). In addition to preventing the synthesis of inactive catalase (compound IICatalase attaches to NADPH, which speeds up the process of converting compound II back to catalase. In G6PD-deficient erythrocytes, H2O2-mediated oxidant sensitivity augmentation was caused by catalase impairment. Catalase activity in G6PD-deficient cells did not decline below 50% of its original level, although it did remain (Scott *et al.* 1993).

Furthermore, It is known that catalase compound II can be converted to compound I without NADPH, albeit at slower rates, and that the quantity of NADPH needed to prevent catalase inactivation in vivo is extremely low (below 0.1 M) (Kirkman *et al.* 1987).

### 2.4.1 G6PD protein structure

The full-grown G6PD protein is comprised of 514 amino acids and has a molecular weight of around 59 kilodaltons. The mature protein seen in human erythrocytes is made up of 515 amino acids, and its N-terminal amino acid is acetylated alanine. During posttranslational processing, the starting methionine is broken down and the subsequent alanine is acetylated (Camardella *et al.* 1995). The equilibrium of dimers and tetramers in the active enzyme is controlled by ion concentrations and pH (Cohen and Rosemeyer 1969).

Mammalian G6PD monomers have a catalytic site and a structural NADP+ binding site, unlike bacterial enzymes (Au *et al.* 2000). Many of the mutations that have an impact on activity are discovered in second structural NADP+ binding site, which is believed to contribute to long-term stability (Wang *et al.* 2008).

The results of several sequence alignments point to a nine-residue peptide that is conserved across animals. (198-RIDHYLGKE-206 in regard to human beings, Kotaka *et al.* 2005). With the aspartate, histidine, and lysine residues playing key roles in binding glucose-6-phospate (Cosgrove *et al.* 2000). The coenzyme NADP+ binding fingerprint, 38-GASGDLA-44, has been linked to nucleotide binding (Levy *et al.* 1996).

### 2.4.2 Genetics and regulation of G6PD

In band Xq28 of the telomeric region of the long arm of the X chromosome, There is a gene called glucose-6-phosphate dehydrogenase (D-glucose 6-phosphate: NADP oxidoreductase, EC 1.1.1.49) that is located in certain organisms (Pai *et al.* 1980, Mason *et al.* 1990). The X-chromosome inactivation hypothesis states that one of the female cell's two X chromosomes becomes inactive inadvertently Lyon Law (1961), was made possible by the usual X-linked gene G6PD (Lyon 1961, Beutler *et al.* 1962).

The gene is about 18.5 kb long and has a total of 13 exons in addition to 12 introns (Martini *et al.* 1986). Exon 1 lacks any coding sequences, and exon 2 has the ATG start site. Although the remaining intron sequences vary between species, the initial intron is substantially conserved (Toniolo *et al.* 1991). About 11 kb long, the second intron is exceptionally lengthy (Chen *et al.* 1991).

The promoter is situated on a CpG island that may be found between nucleotide position -1200 and intron 1.and the methylation of this entire island region is linked to X chromosomal inactivation (Toniolo *et al.* 1988). Numerous binding sites for activator protein 2 (AP-2) and stimulatory protein 1 (Sp1), In the promoter region, there is also an unusual TATA box (ATTAAAT) that may be found at positions -30 to -25 base pairs (Philippe *et al.* 1994, Toniolo *et al.* 1991).

Between nucleotides -147 and +45 is the main promoterThere are inhibitory sequences located between nucleotides -358 and -147, while there are upstream stimulatory sequences located between nucleotides -613 and -358.according to deletion experiments (Philippe *et al.* 1994).

Although the gene is sometimes thought of as a "housekeeping" gene, it has been demonstrated that expression is tissue- and species-dependent. Unknown genetic components affect how quickly a gene is transcribed in various cell types and in response to cellular changes (Luzzatto *et al.* 2001).

Both a lengthier 5' untranslated segment consisting of 69 base pairs and a smaller 3' untranslated area consisting of 655 base pairs are present in the G6PD mRNA. These two regions, when combined, make up all of exon 1 and part of exon 2. The poly (A) tail added to the gene product increases its size to around 2.4 kb (Luzzatto *et al.* 2001). Hormones, diet, and oxidative stress regulate G6PD expression, as reviewed by (Kletzien and coworkers 1994).

The G6PD gene variants have been extensively examined and analyzed, and the most recent database lists 186 mutations (Minucci *et al.* 2012). Historically, there have been as many as 442 different variants (Beutler 1994). However recent breakthroughs in sequencing have revealed that many of the recorded variants were produced by the same mutation. With the exception of Exon 1, two mutations have been discovered in the intronic regions, and 85 percent of the changes are single nucleotide substitutions. The regions of tropical Africa, the Middle East, tropical and subtropical Asia, the Mediterranean area, and Papua New Guinea have the greatest prevalence rates, with the incidence of G6PD polymorphisms reaching up to sixty percent of the population in certain cases (Sodeinde 1992).All cells produce G6PD constitutively, but not consistently, with the basal activity of various organs and tissues changing up to about a 10-fold (Kletzien *et al.* 1994, Corcoran *et al.* 1996).

Glial cells express mRNA and proteins at constitutive quantities inside the brain, as well as neurons in a wide range of animal models, as well as human subjects (Cammer and Zimmerman 1982, Philbert *et al.* 1991, Biagiotti *et al.* 2001).

### 2.4.3 Protective role of G6PD in blood cells

Most G6PD deficient people are often asymptomatic, however they can display a clinical condition in response to an intensified oxidative stress or external stimuli. There is currently no connection between a particular genetic G6PD variant and a single clinical symptom, despite the fact that some defects fall into the Class I of G6PD polymorphisms related with CNSHA. According to Cappellini and Fiorelli (2008), they are unable to enhance G6PD expression in response to oxidative stress due to the fact that they do not have a nucleus. Adult red blood cells, are the only cells that are thought to be affected by G6PD deficiency. Acute hemolysis of red blood cells and anemia, which are typically signs of G6PD deficiency, are brought on by oxidative stress brought on by medications, infections, or exposure to fava beans. The body's defense against oxidative stress depends heavily on G6PD since it is the only generator of NADPH in red blood cells. (Pandolfi *et al.* 1995). When some patients taking the anti-malarial medicine primaquine experienced acute hemolysis, it was shown that they had a G6PD deficit (Beutler 1959).

G6PD is a very significant pharmacogene because various medications have been associated with this manifestation since that discovery (Luzzatto *et al.* 200, McDonagh *et al.* 2012). Hepatitis A and B, cyclomegalovirus, pneumonia, and typhoid fever are among the main and most typical illnesses that might cause oxidative stress and hemolysis in G6PD-deficient patients (Cappellini and Fiorelli 2008). Favism is a distinct form of hemolysis brought on by eating fava beans. It is thought that the bean's divicine, isouramil, and convicine constituents boost the HMP's activity and encourage hemolysis in G6PD-deficient people (Arese and De Flora 1990).

Neonatal jaundice is a different symptom of the disease, and it is yet uncertain why neonates who lack G6PD are more susceptible to it. However, it's possible that these newborns won't be able to conjugate and get rid of bilirubin properly in the liver, albeit the exact mechanism is still unknown (Mason *et al.* 2007). Neonatal jaundice can result in kernicterus, a persistent bilirubin encephalopathy that can harm a child's mental development, if it is not addressed (Mason *et al.* 2007).

G6PD deficiency is thought to be common around the world because of how common malaria is (particularly in regions where outbreaks of the host parasite are a problem).It is well accepted that the malaria-causing Plasmodium falciparum parasite cannot survive or reproduce on G6PD-deficient red blood cells (Hedrick 2011). Based on Carter and Mendis (2002), Leslie *et al.* (2010), Additionally, Plasmodium vivax protection may be provided by G6PD deficiency, which could increase the selective pressure on this enzymopathy's high occurrence. G6PD mutations are "balanced" polymorphisms because of this benefit since they provide a selection advantage despite having the ability to facilitate a disease state (Beutler 1996).

### 2.4.4 G6PD deficiency

Regarding the connection between infant GS and G6PD deficiency, there is uncertainty. Thirty percent of infants with G6PD deficiency exhibit jaundice, which can occasionally be fatal. The pathogenesis of the hyperbilirubinemia in these situations is unknown. Hyperbilirubinemia does not appear to be primarily caused by hemolysis, and GS is not

usually present. Kaplan and colleagues found that neither the mutant UDPGT1 promoter nor the G6PD deficiency combined increase the prevalence of hyperbilirubinemia. In adults with G6PD deficiency, in fabic crises, GS increases hyperbilirubinemia (Iolascon *et al.* 1999).

## 2.5 Hepatitis Virus

The hepatitis B and hepatitis C viruses continue to be a significant danger to the health of people all over the globe. Despite the fact that both viruses cause direct harm to the liver, their pathways are very different. In the world, there are an estimated 250 million HBV carriers, and each year, about 600,000 of them die from liver diseases connected to HBV (Goldstein *et al.* 2005, Ott *et al.* 2011).

In adults, acute HBV infection is typically self-limiting and subclinical, and only around 5% of cases result in chronic infection. This is in contrast to neonatal HBV infection, which results in chronic infection in 90% of instances.

If the infection becomes chronic, it is possible for its covalently closed circular DNA, also known as cccDNA, to integrate into the genome of the host cell making eradication difficult. Non-A or non-B viral hepatitis was initially linked to HCV in 1989 (Choo *et al.* 1989). The findings of this study were published in 2015 by the World Health Organization. 100 million people have serologic evidence of having been exposed to HCV, Moreover, 71 million individuals throughout the globe are living with a chronic case of hepatitis C (WHO 2015).

Cirrhosis occurs in about 20% of those with persistent HCV infection during a 25-year period. 60 to 80 it is estimated that % of individuals with acute HCV infection would become chronic infection. Between 4 and 5 percent of individuals with cirrhosis develop HCC each year. Since HCV is a mutable, unstable RNA virus, HPV undergoes numerous genetic alterations that make it possible for it to evade the immune system and advance rapidly toward chronic illness. 8 Additionally, because to the high amount of HCV

heterogeneity, it has been challenging to develop a vaccine that is both effective and safe (Rustgi *et al.* 2007, Kamal *et al.* 2008).

### 2.5.1 Diagnosis

The signs and symptoms of hepatitis, as well as a patient's medical history, which may include their use of drugs and sexual partners in the past, as well as blood tests, imaging, and a liver biopsy, may all be used to diagnose the disease. The results of the participant's blood tests and their clinical manifestation are typically sufficient to determine if the patient has viral hepatitis or another acute origin of hepatitis. Blood tests might not be useful for identifying other hepatitis causes, especially those that are persistent (Friedman and Dienstag 2015).

The gold standard for diagnosis is a liver biopsy since histopathologic analysis can identify the precise degree and pattern of fibrosis and inflammation in this situation (Friedman 2015). In patients with liver damage and cirrhosis, however, liver biopsies are generally not done as the first diagnostic test because of the invasive nature of the technique and the modest but considerable risk of bleeding that is associated with it (Grant and Neuberger 1999).Blood tests can be used to look for liver enzymes, autoantibodies via serology, hepatitis virus DNA/RNA by nucleic acid testing, blood chemistry, and total blood count (Friedman 2015).

Different patterns of aberrant liver enzymes can be used to determine the precise origins or stages of hepatitis (Pratt *et al.* 2000, Green and Flamm 2002). Whether or not the patient experiences symptoms, in cases of hepatitis, AST and ALT levels are frequently increased. Nevertheless, the frequency of AST vs. ALT elevation, Both the AST/ALT ratio and the degree of the elevation (in other words, the difference between hundreds and thousands) are indicators of the diagnosis (Friedman 2015). Sonography, computed tomography, and magnetic resonance imaging may all detect nodularity on the liver's surface, which is a sign of cirrhosis. In addition to this, steatosis, which refers to fatty alterations, may be seen in the liver tissue (Ito *et al.* 2004, Allan *et al.* 2010).

With CT and MRI in particular, the ability to give more specificity,imaging and characterization of features like arteries and tumors within the liver are made possible Sahani *et al.* (2004), no imaging test can identify liver fibrosis or inflammation (such as hepatitis), in contrast to steatosis and cirrhosis (Friedman 2015). The only reliable diagnostic procedure that can evaluate hepatic fibrosis and inflammation is a liver biopsy (Friedman 2015).

### 2.5.2 Acute hepatitis

Almost of hepatitis A patients totally recover without any issues if they were healthy before the disease. These disorders have a 95-99 percent full recovery rate, just like acute hepatitis B infections. However, ascites, edema, or encephalopathy's initial presenting symptoms or co-morbid medical problems may portend a poorer outcome (Dienstag 2015). Although rates can be greater in some groups, acute hepatitis has a low overall fatality rate of 0.1 percent for both hepatitis A and B patients (women who are pregnant, those who have a super infection consisting of both hepatitis B and D, etc) (Dienstag 2015).

### 2.5.3 Chronic hepatitis

The possibility that an acute hepatitis B infection would advance into a chronic illness declines with age, rates of progression in neonates with vertical transmission exceed 90%, compared to a 1% chance in young people. In moderate cases of chronic hepatitis B, the five-year survival rate is 97%; in severe cases with cirrhosis, it is 55% (Dienstag 2015).

However, chronic infection is significantly more common (80%–90%) and People who have hepatitis B and then develop hepatitis D are more likely to have liver damage (superinfection). Hepatitis D patients who also have hepatitis B often heal without developing a chronic infection (Abbas *et al.* 2015). 20 years after infection, cirrhosis, which is caused by chronic hepatitis C, is thought to be 16 percent frequent (Thein *et al.* 2008).

Hepatocellular carcinoma is a substantial extra long-term consequence that is also the leading cause of death in individuals who have chronic hepatitis. This is the case despite the fact that end-stage liver disease is large primary reasons of mortality in people who have hepatitis C. The underlying liver problem becomes worse, which leads to a rise in the death rate. Those who were diagnosed with compensated cirrhosis as a result of HCV had survival rates of 96%, 91%, and 79% after 3, 5, and 10 years, respectively.

# 3. MATERIALS AND METHODS

## 3.1 Materials

Tools which used in this study such as ELISA, Spectrophotometer, Oven, Light microscope and water bath (Table 3.1).

**Table 3.1** The tools utilized in the current study.

| Instruments and glasses | Company | Country |
|---|---|---|
| ELISA | Labon | China |
| Spectrophotometer | Biobase | India |
| Centrifuge | Memmert | Germany |
| Light microscope | Olympus | Japan |
| Oven | Memmert | Germany |
| Water bath | Memmert | Germany |
| Shaking water bath | Memmert | Germany |
| Syemex Device | Sysmax | Japan |
| Pipette | BioSan | Germany |
| Different glasses | ------ | China |
| Refrigerator | BEKO | Turkey |

Table 3.2, showed the Kits which used in this study and manufacture company with its origin.

**Table 3.2** A list kits and solutions used in this study.

| Kits | Company | Country |
|---|---|---|
| ALT | Biolabo | France |
| AST | Biolabo | France |
| ALP | Biolabo | France |
| Bilirubin | Biolabo | France |
| G6PD | Biolabo | France |
| EDTA solution | Abcam | USA |
| Red safe staining solution | Intron | Korea |
| Agarose | Conda | USA |
| i- genomic BYE DNA extraction Mini kit | Intro biotechnology | Korea |
| TBE buffer 10X | Conda | USA |

## 3.2 Patients

This study included 100 sample from volunteers who attended Al-Salam hospital in Mosul city, from 10/11/2021 to 10/5/2022, who suffer from live disorders, their ages ranged 20-50 years and included 100 volunteers (as control) their ages 22-48 year.

## 3.3 Blood Samples Collection

Each patient had blood drawn by skilled nurses. On whether or not it is necessary to get fresh blood samples, various scientists disagree. As a result, it is no longer required to use recently obtained blood. The five milliliters of venous blood taken from each participant was divided between an EDTA tube (containing one milliliter) and a plain vacutainer tube (4.0 ml).

After chilling the Vacutainer plain tubes in the refrigerator for a short while to enable the blood to coagulate, clear serum samples were extracted from them by centrifuging them at 4000 revolutions per minute for ten minutes. During the analysis, the serum was split into five different tubes, after which they were sealed and kept at a temperature of -20 degrees Celsius. Before usage, the frozen serum samples were gently mixed at room temperature after being gradually defrosted at 4–8 $^{o}$C.

## 3.4 Biochemical Tests

### 3.4.1 Determination of ALT enzyme

Spectrophotometry is used to measure the activity of alanine aminotransferase, as illustrated in Table 3.3.

**Table 3.3** Steps of ALT procedure.

| | |
|---|---|
| Sample | 50 µl |
| Reagent R | 1000 µl |
| Mix and incubate for two minutes. For three minutes, the variation in absorbance (at 340 nm) at 37 °C changes every minute. Estimate the average absorbance increase per minute (ΔA/min). | |

### 3.4.2 Determination of AST enzyme

Spectrophotometry is used to measure the activity of aspartate aminotransferase, as illustrated in Table 3.4.

**Table 3.4** Steps of AST procedure.

| | |
|---|---|
| Sample | 50 µl |
| Reagent R | 1000 µl |
| Mix and incubate for two minutes. For three minutes, the variation in absorbance (at 340 nm) at 37 °C changes every minute. Estimate the average absorbance increase per minute (ΔA/min). | |

### 3.4.3 Determination of alkaline phosphatase (ALP) enzyme

The determination of alkaline phosphatase is made by the use of spectrophotometry, as illustrated in Table 3.5.

**Table 3.5** Steps of ALP procedure.

| | |
|---|---|
| Sample | 50 µl |
| Reagent R | 1000 µl |
| Mix and incubate for two minutes. For three minutes, the variation in absorbance (at 340 nm) at 37 °C changes every minute. Estimate the average absorbance increase per minute (ΔA/min). | |

### 3.4.4 Glucose-6-phosphate dehydrogenase

**Procedure**

- Add 1.0 mL reconstituted R1 reagent (from kit) to the test tube.
- Fill the test tube with 10 mL of blood and the assay solution. By vortexing, thoroughly combine. Allow to rest for five minutes at room temperature.
- On the spectrophotometer, press the F4 button to enable computer control. Launch UVProbe on the connected computer.
- Add 2.0 mL of the R2 reagent (from the kit) to the test tube holding the blood and assay solution. Mix by repeatedly inverting the tube.
- Transfer the solution from the test tube into one to three 1 mL plastic cuvettes. Cuvettes should be placed in a spectrophotometer with a temperature control set to 37 °C. For exactly 5 minutes, incubate.

### 3.4.5 Result calculations

Calculate results using formula given in Pointe Scientific kit insert,

G6PD= ΔA per min X 4839/Hb (g/dl)

### 3.4.6 Total bilirubin (TB)

Spectrophotometry is used to determine total bilirubin, as is shown in Table 3.6.

**Table 3.6** Steps of TB procedure.

| | Assay | Blank |
|---|---|---|
| Reagent R1 | 1000 µl | 1000 µl |
| D.W | | 50 µl |
| Reagent R3 | 50 µl | |
| Mixed and add | | |
| Sample, control | 100 µl | 100 µl |
| Mix well and start a timer when adding specimen. Read absorbance at 550 nm (530-580) against blanks | | |

## 3.5 Genetic Study

### 3.5.1 DNA extraction

All (110) study samples that were included in the study had their blood drawn, and the modified procedure by (Iranpur and Esmailizadeh 2010).

**A- Solutions used for DNA extraction**

**1- EDTA solution (0.5M) pH 8.0**

The solution is autoclaved for 15 minutes at 15 psi and 1 liter of distilled water. First, 800 ml of distilled water is combined with 186.1 g of dry EDTA. The pH is then raised to 8.0 using NaOH granules.

**2- Tris-Hcl (1M) pH 7.6 solution**

After using HCl to get the pH down to 7.6, 121.1 g of Tris base dissolved in 800 ml of purified water. After adding distilled water to the volume to make it (liter), the solution is sanitized by autoclaving it at 15 ºC for 15 minutes.

**3- Red blood cell lysis buffer solution**

800 milliliters of distilled water, this solution was made with 109.54 grams of sucrose, 1.01 grams of magnesium chloride, 10 milliliters of Triton-X100, and 10 milliliters of 1M Tris. The acidity function should then be set to PH 8.0. The solution is autoclaved at 15 psi for ten minutes to sanitize it after one liter of distilled water has been added to the mixture.

**4- Nucleic lysis buffer solution**

This remedy includes

(0.01)Tris-HCl
(0.01)Tris-HCl (1mM) EDTA
(1%) Sodium Dodecyl Sulphate

The following ingredients were combined with 800 ml of distilled water to generate this solution: (10) milliliters of 1M Tris-HCl, pH 7.6, 3.75 grams of dry EDTA, ten grams of SDS, and 2.94 grams of sodium citrate. After that, the pH function was corrected. Using distilled water, the volume is increased to 1 liter at PH 8.0, and the solution is then autoclaved for 15 minutes at 15 $^{o}$C.

**5- TE buffer solution**

To create this solution, combine 5 ml of 1 M tris-HCl (pH 7.6) with 2 ml of 0.5 M EDTA (pH 8.0), add 1 L of distilled water to the mixture, then autoclave at 15 psi for 15 minutes to change the acidity function to pH 8.0.

**6- Chloroform**

**7- Ethanol (100%) at -20 cold ethanol**

**B-Extraction method (DNA)**

1. A blood sample of (500) microliters is collected, placed in a tube with a capacity of 1.5 milliliters, along with one milliliter of RBC lysis solution, and then gently mixed using a Vortex apparatus.
2. 2- The microfuge equipment is used to spin the tube for two minutes while moving at a speed of 7000 revolutions per minute, after which the filtrate is discarded. To eliminate hemoglobin, the previous stages are carried out one to three times. The Alapndrove tube is then placed upside-down on a piece of blotting paper for a few seconds.
3. (400) microliters of Nucleic lysis solution are added to the Alpendorf tube and mixed gently.
4. The Alpendorf tube is filled with (100) microliters of a saturated solution of NaCl (5M), (600) microliters of chloroform, and the mixture is gently stirred before being placed in the microfuge apparatus at a speed of (7000 rpm) for two minutes.

5. Take (400) microliters of the filtrate and place it in an Eppendorf tube that has been cleaned and dried. Add (800) microliters of cold ethanol to the tube and gently stir the contents together. At this point, the DNA bundles start to show up as white threads.
6. The tube is spun at 12000 rpm in the microfuge for one minute, the filtrate is discarded, and the tube is then leaved to dry at normal (room) temperature.
7. The extracted DNA is mixed silently with (50) microliters of TE solution, then stored at (-20) °C until use.

### 3.5.3 The solutions used in the migration process

**1. SB solution with a strength X1**

Weighted sodium hydroxide (0.4 g) is dissolved in 500 ml of distilled water at a low temperature. When the pH value reaches 8.5, additional boric acid is subsequently added to the mixture. After that, the volume is converted to 1 liter and used. The answer is to apply it directly and without dilution.

**2. The loading solution with loading buffer X10**

it is created by combining (50%) glycerol with (0.25) gram of bromophenol blue color, adding (60 mM) EDTA at pH 8.0, and then adding (100 ml) of distilled water to complete the volume..

### 3.5.3 Preparation of agarose gel and DNA electrophoresis

For DNA mobility and detection, an agarose gel with a 1 percent concentration is created. This concentration is made by combining 3 microliters of red safe dye with 0.5 g of agarose powder in 50 ml of X1 TBE, heating to boiling, and allowing to cool to a temperature between (60 and) 50 °C.

After using the appropriate attachment on the comb to carve out wells around the outside borders of the gel, the solution for the gel is poured into the tray of the relay device next. It's crucial to keep in mind to pour softly to avoid bubbles and, if they do, to remove them with a pipette before the gel solidifies. The comb is then progressively raised while the Tray is positioned within an electric relay tank holding the required amount of X1 SB solution. This process is repeated until the desired level of X1 SB solution has been achieved.

Migration samples are prepared by combining five milliliters of DNA sample with three milliliters of loading solution. The relay is then operated by transmitting a five-volt-per-centimeter electric current for an additional one to five hours. The gel is then imaged using a UV Trans illumination gel imaging instrument under ultraviolet light to display the DNA bundles and the conclusion of the PCR procedure.

### 3.5.4 PCR-RFLP reactions

To get the DNA concentration required for PCR reactions, which was 25 ng/microliter per sample, the DNA concentration in each sample was diluted to 25 ng/microliter, TE solution dilution was used to alter the DNA concentration in each of the samples that were tested.

In a 0.2 ml Alpendorf tube supplied by the American firm Promega, the DNA sample and primer for each mutation were combined with the components of the master-mix. To fully mix the reaction components, in a microfuge, the mixture was spun down for between 5 and 3 seconds after the reaction volume was adjusted to 20 l with distilled water. It is important to keep in mind the proper sequencing of the DNA sample and primer for each mutation.

Utilizing the specific thermocycler program created for each reaction, the multiplication process was carried out in the reaction tubes. The PCR reaction product was then placed onto the agarose gel pits, which had been pre-prepared with a 2% solution, and 2

microliters of Red safe dye were added to it. The sample was then incubated with the appropriate trimer enzyme for each mutation for 3 hours. This procedure was aided by ladder DNA volume guidelines from Biolaps, and samples were then migrated by conducting electrophoresis for (90–75) minutes.

### 3.5.5 Molecular diagnosis of a number of genes in patients' blood

Genes from the investigated samples were discovered by amplifying the primers assigned to each gene in the blood-derived genome. In addition, 1 microliter (10 picompl) of each gene-specific primer and 4 microliters (100 nanograms) of template DNA were added to the master mix's components (Table 3.7).

**Table 3.7** Primers used throughout the current research.

| Primer | Sequence | Annealing | Band Size |
|---|---|---|---|
| U1-F | GAGGTTCTGGAAGTACTTTGC | 53 | 250 bp |
| U1-R | CCAAGCATGCTCAGCCAG | | |
| G6PD Aures (143 T→C) | 5′-ACCTGGCCAAGAAGAAGAT-3′ | 56 | 226 bp |
| | 5′-CTCACTCTGTTTGCGGAT G-3 | | |

The multiplication reaction was then carried out in the thermocycler using a specific software designed for the reaction, as indicated Table 3.8.

**Table 3.8** PCR program.

| No. | Stage | Temperature | Time | Cycle number |
|---|---|---|---|---|
| | Initial denaturation | 95 | 6 min. | 1 |
| | denaturation | 95 | 45 sec. | 35 |
| | Annealing | 53,56,61,63 | 1 min. | |
| | Extension | 72 | 1 min. | |
| | Final extension | 72 | 5 min. | 1 |

### 3.5.6 Statistical analysis

The data were statistically examined using Minitab, a statistical analysis tool, and Excel, a spreadsheet program. The data were displayed with their mean and standard deviation. The Dunkin' multiple test and the ANOVA test were used to statistically analyze the findings of the present study in order to compare the arithmetic means of the experimental groups and look for statistically significant differences between them.

If the level of probability was less than 0.5, the data were given as evidence of significant differences, and if it was greater than 0.5, they were presented as evidence of no significant differences. It was used to highlight the presence or absence of substantial changes based on whether the letters were different or similar.

# 4. RESULTS AND DISCUSSION

## 4.1 Demographic Characteristics of Study Groups

### 4.1.1 Age distribution

This study included one hundred volunteers who attended hospitals in Nineveh governorate who suffer from liver disorders, their ages ranged from twenty to fifty years and their average age was 29.29 ±15.92. The study also included one hundred healthy volunteers, their ages ranged from twenty-two to 48 years, and their average age was 26.38 ± 13.88 as shown below in Figure 4.1 and Table 4.1.

**Table 4.1** Age average of the study groups.

| | Control | Volunteers |
|---|---|---|
| Age (years) | 26.38±13.88 | 29.29±15.92 |
| P value | $0.288^{NS}$ | |

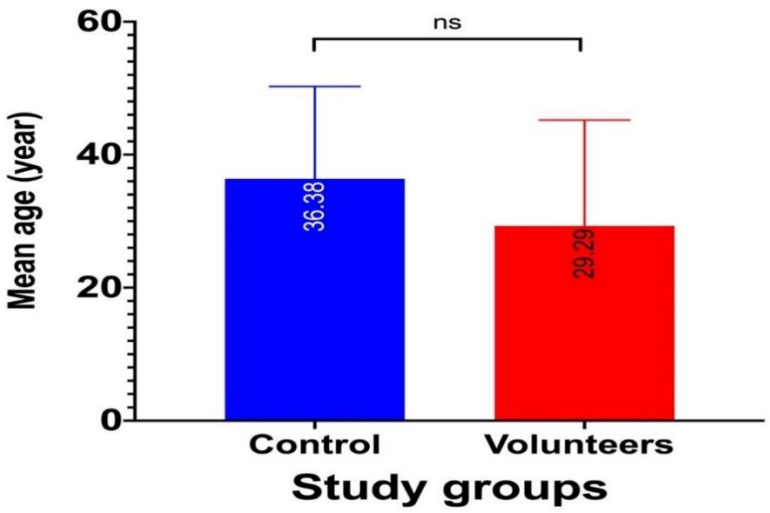


**Figure 4.1** Age average of the study groups.

### 4.1.2 Sex distribution

Table 4.2 shows the distribution of participants during this study according to gender, where the proportion of female was 58%, and male were 42% of the sick volunteers, in contrast, the proportion of males and women was equal to 50% of the healthy group. There is no gender difference that can be considered statistically significant between the two groups.

**Table 4.2** Sex distribution of the study groups.

| | | Control | | Volunteers | |
|---|---|---|---|---|---|
| | | Count | % | Count | % |
| Sex | Female | 50 | 50% | 58 | 58.0% |
| | Male | 50 | 50% | 42 | 42.0% |
| P / value | | $0.302^{NS}$ | | | |

### 4.1.3 Blood groups

Table 3.4 shows the distribution of blood groups between the two study groups, where the O Rh+ group represented the highest percentage of (35%), followed by the A+ group of (33%), while the B group represented the lowest (zero%) in relation to the healthy group. The same group O Rh + represented the highest percentage of (41%), followed by group A Rh + (29%), as well as group B Rh - the lowest percentage of (1%) with regard to the group of volunteer patients. Additionally, In terms of blood types, there is no statistically important difference between the two groups.

**Table 4.3** Blood groups distribution between study groups.

| | | Control | | Volunteers | |
|---|---|---|---|---|---|
| | | Count | % | Count | % |
| Blood group | A Rh - | 3 | 3% | 0 | 0% |
| | A Rh + | 33 | 33% | 29 | 29.0% |
| | AB Rh + | 2 | 2% | 3 | 3.0% |
| | B Rh - | 0 | 0% | 1 | 1.0% |
| | B Rh + | 25 | 25% | 23 | 23.0% |
| | O Rh - | 2 | 2% | 3 | 3.0% |
| | O Rh + | 35 | 35% | 41 | 41.0% |
| P / value | | $0.516^{NS}$ | | | |

## 4.2 Studied Markers

### 4.2.1 GPT

There were a strong difference in the mean concentration of GPT enzyme between the two groups (16.53 ± 3029 IU/L), (24.83 ± 10.33 IU/L) healthy and sick groups, respectively. Also, a significant difference was found between these groups (p-value <0.001), during the current study, as shown as Table 4.4 and Figure 4.2 below.

**Table 4.4** Serum concentration of GPT enzyme between groups of study.

| Parameter | Control<br>Mean ± Std. Deviation | Volunteers<br>Mean ± Std. Deviation |
|---|---|---|
| GPT IU/L | 16.53 ± 3.29 | 24.83 ± 10.33 |
| P / value | <0.001 | |

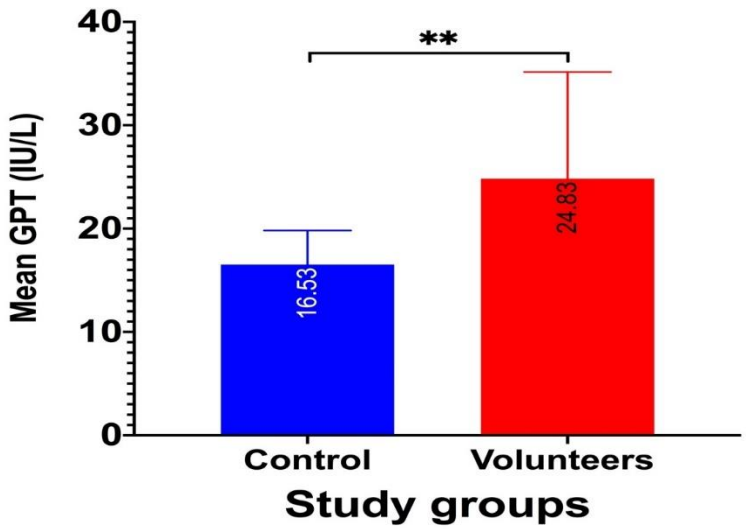


**Figure 4.2** Serum concentration of GPT enzyme between study groups.

### 4.2.2 GOT

The healthy group had a mean concentration of GOT enzyme that was (16.53 ±30.29 IU/L), whereas the ill group had a mean concentration of (24.83± 10.33 IU/L). This was a key area where the two groups differed significantly. During this investigation, a substantial difference was discovered between these groups (p-value <0.001), as can be shown in Table 4.5 and Figure 4.3 below.

**Table 4.5** Serum concentration of GOT enzyme between groups of study.

| Parameter | Control<br>Mean ± Std. Deviation | Volunteers<br>Mean ± Std. Deviation |
|---|---|---|
| GOT IU/L | 20.58±5.47 | 27.52 ± 12.77 |
| P/value | <0.001 | |

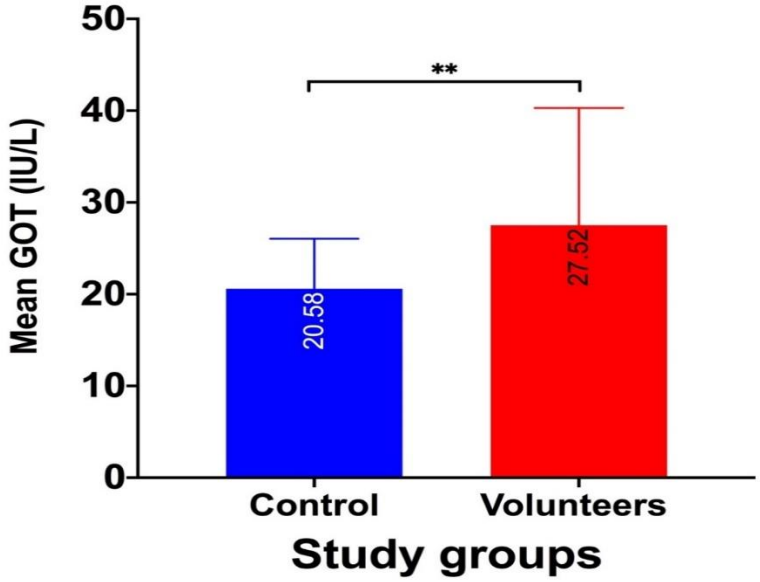


**Figure 4.3** Serum concentration of GOT enzyme between groups of study.

### 4.2.3 ALP

There was a strong difference in the mean concentration of ALP enzyme between the two groups (11 ± 15.94 IU/L), (121 ± 58.6 IU/L) healthy control and patient's groups, respectively. Also, a significant difference was found between these groups (p-value <0.001), during the current study, as shown as Table 4.6 and Figure 4.4 below.

**Table 4.6** Serum concentration of ALP enzyme between study groups.

| Parameter | Control<br>Mean ± Std. Deviation | Volunteers<br>Mean ± Std. Deviation |
|---|---|---|
| ALP IU/L | 11 ± 15.94 | 121 ± 58.6 |
| P value < | <0.001 | |

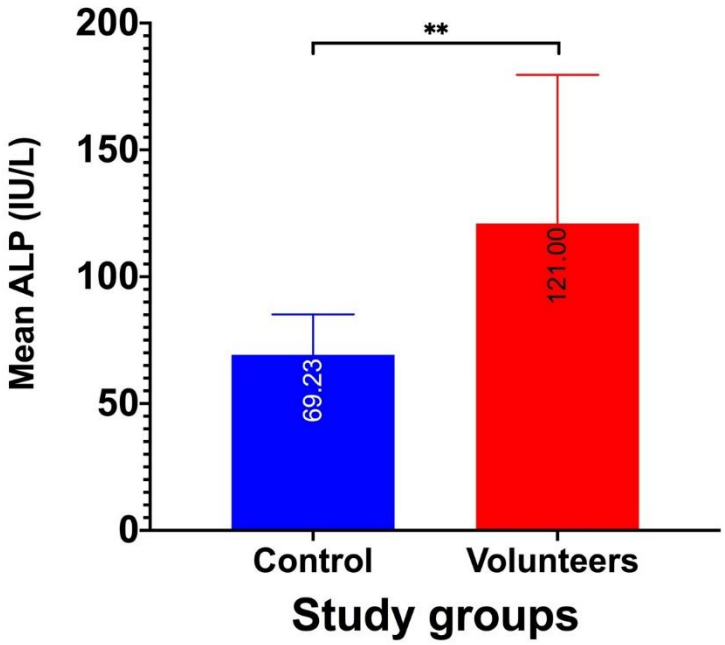


**Figure 4.4** Serum concentration of ALP enzyme between study groups.

### 4.2.4 Bilirubin

Between the two groups, the healthy control group's mean serum total bilirubin concentration was lager in the patient group (1.54 ±0.95 mg/dl), whereas it was lower in the healthy control group (0.94 ±0.15 mg/dl). Additionally, throughout the present investigation, A substantial difference (p-value of 0.025) was found to exist between these two groups, as can be seen in Table 4.7 and Figure 4.5, which are provided below.

**Table 4.7** Serum concentration of total bilirubin between groups of study.

| Parameter | Control<br>Mean ± Std. Deviation | Volunteers<br>Mean ± Std. Deviation |
|---|---|---|
| Bilirubin<br>mg/dl | 0.94 ± 0.15 | 1.54 ± 0.95 |
| P / value | 0.025 | |

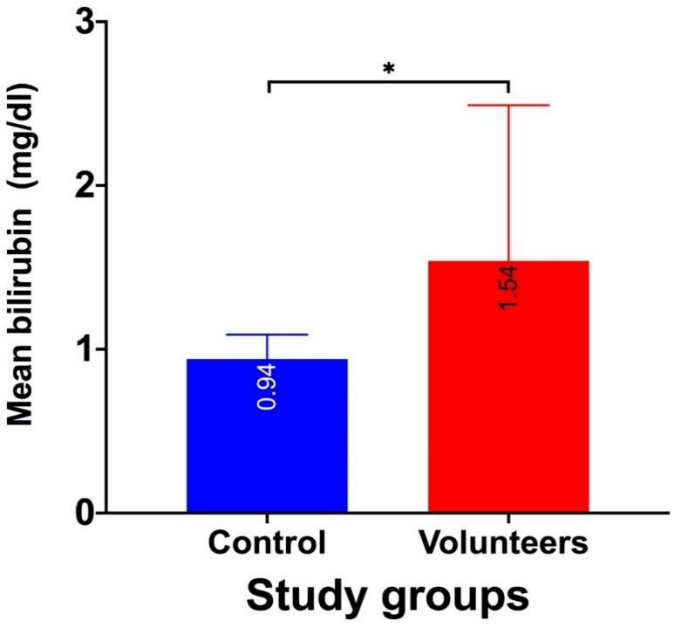


**Figure 4.5** Serum concentration of total bilirubin between study groups.

### 4.2.5 G6PD

The patient group had a mean serum G6PD enzyme concentration that was greater than the healthy control group (12.05 ±5.61 IU/L), whereas the healthy control group had a mean serum G6PD enzyme concentration that was lower than the patient group (9.47± 3.17 IU/L). In addition, over the course of this inquiry, It was discovered that these groups differ significantly from one another. (The p-value for this difference is 0.006), as can be seen in Table 4.8 and Figure 4.6 below.

**Table 4.8** Serum concentration of G6PD enzyme between groups of study.

| Parameter | Control<br>Mean ± Std. Deviation | Volunteers<br>Mean ± Std. Deviation |
|---|---|---|
| G6PD IU/L | 9.47 ± 3.17 | 12.05 ± 5.61 |
| P value | 0.006 | |

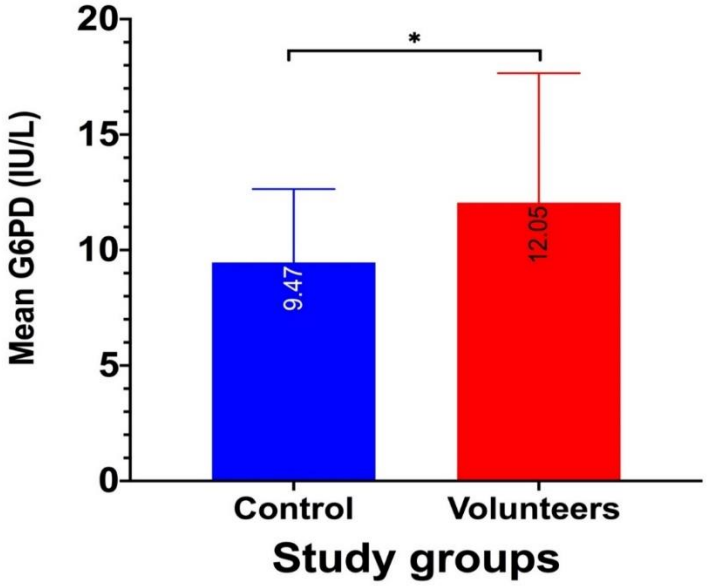


**Figure 4.6** Serum concentration of G6PD enzyme between study groups.

### 4.2.6 Controversies in opinion about bilirubin

A mutation in human chromosome two's upstream promoter region, which changes how bilirubin is glucuronidated, causes Gilbert syndrome (Bosma *et al.* 1995). This condition has a number of different mutational variations and is autosomal recessive. It is identified on routine liver function tests as moderatebenign, hereditary, unconjugated hyperbilirubinemia (serum bilirubin levels between 1 and 5 mg/dl), hyperbilirubinemia not associated with hemolysis (King and Armstrong 2019).It is a condition that is quite prevalent globally, with prevalence rates of 12.4% from Germany and 8% from Egypt (Kamal *et al.* 2019). Genetic variation in the prevalence of the illness explains why there are various epidemiologies around the world (Borlak *et al.* 2000).

According to Zhang *et al.* (2020). Total bilirubin (73.5 mol/L, normal: 0-25 mol/L), direct bilirubin (21.2 mol/L, normal: 0-7 mol/L), and alanine aminotransferase (60 U/L, normal: 9-50 U/L) were all abnormally high in the liver function tests of Gilbert syndrome patients. The current study's findings contradict these findings.

Patients with Gilbert syndrome in the current study exhibited bilirubin levels that were noticeably greater than those of healthy people. In GS, UGT1A1 activity can be lowered by 70%. According to (Cuperus *et al.* 2009). Which causes sporadic and moderately elevated unconjugated bilirubin levels that are normally between 20 and 50 mol/L but occasionally approach 70 mol/L (Hirschfield *et al.* 2006, Erlinger *et al.* 2014).

Currently, GS is primarily diagnosed through exclusion, the predominant clinical symptom of this condition is a rise in blood indirect bilirubin that is only slightly elevated, in contrast to the absence of this sign in other forms of unconjugated hyperbilirubinemia (Maruo *et al.* 2016).Physical tests, biochemical testing, imaging analyses, and liver histology are all part of the usual GS procedures (which is typically not required). Notably, In order to identify GS from other hereditary illnesses associated with hyperbilirubinemia, such as Crigler-Najjar syndrome, genetic testing is becoming increasingly crucial (Radu and Atsmon 2001).

The findings of the current study corroborate those of Mousavi *et al.* (2005) who noted that individuals with Gilbert syndrome had blood bilirubin levels, both total and unconjugated, greater than 2.4 mg/dL. These measures can indicate GS with great specificity and sensitivity.

There have only been a few of research that have investigated the possible link between bilirubin levels and psychopathy. Yamaguchi *et al.* (2002) discovered a link between emotional stressors and an uptick in the oxidative bilirubin metabolites in human urine in 2005 (Miyaoka *et al.* 2005). The presence of higher bilirubin oxidative metabolites (biopyrrins) in the urine of people with mental health difficulties has been found (schizophrenia and depression). Additionally, bilirubin levels were linked to positive symptoms in those with schizophrenia (Widschwendter *et al.* 2016).

Tang *et al.* (2013) found a connection between high bilirubin levels and post-stroke depression. These findings suggest that oxidative bilirubin metabolite levels in human urine are correlated with behavioral states. There is still debate over the potential relationship between increased blood bilirubin levels and neurological disorders (Gazzin *et al.* 2016). According to research by Kao *et al.* (2012) higher blood total bilirubin levels were linked to a decreased risk of functional reliance when it came to the condition in older persons.

Oren *et al.* (2002) found that seasonal depressive disorder patients had lower nighttime bilirubin levels than controls. Despite these neurochemical routes, social effects of a potential clinical presentation with GS must be considered carefully. Example, a person's personality qualities may be impacted by the stigma attached to chronic or intermittent jaundice in GS patients.

### 4.3 Genetic Study

Blood samples from infants with normal and hyperbilirubinemia conditions were processed to extract genomic DNA within 24 to 48 hours of aspiration. This technique

dispenses with the requirement for DNA precipitation and organic solvent extractions, enabling the quick purification of numerous samples at once.

It was found that the concentration and band integrity of the extracted genomic DNA varied depending on the number of RBCs in the sample, as well as its volume and purity.

Genomic DNA should be used as soon as practical because it has been demonstrated that using fresh blood samples performed better than those that had been stored at 20 °C for several days. The presence and integrity of the isolated genomic DNA were verified using agarose gel electrophoresis, which was detected by staining and then made visible by exposure to UV light (O'Leary *et al.* 1997, Brinkmann 1998).

The fluorescent dye ethidium bromide is used to stain agarose gels, which is the simplest and most used method for observing DNA in them. Viewing the DNA that has been extracted was done using this. (Figure 4.7) depicts the genomic DNA bands from the normal and hyperbilirubinemia blood samples used in this experiment.

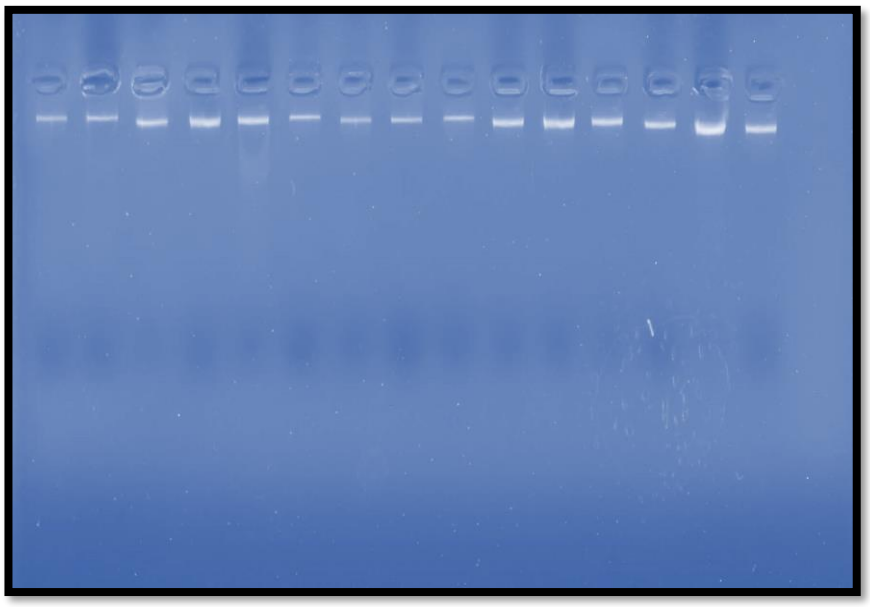

**Figure 4.7** Genome extracted from blood samples and migrated with 1% agarose gel.

### 4.3.1 G6PD Aures (143 T→C) frequency between study groups

Table 4.9 showed that genotype (TC) had the highest frequency of 10 (ten percent) in patients versus a lower frequency through control group 1 (one percent), whereas genotype (TT) had the frequency of 90 (ninety percent) in patients compared to the highest frequency of 99 (ninety percent) in the healthy control group. In addition, genotype (CC) was absent in 0% (zero percent) in both groups (Figure 4.8).

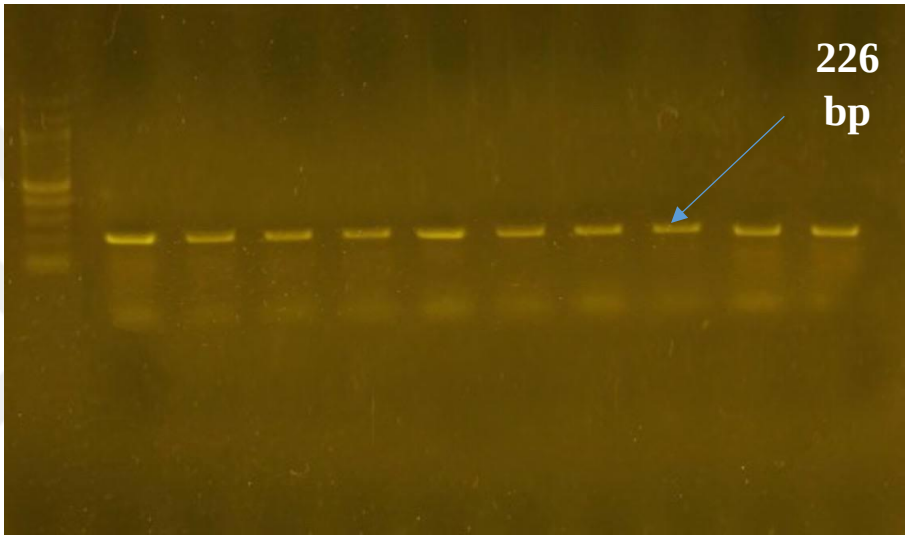


**Figure 4.8** The PCR product for the G6PD Aures (143 T→C) gene is shown in blood samples with a reaction product of 450 bp and carried over with 2% agarose gel.

**Table 4.9** The frequency of G6PD Aures (143 T→C) polymorphism between study groups.

| G6PD Aures (143 T→C) | | Control | | Volunteers | | P value | Odds ratio (95% CI) |
|---|---|---|---|---|---|---|---|
| | | Count | % | Count | % | | |
| Genotype | CC | 0 | 0% | 0 | 0% | 0.009* | 11 (1.8-120.7) |
| | TC | 1 | 1% | 10 | 10% | | |
| | TT | 99 | 99% | 90 | 90% | | |
| Allele | C | 1 | 0.5% | 10 | 5% | 0.018* | 10.47 (1.79-114.5) |
| | T | 199 | 99.5% | 190 | 95% | | |

### 4.3.2 UGT1 gene (position 179 G>C) frequency between study groups

Table 4.10 showed that genotype (GC) had the highest frequency of 6 (six percent) in patients versus control group 0 (zero percent), whereas genotype (GG) had the frequency of 94 (ninety-four percent) in patients compared to the highest frequency of 100 (hundred percent) in the healthy control group. In addition, genotype (CC) was absent in 0 (zero percent) in both groups (Table 4.9).

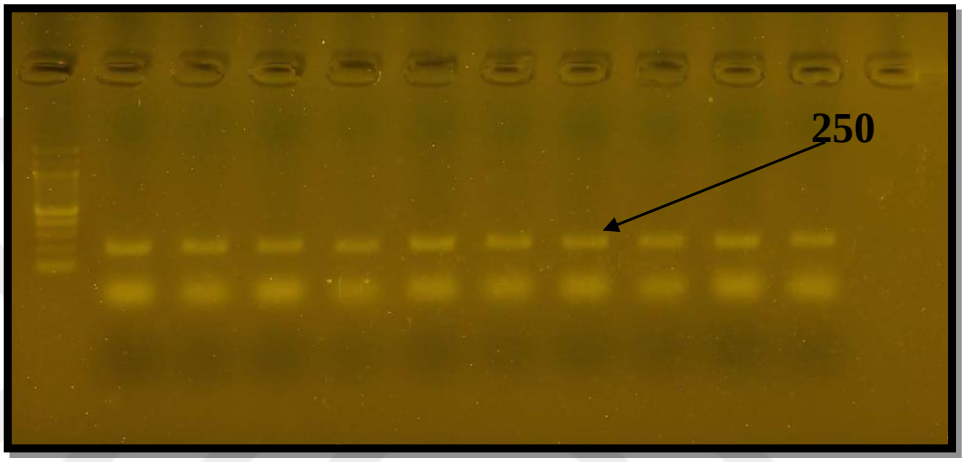


**Figure 4.9** The PCR product for the UGT1 gene is shown in blood samples with a 250 bp reaction product and carried over with 2% agarose gel.

**Table 4.10** The frequency of UGT1 gene (position 179G>C) polymorphism between study groups.

| Position 179 UGT1 gene | | Control | | Volunteers | | P value | Odds ratio (95% CI) |
|---|---|---|---|---|---|---|---|
| | | Count | % | Count | % | | |
| Genotype | CC | 0 | 0% | 0 | 0% | 0.077 | 13 (0.72-239) |
| | GC | 0 | 0% | 6 | 6% | | |
| | GG | 100 | 100% | 94 | 94% | | |
| Allele | C | 0 | 0% | 6 | 3% | 0.074 | 13.7 (0.79-237.9) |
| | G | 200 | 100% | 194 | 97% | | |

### 4.3.3 Association between G6PD Aures (143 T→C) polymorphism and studied markers

Although there was a considerable variation in the values of means between genotypes TT and TC, 0.18, 0.796 and 0.157, respectively). On the other hand, there was a significant association between the blood levels of bilirubin, ALP, and the G6PD Aures (143 TC) polymorphism (p= value 0.006 and 0.015, respectively) (Table 4.11).

**Table 4.11** Reveal that there is no significant connection between blood levels of GOT, GPT and G6PD and G6PD Aures (143 TC) poly morphism (p= value.

| Parameters | G6PD Aures (143 T→C) | | Mean | Std. Deviation | P/value |
|---|---|---|---|---|---|
| Bilirubin (mg/dl) | Genotype | TC | 1.93 | 1.37 | 0.006 |
| | | TT | 0.38 | 0.31 | |
| | Allele | C allele | 1.93 | 1.37 | 0.001 |
| | | T allele | 0.46 | 0.55 | |
| GPT (IU/L) | Genotype | TC | 29.00 | 7.13 | 0.180 |
| | | TT | 24.37 | 10.56 | |
| | Allele | C allele | 29.00 | 7.13 | 0.190 |
| | | T allele | 24.61 | 10.41 | |
| GOT (IU/L) | Genotype | TC | 29.30 | 9.46 | 0.796 |
| | | TT | 27.32 | 23.81 | |
| | Allele | C allele | 29.30 | 9.46 | 0.800 |
| | | T allele | 27.43 | 23.21 | |
| ALP (IU/L) | Genotype | TC | 96.50 | 26.32 | 0.015 |
| | | TT | 124.29 | 60.63 | |
| | Allele | C allele | 96.50 | 26.32 | 0.014 |
| | | T allele | 122.83 | 59.45 | |
| G6PD (IU/L) | Genotype | TC | 13.85 | 7.14 | 0.157 |
| | | TT | 10.31 | 4.60 | |
| | Allele | C allele | 13.85 | 7.14 | 0.172 |
| | | T allele | 10.50 | 4.79 | |

In spite of there was clear difference in means values between both alleles “C” and “T “respectively, there was no significant association between GOT, GPT and G6PD (p=value 0.190, 0.800 and 0.172), respectively and both alleles. While significant association was found between bilirubin and ALP (p=value 0.001 and 0.014), respectively and both alleles.

### 4.3.4 Association between UGT1 gene (Position 179 G>C) polymorphism and studied markers

Tables 4.11 show that there is no significant correlation between serum concentrations of GOT, GPT, and UGT1 gene (Position 179 G>C) polymorphism (p= value 0.193 and 0.625 respectively), despite the fact that there was a large variance in the values of means between genotypes GC and GG.

**Table 4.12** Relationship between the investigated markers and the G6PD Aures (143 T→C) polymorphism.

| Parameters | **UGT1** 179G>C polymorphism | | Mean | Std. Deviation | p/value |
|---|---|---|---|---|---|
| Bilirubin (mg/dl) | Genotype | GC | 2.38 | 1.64 | <0.001 |
| | | GG | 0.42 | 0.35 | |
| | Allele | C | 2.38 | 1.64 | <0.001 |
| | | G | 0.48 | 0.55 | |
| GPT (IU/L) | Genotype | GC | 30.17 | 6.18 | 0.193 |
| | | GG | 24.49 | 10.47 | |
| | Allele | C | 30.17 | 6.18 | 0.198 |
| | | G | 24.66 | 10.37 | |
| GOT (IU/L) | Genotype | GC | 29.50 | 8.38 | 0.625 |
| | | GG | 27.39 | 23.40 | |
| | Allele | C | 29.50 | 8.38 | 0.607 |
| | | G | 27.46 | 23.02 | |
| Alkaline Phosphatase (IU/L) | Genotype | GC | 92.67 | 24.45 | 0.027 |
| | | GG | 123.35 | 59.76 | |
| | Allele | C | 92.67 | 24.45 | 0.029 |
| | | G | 122.40 | 59.04 | |
| G6PD (IU/L) | Genotype | GC | 15.28 | 7.24 | 0.018 |
| | | GG | 10.37 | 4.70 | |
| | Allele | C | 15.28 | 7.24 | 0.02 |
| | | G | 10.52 | 4.84 | |

On the other hand, there was a substantial correlation (p= value 0.001, 0.027, and 0.018, respectively) between the serum concentrations of bilirubin, ALP, and the G6PD with UGT1 gene (Position 179 G>C) polymorphism. In spite of the fact that there was a discernible difference in mean values between both alleles "C" and "G" respectively, there was not a significant correlation between GOT and GPT (p=value 0.198, and 0.607), respectively, and any of the alleles. Notwithstanding the fact that a significant association was identified between both alleles and bilirubin, ALP, and G6PD (p-values of less than 0.001, 0.029, and 0.02), respectively.

Premature UDPGT enzyme activity is often the source of unconjugated hyperbilirubinemia, a physiological condition that occurs in 60% of full-term neonates during the first week of life. Statistics show that our study cannot establish that the elevated bilirubin levels are caused by the UGT1A1 gene alterations. Our findings corroborate those of (Akaba *et al.* 1999, Maruo *et al.* 1999, Yamamoto *et al.* 2002, Sutomo *et al.* 2004, Dastgerdy *et al.* 2012). Who discovered that neonates with severe hyperbilirubinemia had a higher frequency of the G71R mutation than neonates without hyperbilirubinemia.

In conclusion, the UGT1A1 gene mutation may have contributed to infant hyperbilirubinemia in Iraq. We made the assumption that variables other than the G71R mutation in the UGT1A1 gene could possibly play a role in the development of newborn hyperbilirubinemia because the frequency of the G71R mutation was not significantly greater in the hyperbilirubinemia group compared to the control group.

UGT1A1 was shown to have a heterozygous mutation in the research population, however no homozygous variant could be identified (Innocenti *et al.* 2009). trial, which covered African American patients and was conducted in the USA, did not uncover any homozygous polymorphism. Other studies (Côté *et al.* 2007, Innocenti *et al.* 2009, Horsfall *et al.* 2011). Discovered that the homozygous variant prevalence was 13% among American Whites, 7% in France, and 4% in the UK (7 percent).

Another study found that the prevalence of variant alleles was 26%, 8%, and 36%, respectively, in Europeans, Asians, and Africans (Fujiwara and Minami 2010). Although the causes of the observed differences between the current study and other studies are unclear, they may be due to ethnicity-related differences or a small sample size of the community being studied (Premawardhena *et al.* 2003, Horsfall *et al.* 2011).

# 5. CONCLUSIONS AND RECOMMENDATIONS

## Conclusıons

1. The blood groups O and A has highest frequencies among patients, this indicated that these blood groups may be more susceptible in elevation bilirubin in patients with liver disorders.
2. The differences in concentrations of liver functions between patients with liver disorders and healthy subjects indicated that abnormal elevation may have related to size of effects which occur in hepatocytes which lead to these abnormalities in liver biomarkers.
3. The differences in serum level of G6PD between study groups referred that this enzyme may have important role in hyperbilirubinemia condition among patients.
4. High frequency of genotypes TC of G6PD Aures (143 T→C) and GC of UGT1A1 (position 179G>C) genes polymorphisms in patients group in comparison with control, indicated that these genotypes may be play important role variation in level of G6PD, abnormality in liver enzymes as well as hyperbilirubinemia.

## Recommendatıons

1. We need additional studies focusing on the groups of patients with liver disorders, each group separately, whether it was viral infections, autoimmune disorders or genetic syndromes, and measuring liver functions and comparing them between those groups.
2. Also, intensive studies are needed regarding different types of mutations and polymorphisms in the G6PD enzyme gene, and to examine their relationship to elevated levels of bilirubin in patients with hyperbilirubinemia and variations levels in G6PD enzyme.
3. Other studies related to changes in the level of the UGT gene in general and to investigate its role in the case of excessive bilirubin level and elevated liver enzymes and its relationship to the development of inherited diseases.

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