Deanship of Graduate Studies

Al-Quds University

Molecular Characterization of β-Thalassemia Intermedia in the West Bank

Rashail Ibrahem Hasan Faraon

M.Sc. Thesis

Jerusalem-Palestine

1439 /2017

Molecular Characterization of β-Thalassemia Intermedia in the West Bank

Prepared By:

Rashail Ibrahem Hasan Faraon

B.Sc. Medical Technology from Al-Quds University – Palestine

Supervisor: Dr. Mahmoud A. Srour

A thesis submitted in partial fulfillment of requirements for the Degree of Master in Medical Laboratory Sciences/ Hematology Track, Faculty of Health Professions – Al-Quds University

1439 / 2017

Al-Quds University

Deanship of Graduate Studies

Department of Medical Laboratory Sciences

Thesis Approval

Molecular Characterization of β-Thalassemia Intermedia in the West Bank

Prepared by: Rashail Ibrahem Hasan Faraon

Registration Number: 21510024

Supervisor: Dr. Mahmoud A. Srour

Master thesis submitted and accepted, Date: Saturday, 11/11/2017

The names and signatures of the examining committee members are as follows:

1. Head of committee: Dr. Mahmoud A. Srour Signature .....
2. The Internal examiner: Dr Khalid Younis Signature .....
3. The External examiner: Dr. Fekri Samarah Signature .....

11/11/2017

Jerusalem / Palestine

1439 / 2017

Dedication

To my God

To my supervisor

To my parents

To my Husband

To my children

To my mother in low

To my sisters and brothers

To all my friends

For their love and support

Rashail Ibrahem Hasan Faraon

Declaration

I certify this thesis submitted for the degree of Master of Medical Laboratory Sciences/ Hematology track, is the result of my own research, except where otherwise acknowledged, and that this study has not been submitted for higher degree to any other university or institution.

Signed:……..

Rashail Ibrahem Hasan Faraon

Date: Saturday, 11/11/2017

Acknowledgement

I would like to express all my gratitude to Allah, the most merciful, for the power and endurance he gave me throughout my life.

I really want to thank my supervisor Dr. Mahmoud Srour, who was always with me in this research step by step, supporting and advising me, and providing assistance in troubleshooting of all types of technical problems in this project.

I want to thank Al-Quds University for giving me opportunity to be one of its students, especially the Department of Medical Laboratory Sciences represented by their kind and compassionate faculty, who had always supported and encouraged me.

I am grateful to the Palestinian Thalassemia Patients' Friends Society (TPFS) for funding this study, especially its president Dr. Bashar Karmi and its director Jihad Abu-Ghoush. Also, sincere thanks to TPFS staff for assistance in recruitment of patients in particular Mahmoud Daraghmah, Wala' Shamasneh, Amani Shokri and Fatimah Rummanah.

I would like to express my sincere thanks for those who helped me to accomplish this work either for patients' recruitment and sample collection, especially Mahmoud Daraghma, Seham Badran, Hasan Abu Esnainah, Osama Barham and Osama Dudeen.

I would like to thank also, Dr. Khalid Shiha and Dr. Nahed Halabia for their support.

I greatly thank my parents, brothers, sisters, friends and my mother in low for their continuous support and for giving me the strength that kept me standing and this accomplishment would not have been possible without them.

I would like also to express my gratitude for the patients and their parents in case of minors who accepted to participate in this study.

Finally, I would like to thank my husband, children for their support, love and patience without any complaint.

Thanks all.

Abstract:

Thalassemia is one of the most common genetically autosomal recessive disorders in the world as well as in Palestine. There are more than 200 genetic mutations responsible for the decreased production of β-globin chain. β-Thalassemia intermedia (βTI) is a type of thalassemia that lies between the two extreme transfusion dependent Thalassemia Major TM and transfusion independent Thalassemia Trait (TT). βTI shows a considerably variable clinical picture ranging from mild to severe anemia requiring occasional blood transfusion. Genetically, βTI is a heterogeneous group that is attributed to mutations in β-globin gene alone or co-inheritance of mutations in the β-globin gene and α- and/or γ-globin genes. The genetic determinants of βTI may be different in different regions and in particular in different ethnic groups and is affected by the different types of thalassemia alleles common in each area and ethnic group. We aimed to determine the spectrum of β- and α – globin gene mutations and *Xmn* I polymorphism of Gγ-globin gene in βTI patients in the West Bank region of Palestine as well as to evaluate the management practices of those patients. A total of 51 cases of βTI were enrolled. Complete Blood Count (CBC) and Hb electrophoresis were evaluated. DNA sequencing was used to analyze β-globin gene mutations. Common α-globin gene mutations were screened by Gap-PCR ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $--^{MED}$, $\alpha\alpha\alpha^{anti3.7}$) or DNA sequencing (α2-IVS II 5 nt del). *Xmn* I polymorphisms of Gγ-globin gene was determined by RFLP-PCR. Seven β-globin gene mutations were observed among the βTI patients, namely IVS-I -6 C>T, IVS-I-110 G>A, IVS-II-1 G>A, IVS-I-1 G>A, Codon 37 Trp>Stop, B -101 and IVS-II-848 C>A. Ten genotypes were observed. Homozygosity for IVS-I-6 C>T accounted for the majority of βTI cases with a frequency of 74.5%. The second common β-globin gene genotype among study patients were homozygote IVS-I-110 G>A (5.8%) and homozygote IVS-II-1 G>A (5.8%). The remaining seven genotypes were each detected in about 2% of βTI patients. α-Thalassemia mutations were seen in five patients (9.8%), and included ($-\alpha^{3.7}$, $\alpha\alpha\alpha^{anti3.7}$ and α2-IVSI 1-5 nt del). *Xmn*I polymorphism of Gγ-globin gene was observed in four patients (7.84%), three homozygotes and one heterozygote for this SNP. The inheritance of the mild β-globin gene homozygote IVS-I-6 allele was the major contributing factor for the βTI phenotype among the study subjects. The role of *Xmn* I -158 C>T SNP of $^{G}\gamma$-globin gene and α-thalassemia mutations in ameliorating the thalassemia phenotype was observed in few

patients for each factor. In conclusion, homozygosity for β-globin gene mutation IVS-I-6 C>T is the most common genotype among Palestinian βTI patients. The beta -101 C>T mutation was diagnosed in one patient in homozygote state for the first time in Palestine. The study results will positively affect the health management of βTI patients in Palestine and provide more data on the origin and distribution of β-globin gene alleles.

Table of Contents

List of Figures

List of Tables

List of Appendices

List of Abbreviations

TI	Thalassemia Intermedia
TM	Thalassemia Major
TT	Thalassemia Trait
EDTA	Ethylene Diamine TetraAcetic Acid
PCR	Polymerase Chain Reaction
UDPG	Uridine 5`-diphospho-alpha-d-glucose
Bp	Base pair
DMSO	DiMethyl SulfOxide
DEPC	Diethyl pyrocarbonate
RFLP	Restriction Fragment Length Polymorphism
Hb	Hemoglobin
Hb F	Fetal hemoglobin
Hb S	Sickle cell hemoglobin
MCH	Mean Corpuscular Hemoglobin
MCV	Mean Corpuscular Volume
ARMS PCR	Amplified Refractory Mutation System
MED	Mediterranian
RBC	Red Blood Cells
α - thalasseima	Alpha thalassemia
β - thalassemia	Beta thalassemia
Nt	Nucleotide
UTR	Un Translated region
HS	HyperSensitivity
LCR	Locus Control Region
HPFH	Heridatary Persistence Fetal Hemoglobin
EMH	Extra Medullary Hematopoiesis
MOH	Ministry Of Health
MRI	Magnetic Resonance Imaging
FDA	Food and Drug Administration
BMT	Bone marrow transplantation
HU	Hydroxyurea

MLPA	Multiplex Ligation-dependent Probe Amplification
SNP	Single Nucleotide Polymorphism
mRNA	Messenger Ribo Nucleic Acid
DNA	Deoxyribo Nucleic Acid
IVS	InterVening Sequence
HH	Hereditary Hemochromatosis
CHF	Congestive Heart Failure
DVT	Deep Venous Thrombosis

Chapter one

Introduction

1.1. Thalassemia

Thalassemia is a genetic disorder characterized by inadequate synthesis of globin chain subunits of the hemoglobin (mostly α or β globin chains), while the globin chain is structurally normal. Thalassemia is classified into three different subclasses: thalassemia major (βTM) the most severe form of thalassemia which is transfusion dependent, thalassemia intermedia (βTI) and the asymptomatic thalassemia trait (βTT) (M. Karimi, Cohan, De Sanctis, Mallat, & Taher, 2014).

Thalassemia is the most common autosomal recessive disorder in the world, especially in the Mediterranean, Middle-East, Transcaucasia, Central Asia, Indian subcontinent and Far East (Cao & Galanello, 2010).

1.2. Beta Thalassemia Intermedia

Beta-thalassemia intermedia (βTI) result from a group of heterogeneous mutations affecting the β-globin gene and the severity of the disorder can't generally be predicted from the genotype. Some patients with βTI genotype are treated as thalassemia major because they present a relatively severe clinical disease, others may be classified as having thalassemia trait because of the asymptomatic or mild nature of their clinical condition (Cao & Galanello, 2010).

βTI shows a considerably variable clinical picture ranging from mild to severe anemia requiring occasional blood transfusion. Genetically, βTI is a heterogeneous group that is attributed to mutations in β-globin gene alone or co-inheritance of β-globin gene mutations and other globin genes such as α- or γ-globin genes. The genetic determinants of βTI may be different in different regions and in particular in different ethnic groups and is affected by the different types of thalassemia alleles common in each area and ethnic group. Fortunately, from the more than 200 mutations causing β-thalassemia, only less than 20 mutations are usually

responsible for the β-thalassemia in each specific ethnic group including 15 mutations that occur in the intervening sequences (Agouti et al., 2007).

βTI was first described in 1955 by Rietti-Greppi-Micheli, where he used the term βTI to describe patients having less severe clinical courses than βTM and more severe courses than βTT by their phenotype and hematological tests. βTI patients generally experience mild anemia with Hb level between 7-10 g/dl, survive well without regular transfusion, show normal growth development and generally onset of disease occurs between 2 and 6 years of age (Maria D. Cappellini, Musallam, & Taher, 2009; A. Taher, Isma'eel, & Cappellini, 2006).

1.3. Clinical features of β thalassemia intermedia

Patients with βTI show heterogeneous clinical picture. The clinical symptoms are pallor, extramedullary hematopoiesis, cholelithiasis, jaundice, leg ulcers; moderate to severe skeletal changes such as expansion of the facial bones and maxillary sinuses that causes protrusion of the upper jaw, more severe red blood cells morphology, hemolysis, hepatosplenomegally, osteoporosis and thrombotic complication. Patients also suffer from iron over load mainly from increased intestinal absorption of iron and the occasional blood transfusion (Maria D. Cappellini et al., 2009).

1.3.1. Extramedullary hematopoiesis (EMH)

The ineffective red blood cell production from the bone marrow forces the hematopoietic tissue to expand outside the marrow and leads to hematopoietic compensatory mechanism. Most body organs are involved including the liver, spleen, lymph nodes, thymus, breasts, heart, kidneys, adrenal glands, prostate, pleura, skin, retroperitoneal tissue, cranial nerves and spinal canal. The incidence of extramedullary hematopoiesis in βTI patients reaches up to 20 % compared with βTM that reaches < 1 %. The early diagnosis of EMH affects the course of management and reduces the irreversible neurologic damage. Magnetic resonance imaging (MRI) is the method of choice for the diagnosis of EMH (Haidar, Mhaidli, & Taher, 2010)

1.3.2. Pulmonary hypertension

Pulmonary hypertension is a common complication in 59.1% of βTI patients and considered primary cause of congestive heart failure (CHF). The mechanism causing pulmonary hypertension in βTI patients is unclear; but some evidences for the mechanism of pulmonary hypertension include; the endothelial dysfunction that increases the apoptosis and inflammation, pulmonary hemosiderosis, local thrombosis, decreased nitric oxide and nitric oxide synthase production. Pulmonary hypertension is reversible by blood transfusion and can be treated with aspirin and warfarin (A. Taher et al., 2006).

1.3.3 Thrombophilia

The βTI patients have a high risk of thrombosis compared with βTM, 4 % of βTI have a risk of thrombosis compared with 0.8% in βTM. Deep vein thrombosis (DVT), portal vein thrombosis, stroke and pulmonary embolism are the most common complications of thrombophilia. Splenectomized patients had higher incidence of thrombosis than non-splenectomized patients; due to procoagulant activity of damaged circulating RBCs, where the RBC remnants expose negatively charged phosphatidyl-serine through the "Flip-Flop" phenomenon and initiate thrombosis (A. Taher et al., 2006).

1.3.4 Endocrine diseases and pregnancy

Osteoporosis due to bone marrow expansion and vitamin D deficiency are highly prevalent in βTI patients. Fertility is usually normal, delayed puberty is common, hypothyroidism and diabetes mellitus are rare (Musallam et al., 2011).

Pregnant women with βTI have a high risk of abortion, pre-term delivery, caesarean delivery, thromboembolic events and intrauterine growth restriction. Splenomegaly interferes with the enlargement of uterus that may be complicated by hypersplenism and thus splenectomy is necessary after delivery as well as the administration of anticoagulant therapy (Nassar et al., 2006).

1.3.5 Leg ulcers

It is unclear why some patients of βTI develop leg ulcers. However it`s more common in elderly βTI patients than younger patients. The skin of the extremities is thin due to reduced tissue oxygenation that makes tissues fragile and increases the risk of ulcers. These ulcers are indolent and painful. Keeping the patients leg above the level of the heart decreases the pain and can be beneficial (Musallam et al., 2011).

1.3.6 Gallstones

Gallstones are more common in βTI than βTM as a result of ineffective erythropoiesis and hemolysis. Uridine 5`-diphospho-alpha-d-glucose (UDPG) deficiency increases the risk of gallstones formation. Cholecystectomy should be performed in symptomatic gallstones patients to prevent cholecystitis which is a serious problem in splenectomized patients (Maria D. Cappellini et al., 2009).

1.4 Molecular biology of βTI

The β-globin gene is located on the short arm of chromosome 11 within the β-globin gene cluster. This region also contains δ-gene, embryonic gene (ε-gene) and the $^{A}\gamma$-gene, $^{G}\gamma$-gene, and the pseudogene (ψβ1) gene. While the α-globin gene cluster is located on chromosome 16 (Figure 1).

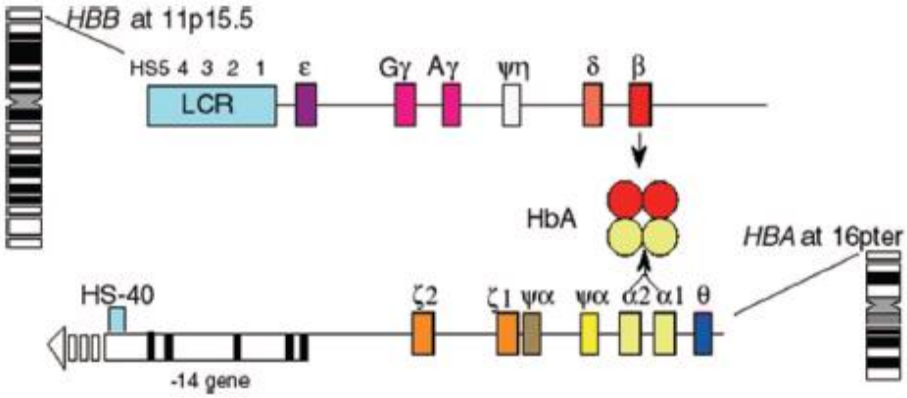

Figure 1. The localization of β-and α-gene clusters on chromosomes 11 and 16, respectively (Cao & Galanello, 2010).

The β-globin gene contains three exons and both the 5` and 3` untranslated region (UTRs), which spans 1.6 Kb. The gene is regulated by a 5` promoter that contains TATA, CAAT and duplicated CACCC boxes. A major regulatory region contains a strong enhancer that maps 50Kb from the beta gene, this region contains the locus control region (LCR) that includes four HS sites (HS-1 to HS-4), and the erythroid specific DNase hypersensitivity sites (HSs) (Cao & Galanello, 2010).

The majority of mutations causing β-thalassemia are single nucleotide substitutions or insertions of oligonucleotide that lead to frame shift or deletions. Point mutations belong to three categories: mutations in the promoter and 5` UTR (defective gene transcription), mutations affecting the mRNA processing, and mutations that result in abnormal mRNA translation (Cao & Galanello, 2010).

The genetic basis of βTI for phenotype diversity is explained by three terms primary, secondary, and tertiary. The primary modifiers reflect the broad spectrum of mutations affecting the β-gene and range from mild promoter mutations (the slight reduction in β-gene

production) to many different mutations that result in β^0-thalassemia. The secondary modifiers include modifiers that make imbalance between the globin chains; the coinheritance of α-thalassemia has this effect, also the production of more γ-chains of fetal hemoglobin after birth. The tertiary modifiers include those that are not related to globin chains but have important effects in the complications of the disease. Figure 2 represents several genetic profiles that lead to βTI phenotype (Musallam et al., 2011).

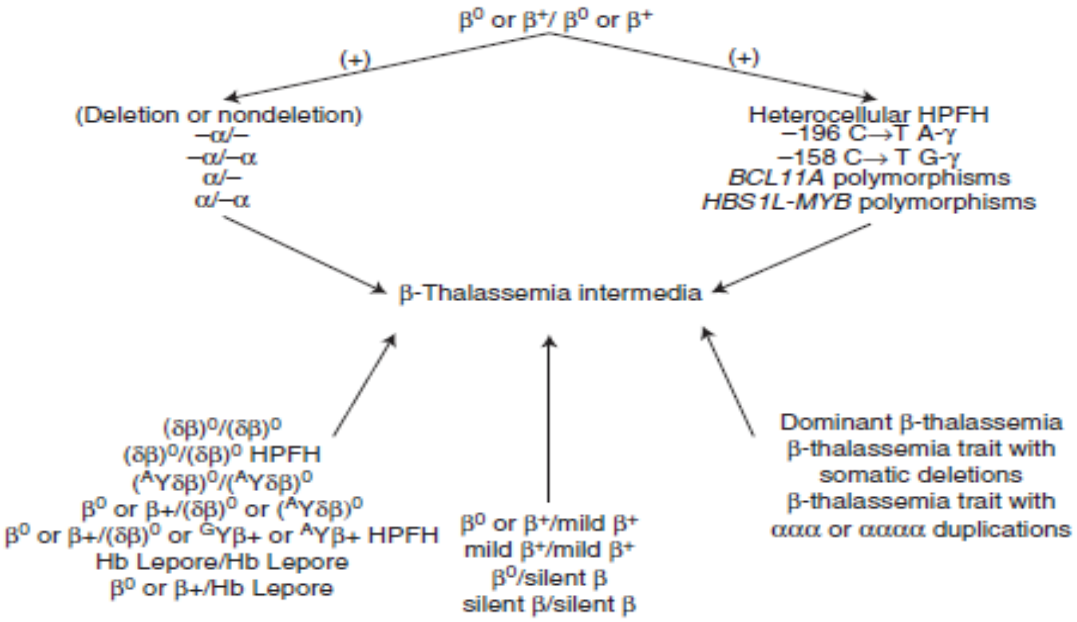

Figure 2: Genetic profiles that may lead to thalassemia intermedia phenotype. HPFH: Hereditary persistence of fetal hemoglobin; Hb: hemoglobin (Musallam et al., 2011).

The molecular basis of βTI can be classified into the following categories:

- Homozygosity or compound heterozygosity for mild mutations.
- Homozygosity or compound heterozygosity for severe mutations plus α-thlassemia or heterocellular HPFH
- Compound heterozygosity for β-thalassemia and (δβ-thalassemia and deletion or non-deletion HPFH)
- Heterozygosity for β-thalassemia and triplicate α-locus ($\alpha\alpha\alpha^{anti3.7}$)
- Heterozygosity for a dominant form of β-thalassemia (Camaschella et al., 1995)

1.4.1 Homozygous β-thalassemia intermedia

Portuguese thalassemia was the first description of mild thalassemia mutation due to the homozygosity of IVS-I-6 T>C mutation. This substitution creates alternative splice sites in vitro that produces a normal β chains; and it is mild mutation in vivo. Other mild mutations are -87 C>G and -101 C>T that are associated with decreased production of β-gene transcription (Camaschella et al., 1995).

The most important mechanism of βTI is the coinheritance of homozygosity or compound heterozygosity for β-thalassemia alleles. The most common mutation in Mediterranean area is the mild mutation IVS-I-6 T>C and IVS-I-110 G>A. Other mild mutations include the coinheritance of homozygous β-thalassemia with α-thalassemia by reducing the imbalance between alpha and beta chains. Other mild mutations include the continuous production of gamma chain in adults, which reduces the imbalance between alpha and non-alpha chains, where the increase in gamma chain compensates for the decrease in beta chain and produces mild thalassemia (Cao & Galanello, 2010).

1.4.2 Heterozygous β-thalassemia intermedia

Heterozygous beta-thalassemia either beta+ or beta^0 is asymptomatic and characterized by increased RBC count, microcytosis, hypochromia and increased HbA_2 level. There are two mechanisms that may increase the severity of clinical and hematological manifestations. The first mechanism includes; the co-inheritance of heterozygous beta globin gene mutation and triplicated or quadruplicated α-globin gene rearrangement which increases the imbalance between α-globin and non-α-globin chain synthesis. The second is the presence of other mutations in the β-globin chain that cause instability of β-chains. Unstable β-globin chains bind to heme and precipitate in the red blood precursors before assembly with alpha chains and lead to production of inclusion bodies. Precipitation of defective β-chains occurs not only in mature erythrocytes but also in erythroblasts causing ineffective erythropoiesis (Agarwal, Sarwai, Agarwal, Gupta, & Phadke, 2002; Camaschella et al., 1995; Gasperini, Perseu, Melis, & Maccioni, 1998).

1.4.3 Interaction of α-globin gene triplication with β-globin gene

The inheritance of α-thalassemia with β-thalassemia reduces the imbalance between alpha and beta gobin chain. The triplication of α-globin gene ($\alpha\alpha\alpha^{anti\ 3.7}$) is due to unequal crossing over between misaligned homologous segments during meiosis in the globin gene cluster. The clinical and hematological phenotype may vary from mild anemia in case of (-α/αα) to severe anemia in case of (αα/ααα). The coinheritance of $\alpha\alpha\alpha^{anti\ 3.7}$ allele aggravates the mild β-thalassemia carrier phenotype to βTI phenotype (Farashi et al., 2015).

1.4.4 Mutations in γ-globin gene

Continuous production of γ-globin chains is due to coinheritance of genetic determinants; which reduce the imbalance of alpha/non-alpha chains. The increased production of gamma chains -196 C→T A-gamma compensates for the absence of beta chain, compound heterozygosity for this determinants and beta thalassemia produces a βTI phenotype. The -158 C>T $^{G}\gamma$-globin gene is silent in normal subjects and heterozygote beta thalassemia but results in increased Hb F production due to hematopoietic stress. The -158 C>T G-gamma SNP has been reported in association with IVS-II-I (G→A), frame shift 8 (A. T. M. Taher, K. M. Karimi, M. El-Beshlawy, A. Belhoul, K. Daar, S. et al), frame shift 6 (-A), and codon 39 nonsense mutations and this explains the mild phenotype with these mutations (Cao & Galanello, 2010).

1.4.5 Novel mutations in the second intervening sequence

Agouti et al have identified a thalassemia intermedia patient characterized by increased HbA_2 levels and increased HbF level with moderate anemia. This hematological phenotype is typical for homozygote or compound heterozygote for mild β^+; but DNA sequencing revealed that this patient was homozygote for IVS-II-726 A>G substitution. This mutation is responsible for the β^+ phenotype. This mutation creates a cryptic splice signal (normal splice junctions

and the cryptic splice sites not themselves changed) that is used as an alternative site to the existing sites (Agouti et al., 2007; Murru, 1991).

1.4.6 New insertions/ frame shifts mutations

Williamson et al, have studied a mild thalassemia intermedia patient with moderate anemia, this patient had a severe red cell morphology, significant reticulocytosis, increased HbF and iron over load. A frame shift in the β-globin gene was found affecting the exon 2 at the region of amino acid codon 100. There were an insertion of eight nucleotides in the β-globin gene that results in a shift of the normal protein reading frame and an abnormal amino acid sequence before codon 99. The final product results in extended polypeptide chain of 159 amino acid long. The excess unstable β-chain and unpaired to α-chain leads to extensive cell damage (erythroblast destruction and ineffective erythropoiesis) (Williamson, Brown, Langdown, & Baglin, 1997).

1.4.7 Other modifiers

The phenotype of thalassemia always results from the allelic heterogeneity of beta globin gene and the effect of alpha and gamma globin gene mutations. Also the phenotype could be modified by other genetic factors mapping outside the globin gene cluster and not influenced with HbF. These factors almost affect bilirubin, iron, and bone metabolism. The rapid turnover of red cells in thalassemia patients results in jaundice and the probability to form gallstones. Bilirubin and gallstones are related to polymorphic motifs at the promoter of the gene in hepatic glucuronidation of bilirubin. In normal conditions the promoter has six TA repeats in the TATA box $(TA)_6$, in thalassemia patients that develop hyperbilirubinemia, jaundice and gallstones have the more common and less efficient TA_7 motif (Cao & Galanello, 2010).

A common mutation of the *HFE* gene (C282Y) that cause the common type of Hereditary Hematochromatosis (HH) may be involved in determining the variability of iron overload in TI patients (Cao & Galanello, 2010).

A polymorphism at the genetic loci involving bone metabolism mainly vitamin D receptor and the *COLIAI* gene, is associated with the development of marked osteoporosis that depends on

many factors including hypogonadism and the extent of iron chelation (Cao & Galanello, 2010).

1.5. Clinical management of βTI

Treatment of βTI is based on the symptoms and folic acid supplement and splenectomy (Cao & Galanello, 2010). Guidelines for the management of βTI have been published by the Thalassemia International Federation (M. Karimi et al., 2014).

1.5.1. Transfusion therapy

Transfusion is not a routine treatment of βTI patients. Occasional transfusion is recommended in pregnancy, surgery, and infection. The initiation of transfusion therapy is based on the severity of signs and symptoms of anemia including failure of growth and development, enlargement of spleen and poor performance in school. Alloimmunization is observed in βTI patients, although the risk is decreased when transfusion is initiated before 12 months of age. Testing for Rh antigens (D, C, E, c, e) and K1 (partial K) is recommended before transfusion therapy. Some physicians administrate steroids therapy for 3-5 days. The increased intestinal iron absorption leads slowly to iron overload. In addition, transfusion therapy increases the risk of iron overload (A. T. M. Taher, K. M. Cappellini, M. D. Weatherall, D. J., 2011; Vichinsky, 2016).

1.5.2. Iron chelation therapy

Iron overload is the most common complication in βTI patients. Iron accumulates and damages the tissues and cells. Excess iron causes a peroxidative injury to the phospholipids of mitochondria and lysosomes that produce free radicals (the most pathogenic factor). Hepcidin that regulates excess iron is low in thalassemia patients and result in excessive gastrointestinal absorption of iron even in the presence of iron overload. Ferritin can be used to measure iron overload, but recently the most reliable measurement and considered the gold standard for evaluation the iron overload is the liver biopsy by Magnetic Resonance Imaging (MRI) technique and particular R2 and R 2* parameters that measure the correlation between liver iron and body iron. Iron chelating is necessary to reduce the risk of hemosiderosis. Recently; there are three chelating drugs available: Desferal that is administrated subcutaneously at a

median dose of 40 mg/kg over 8-12 hours. Ferriprox (L1) that is an oral drug administrated in a dose of 75-100 mg/kg/day in three sub-doses given one hour before meal. Exjade is an oral drug that has been approved by the Food and Drug Administration (FDA) and by the European Agency for the Evaluation of Medical Products (EMEA). The dosage is 10-30 mg/kg in one morning dose. The oral chelating drugs are more efficient in chelating the excess iron pools of cardiomyocytes; attenuate reactive oxygen labile formation and binding labile iron (Borgna-Pignatti, 2007; Haddad, Tyan, Radwan, Mallat, & Taher, 2014).

1.5.3 Bone marrow transplantation (stem cell transplantation)

Bone marrow transplantation (BMT) means that the marrow of affected patient is replaced with marrow of unaffected patient (healthy patient). Stem cell transplant is rare in βTI patients and is probably considered for severely transfusion dependent patients. Successful BMT results in cure, but if it fails it may leads to other complications including graft versus host disease (GVHD) growth impairment, and neurologic complications and may lead to death. The decision to make BMT depends on the quality of life, the expected survival time, availability of matched donor and this is relevant for βTI patients especially who are mildly affected (Borgna-Pignatti, 2007; A. Taher et al., 2006).

1.5.4. Modulation of fetal hemoglobin

Enhancing γ-globin chain synthesis reduces the imbalance between α- and β-globin chains and potentially leads to improved RBC survival and alleviates anemia. There are several pharmacological agents that increase γ-globin chain production by increasing Hb F. Therefore such agents or drugs may become a potential therapy for patients with βTI. Hydroxycarbamide known as hydroxyurea HU is an S-phase-specific and non-DNA hypomethylating chemotherapeutic agent that induces HbF synthesis. In a series study in Iran, the effects of HU in βTI patients were studied and found an increase in hemoglobin level in non transfused βTI patients, and the transfusion-dependent βTI patient became transfusion-independent (Borgna-Pignatti, 2007).

Butyric acid and its derivatives are also postulated to augment HbF via their action at the sequences near the transcriptional starting sites of the γ-globin gene promoter. In βTI patients the response was limited and not predictable (Carolyn Hoppe, 1999).

1.5.5. Splenectomy

The size of spleen increases with time in non-transfused βTI patients and could aggravate the anemia and sometimes causes neutropenia and thrombocytopenia. Splenectomy allows the discontinuation of transfusion in the majority of the patients. If gallstones are present, Cholecystectomy should be performed at the time of splenectomy to avoid two surgical interventions. Complications following splenectomy include increased risk of infections and thrombophilia. Splenectomy should be avoided in children less than five years old because they have a high risk of fulminate post-splenectomy and sepsis (Borgna-Pignatti, 2007; Haddad et al., 2014; Hashemieh, 2016).

1.5.6. Anticoagulant therapy

The role of anticoagulant therapy is for the prevention of vascular diseases in βTI patients. Aspirin should be administrated to splenectomized patients with platelets $>500*10^9$/L and Coumadin to patients who have experienced thromboembolic events (Musallam et al., 2011).

1.6. Recommendations for the management of thalassemia intermedia

Despite the availability of several treatment options, there are no clear guidelines for managing βTI. Management of βTI depends on two categories: first manage the complication, second what is recommended to prevent development of these complications. The recommendations are:

- A guarded approach for the need of splenectomy and delay the initiation of transfusion until it is necessary.
- Early initiation of transfusion and iron chelating if there is growth abnormality, poor performance and facial deformities.
- Regular follow up with endocardiodoppler for cardiac complications.
- Regular follow up of liver iron concentration with MRI.
- Avoid smoking; prolong immobilization and use of oral contraceptives (A. Taher et al., 2006; A. T. M. Taher, K. M. Karimi, M. El-Beshlawy, A. Belhoul, K. Daar, S. et al, 2010).

1.7. Prevalence and Geographic Distribution

βTI is a clinical condition that is less severe than βTM and more severe than βTT. The prediction of phenotype from genotype in βTI is difficult due to genetic and environmental modifying factors. A cross sectional study was conducted in China (2010), where the authors analyzed the genotype of 117 βTI patients that were diagnosed after the second year of age and their hemoglobin levels were (6-10 g/dl). The common genetic defects in Chinese βTI patients fall into two types: type 1 β-thal homozygotes or compound heterozygotes for β-globin gene mutation and other β-globin defects were reported in 82.9% (97/117) of the study subjects in which β^{+}-thalassemia mutations were the most common (49/97), HbE variant was the second (27/97) and deletional HPFH or δβ-thalassemia was the third (11/97); and type II β-thalassemia heterozygotes for β-globin gene mutations reported in 17.1% of patients. In βTI patients heterozygotes for β-globin gene mutations, only 5 of 20 patients co-inherited $\alpha\alpha\alpha^{anti\text{-}3.7}$/ triplication, 14 patients had normal α-globin genes and 1 patient had a dominant frame shift β-thalassemia mutation at codon 53 (Chen et al., 2010).

βTI show a considerable heterogeneity in India. Researchers analyzed 73 cases of βTI in order to study the prevalence and the effect of common molecular determinants in βTI. The 73 βTI patients were grouped into: homozygous β-thalassemia (n=50) and heterozygous β-thalassemia (n=23). The authors reported that the milder form of βTI is attributed to co-inheritance of α-globing deletions (15/50), homozygous Omni polymorphism (17/50), both factors (3/50) and milder β-allele (9/50) in homozygous β-thalassemia (total 50 cases). While the heterozygote's of β-thalassemia and $\alpha\alpha\alpha^{anti\text{-}3.7}$/ triplication were the predominant factor (14/23 cases) (Inusha Panigrahi, 2006).

Sollaino et al. (2009) have studied the association of α-globin gene quadraplication and heterozygous β-thalassemia in patients with βTI in Italy. The authors analyzed the genotypes of 10 patients of βTI. All patient had moderate to severe microcytic anemia (Hb 7-9.8 g/dl), increased HbA_2 (>4.1%) and HbF (2.7-19.4%). All patients had shown one mutation, that is codon 39 C>T nonsense mutation in one β-globin gene and a complete normal sequence in the other β-globin gene. Multiplex Ligation-dependent Probe Amplification (MLPA) analysis of α-globin cluster revealed a complete duplication of α-globin gene in one family covering 170 Kb from the telomere while other families showed larger duplications. The duplication includes the regulatory elements MCS-R1, MCS-R2 (HS40), MCS-R3 and MCS-R4. This causes a more severe imbalance of the α/β globin chain ratio; the increase in imbalance enhances the ineffective erythropoiesis resulting in evident clinical symptoms. Thus, the co-inherited triple α-globin arrangement, converts the typical clinically asymptomatic β-thalassmia carrier state into that of β-thalassemia intermedia phenotype (Maria Caria Sollaino, 2009).

Maragoudaki (1999) has studied the molecular, hematological and clinical manifestation of the -101 C>T substitution of the β-globin gene promoter in 25 βTI patients in Athens, Greece. All patients had moderate anemia with Hb levels between 8.7 and 10.5 g/dl. Genotyping revealed that all patients were double heterozygotes for the -101 to C>T mutation and a second severe β-thal mutation in *Trans* with normal α-globin genotype in 24 patients and presenting with mild βTI phenotype. While one patient who shared the same β-globin genotype was transfusion depended because it has an additional α-globin gene mutation. All 25 patients were normal for the *Xmn* I polymorphism in $^{G}\gamma$-globin gene (Maragoudaki, 1999).

Al-Allawi (2014) has studied the molecular basis of βTI in 74 patients from North Iraq. Patients showed Hb levels between 6.5 and11.6 g/dl and HbF ranging from 3.3 to 98.5% and Hb A_2 ranges from 1.5 to 8.5%. The most common mutation was IVS-I-6 (T>C) followed by IVS-II-1(G>A), codon 82/83 (-G) and codon 8 (-AA). Also the inheritance of β-thalassemia mutations and *Xmn* I polymorphism are the most important mechanism implicated in these βTI patients (Al-Allawi, Jalal, A. M. Omer, & S. Q. Markous, 2014).

El-Shanshory (2014) has studied the different β-globin gene mutations in 158 βTM patients and 42 βTI patients from Egypt. The most common mutations were IVS-I-110 (G>A), IVS-I-6 (T>C), IVS-I-1 (G>A), IVS-I-5(G>C), IVS-II-848(C>A), IVS-II-745 (C>G), IVS-II-I(G>A) (El-Shanshory, 2014).

Ebrahim Miri-Moghaddam (2016) has studied the genotypes of β- and α-globin genes in 50 cases with βTI including 5 patients with hemoglobin variants. The most common mutations were: IVS-I-5 G>C, and IVS-II-1 G>A and the predominant genotypic combinations were βº/βº (68.9%). Coinheritance of α-thalassemia was observed in 33% of the patients with the -$\alpha^{3.7}$ as the most common allele and one patient with the $-\alpha^{4.2}$ allele and another patient with the $-^{MED}$ allele. However, the alleviating mechanism for the intermediate thalassemia phenotype was not explainable in more than half of the cases with the β°/β° genotype (Miri-Moghaddam, 2016).

Genetic analysis of β-Thalassemia Intermedia has been studied in Israel, Rund has analyzed 95 βTI patients from 60 families including Arabs, Samaritan and Druze families. In 10 families, the mild βTI phenotype was attributed to compound heterozygosity of -101 C>T or coexistence of triplicate α-globin genes with β-thalassemia trait. In 39 families, the sever phenotype of βTI was attributed to inheritance of sever β-thaassemia mutations. No beneficial effect for coinheritance of α-thalassemia in 11 families was studied. Elevated HbF levels ameliorated the disease in some patients with a severe genotype (Rund, 1997).

1.8. Problem statement

β-Thalassemia intermedia shows a considerably variable clinical picture ranging from mild to severe anemia requiring occasional blood transfusion. Genetically, βTI is a heterogeneous group that is attributed to mutations in β-globin gene alone or co-inheritance of β-globin gene mutations and other globin genes such as α- or γ-globin genes. The genetic determinants of β-TI may be different in different regions and in particular in different ethnic groups and is affected by the different types of thalassemia alleles common in each area and ethnic group. Fortunately, from the more than 200 mutations causing β-thalassemia, only less than 20 mutations are usually responsible for the β-thalassemia in each specific ethnic group.

The proper diagnosis of βTI is challenging due to its genetic heterogeneity and thus requires careful considerations, so patients are correctly identified and given the appropriate treatment. A definitive diagnosis for βTI is possible through DNA analysis.

1.9. Study justification

Precise diagnosis and management are essential in β-thalasemia intermedia patients for prevention of later clinical complications and not to be confused with β-thalasemia major. β-Thalassemia intermedia patients in West Bank region, were mostly diagnosed based on CBC, hemoglobin electrophoresis and clinical symptoms which do not completely resolve it from β-Thalassemia major, and thus they most likely do not receive the appropriate clinical management. Misdiagnosis of βTI patients as βTM makes them vulnerable to inappropriate transfusion that loads their bodies with iron and increases their suffering from disease complications and compromises their life quality.

1.10. Aim of the study

The aim of this research is to study the molecular and hematological characteristics of β-thalasemia intermedia in the West Bank, in order to provide a proper diagnosis and management of patients.

1.11. Objectives

Palestine, like other countries in the region, has around 800 BTM patients and of whom more than 400 patients in the West Bank region. While the exact number of BTI patients is not exactly known. So far, no study has been conducted to determine the genetic mutations contributing to βTI. Therefore, the objectives of the present study were;

To determine the spectrum of genetic mutations that causes β-thalassemia intermedia in the West Bank region, Palestine.

To investigate the hematological characteristics (Hb, Hb A_2 and HbF levels) in βTI patient`s, and the level of clinical management of β-thalassemia intermedia in the West Bank region, Palestine.

Provide background data that should improve the understanding of the genetic modifiers that contribute the phenotype of BTI in West bank region and to make an efficient use of health care resources.

1.12. Hypothesis

H0: β-thalassemia intermedia patients have a variant genetic mutation in the West Bank region, Palestine.

Chapter two

Materials and Methods

2.1. Materials

All reagents, chemicals and instruments used in this study are shown in table (2.1).

Table 2.1: List of instruments and materials used in this study

Item	Manufacturer/country
Syringes	Medi-Plus. China
Needles	Medi-Plus. China
EDTA tubes	Sigma
DNA extraction kits from whole blood	Genomic DNA Mini kit, Geneaid, Cat #: GB100
Lyophilized PCR master mix	AccuPower® HotStart PCR PreMix, BIONEER, Korea
Gel purification kit	AccuPower® Gel Purification kit, BIONEER, Korea
PCR primers	Metabion, Germany
1kb DNA ladder	Gene Direx ®
50 bp DNA ladder	Gene Direx®
Agarose	Hy-labs, Israel
Ethidium bromide	Hy-labs, Israel
Thermal cycler	Esco Healthcare Ltd. USA
Gel documentation system	Bio-RAD GEL DOC 2000. USA
Tris base	Sigma
DNAase free nuclease water	Hy-labs
DiMethyl SulfOxide (DMSO)	Sigma
Xmn I enzyme,	New England Biolabs, UK

2.2 Methods

2.2.1. Study population and Design

This was a case series retrospective multi-center study. Thalassemia patients are registered in eight major centers in the major hospitals administered by the Palestinian Ministry of Health (MOH). These thalassemia centers are located in eight major cities in the West Bank of Palestine namely: Al-Watani Hospital (Nablus), Thabet-Thabet Hospital (Toulkarem), Sulieman Khalil Hospital (Jenin), Qalqiliyah Hospital (Qalqilia), Palestine Medical Complex (Ramallah), Alia Hospital (Hebron), Biet Jala Hospital (Beit Jala) and Jericho Hospital (Jericho).

Patients' medical files were reviewed retrospectively in the first visit by the researcher. A special questioner was used to collect demographic and medical data including patient`s age, sex, age on first blood transfusion, diagnosis, history of splenectomy / splenomegaly, onset of disease, serum ferritin level and any other relevant health complications.

Data were analyzed and patients that met the inclusion criteria (see below) for β-thalassemia intermedia were identified and considered eligible for inclusion in this study.

2.2.2. Inclusion criteria for β-thalassemia intermedia

1. Diagnosis of TI.
2. Age at diagnosis or initiation of transfusion ≥2 years.
3. Frequency of blood transfusion, once every 2-3 months or even larger intervals.
4. Questionable TM diagnosis like a patient who is more than 30 years old or has high HbA2 levels.

2.2.3. Questionnaire

Patients who fulfilled the inclusion criteria listed in the previous section were contacted by telephone and briefed about the study objectives and asked for participation in the study. The questionnaire developed for this study included two parts, the first part was used to collect data from medical files and the second part was used to collect data from the patient directly via a short interview Appendix 4. The questionnaire aimed to collect demographic information

and medical history of the patients. Patients who accepted to participate in the study, were asked to attend to the nearest clinical care center. Patients were asked to provide information to complete the second part of the questionnaire as well as to provide a written consent. For patients younger than 18 years old, the guardian (either father or mother) were asked to provide the information needed to complete the questionnaire and to provide the written consent form.

2.2.4. Specimen collection, transport and preservation

Two samples of venous blood were collected in K_3EDTA tubes; each tube contained one to three milliliters of blood. One blood sample was used for CBC analysis using an automated hematology analyzer and the rest of the sample was used for Hb electrophoresis. The second sample was kept closed and used for DNA analysis.. Thus, study samples were analyzed using one of three different hematology analyzers (Nihon Kohden, Celltac and Abcus 380) were used for CBC analysis based on availability of the machine in the Health care centers where samples were collected. Samples were sent to the research laboratory at Al-Quds University within 8 hours in a refrigerated box. One sample (same one used for CBC) was used for Hb electrophoresis that was performed using HPLC method on the D-10 machine (Biorad). The second sample was sent to Al-Quds University using a refrigerated box and stored at $6 \pm 2^{\circ}C$ and used for preparation of genomic DNA.

2.2.5. Preparation of genomic DNA

Genomic DNA was prepared by two methods: first, whole blood was used using a commercial kit (Genomic DNA Mini kit, Geneaid, USA). Briefly, 300 µL of whole blood were transferred into 1.5 mL micro centrifuge tube, followed by addition of 900 µL RBC lysis buffer, mixed by inversion, incubated for 10 minutes at room temperature then centrifuged at 3000 xg for 5 minutes. The supernatant was removed and the pellet was resuspended in 100 µL of RBC lysis buffer, mixed well, 200µL of GB buffer was added, mixed vigorously and incubated for at least 10 minutes at 60°C. Then 200µL of absolute ethanol was added, mixed for ten seconds and the mixture was transferred to a GD column followed by centrifugation at 13000 xg for five minutes. The 2 ml collection tube and the through filtrate were discarded. The column was then washed with 400 µL of W1 buffer and centrifuged at 13000 xg for 60 second. A

second wash was performed using 600 µL of washing buffer followed by centrifugation at 13000 xg for 60 second. Then, the GD column was dried by centrifugation at 13000 xg for 3 minutes. After that, the GD column was transferred to a clean 1.5 mL microcentifuge tube, 100 µL of preheated elution buffer was added, let stand at least three minutes then centrifuged at 13000 xg for 60 seconds to elute DNA. Purified DNA was stored at -20°C.

Second: genomic DNA was prepared from **Buffy coat** to increase the yield of genomic DNA using a commercial kit (Genomic DNA Mini kit, Geneaid, USA). Briefly, whole blood was centrifuged at 3500 xg for 5 minutes. 200-300 µL of the buffy coat were transferred to 1.5 mL micro centrifuge tubes and mixed with 900 µL of RBC Lysis solution. Then the procedure was continued as described above for the whole blood.

2.2.6 Assessment of DNA quality and quantity

The quality and quantity of the DNA samples were assessed by loading them on 1% agarose gel parallel to 1 kB DNA ladder. Electrophoresis was performed at 100 Volts and gels were visualized using a UV transilluminator. DNA samples were stained using ethidium bromide.

2.2.7.1 Amplification of β-globin gene

The 5' part of β-globin gene was amplified using primer pair: Bglob-F1 and Bglob-R1 yielding a 916-bp amplicon table (2.2). The 3' end of the β-globin gene was amplified using primer pair: Bglob-F2 and Bglob-2 yielding a 667-bp amplicon. The latter two pairs of primers were described earlier(Clark & Thein, 2004). Gene amplification was done using Polymerase Chain Reaction (PCR) technique and the lyophilized Hot-start PCR master mix (AccuPower® HotStart PCR PreMix, BIONEER, Korea). This lyophilized master mix contained hot start Taq DNA polymerase, Buffer, $MgSO_4$, dNTPs and the loading buffer. Primers, DNA samples and nuclease free water were added to the master mix as shown in Table (2.3) Thermal cycling was performed using a thermal cycler from Esco Healthcare Ltd (USA) as shown in table (2.4).

Table 2.2: Sequence and location of PCR primers used for analysis of α- and β-globin genes as well as *Xmn* I polymorphism of $^{G}\gamma$-globin gene.

Primer	Sequence (5´→3´)	GenBank Accession no.	Coordinates	Gene
Bglob-F1	CGA TCT TCA ATA TGC TTA CCA A	U01317.1	61830 61851	B
Bglob-R1	CAT TCG TCT GTT TCC CAT TCT A	U01317.1	62745 – 62724	B
Bglob-F2	CAA TGT ATC ATG CCT CTT TGC A	U01317.1	63214 – 63245	B
Bglob-R2	TGC AGC CTC ACC TTC TTT CAT	U01317.1	63870 63890	B
P51	CTGCACAGCTCCTAAGCCAC	J00153	7254-7273 11072-11090	α_2 α_1
P52	CCTCCATTGTTGGCACATTCC	J00153	7531-7551	α_2
P54	CTCAAAGCACTCTAGGGTCC A	J00153	11497-1517	α_1
P55	GTCCACCCCTTCCTTCCTCA	J00153	5687-5706 9247-9266	$Y2^1$ $Y1^1$
P59	CTCTAGGTCACCCTGTCATCA	J00184	26-46	Ψζ1
P60	CTCTGTCGTGTAGACGCCGA	M33022	423-442	θ2
P71	TACCCATGTGGTGCCTCCAT G	J00153	3068-3088	$\Psi\alpha_1$
P72	TGTCTGCCACCCTCTTCTGAC	J00153	8894-8915	$NH\text{-}II^2$

IVSID52 W	GGAGGCCCTGGAGACGTGAG	Z84721	33847-33877	α_2
A2D5M	GTATGGTGCGGAGGCCCTGG AGACGC	Z84721	33856-33875	α_2
A2R904	GTCTGAGACAGGTAAACACC TCCAT	Z84721	34642-34618	α_2
α-CF	GGAGGGTGGAGACGTCCTG	Z84721	33538-33556 37344-37360	α_2 α_1
X4GG158F	AAC TGT TGC TTT ATA GGA TTT	U01317.1	33862-33882	$^{G}\gamma$
XGG-158R	TTT TAT TCT TCA TCC CTA GC	U01317.1	34453-34434	$^{G}\gamma$

[1]Y1 and Y2 refer to homologous regions Y1 and Y2 overlapping α_2 and α_2-globin genes.

[2]NH-II: non-homologous region 2.

Table 2.3: Amplification of β-globin gene

Reaction components	**Volume (µL)**
Lyophilized master mix	0
DNA sample (~30 ng/µL)	3
Forward primer (10 µM)	0.5
Reverse primer (10 µM)	0.5
DEPC-Water	16
Total volume	20

Table 2.4: Amplification of β-globin gene using Thermal cycle program

Cycles	Step	Duration	Temperature
1X	Initial denaturation	5 min	94°C
35X	Denaturation	45 sec	94°C
	Annealing	45 sec	55°C
	Extension	80 sec	72°C
1X	Final extension	5 min	72°C

The PCR products were analyzed by running them on 1% agarose gel.

2.2.7.2 Gel purification

The gel was purified by cutting the target DNA fragment from the agarose gel and the DNA fragment was purified using the AccuPower Gel Purification kit (Bioneer, Korea) per the manufacturer's instructions. Briefly, the gel slice was weighed in a clean 1.5 mL micro-centrifuge tube. Three volumes of the gel binding buffer were added to gel slice, incubated at 60°C for 10 minutes and the mixture was mixed every 3 minutes. One volume of absolute isopropanol was added to the mixture, mixed gently, the mixture was transferred to the DNA binding column tube and centrifuged for 1 minute at 13,000 xg The flow through was poured off and the DNA binding column was washed with 500 µL of buffer 2, centrifuged for 1 minute at 13,000 xg, the flow was poured off and this step was repeated twice. The DNA binding column was dried by centrifugation at 13,000 xg for 3 minutes to remove the residual propanol. The DNA binding filter was transferred to a new 1.5 mL micro centrifuge tube, 45 µL of elution buffer were added to the center of the binding column, incubated at least 1 minute at room temperature, and then the DNA fragment was eluted by centrifugation at 13,000 xg for 2 minutes. Purified DNA fragments were used for DNA sequencing.

The amplification products were analyzed by running the product on 1% agarose gel.

2.2.7.3. DNA sequencing

The purified DNA fragments or PCR products were sequenced using the Forward and reverse primers. For this purpose the PCR products along with either Forward or reverse primers were sent for DNA sequencing to Hy-labs laboratories in Jerusalem. The DNA sequence results were analyzed visually and then using the BLAST bioinformatics tool.

2.2.8.1 Analysis of α-globin gene deletion mutations

The primers P51 to P72 (Table 2.2) were used for gap PCR analysis of deletion mutations of the α-globin genes (Oron-Karni, Filon, Oppenheim, & Rund, 1998). The primers P52 to P72 were used in four different reaction mixtures (reactions A to D) for detection of the four common α-globin gene deletion mutations: $-\alpha^{3.7}$, $-\alpha^{4.2}$, --MED/ and $\alpha\alpha\alpha^{anti3.7}$. Reaction A is a multiplex reaction which detects the ⁻MED/ mutation and contains the following primers: P51 (60 ng), P52 (40 ng), P54 (60 ng), P59 (40 ng), and P60 (40 ng). Reaction B detects the - $\alpha^{3.7}$/ mutation and contains the following primers: P55 (60 ng) and P54 (60 ng). Reaction C detects the -$\alpha^{4.2}$/ mutations and contains the following primers: P71 (40 ng), P72 (60 ng), and P52 (20 ng). Reaction D detects the $\alpha\alpha\alpha^{anti3.7}$ triplication and contains the following primers: P55 (59 ng), P52 (63.08 ng). All PCR reactions (reactions A to D) were performed with a Hot Start ready PCR mix (Bioneer) using ~100 ng of genomic DNA and 5 % DMSO. Each 20 μL reaction contained the manufacturer's buffer (1X), 1.5 mM $MgCl_2$, 0.25 mM of each dNTP and Hot-Start Taq DNA Polymerase. Amplification was performed with an initial heat activation step of 5 minutes at 94°C followed by 35 cycles, each consisting of 94°C 1 minute, 58°C for 1 minute, and 72°C for 2.5 minutes, and a final extension step of 72°C for 10 minutes. The PCR products of reactions A to D were analyzed on 2% agarose gel and interpreted as described in Table (2.5).

Table 2.5: The α-globin gene genotypes detected using the multiplex gap-PCRs and the size of the corresponding amplicons in bp.

PCR reaction							
	---MED A			$-\alpha^{3.7}$ B	$-\alpha^{4.2}$ C		$\alpha\alpha\alpha^{anti3.7}$ D
Primers→ Alleles |	P59-P60	P51-P52	P51-P54	P55-P54	P71-P72	P71-P52	P55-P52
Aα	-	298	446	2271		233	1865
-MED	561	-	-	-		-	-
$-\alpha^{4.2}$	-		446	2271	1596	-	-
$-\alpha^{3.7}$	-		446	2013		233	-
$A\alpha\alpha^{anti3.7}$							1865-2123

For detection of the IVS2-5nt del mutation, ARMS PCR using the following primers IVSID52W, A2D5M and A2R904 was used as described by (Lacerna et al, 2007). The mutant forward primer A2D5M was slightly modified and the forward wild type primer IVSID52W was redesigned to increase the specificity of the RCR. The PCR reaction was performed with a Hot-start ready PCR mix (Bioneer) using ~100 ng of genomic DNA and 5 % DMSO. Each 20-µL reaction contained the manufacturer's buffer (1X), 1.5 mM $MgCl_2$, 0.25 mM of each dNTP, 0.25 µM of each forward and reverse primers and Hot-start Taq DNA Polymerase. The mutant sequence was amplified using the forward primer A2D5M and the common reverse primer A2R904. The Wild type sequence was amplified using the forward primer IVSID52W and the common reverse primer A2R904. Amplification was performed with an initial heat activation step of 5 minutes at 94°C followed by 30 cycles, each consisting of denaturation at 94°C for 50 seconds, annealing at 60°C for wild type reaction or 62°C for the mutant reaction for 50 seconds, and extension at 72°C for 1 minute and a final extension step of 72°C for 5 minutes. However, this ARMS PCR generated non-specific fragments that made accurate

discrimination of mutant and wild type alleles difficult and thus we decided to detect the IVS2-5nt del by DNA sequencing of the α2-globin gene.

For DNA sequencing, the α2-globin gene was amplified using PCR primers, forward primer P52(Oron-Karni et al., 1998) and reverse primer α-CF (Clark & Thein, 2004)). The α2-globin gene was amplified using the primer pair: α-CF and α2-R2, which yields an amplicon of 1084 bp long. PCR amplification was performed using Hot-start ready PCR mix (Bioneer) using ~100 ng of genomic DNA and 7.5 % DMSO. Each 20-µL reaction contained the manufacturer's buffer (1X), Hot-Start Taq DNA polymerase, 1.5 mM MgCl2, 0.25 mM of each dNTP and 0.25 µM of each primer. Amplifications were performed with an initial heat activation step of 5 minutes at 94°C followed by 35 cycles, each consisting of 94°C for 1 minute, 55°C for 1 minute and 72°C for 1.5 minute, followed by a final extension step of 72°C for 5 minute. The PCR product was analyzed on 1% agarose gel and purified from the gel as described in section (2.2.8.2). DNA sequencing was performed as described as in section (2.2.8.3)

2.2.9. Detection of XmnI polymorphism in $^{G}\gamma$-globin gene

The *Xmn* I SNP (-158 C>T) at the 5'end of $^{G}\gamma$-globin gene was detected using RFLP-PCR. For this purpose the 5' end of the $^{G}\gamma$-globin gene flanking the *Xmn* I SNP (-158 C>T) was amplified using the primers X4GG158F (Nadir Ali a, 2015) and XGG-158R (Rujito et al., 2016)

PCR amplification was performed using Hot-start ready PCR mix (Bioneer) using ~100 ng of genomic DNA. Each 20-µL reaction contained the manufacturer's buffer (1X), Hot-Start Taq DNA polymerase, 1.5 mM MgCl2, 0.25 mM of each dNTP and 0.25 µM of each primer. Amplifications were performed with an initial heat activation step of 5 minutes at 94°C followed by 35 cycles, each consisting of 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute, followed by a final extension step of 72°C for 5 minute.

Ten µL of the PCR product (592-bp long) was digested with 5 Units of *Xmn* I enzyme for 2 hours at 37°C. The *Xmn* I reaction was analyzed using 3% agarose gel. *Xmn* I digestion

generates 3 possible genotypes: 147, 445 bp **(+, C allele)**; 592 **(-, T allele) and** 147, 445, 592 bp **(+/-, CT alleles).**

For confirmation of the RFLP PCR, 2 samples were analyzed by DNA sequencing of the PCR product.

2.2.10. Ethical consideration

The study protocol was approved by the Research ethics committee at Al-Quds University. A copy of the ethical approval is shown in Appendix 2. Also permission from the Ministry of Health was obtained to review the medical files of patients at Thalassemia care centers. A copy of the MOH approval is shown in Appendix 3.

An informed consent was obtained from individual study participants or their guardians in case of minors.

2.2.11. Statistical analysis

Descriptive statistics including mean and standard deviation were calculated using SPSS version 22.

Chapter three

Results

3.1 Study samples

This study aimed to determine the spectrum of genetic mutations associated with β-thalassemia intermedia as well as to investigate the hematological characteristics and the level of clinical management of β-thalassemia intermedia in the West Bank region, Palestine. In order to determine the patients diagnosed with βTI, the researchers visited all eight Thalassemia Health Care centers in the West Bank and collected data about potential 72 cases of BTI that met the inclusion criteria of this study. All patients were contacted and asked to participate, but only 55 potential βTI patients accepted to participate in this study. Samples were collected in the period September 2016 to April 2017. From the 55 cases, 3 cases were found to have Sickle β-thalassemia and 1 case with Sickle Cell C disease and thus were excluded, leaving 51 unrelated cases of βTI for further investigation and analysis.

The geographic distribution of βTI cases in the West Bank is shown in Figure (3.1). βTI cases were collected from 6 governorates, while none of the thalassemia patients at Jericho Hospital and Beit Jala Hospital fulfilled the inclusion criteria. The highest number of cases has been collected from Hebron and Nablus, respectively.

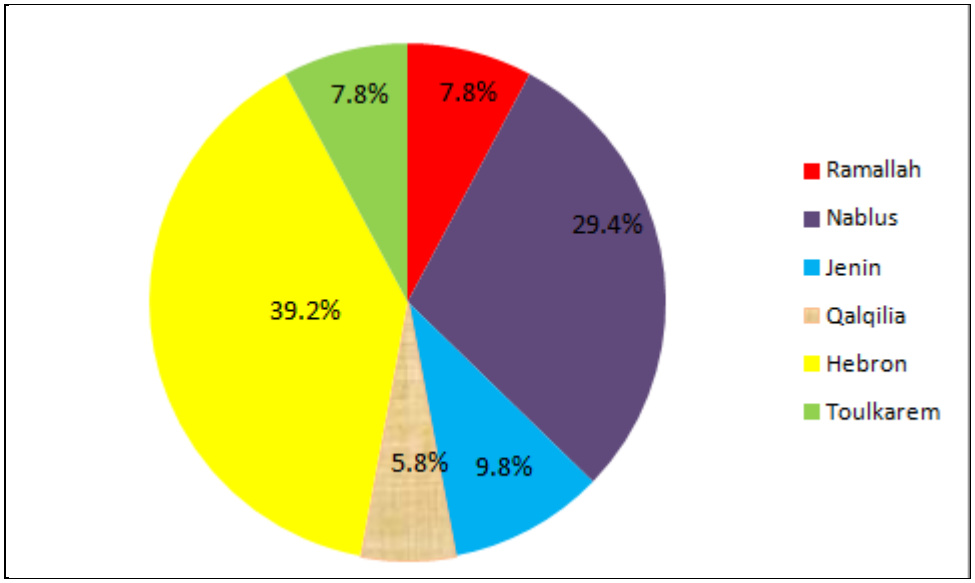

Figure (3.1): Distribution of βTI cases based on residence place.

3.2 Clinical findings

Fifty-one of βTI patients were enrolled in this study. The general characteristics of the 51 βTI patients are shown in Table 3.1. This study included 27 males and 24 females. The patients' age ranged from 4 years to 71 years with a mean 18.9 years old. The low age mean of patients, shows that this is a young population where 26 patients (50.9%) are below 18 years old and 25 patients (49%) are above 18 years old. The age at diagnosis varied between one month and 25 years with mean 2.9. Among the 51 βTI patients, 9 patients (17.6%) were never transfused, also 9 patients (17.6%) received occasional transfusions (once or twice a year), while the remaining 33 patients (64.7%) were receiving regular transfusion (three to four times per year) (Table 3.1).

Analysis of the general health status of βTI patients revealed that 19 patients (37.2%) were splenectomized, while 9 patients (17.6%) suffer from splenomegaly. From the βTI patients who were never transfused (n=9), five patients were splenectomized; three patients were suffering from splenomegaly while one patient did not show symptoms of splenomegaly at time of enrollment in this study.

From the 51 βTI patients, 31 patients have received Iron chelation therapy for at least one year at the time of enrollment. Iron chelating drugs that were used by the βTI patients included deferoxamine (Deferral) in eight patients, deferasirox (Exjade) in nineteen patients and, four patients used a combination of the latter two drugs. Twenty βTI patients did not use iron chelation therapy.

Most βTI patients were the result of consanguineous marriages, as the parents of all patients were relatives except three. None of the patients had ever leg ulcers, diabetes, hypertension (chronic disease) or documented venous thromboses.

The level of Hb in βTI patients ranged between 6.4 and 13.9 g/dL with a median of 8.3 g/dL at the time of enrollment. The serum ferritin levels were taken from the medical files and were available for 31 patients only. Ferritin levels ranged from 92 to 8600 ng/mL with a median of 1800 ng/mL.

Table 3.1: General characteristic of the 51 Palestinian β-thalassemia intermedia patients

Parameter	**Value**
Males, n (%)	27 (52.9%)
Females, n (%)	24 (47.1%)
Age, years	Mean: 18.9 95% CI: 15.9 – 21.8
Age at diagnosis, years	Mean: 2.9 95% CI: 2.0 – 3.7
Hb, g/dL	Median:8.3 Range: 6.4-13.9
Serum Ferritin*, ng/ml	Median: 1800 Range: 92-8600
Splenectomy , n (%)	19 (37.3%)
Splenomegaly , n (%)	9 (17.6%)
Transfusion frequency, n (%) ▪ Never ▪ Occasionally (1-2 times / year) ▪ Regular (3-4 times per year)	 9 (17.6%) 9 (17.6%) 33 (64.7%)
No. of patients on iron chelation therapy, n (%)	31(60.8%)

*Ferritin levels are reported for 31 patients only.

3.2 Hematological findings

The level of Hb in βTI patients ranged from 6.4 to 13.9 g/dL with a median of 8.3 (mean of 8.4 g/dL; 95% CI: 8.0 – 8.9) at the time of enrollment (Table 3.1). The serum ferritin levels were available for 31 patients and ranged from 92 to 8600 ng/mL with a median of 1800 ng/mL (Table 3.1). In addition two patients who were never transfused, had high serum ferritin (>2700 ng/mL) and one patient was diagnosed at three years of age with the genotype β°/β°, has never been transfused and has an elevated level of serum ferritin (490 ng/mL).Twenty two patients who had regular and occasional transfusion had high serum Ferritin >1000 ng/mL.

Hemoglobin electrophoresis was performed for all study samples (Table 3.3). Since most patients have a regular or occasional blood transfusion, patients were asked to donate blood samples for the study just before taking the next transfusion, mostly around 3 months after the last blood transfusion. HbF ranged between 1.3 and 83% While HbA_2 ranged between 2.3 and 8.5 %. The level of HbF and HbA2 showed large variations reflecting the different genetic mutations responsible for the thalassemic phenotype.

3.3 β-thalassemia mutations

Identification of the β-Thalassemia intermedia mutations among Palestinians was performed by Sanger DNA sequencing (Figure 3.2). A total of seven different mutations of β-globin gene were detected and comprised null mutations (β^0) as well as mild mutations (β^+) allowing reduced synthesis of β-globin chain (Table 3.2). These seven β-globin gene mutations generated 10 genotypes, that in turn were responsible for the phenotype of βTI in our study subjects (Table 3.3). The most common allele of β-globin gene encountered was IVS-I-6 (T>C) with a frequency of 76.5% followed by IVS-I-110 (G>A) with a frequency of 7.8% IVS –II-I (G>A) with a frequency 6.9% and the remaining 4 alleles IVS-I-I, codon 37, IVS-II-848 and -101, accounted each for a frequency of 1-3% (Table 3.2). The most frequent single genotype was IVS-I-6 (T>C)/IVS-I-6 (T>C) with a frequency 74.5%, followed by IVS-II-I(G>A)/IVS-II-I (G>A) with a frequency 5.8% and IVS-I-110 (G>A)/IVS-I-110 (G>A) with a frequency 5.8%, and the remaining 7 genotypes each accounted for about 2% (Table 3.3).

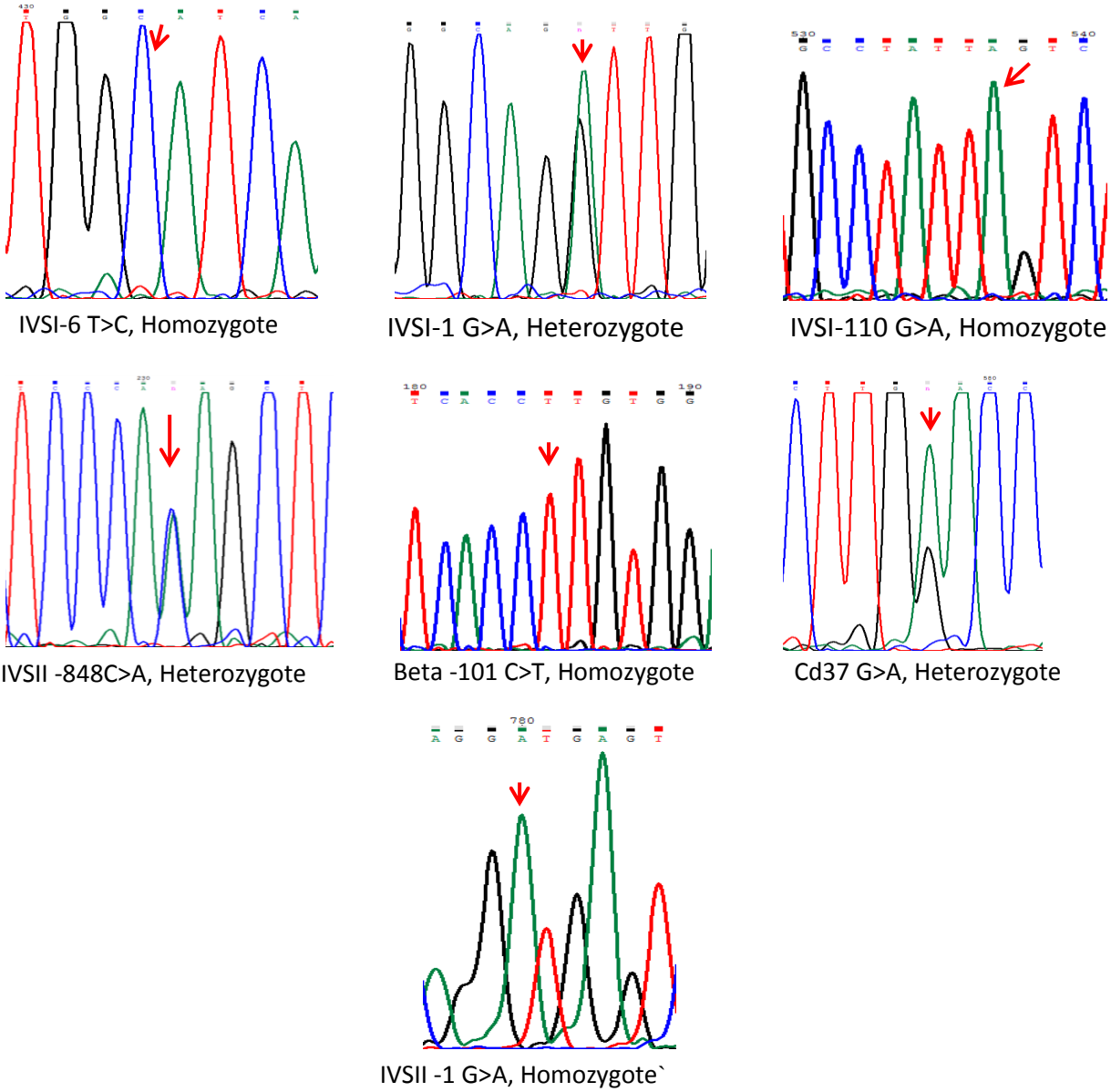

Figure 3.2: Representative chromatograms of the seven β-globin gene mutations detected in this study.

Table (3.2): β-Globin gene mutations detected in Palestinian β-thalassemia intermedia patients. Allele frequencies were calculated based on 102 chromosomes from 51 patients.

Mutation	Nt. Substitution	Type of mutation	HGVS nomenclature	Allele frequency (%)*
IVS-I-6	T>C	β^{+}	HBB:c.92+6T>C	76.5
IVS-I-I	G>A	β^{0}	HBB:c.92+1G>A	2.0
IVS-I-110	G>A	β^{+}	HBB:c.93-21G>A	7.8
Codon 37	TGG>TGA	β^{0}	HBB:c.114G>A	3.0
IVS-II-I	G>A	β^{0}	HBB:c.315+1G>A	6.9
IVS-II-848	C>A	β^{+}	HBB:c.316-3C>A	1.0
Beta -101	C>T	β^{++}	HBB:c.-151C>T	2

*The normal allele (β^{A}) is present in one case from 51 patients and is not shown in the above table, making the sum of allele frequency 99.6 rather 100%.

To enable analysis of phenotype-genotype correlations, all the βTI patients were grouped into four major genotypes (table 3.3)

First: The **mild** genotype **β^{+}/β^{+}** was further classified into **four subgroups (Table 3.3)**.

Group I: Homozygosity for IVS-I-6 C>T. This group included 38 patients showed a variable clinical picture with a mean Hb level of 8.4 g/dl. From 38 patients, 24 patients had a regular blood transfusion, 7 patients had occasional transfusion, while the last seven had never been transfused. In this group, 15 patients were splenectomized while 6 patients suffer from splenomegaly.

Group II: Compound heterozygous for IVS-I-6 C>T / IVS-I-110 G>A with $\alpha^{IVSI(-5nt)}\alpha/\ \alpha\alpha$. This group included one patient with Hb vale of 8.7 g/dL. Hb electrophoresis was not determined because he had blood transfusion one month before sample collection. This patient had regular blood transfusion, was not splenectomized and showed no symptoms of splenomegaly at time of enrollment.

Group III: Homozygosity for IVS-I-110 G>A. This group included three patients. One of them had $\alpha^{3.7}/\ \alpha\alpha$, the other two had normal α genotype. Two patients had regular blood transfusion while one had occasional transfusion. In addition, one patient was splenectomized; one suffered from splenomegaly and the third patient showed no symptoms of splenomegaly.

Group IV: Homozygosity of the silent mutation beta -101 C>T. This group included one patient and showed the highest Hb (13.9 g/dL) value among all study patients. The patient aged 13 years old and was never transfused.

Among all 4 subgroups, group I showed the highest percentage of HbF (10.2 ±6.2%).

The **second** genotype **β^{+}/β°** was classified into three different heterozygote subgroups, each containing one patient. Hb values ranged from 6.5 to 7.1 g/dL. One patient (β-globin genotype: IVS-II-I G>A/ IVS-II -848 C>A) was heterozygote for α-thalassemia ($-\alpha^{3.7}$/ αα) and $^{G}\gamma$-globin gene *Xmn* I SNP and showed the highest HbF percentage. All 3 patients with this $\beta^{+}\backslash\beta^{\circ}$ genotype had regular blood transfusion.

The **third** genotype **β^{+}/β^{A}** was seen in one patient in association with α-globin gene triplication ($\alpha\alpha/\alpha\alpha\alpha^{anti3.7}$), this patient had occasional blood transfusion.

Table (3.3): Hematological data, frequency of β- thalassemia mutations, α- thalassemia genotypes as well as *Xmn* I polymorphism of Gγ-globin gene among Palestinian β-thalassemia intermedia patients.

β- thalassemia genotypes	N (%)	*Xmn*I SNP	α-thalassemia	Hb g/dL	Hb A2 %	Hb F %
β^{+}/β^{+}						
IVS-I-6 / IVS-I-6	38 (74.5)	-/-	αα/ αα	8.4 ± 1.1	6.4 ± 1.4	10.2 ± 6.2
IVS-I-6 / IVS-I-110	1 (1.96)	-/-	$\alpha^{IVSI(-5nt)}\alpha$/ αα	8.7	ND	ND
IVS-I-110 / IVS-I-110	3 (5.88)	-/-	$\alpha^{3.7}$/ αα (1:0:0)	9/11.7/9.5	3.3/3.2/8.5	1.7/6.4/17
Beta -101/ Beta -101	1 (1.96)	-/-	αα/αα	13.9	4.6	7.4
Subtotal	43 (84.3)					
β^{+}/β°						
IVS-I-6 / Cd37	1 (1.96)	-/-	αα/ αα	6.9	3.3	6.6
IVS-I-110 / Cd37	1 (1.96)	-/-	αα/ αα	6.5	2.5	30.9
IVS-II-I / IVS-II -848	1 (1.96)	+/-	$-\alpha^{3.7}$/ αα	7.1	3	49.1
Subtotal	3 (5.88)					
$\beta^{\circ}/\beta^{\circ}$						
IVS-II-I / IVS-II-I	3 (5.88)	+/+	αα/ αα	8.7 ± 0.86	2.1 ± 0.23	72.7 ± 7.3
IVS-I-I / Cd37	1 (1.96)	-/-	$\alpha^{IVSI(-5nt)}\alpha$/ αα	8.7	2.9	46.8
Subtotal	4 (7.84)					
β^{+}/β^{A}						
IVS-I-I / β^{A}	1 (1.96)	-/-	αα/ $\alpha\alpha\alpha^{anti3.7}$	ND	3.8	6.3
Total	51 (100)					

ND: Not determined.

The **forth** genotype is **β°/β°**. This group was comprised of four patients with Hb values ranging from 7.7 to 9.8 g/dL. Usually, β-thalassemia patients with β°/β° genotype and normal α- globin gene show the βTM phenotype Three patients were homozygout for $^{G}\gamma$-globin gene *Xmn* I SNP and one patient was heterozygote for α-thalassemia ($\alpha^{IVSI(-5nt)}\alpha$/ αα). Three patients had regular blood transfusion and one was never transfused. One patient was splenectomized and one showed symptoms of splenomegaly (Table 3.4). This group also showed the highest percentage of HbF among the four groups listed in Table 3.3.

Table (3.4): Hematologic data and genetic modifiers for the 4 βTI patients with β^0/β^0 genotype.

Patient	**1**	**2**	**3**	**4**
Age / age at diagnosis	17/6	16/5	29/3	39/1
Age at first transfusion, years	6	5	<1	1
Hb, g/dL	7.7	8.6	9.8	8.7
MCV, fL	66.5	91.8	76.4	99.0
MCH, pg/cell	23.1	26.1	26.2	28.6
HbF, %	68.0	67.0	83.0	46.8
HbA2, %	2.3	1.8	2.3	2.9
β-thalassemia genotype	IVS-II-I/ IVS-II-I	IVS-II-I/ IVS-II-I	IVS-II-I/ IVS-II-I	IVS-I-I/ Cd37
*Xmn*I SNP γ^{G} -158 C>T	+/+	+/+	+/+	-/-
α- thalassemia genotype	αα/αα	αα/αα	αα/αα	$\alpha^{IVSI(-5nt)}\alpha$/ αα

3.4. α-thalassemia mutations

The five common α- thalassemia deletion mutations were detected using gap-PCR (-$\alpha^{3.7}$/, -$\alpha^{4.2}$/, --^{MED}/ and αα$\alpha^{anti\ 3.7}$) or DNA sequencing (α2-IVSI-5nt del). Four different multiplex gap-PCR reactions (reaction A to D) were performed for the detection of four deletion mutations affecting the α-globin genes: -$\alpha^{3.7}$/, -$\alpha^{4.2}$/, --^{MED}/ and αα$\alpha^{anti\ 3.7}$, respectively. These mutations represent the most prevalent deletion mutations affecting the α-globin genes in the Mediterranean region. Representative agarose gels for reactions A to D are shown in Figures 3.3-3.6

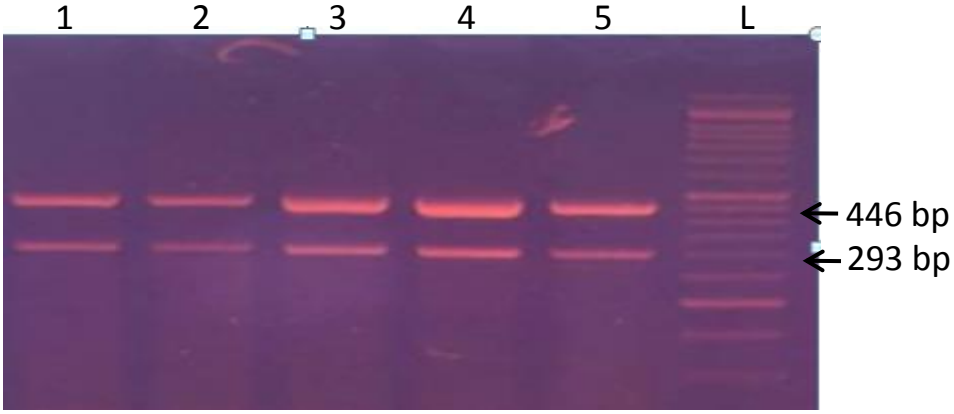

Figure 3.3 : Representative agarose gel for the –^{MED}/ multiplex PCR reaction.
Lanes 1-5: negative for –^{MED}/αα mutation; Lane L: 50 bp DNA ladder.

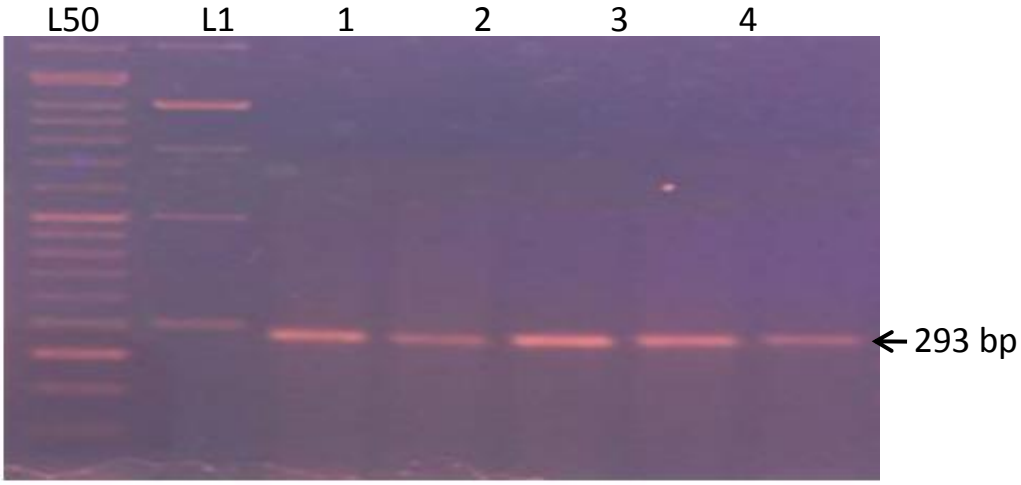

Figure 3.4: Representative agarose gel for the –$\alpha^{4.2}$ PCR reaction. Lanes 1-5: negative for –$\alpha^{4.2}$ mutation; Lane L50: 50 bp DNA ladder; Lane L1: 1kb DNA ladder.

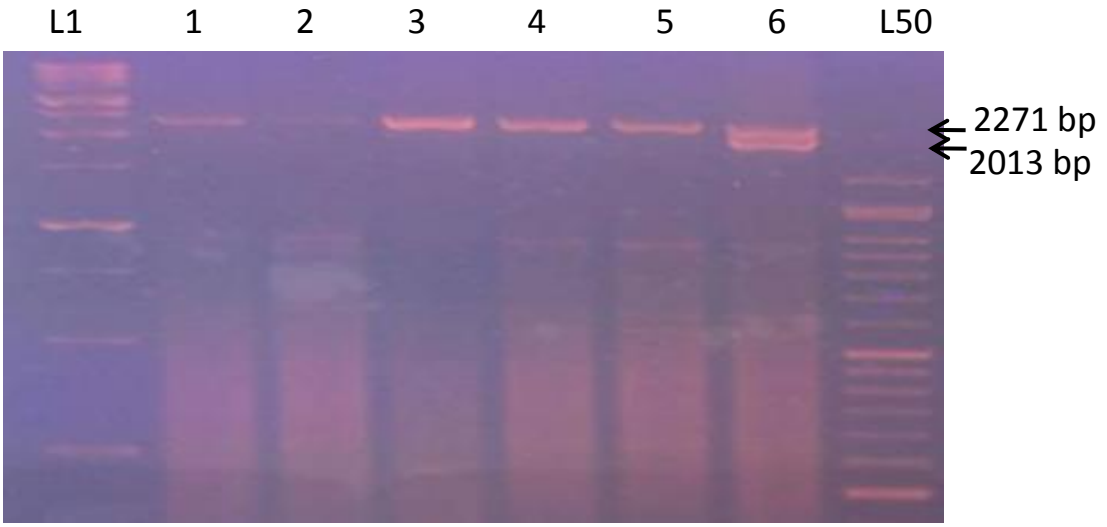

Figure 3.5: Representative agarose gel for the $-\alpha^{3.7}$ PCR reaction. Lanes 1-5: negative for $-\alpha^{3.7}$/ mutation, lane 6: $-\alpha^{3.7}/\alpha\alpha$ genotype; L1: 1 kb DNA ladder; L50: 50 bp DNA ladder.

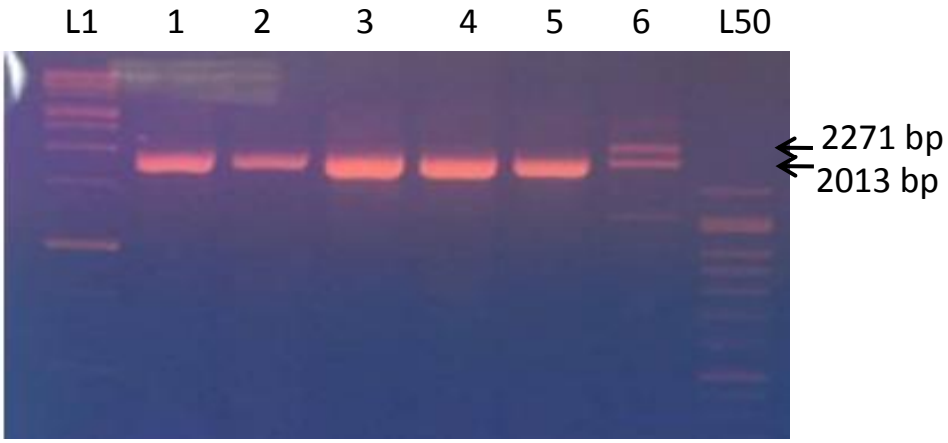

Figure 3.6: Representative agarose gel for the $\alpha\alpha\alpha^{anti\ 3.7}$ PCR reaction. Lanes 1-5: negative for $\alpha\alpha\alpha^{anti\ 3.7}$ triplication; lane 6: $\alpha\alpha\alpha^{anti\ 3.7}$ triplication; L1: 1 kb DNA ladder; L50: 50 bp DNA ladder.

For detection of the $\alpha^{IVSI(-5nt)}$ mutation, the 5' region of the α2-globin gene including exons 1 and 2 were amplified by PCR and analyzed by Sanger DNA sequencing. Two cases of βTI were found heterozygote for this mutation. A representative chromatogram showing this mutation is shown in Figure 3.7.

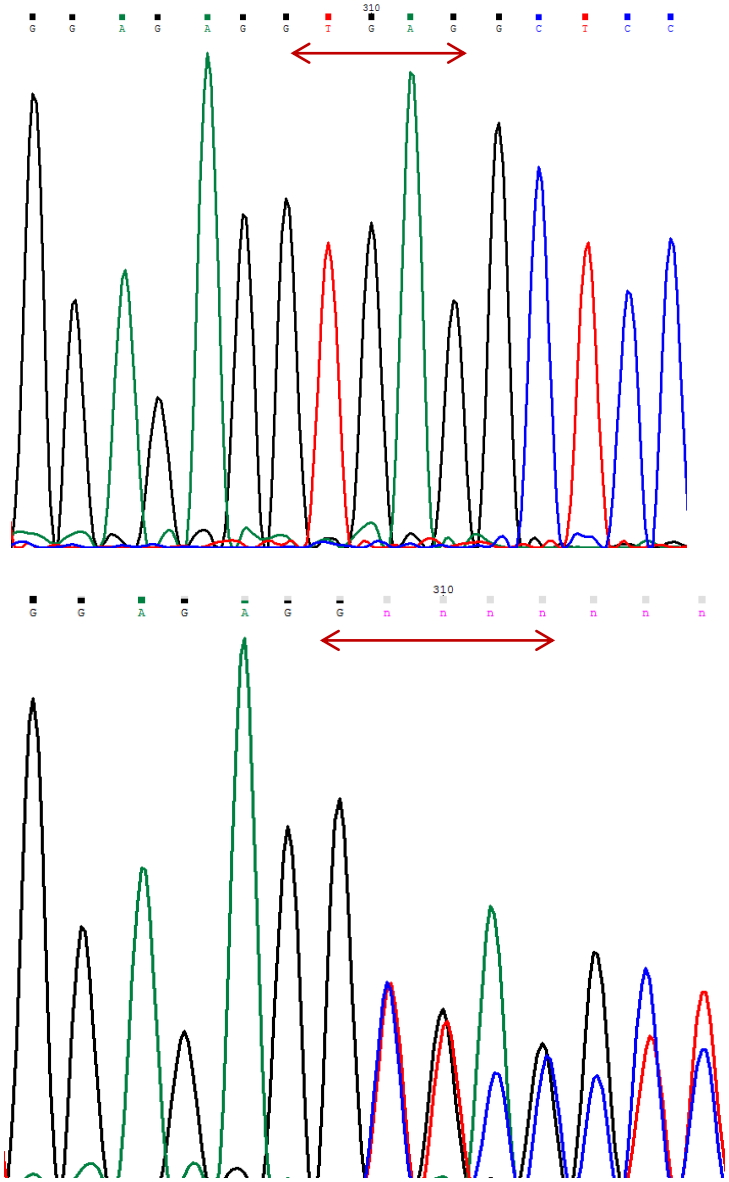

Figure 3.7: Identification of α2-IVSI-5nt del mutation by DNA sequencing. (A) Wild type allele. (B) Mutant allele. The double headed arrow indicates the 5 nucleotides deleted in this mutation.

From the 51 patients` samples analyzed, 5 samples (9.8%) were found to have α-thalassemia mutations. Two patients were heterozygote for $-\alpha^{3.7}/$ mutation, two patients were heterozygous for α2-IVSI -5nt/ del mutation and one patient was heterozygote for the $\alpha\alpha\alpha^{anti\ 3.7}/$ mutation. The $-\alpha^{4.2}/$ and $--^{MED}/$ mutations were not detected in this study. Table (3.5) summarizes the α-thalassemia genotypes detected in this study

Table (3.5): α-thalassemia genotypes detected among 51 βTI patients.

Mutation	**No.**	**Percent (%)**
$-\alpha^{3.7}/$	2	4
$\alpha\alpha\alpha^{anti\ 3.7}/$	1	2
$-\alpha^{4.2}/$	0	0
$--^{MED}/$	0	0
α2-IVSI -5nt del/	2	4
Total	5	10

3.5. *Xmn* I polymorphism of the Gγ-globin gene

The *Xmn* I SNP (-158 C>T, rs7482144) of Gγ-globin gene was detected by RFLP PCR. Out of 51 βTI patients, four patients (7.8%) were positive for ^{G}γ-globin gene *Xmn* I SNP. A representative agarose gels for this mutation is shown in Figure 3.8.

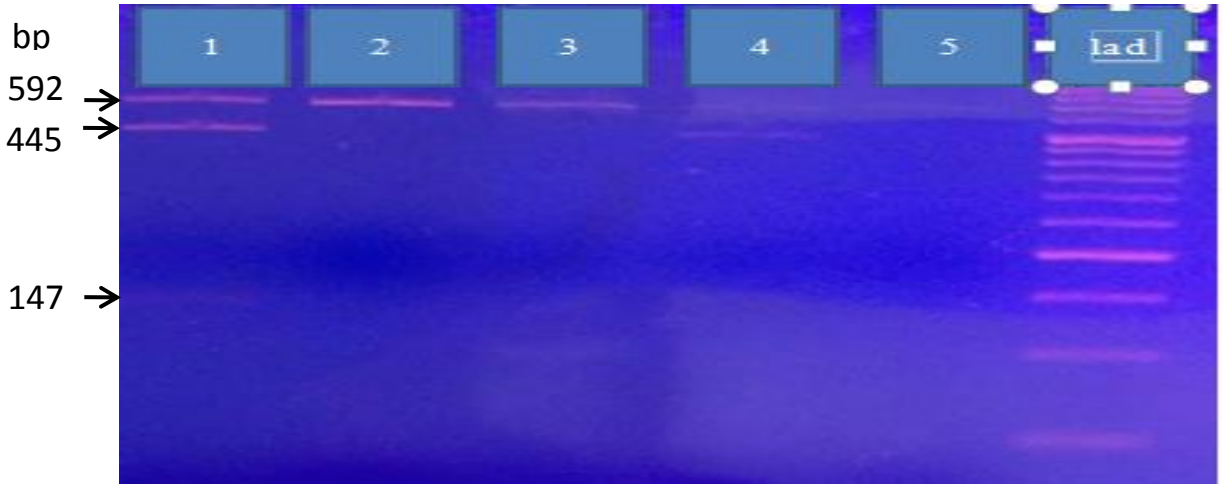

Figure 3.8: Representative agarose gel of RFLP-PCR for the ^{G}γ-globin gene *Xmn* I SNP. Lanes 2, 3,5: negative for *Xmn* I polymorphism (-/-); lanes 1, 4: heterozygous for *Xmn* I (+/-), lad: 50-pb DNA ladder.

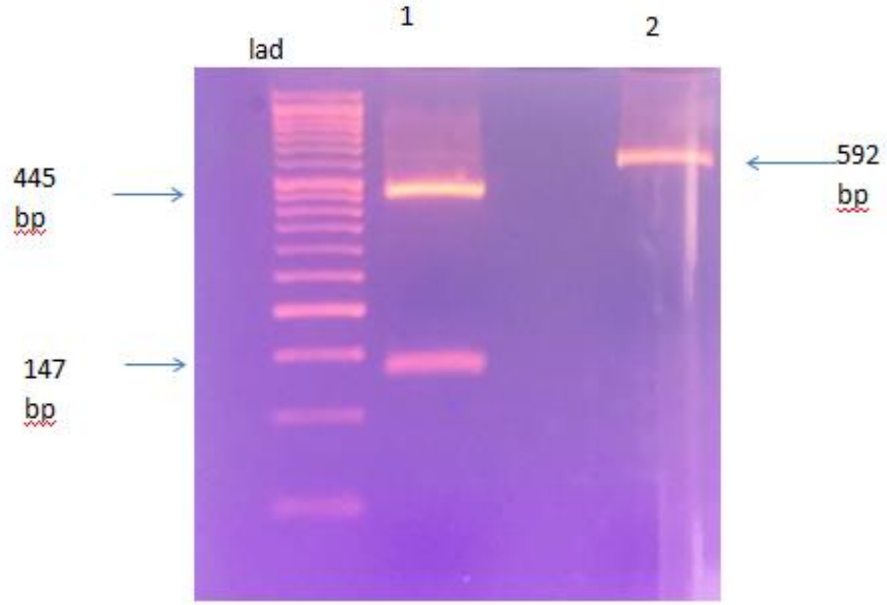

Figure 3.9: Representative agarose gel of RFLP-PCR for the ^{G}γ-globin gene *Xmn* I SNP. Lane 2: negative for *Xmn* I polymorphism (-/-); lanes 1: homozygous for *Xmn* I (+/+), lad: 50-pb DNA ladder.

For confirmation of the RFLP PCR, 2 samples were analyzed by DNA sequencing of the PCR product. A representative chromatogram showing this mutation is shown in Figure 3.7.

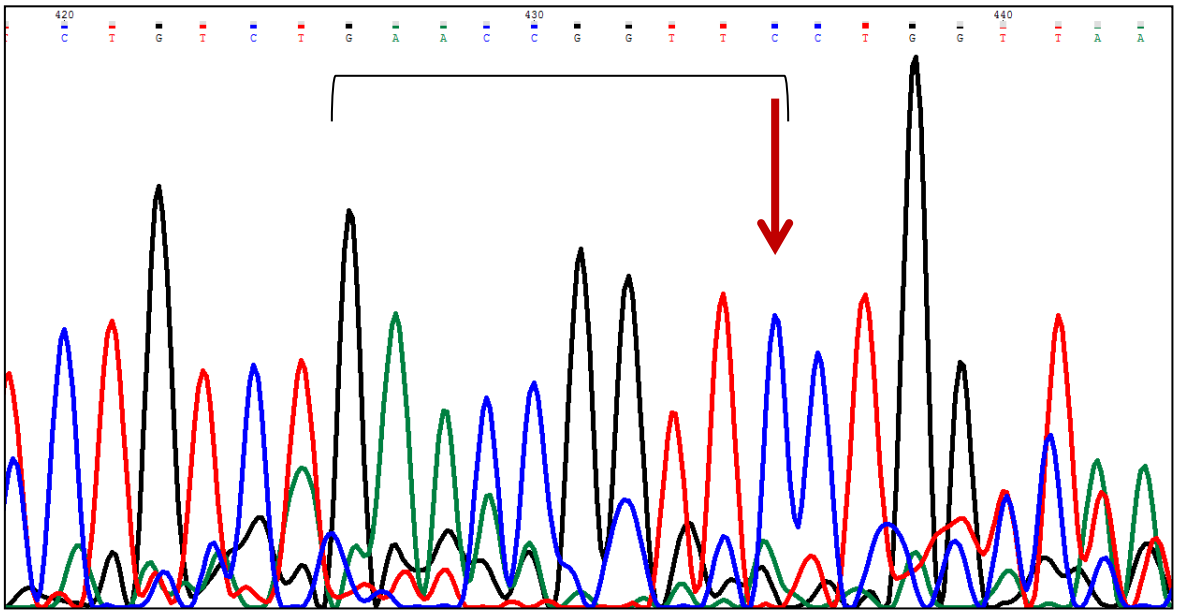

Figure 3.10: Identification of $^{G}\gamma$-globin gene *Xmn* I SNP by DNA sequencing.

3.6 Genetic modifiers of HbF levels

Several genetic modifiers for HbF levels in β-thalassemia have been reported. In this study the $^{G}\gamma$-globin gene *Xmn* I SNP and α-thalassemia mutation were examined (Table 3.6). The 3 βTI patients who were homozygote for the *Xmn* I SNP showed the highest average of HbF compared to HbF percentage of all samples or that heterozygote for α-thalassemia (Table 3.6). One sample was heterozygote for *Xmn* I SNP and α-thalassemia showed HbF percentage that is higher than the average of samples heterozygote for α-thalassemia, but lower than that of samples homozygote for *Xmn* I SNP. However, the limited number of samples positive for either *Xmn* I SNP or α-thalassemia does not permit a reliable statistical comparison of means among these groups.

Table 3.6: Genetic modifiers of HbF level among βTI patients. HbF percentages of all βTI patients are compared with samples homozygous and heterozygote for *Xmn* I SNP, α-thalassemia or a combination of both.

	All samples	***Xmn* I SNP (+/+)**	**α-thalassemia Heterozygotes**	***Xmn* I SNP & α-thalass**
N	51	3	5	1
HbF %				
Median	10.4	67.5	26.6	49.1
Range (min– max)	1.7 – 83.0	49.1 – 83.0	1.7 – 49.1	--
Hb g/dL				
Median	8.3	8.6	8.7	7.1
Range (min-Max)	6.4 – 13.9	7.7 – 9.8	7.1 – 9.0	--

Chapter Four

Discussion

Thalassemia is one of the most common genetically autosomal recessive disorders in the world as well as in Palestine. There are more than 200 genetic mutations responsible for the decreased production of β-globin chain (Georgiou et al., 2003). β-Thalassemia intermedia (βTI) is a type of thalassemia that lies between the two extreme transfusion dependent βTM and transfusion independent βTT. Different genetic mutations are responsible for βTI phenotype, including the inheritance of severe, mild or silent β-thalassemia alleles, coinheritance of α-thalassemia, and certain polymorphisms in γ-globin genes (Al-Allawi et al., 2014). The aim of this research is to study the molecular and hematological characteristics of βTI in the West Bank region, in order to establish the common genetic basis for βTI prevailing among Palestinian βTI patients and to provide a better diagnosis and management of patients.

A total of 51 βTI patients were investigated in this study. Most of the patients were from Hebron (39.2%), followed by Nablus (29.4%), Jenin (9.8%), Qalqilia (7.8%), Toulkarem (7.8%) and Ramallah (5.8%). The average age of βTI patients was 18.9 years and almost half of them (50.9%) were less than 18 years old (Table 3.1) which indicate that these patients are mostly a young population.

Analysis of the health data of the study patients showed that 64.7% of the patients had regular blood transfusion and the average Hb was 8.4 g/dL. Hb level should not be an indicator for initiation of transfusion therapy, except in patients with considerably severe anemia (Hb level <5 g/dL)(A. Taher, Vichinsky, Musallam, Cappellini, & Viprakasit, 2013)

In addition, the median serum ferritin among the study patients was 1800 ng/mL (range 92–8600 ng/mL) and only 31 patients received iron chelation therapy. The transfusion frequency is inappropriate and should be reviewed case by case to determine which case needs reduction of transfusion frequency. Since transfusion therapy should be restricted and used carefully to avoid complications associated with iron overload. In this regard, previous reports (Olivieri et al., 1994) showed that there is a significantly reduced risk for cardiac diseases and death in two-thirds of cases when ferritin levels were maintained

below 2500 ng/mL. In addition, other studies showed that a better prognosis for survival was associated with a low serum ferritin levels (Borgna-Pignatti, 2007; Rajaeefard et al., 2015; Roudbari, Soltani-Rad, & Roudbari, 2008). Several factors contribute to accumulation of iron in body tissues or iron overload including ineffective erythropoiesis, anemia and hypoxia leading to a compensatory increase in erythropoietin and a decrease in serum hepcidin. This in turn will increase intestinal iron absorption and liver iron accumulation(M. D. Cappellini, Cohen, Porter, Taher, & Viprakasit, 2014).

HbF values among our βTI patients ranged between 1.7 and 83% While HbA_2 values ranged between 2.3 and 8.5 %. The levels of HbF and HbA2 showed large variations reflecting the different genotypes responsible for the thalassemic phenotype among the study patients.

A total of seven different mutations of *HBB* gene were detected and comprised null mutations (β^0) as well as mild mutations (β^+) allowing reduced synthesis of β-globin chain. The most common allele of *HBB* gene encountered was IVS-I-6 (T>C) with a frequency of 76.5%, followed by, in decreasing order: IVS-1-110 (G>A), IVS –II-1 (G>A), Cd37 (G>A), IVS-I-1 (G>A), beta -101 (C>T) and IVS-II-848 (C>A) (Table 3.2). These results are interesting because the IVS-I-6 (T>C) allele was detected in a high percentage among our βTI patients.

In consistence with our results, this allele was found to be the most frequent allele among Israeli Arabs with βTI (or Palestinians in Israel; (57.7%)) (Rund, 1997), as well as among β-thalassemia patients from West Bank region (28.7%) (Darwish, El-Khatib, & Ayesh, 2005) and Southern region of West Bank (48.5%) (El-Latif, Filon, Rund, Oppenheim, & Kanaan, 2002) as shown in Table 4.1. While, the IVS-I-6 (T>C) allele was the third frequent allele among Palestinian β-thalassemia patients from Gaza strip (Sirdah et al., 2013). The current study included only βTI patients whose genotypes are mostly contributed by mild *HBB* genotypes (β+). This in turn may partially explain the different frequencies of the IVS-I-6 (T>C) allele among our βTI patients compared to the other two studies from the West Bank (Darwish et al., 2005; El-Latif et al., 2002) that used a mixed sample mostly comprised of βTM patients. In addition, the IVS-I-6 (T>C) allele is a mild allele and is probably less frequent among βTM patients compared to βTI patients. In

addition, the different frequencies of *HBB* mutations among our βTI patients compared to β-thalassemia patients from Gaza strip (Sirdah et al., 2013) reflects different genetic background of the Palestinian tribes in both regions of Palestine.

In contrast, analysis of the frequency of IVS-I-6 (T>C) allele among β-thalassemia patients in neighboring Arab countries showed that this allele was ranked as the second, third, fifth or sixth common allele, among other *HBB* alleles, in Iraqi Arabs (Al-Allawi, Puehringer, Raheem, & Oberkanins, 2015), Egyptians (Jiffri, Bogari, Zidan, Teama, & Elhawary, 2010), Lebanese (Makhoul et al., 2005), Jordanians (Sadiq, Eigel, & Horst, 2001) and Syrians (Jarjour, Murad, Moasses, & Al-Achkar, 2014), respectively.

Among our TI patients, 10 genotypes of *HBB* gene were detected. Of these genotypes, the most frequent single genotype was IVS-I-6 (T>C)/IVS-I-6 (T>C) and was encountered in 74.5% of βTI patients, followed by IVS-II-1(G>A)/IVS-II-1 (G>A) and IVS-I-110 (G>A)/IVS-I-110 (G>A) that were each encountered in 5.9% of βTI patients Table (3.3). The remaining six genotypes were each detected in 2% of βTI patients. These results indicate that the major contributing factor for βTI among our study population is the inheritance of the mild *HBB* allele (β+) and namely IVS-I-6 (T>C) allele.

The second common mutation identified in this study was the Mediterranean IVS-I-110 G>A with allele frequency of 7.8%. This allele was found to be the most frequent allele among β-thalassemia patients in Gaza strip (33.9%) (Sirdah et al., 2013). In addition, earlier reports from Palestine, found that the IVS-I-110 G>A allele was the second frequent allele among β-thalassemia patients in West Bank (17.1 %) (Darwish et al., 2005), and the third frequent allele among β-thalassemia patients from Southern region of West Bank (9.5 %) (El-Latif et al., 2002).

In contrast, analysis of the frequency of IVS-I-110 G>A allele among β-thalassemia patients in neighboring Arab countries showed that this allele was ranked as the first common allele in Egyptians (Jiffri et al., 2010), Lebanese (Makhoul et al., 2005), Jordanians (Sadiq et al., 2001), Greece (Georgiou et al., 2003), and Syrians (Jarjour et al., 2014), while it is the third frequent allele in Iraqi Arabs (Al-Allawi et al., 2015).

The third frequent mutation was IVS-II-1 G>A with an allele frequency of 6.9%, which is considered as a severe *HBB* allele (β°). Earlier reports from Palestine, reported this IVS II-1 G>A allele as the eighth and fifth frequent allele among β-thalassemia patients from West Bank (2.9 %) (Darwish et al., 2005), and from Southern region of West Bank (4.4 %) (El-Latif et al., 2002). However, this allele was not detected in Gaza strip (Sirdah et al., 2013). It is the most common mutation in Iraqi Arabs 41.2% (Al-Allawi et al., 2015), and the second frequent allele in Israeli Arabs (Rund, 1997).

The next two alleles (IVS-I-1 and Codon 37) were found in our study at a lower frequency compared to previous reports from Palestine (El-Latif et al., 2002) (Darwish et al., 2005; Sirdah et al., 2013). A previous study from Gaza Strip reported the IVS-I-1 allele as the most frequent allele and even showed a milder severity compared to the IVS-I-110 (Ghoti, Fibach, Rachmilewitz, Jeadi, & Filon, 2017).

The allele (IVS-II-848 C>A) was reported earlier in West Bank (Darwish et al., 2005) and at very low frequency among Jordanians (Sadiq et al., 2001), Syrians (Jarjour et al., 2014) and Iraqi Arabs (Al-Allawi et al., 2014).

The allele beta -101 C>T was found at a low frequency in our study (2%) and this is the first report of this allele in Palestine. This allele was reported at a lower frequency in Syria (Jarjour et al., 2014) and Lebanon (Makhoul et al., 2005).

Table (4.1): The allele frequencies of β-thalassemia mutations in the current study compared to earlier studies from Palestine, other neighboring and some Middle Eastern countries.

Nationality/ country	Palestinians				Israeli Arabs	Jordan	Syria	Lebanon	Egypt	Iraqi Arabs	Greece
Region	WB	WB	WB/s	Gaza							
Phenotype	TI	TM, TI[1]	Mixed[a2]	TM, TT	TI	Mixed[a5]	TM, TT	Mixed[a]	TM	TI	TM, TI
No. of alleles analyzed	102	279	136	274	53	240	331	520	188	204	1179
Mutations											
IVSI-6 T>C	76.5	28.7	48.5	13.1	57.7	8.3	4.8	14.4	20.2	24	7.2
IVSI-110 G>A	7.8	17.6	9.5	33.9	8.5	25	15.7	34.2	57.4	11.3	42.5
IVSII-1 G>A	6.9	2.9	4.4	-	11.3	15	9.1	8.6	5.3	41.2	2
Cd37 TGG>TGA	3.0	10.4	11.3	2.6	1.4	6.3	2.1	-		0.5	-
IVSI-1 G>A	2.0	9.0	4.4	21.2	-	10	13.5	15	9.6	2	13.2
Beta -101 C>T	2.0	-	-	-	-	-	0.6	-	-	0.5	-
IVSII-848 C>A	1.0	2.5	-	-	-	1.3	0.6	-	-	1.5	-
Cd106/107 (+G)	-	6.8	-	-	-	-	-	-	-	-	-
Cd39 C>T	-	4.6	2.2	9.5	2.8	4.6	13.3	0.2	2.1	0.5	16.9
Cd5 (-CT)	-	2.5	8.1	0.4	-	3.8	4.5	5	-	0.5	1.12
Not-genotyped	-	8.2	3.7	-	5.6	1.6	7.8	0.2	-	-	-
Wild	1.0	-	-		4.2	-	-			1.5	-
Total No. of mutations	7	17	10	15	7	19	31	20	7	22	10
References		(Darwish et al., 2005)	(El-Latif et al., 2002)	(Sirdah et al., 2013)	(Rund, 1997)	(Sadiq et al., 2001)	(Jarjour et al., 2014)	(Makhoul et al., 2005)	(Jiffri et al., 2010)	(Al-Allawi et al., 2014)	(Georgiou et al., 2003)

WB: West Bank region; WB/s: southern part of WB. TI: β-thalassemia intermedia; TM: β-thalassemia major; TT: β-thalassemia trait; [a]Mixed: included TM, TI and TT and/or Sickle B-thalassemia but did not specify the allele frequency in each subgroup.

The coinheritance of α-thalassemia in association with homozygous or compound heterozygous β-thalassemia would reduce the excess alpha chains, and thus leads to a less severe phenotype. α-Thalassemia mutations were seen in five patients only (9.8%); one of these five patients, whose *HBB* genotype was β^{o}/β^{+} and *HBA2* genotype was $-\alpha^{3.7}/\alpha\alpha$ was also heterozygous for the $^{G}\gamma$-globin gene *Xmn* I SNP, two were β^{+}/β^{+} (one with α2-IVSI-5nt and the other with-$\alpha^{3.7}/\alpha\alpha$); one β^{o}/β^{o} with α2-IVSI-5nt and the last one was β^{+}/β^{A} with α-triplication ($\alpha\alpha/\alpha\alpha\alpha^{anti3.7}$).

Similar findings were reported from regional and neighboring Arab countries, where 7.8% (4/51) of Iraqi (Al-Allawi et al., 2014) and 9.8% (5/52) of Iranian βTI patients (Neishabury et al., 2008) showed coinheritance of α-thalassemia mutations. The βTI phenotype of another patient whose *HBB* genotype was β^{0}/β^{0} which is supposed to be severe, is probably ameliorated by co-inheritance of at least two genetic modifiers, namely the existence of an α-thalassemia mutation ($\alpha^{IVSI(-5nt)}\alpha/\ \alpha\alpha$) and existence his high HbF level (46.8%). In the latter case, the high levels of HbF is probably caused by genetic modifiers influencing HbF production (Lai et al., 2016) (Mejri et al., 2016).

In the other two patients whose *HBB* genotype was β^{+}/β^{+} and co-inherited an additional α-thalassemia mutation, their Hb level is slightly above the average Hb of the study sample and thus the α-thalassemia mutation has probably slightly influenced their phenotype. Similar findings were reported in two of four members of a Jordanian family whose *HBB* was β^{+}/β^{+} and *HBA* genotype $-\alpha^{3.7}/\alpha\alpha$, they were reported as non-transfusion dependent (A Al Qaddoumi, 2006).

One patient was heterozygous for the *HBB* gene (β^{+}/β^{A}), but he co-inherited the α-triplication ($\alpha\alpha/\alpha\alpha\alpha^{anti3.7}$), which explains his βTI phenotype. Since such alpha triplication increases the globin chain imbalance among β- thalassemia heterozygote and shifts the disease severity from βTT toward the βTI phenotype (Farashi et al., 2015; Thein, 2005; Traeger-Synodinos et al., 1996). A similar findings in Israel was reported for a βTT patient, who showed severe anemia and splenomegaly, although his *HBB* genotype was β^{+}/β^{A} but his *HBA* genotype was $\alpha\alpha/\alpha\alpha\alpha^{anti3.7}$ (Steinberg-Shemer et al., 2017).

Analysis of the Gγ-globin gene *Xmn* I SNP showed that three patients were homozygous and one patient was heterozygous for this SNP, three of them have the β^{0}/β^{0} genotype and one has the

β^0/β^+ (in addition to $-\alpha^{3.7}/\ \alpha\alpha$) and the effect of this SNP was clearly illustrated by the high levels of HbF in these patients. The role of Gγ-globin gene *Xmn* I SNP in increasing HbF levels and moderation of thalassemia phenotype is widely known and reported in many earlier studies (Ali et al., 2015) (Rujito et al., 2016) (Al-Allawi et al., 2014). The *Xmn* I polymorphism is one of three major HbF quantitative trait loci (QTLs) responsible for HbF variation, and it leads to a less severe phenotype by increasing γ-chain production, which helps to neutralize unbounded α-chains (Borgna-Pignatti, 2007; Chan, Lau, Cheng, Chan, & Ng, 2016).

Analysis of the $^{G}\gamma$-globin gene *Xmn* I polymorphism, showed that this SNP was found in 4/51 patients (7.84%). Three homozygous patients showed the β°/β° genotype and one heterozygous patient (1.96%) showed the β°/β^+ genotype, but it was not detected in any patient with the genotype β^+/β^+ or the β^+/β^A. The latter findings are consistent with earlier reports that the *Xmn* I SNP is the commonest ameliorating factor in cases with β° mutations and in particular with β° allele but not β^+ (Al-Allawi et al., 2014; Arab et al., 2011). However, in our study the percentage of βTI patients having the *Xmn* I SNP was low, in consistence with an earlier report from Barzil (9.7%)(Fonseca et al., 1998) but inconsistent to previous reports from Iraq (56.8%)(Al-Allawi et al., 2014) Iran (51.9%); (Neishabury et al., 2008) Pakistan (23%); (Ali et al., 2015) and China (26.5%) (Chen et al., 2010).

It is interesting that the three patients with the β^0/β^0 (IVS II-1/ IVS II-I) and homozygous for *Xmn* I SNP not only have a high level of HbF but also have Hb levels (mean ± SD= 8.7 ± 0.86) above the average levels of the study patients. A recent study on a cohort of Palestinian β-thalassemia patients from Gaza strip reported a milder phenotype for the homozygous IVS-I-I allele and association between this allele and the high expression level of HbF (Ghoti et al., 2017). Ghoti el al 2017, found *Xmn* I SNP in only 7 out of 15 patient's homozygotes for the IVS-I-I allele, thus other genetic factors probably contribute to the high levels of HbF in association with null allele, which yet have to be determined. In addition, other studies have also reported an amelioration of the *HBB* β^0 allele by inheritance of either the *Xmn* I SNP, including reports from Egypt, that showed that patients with the IVS-II-I allele have relatively higher *Xmn*I polymorphism frequency (50%) than IVS-I-6 and IVS-I-110 (Said & Abdel-Salam, 2015), and a report from Southern Iran showed that 87.5% of patients with the IVS-II-1 allele were homozygous for the *Xmn* I SNP (Mehran Karimi et al., 2002).

Further studies with a larger number of patients and analysis of major QTL, *HBB* haplotypes and detailed analysis of the promoters of γ-globin genes may help reveal the genetic modifiers contributing to the variations in HbF and milder phenotype associated with the IVS-II-I allele.

In conclusion, this study is the first one to report the molecular characterization of βTI in Palestine. In βTI patients Hb level is adequate; the transfusion frequency is inappropriate and should be reviewed case by case to determine which case needs reduction of transfusion frequency. Since Transfusion therapy should be restricted and used carefully to avoid iron overload. Genotyping of the *HBB* gene detected 7 different mutations and 10 genotypes. The IVS-I-6 allele was the major allele with a remarkably high allele frequency of 76.5%. The beta -101 C>T mutation was diagnosed in one patient in homozygote state for the first time in Palestine. The inheritance of the mild *HBB* homozygote IVS-I-6 allele was the major contributing factor for the βTI phenotype among study subjects. The role of *Xmn* I SNP and α-thalassemia mutations in ameliorating the thalassemia phenotype was observed in few patients for each factor.

Recommendation

Further studies with larger number of patients and analysis of more genetic factors inside and outside the globin genes could provide more data on the genotype-phenotype correlation of certain null *HBB* genotypes associated with milder phenotype.

References

A Al Qaddoumi, A. (2006). *Co-inheritance of alpha and beta-thalassemia in a Jordanian family* (Vol. 19).

Agarwal, S., Sarwai, S., Agarwal, S., Gupta, U. R., & Phadke, S. (2002). Thalassemia Intermedia: Heterozygous β-Thalassemia and co-inheritance of an α gene triplication *Hemoglobin, 26*(3), 321-323.

Agouti, I., Bennani, M., Ahmed, A., Barakat, A., Mohamed, K., & Badens, C. (2007). Thalassemia Intermedia Due to a Novel Mutation in the Second Intervening Sequence of the β-Globin Gene. *Hemoglobin, 31*(4), 433-438.

Al-Allawi, N. A., Jalal, S. D. M., A. M. Omer, & S. Q. Markous, R. S. (2014). beta -thalassemia intermedia in Northern Iraq: a single center experience. *Biomed Res Int, 2014*, 262-853.

Al-Allawi, N. A., Puehringer, H., Raheem, R. A., & Oberkanins, C. (2015). Genetic Modifiers in beta-Thalassemia Intermedia: A Study on 102 Iraqi Arab Patients. *Genet Test Mol Biomarkers, 19*(5), 242-247.

Ali, N., Ayyub, M., Khan, S. A., Ahmed, S., Abbas, K., Malik, H. S., & Tashfeen, S. (2015). Frequency of Ggamma-globin promoter -158 (C>T) XmnI polymorphism in patients with homozygous/compound heterozygous beta thalassaemia. *Hematol Oncol Stem Cell Ther, 8*(1), 10-15.

Arab, A., Karimipoor, M., Rajabi, A., Hamid, M., Arjmandi, S., & Zeinali, S. (2011). Molecular characterization of β-thalassemia intermedia: a report from Iran. *Molecular Biology Reports, 38*(7), 4321-4326.

Borgna-Pignatti, C. (2007). Modern treatment of thalassaemia intermedia. *Br J Haematol, 138*(3), 291-304.

Camaschella, C., Mazza, U., Roetto, A., Gottardi, E., Parziale, A., Travi, M., . . . Cappellini, M. D. (1995). Genetic interactions in thalassemia intermedia: analysis of beta-mutations, alpha-genotype, gamma-promoters, and beta-LCR hypersensitive sites 2 and 4 in Italian patients. *Am J Hematol, 48*(2), 82-87.

Cao, A., & Galanello, R. (2010). Beta-thalassemia. *Genet Med, 12*(2), 61-76.

Cappellini, M. D., Cohen, A., Porter, J., Taher, A., & Viprakasit, V. (2014). Guidelines for the Management of Transfusion Dependent Thalassaemia (TDT) (3rd ed.). Nicosia (CY).

Cappellini, M. D., Musallam, K. M., & Taher, A. T. (2009). Insight onto the Pathophysiology and Clinical Complications of Thalassemia Intermedia. *Hemoglobin, 33*(sup1), S145-S159.

Carolyn Hoppe, e. a. (1999). Hudroxyurea and sodium Phenylbutyrate therapy in Thalassemia Intermedia *American Journal of Hematology, 62:221-227*.

Chan, N. C., Lau, K. M., Cheng, K. C., Chan, N. P., & Ng, M. H. (2016). A Multi-locus Approach to Characterization of Major Quantitative Trait Loci Influencing Hb F Regulation in Chinese beta-thalassemia Carriers. *Hemoglobin, 40*(6), 400-404.

Chen, W., Zhang, X., Shang, X., Cai, R., Li, L., Zhou, T., . . . al, e. (2010). The molecular basis of beta-thalassemia intermedia in southern China: genotypic heterogeneity and phenotypic diversity. *BMC Med Genet, 11*, 31.

Clark, B. E., & Thein, S. L. (2004). Molecular diagnosis of haemoglobin disorders. *Clin Lab Haematol, 26*(3), 159-176.

Darwish, H. M., El-Khatib, F. F., & Ayesh, S. (2005). Spectrum of beta-globin gene mutations among thalassemia patients in the West Bank region of Palestine. *Hemoglobin, 29*(2), 119-132.

El-Latif, M. A., Filon, D., Rund, D., Oppenheim, A., & Kanaan, M. (2002). The beta+-IVS-I-6 (T-->C) mutation accounts for half of the thalassemia chromosomes in the Palestinian populations of the mountain regions. *Hemoglobin, 26*(1), 33-40.

El-Shanshory, M. H., A. Shebl, S. Badria, I. Abd Elhameed, A.Abd El-Bar, E. et al. (2014). Spectrum of Beta Globin Gene Mutations in Egyptian Children with beta-Thalassemia. *Mediterr J Hematol Infect Dis, 6*(1), e2014071.

Farashi, S., Bayat, N., Faramarzi Garous, N., Ashki, M., Montajabi Niat, M., Vakili, S., . . . Azarkeivan, A. (2015). Interaction of an α-Globin Gene Triplication with β-Globin Gene Mutations in Iranian Patients with β-Thalassemia Intermedia. *Hemoglobin, 39*(3), 201-206.

Fonseca, S. F., Kerbauy, J., Escrivao, C., Figueiredo, M. S., Cancado, R., Arruda, V. R., . . . Costa, F. F. (1998). Genetic analysis of beta-thalassemia major and beta-thalassemia intermedia in Brazil. *Hemoglobin, 22*(3), 197-207.

Gasperini, D., Perseu, L., Melis, M., A. L., & Maccioni, e. a. (1998). Heterozygous B-Thalassemia with Thalassemis intermedia phenotype. *American Journal of Hematology, 57;43-47*.

Georgiou, I., Makis, A., Chaidos, A., Bouba, I., Hatzi, E., Kranas, V., . . . Bourantas, K. L. (2003). Distribution and frequency of beta-thalassemia mutations in northwestern and central Greece. *Eur J Haematol, 70*(2), 75-78.

Ghoti, H., Fibach, E., Rachmilewitz, E. A., Jeadi, H., & Filon, D. (2017). New Insights on beta-Thalassemia in the Palestinian Population of Gaza: High Frequency and Milder Phenotype Among Homozygous IVS-I-1 (HBB: c.92+1G>A) Patients with High Levels of Hb F. *Hemoglobin, 41*(2), 144-146.

Haddad, A., Tyan, P., Radwan, A., Mallat, N., & Taher, A. (2014). beta-Thalassemia Intermedia: A Bird's-Eye View. *Turk J Haematol, 31*(1), 5-16.

Haidar, R., Mhaidli, H., & Taher, A. T. (2010). Paraspinal extramedullary hematopoiesis in patients with thalassemia intermedia. *European Spine Journal, 19*(6), 871-878.

Hashemieh, M. A., A. Sheibani, K. (2016). A Comparison of Hemostatic Changes in Splenectomized and Nonsplenectomized beta-Thalassemia Intermedia Patients. *J Pediatr Hematol Oncol, 38*(8), 636-641.

Inusha Panigrahi, S. A., Mandakini Pradhan, Dharma R Choudhry, Renu Saxena. (2006). Molecular characterization of thalassemia intermedia in Indians. *Haematologica, 09*, 1279-1280.

Jarjour, R. A., Murad, H., Moasses, F., & Al-Achkar, W. (2014). Molecular update of beta-thalassemia mutations in the Syrian population: identification of rare beta-thalassemia mutations. *Hemoglobin, 38*(4), 272-276.

Jiffri, E. H., Bogari, N., Zidan, K. H., Teama, S., & Elhawary, N. A. (2010). Molecular Updating of β-Thalassemia Mutations in the Upper Egyptian Population. *Hemoglobin, 34*(6), 538-547.

Karimi, M., Cohan, N., De Sanctis, V., Mallat, N. S., & Taher, A. (2014). Guidelines for diagnosis and management of Beta-thalassemia intermedia. *Pediatr Hematol Oncol, 31*(7), 583-596.

Karimi, M., Yarmohammadi, H., Farjadian, S., Zeinali, S., Moghaddam, Z., Cappellini, M., & C Giordano, P. (2002). *β-Thalassemia intermedia from southern Iran: IVS-II-1 (G→ A) is the prevalent thalassemia intermedia allele* (Vol. 26).

Lai, Y., Chen, Y., Chen, B., Zheng, H., Yi, S., Li, G., . . . Zheng, C. (2016). Genetic Variants at BCL11A and HBS1L-MYB loci Influence Hb F Levels in Chinese Zhuang β-Thalassemia Intermedia Patients. *Hemoglobin, 40*(6), 405-410.

Makhoul, N. J., Wells, R. S., Kaspar, H., Shbaklo, H., Taher, A., Chakar, N., & Zalloua, P. A. (2005). Genetic Heterogeneity of Beta Thalassemia in Lebanon Reflects Historic and Recent Population Migration. *Annals of Human Genetics, 69*(1), 55-66.

Maragoudaki, E. K., E. Traeger-Synodinos, J. Vrettou, C. Tzetis, M. Metaxotou-Mavrommati, A. Kattamis, C. (1999). Molecular, haematological and clinical studies of the -101 C --> T substitution of the beta-globin gene promoter in 25 beta-thalassaemia intermedia patients and 45 heterozygotes. *Br J Haematol, 107*(4), 699-706.

Maria Caria Sollaino, M. E. P., Lucia Perseu, Nicolina Giagu, Daniela Loi, and Renzo Galanello. (2009). Association of a globin gene quadruplication and heterozygous B thalassemia in patients with thalassemia intermedia. *Haematologica, 94(10)*, 1445-1448.

Mejri, A., Mansri, M., Hadj Fredj, S., Ouali, F., Bibi, A., Hafsia, R., . . . Siala, H. (2016). First description of the rs45496295 polymorphism of the C/EBPE gene in β-thalassemia intermedia patients. *Hemoglobin, 40*(6), 411-416.

Miri-Moghaddam, E. B., S. Naderi, M. Bazi, A. Karimipoor, M. (2016). Molecular Characterization of beta-Thalassemia Intermedia in Southeast Iran. *Hemoglobin, 40*(3), 173-178.

Murru, S. L., G. Deiana, M. Camaschella, C. Sciarratta, G. V. Agosti, S. Parodi, M. I.et al. (1991). Molecular characterization of beta-thalassemia intermedia in patients of Italian descent and identification of three novel beta-thalassemia mutations. *Blood, 77*(6), 1342-1347.

Musallam, K. M., Taher, A. T., Duca, L., Cesaretti, C., Halawi, R., & Cappellini, M. D. (2011). Levels of growth differentiation factor-15 are high and correlate with clinical severity in transfusion-independent patients with beta thalassemia intermedia. *Blood Cells Mol Dis, 47*(4), 232-234.

Nadir Ali a, Muhammad Ayyub b, Saleem Ahmed Khan a, Suhaib Ahmed c, Kazim Abbas a,Hamid Saeed Malik a, Sunila Tashfeen. (2015). Frequency of Gc-globin promoter 158 (C>T) XmnI polymorphism in patients with homozygous/compound heterozygous beta thalassaemia. *Hematol Oncol Stem Cell Ther, 8(1): 10–15*.

Nassar, A. H., Usta, I. M., Rechdan, J. B., Koussa, S., Inati, A., & Taher, A. T. (2006). Pregnancy in patients with beta-thalassemia intermedia: outcome of mothers and newborns. *Am J Hematol, 81*(7), 499-502.

Neishabury, M., Azarkeivan, A., Oberkanins, C., Esteghamat, F., Amirizadeh, N., & Najmabadi, H. (2008). Molecular mechanisms underlying thalassemia intermedia in Iran. *Genet Test, 12*(4), 549-556.

Olivieri, N. F., Nathan, D. G., MacMillan, J. H., Wayne, A. S., Liu, P. P., McGee, A., . . . Cohen, A. R. (1994). Survival in medically treated patients with homozygous beta-thalassemia. *N Engl J Med, 331*(9), 574-578.

Oron-Karni, V., Filon, D., Oppenheim, A., & Rund, D. (1998). Rapid detection of the common mediterranean α-globin deletions/rearrangements using PCR. *American Journal of Hematology, 58*(4), 306-310.

Rajaeefard, A., Hajipour, M., Tabatabaee, H. R., Hassanzadeh, J., Rezaeian, S., Moradi, Z., . . . Soltani, M. (2015). Analysis of survival data in thalassemia patients in Shiraz, Iran. *Epidemiol Health, 37*.

Roudbari, M., Soltani-Rad, M., & Roudbari, S. (2008). The survival analysis of beta thalassemia major patients in South East of Iran. *Saudi Med J, 29*(7), 1031-1035.

Rujito, L., Basalamah, M., Siswandari, W., Setyono, J., Wulandari, G., Mulatsih, S., . . . Sutaryo, S. (2016). *Modifying Effect of XmnI, BCL11A, and HBS1L-MYB on Clinical Appearances: A Study on β-Thalassemia and Hemoglobin E/β-Thalassemia Patients in Indonesia* (Vol. 9).

Rund, D. O.-K., V. Filon, D. Goldfarb, A. Rachmilewitz, E.Oppenheim, A. (1997). Genetic analysis of beta-thalassemia intermedia in Israel: diversity of mechanisms and unpredictability of phenotype. *Am J Hematol, 54*(1), 16-22.

Sadiq, M. F., Eigel, A., & Horst, J. (2001). Spectrum of beta-thalassemia in Jordan: identification of two novel mutations. *Am J Hematol, 68*(1), 16-22.

Said, F., & Abdel-Salam, A. (2015). XmnI polymorphism: Relation to β-thalassemia phenotype and genotype in Egyptian Children. *Egyptian Journal of Medical Human Genetics*, *16*(2), 123-127.

Sirdah, M. M., Sievertsen, J., Al-Yazji, M. S., Tarazi, I. S., Al-Haddad, R. M., Horstmann, R. D., & Timmann, C. (2013). The spectrum of beta-thalassemia mutations in Gaza Strip, Palestine. *Blood Cells Mol Dis, 50*(4), 247-251.

Steinberg-Shemer, O., Ulirsch, J. C., Noy-Lotan, S., Krasnov, T., Attias, D., Dgany, O., . . . Tamary, H. (2017). Whole exome sequencing identifies an alpha-globin cluster triplication resulting in increased clinical severity of beta-thalassemia. *Cold Spring Harb Mol Case Stud*.

Taher, A., Isma'eel, H., & Cappellini, M. D. (2006). Thalassemia intermedia: revisited. *Blood Cells Mol Dis, 37*(1), 12-20.

Taher, A., Vichinsky, E., Musallam, K., Cappellini, M. D., & Viprakasit, V. (2013). Guidelines for the Management of Non Transfusion Dependent Thalassaemia (NTDT)In D. Weatherall (Ed.). Nicosia, Cyprus.

Taher, A. T. M., K. M. Cappellini, M. D. Weatherall, D. J. (2011). Optimal management of beta thalassaemia intermedia. *Br J Haematol, 152*(5), 512-523.

Taher, A. T. M., K. M. Karimi, M. El-Beshlawy, A. Belhoul, K. Daar, S. et al. (2010). Overview on practices in thalassemia intermedia management aiming for lowering complication rates across a region of endemicity: the OPTIMAL CARE study. *Blood, 115*(10), 1886-1892.

Thein, S. L. (2005). Pathophysiology of beta thalassemia--a guide to molecular therapies. *Hematology Am Soc Hematol Educ Program*, 31-37.

Traeger-Synodinos, J., Kanavakis, E., Vrettou, C., Maragoudaki, E., Michael, T., Metaxotou-Mavromati, A., & Kattamis, C. (1996). The triplicated alpha-globin gene locus in beta-thalassaemia heterozygotes: clinical, haematological, biosynthetic and molecular studies. *Br J Haematol, 95*(3), 467-471.

Vichinsky, E. (2016). Non-transfusion-dependent thalassemia and thalassemia intermedia: epidemiology, complications, and management. *Current Medical Research and Opinion, 32*(1), 191-204.

Williamson, D., Brown, K. P., Langdown, J. V., & Baglin, T. P. (1997). Mild Thalassemia Intermedia Resulting from a New Insertion/Frameshift Mutation in the β-Globin Gene. *Hemoglobin*, *21*(6), 485-493.

Appendix 1

Table1: Hemoglobin, Hb electrophoresis and genetic profiles for the study samples

Number	β Genotype	Xmn I	α-Genotype	Age (yr)	Sex	Age at diagnosis (Yr)	Hgb(g/dl)	Hb A2 (%)	Hb F (%)
1	IVS-I-6 / IVS-I-6	x-/-	αα/αα	11	M	5	8.3	7.7	5.3
2	IVS-I-6 / IVS-I-6	x-/-	αα/αα	11	F	8	7.6	5.8	13.8
3	IVS-I-6 / IVS-I-6	x-/-	αα/αα	7	M	5	7.7	5.3	10.4
4	IVS-I-6 / IVS-I-6	x-/-	αα/αα	34	F	2	8.2	8.2	5.3
5	IVS-I-6 / IVS-I-6	x-/-	αα/αα	36	F	4	7.7	7.7	11.3
6	IVS-I-6 / IVS-I-6	x-/-	αα/αα	13	M	0.08	7.8	4.8	8.3
7	IVS-I-6 / IVS-I-6	x-/-	αα/αα	35	M	4	8.7	6.2	24.2
8	IVS-I-6 / IVS-I-6	x-/-	αα/αα	4	F	0.66	9	6.4	29
9	IVS-I-6 / IVS-I-6	x-/-	αα/αα	60	M	25	7.1	4.7	1.3
10	IVS-I-6 / IVS-I-6	x-/-	αα/αα	54	F	16	7.5	8.6	6.6
11	IVS-I-6 / IVS-I-6	x-/-	αα/αα	13	F	0.58	9	4.5	5.3
12	IVS-I-6 / IVS-I-6	x-/-	αα/αα	12	M	0.75	8.1	7.1	3.3
13	IVS-I-6 / IVS-I-6	x-/-	αα/αα	22	M	8	9.2	6.2	17.1
14	IVS-I-6 / IVS-I-6	x-/-	αα/αα	18	M	1	10	7.9	9.7
15	IVS-I-6 / IVS-I-6	x-/-	αα/αα	10	F	1	7.8	5.3	4.3
16	IVS-I-6 / IVS-I-6	x-/-	αα/αα	71	M	25	7.1	7.3	2.3
17	IVS-I-6 / IVS-I-6	x-/-	αα/αα	4	M	0.66	8.9	3.8	6.9
18	IVS-I-6 / IVS-I-6	x-/-	αα/αα	23	F	0.25	7.6	7.3	5.8
19	IVS-I-6 / IVS-I-6	x-/-	αα/αα	4	F	2	6.5	8.1	15.8
20	IVS-I-6 / IVS-I-6	x-/-	αα/αα	20	M	0.08	9.3	8	17.1
21	IVS-I-6 / IVS-I-6	x-/-	αα/αα	25	M	0.5	9.2	7.7	12.3
22	IVS-I-6 / IVS-I-6	x-/-	αα/αα	30	F	3	10.7	7.3	5.1
23	IVS-I-6 / IVS-I-6	x-/-	αα/αα	14	F	2	7.6	2.8	7.6
24	IVS-I-6 / IVS-I-6	x-/-	αα/αα	11	M	5	8.4	5.4	7.9
25	IVS-I-6 / IVS-I-6	x-/-	αα/αα	18	M	2	9.9	5.9	6.1
26	IVS-I-6 / IVS-I-6	x-/-	αα/αα	14	F	2	7.6	7.6	3.4
27	IVS-I-6 / IVS-I-6	x-/-	αα/αα	22	M		8.6	6.3	12.3
28	IVS-I-6 / IVS-I-6	x-/-	αα/αα	37	F	2	7.2	6.1	9.3
29	IVS-I-6 / IVS-I-6	x-/-	αα/αα	54	M	25	9.4	5.9	19.5
30	IVS-I-6 / IVS-I-6	x-/-	αα/αα	27	M	1	8.9	7.4	10.3
31	IVS-I-6 / IVS-I-6	x-/-	αα/αα	18	M	5	8.3	7.5	10.1
32	IVS-I-6 / IVS-I-6	x-/-	αα/αα	14	F	0.08	9.5	5.8	10.6
33	IVS-I-6 / IVS-I-6	x-/-	αα/αα	17	M	3	6.4	7.4	8.6

34	IVS-I-6 / IVS-I-6	x-/-	αα/αα	23	M	12	11.1	6.6	6
35	IVS-I-6 / IVS-I-6	x-/-	αα/αα	34	M	0.08	7.6	6.7	13.6
36	IVS-I-6 / IVS-I-6	x-/-	αα/αα	18	F	1	9	4.6	10
37	IVS-I-6 / IVS-I-6	x-/-	αα/αα	21	F	0.75	9	4.3	24.7
38	IVS-I-6 / IVS-I-6	x-/-	αα/αα	20	M	0.5	8.4	7.3	7.8
39	IVS-I-6 / IVS-I-110	x-/-	α2-IVSI-5nt	10	F	3	8.7	ND	ND
40	Beta -101/-101	X-/-	αα/αα	13	M	5	13.9	4.6	7.4
41	IVS-II-I /IVS-II-I	x+/+	αα/αα	16	M	6	7.7	2.3	68
42	IVS-II-I /IVS-II-I	x+/+	αα/αα	29	M	3	9.8	2.3	83
43	IVS-II-I /IVS-II-I	x+/+	αα/αα	16	F	5	8.6	1.8	67
44	IVS-I-110 /IVS-I-110	x-/-	$-\alpha^{3.7}/\alpha\alpha$	7	F	0.6	9	3.3	1.7
45	IVS-I-110 /IVS-I-110	x-/-	αα/αα	4	F	1	11.7	3.2	6.4
46	IVS-I-110 /IVS-I-110	x-/-	αα/αα	22	M	3	9.5	8.5	17
47	IVS-I-I / β^{A}	x-/-	αα/ααα	20	F	4	NA	3.8	6.3
48	IVS-II-I G>A / IVS-II -848	x+/-	$-\alpha^{3.7}/\alpha\alpha$	38	F	6	7.1	3	49.1
49	IVS-I-110 / Codon 37 TGG>TGA	x-/-	αα/αα	24	F	11	6.5	2.5	30.9
50	IVS-I-I / Codon 37 Trp>Stop	x-/-	α2-IVSI-5nt	39	F	1	8.7	2.9	46.8
51	IVS-I-6 / Codon 37 TGG>TGA	x-/-	αα/αα	11	M	1	6.9	3.3	6.6

Appendices2: Ethical Approval of Al-Quds University

Al-Quds University
Jerusalem
Deanship of Scientific Research

بسم الله الرحمن الرحيم

جامعة القدس
القدس
عمادة البحث العلمي

Research Ethics Committee
Committee's Decision Letter

Date: 26/11/2016
Ref No: 2/REC/2016

Dear Dr. Mahmoud A. Srour,

Thank you for submitting your application for research ethics approval. After reviewing your application entitled **"Molecular characterization of thalassemia intermedia in West Bank, Palestine"** the Research Ethics Committee (REC) confirms that your application is in accordance with the research ethics guidelines at Al-Quds University.

Please inform us if there will be any changes in your research methodology/subjects/ plan and we would appreciate receiving a copy of your final research report. Thank you again and wish you a productive research that serves the best interests of your subjects.

Dr. Dina M. Bitar
Research Ethics Committee Chair

cc. Prof. Imad Abu Kishek - President
cc. Members of the committee
cc. file

Abu-Dies, Jerusalem P.O.Box 20002
Tel-Fax: #970-02-2791293
research@admin.alquds.edu
أبوديس، القدس ص.ب. 20002
تلفاكس: #970-02-2791293

Appendices3: Ethical Approval of Ministry Of Health

20. JUL. 2016 9:51 Fax J. H. U page 1

Al-Quds University
Faculty of Health Professions
Medical Laboratory Sciences Department
Jerusalem - Abu Dies

جامعة القدس
كلية المهن الصحية
دائرة العلوم الطبية المخبرية
القدس – أبوديس

التاريخ: 18.07.2016

حضرة الدكتور حمدي القايلمي المحترم،،
مدير عام المستشفيات
وزارة الصحة

الموضوع: الوصول الى ملفات مرضى التلاسيميا في مستشفيات وزارة الصحة

تحية طيبة وبعد،،

تقوم طالبة الماجستير راشيل فرعون من برنامج الماجستير في العلوم الطبية المخبرية في جامعة القدس بالاعداد لدراسة علمية حول مرضى التلاسيميا في فلسطين وبالتعاون مع جمعية أصدقاء مرضى التلاسيميا في فلسطين، وعنوان الدراسة:

- Characterization of Thalassemia Intermedia in West Bank, Palestine

وتهدف الدراسة الى تحديد مرضى التلاسيميا المشخصين او المصنفين "مرضى تلاسيميا متوسطة" ومن ثم إعادة تشخيص المرضى باجراء الفحوصات المخبرية الضرورية مثل تعداد الدم الكامل (CBC) والترحيل الكهربائي لخضاب الدم (Hb electrophoresis) ومن ثم تحليل المادة الوراثية (DNA analysis) للوصول الى تشخيص نهائي ودقيق مما يتيح لهم الحصول على العلاج المناسب، اضافة الى التعرف على نوع الطفرات التي تسبب التلاسيميا المتوسطة في فلسطين مما يسهل تطوير بروتوكول علاجي وتشخيصي لهؤلاء المرضى في المستقبل.

وبناء على ذلك نحن بحاجة الى الوصول الى ملفات جميع مرضى التلاسيميا في ثمان مراكز او مستشفيات مسؤولة عن متابعة مرضى التلاسيميا في الضفة الغربية، حيث سنقوم في الزيارة الاولى بحصر مرضى التلاسيميا في كل مركز وتسجيل البيانات الخاصة بهم (العمر، الجنس، تاريخ التشخيص وتاريخ أول عملية نقل دم وعنوان المريض) من أجل تحديد من هم مرضى التلاسيميا المتوسطة المحتملين، ومن ثم سيتم الاتصال بالمرضى والطلب منهم المشاركة في الدراسة والتبرع بعينة دم (5 ملليلتر) خلال الزيارة الثانية والاخيرة من أجل اجراء التشخيص النهائي والوصول الى تشخيص نهائي وقاطع.

Al-Quds University
Faculty of Health Professions
Medical Laboratory Sciences Department
Jerusalem - Abu Dies

جامعة القدس
كلية المهن الصحية
دائرة العلوم الطبية المخبرية
القدس – أبوديس

وسف تقوم بزيارة المراكز أو المستشفيات المحددة أدناه طالبة الماجستير راشيل فرعون من جامعة القدس ويرافقها من جمعية أصدقاء مرضى الثلاسيميا السيدة أماني شكري أو السيدة ولاء شماسنة.

والمستشفيات المطلوب زيارتها هي: المركز الطبي في نابلس، مستشفى ثابت ثابت في طلكرم، مستشفى خليل سليمان في جنين، مستشفى قلقيلية في قلقيلية، مستشفى رام الله في رام الله، مستشفى عالية في الخليل، مستشفى بيت جالا في بيت جالا، ومستشفى اريحا.

مع التأكيد ان جمع العينات والمعلومات عن المرضى هو لأغراض البحث العلمي فقط وسيتم التعامل مع المعلومات الخاصة بالمرضى والعينات بالسرية التامة وحسب اخلاقيات البحث العلمي.

يرجى التكرم من حضرتكم السماح للباحثين المذكورين أعلاه بالوصول الى منازل مرضى الثلاسيميا في المراكز المحددة أعلاه وتسهيل مهماتهم.

وتفضلوا بقبول فائق الاحترام والتقدير،،

الدكتور محمود سرور
المشرف على الدراسة

Appendix 4: Arabic questioner

رقم العينة: ..... اسم المستشفى: ..... التاريخ: .....

استبانة بحثية

(مقابلة مع المرضى)

عنوان الدراسة: توصيف مرضى التلاسيميا المتوسطة في الضفة الغربية، فلسطين

التلاسيميا –نوع بيتا هي مرض وراثي ناتج عن خلل كمي في تصنيع خضاب الدم، وتقسم سريريا حسب شدة المرض الى ثلاثة أقسام. صفة التلاسيميا نوع بيتا: حيث يحمل الشخص المورثة المسؤولة عن المرض ولكن لاتظهر عليه أعراض المرض. التلاسيميا المتوسطة – نوع بيتا: وتظهر على المريض اعراض متوسطة الشدة وتختلف شدتها من شخص لآخر ويحتاج المريض الى علاج على شكل نقل دم على فترات متباعدة. وأخيرا التلاسيميا الرئيسة –نوع بيتا: حيث يعتمد المريض بشكل أساسي ودائم على نقل الدم. وعادة يحدث تداخل في تشخيص مرضى التلاسيميا المتوسطة والرئيسية لصالح الرئيسية مما يؤدي الى تشخيص التلاسيميا المتوسطة كالثلاسيميا رئيسية وبالتالي اخضاعهم بشكل غير مبرر طبيا الى نقل دم زائد مما يؤثر سلبا عليهم ومع تكرار نقل الدم بشكل كبير يعاني المرضى من مضاعفات تقترب من مضاعفات التلاسيميا الرئيسية ونقل الدم.

وتهدف الدراسة الى تحديد مرضى التلاسيميا المشخصين او المصنفين "مرضى تلاسيميا متوسطة" ومن ثم إعادة تشخيص المرضى باجراء الفحوصات المخبرية الضرورية مثل تعداد الدم الكامل (CBC) والترحيل الكهربائي لخضاب الدم (Hb electrophoresis) ومن ثم تحليل المادة الوراثية (DNA analysis) للوصول الى تشخيص نهائي ودقيق مما يتيح لهم الحصول على العلاج المناسب، اضافة الى التعرف على نوع الطفرات التي تسبب التلاسيميا المتوسطة في فلسطين مما يسهل تطوير بروتوكول علاجي وتشخيصي لهؤلاء المرض في المستقبل.

ولذلك نرجو من حضرتك التعاون معنا من خلال المشاركة في هذه الدراسة (وفي حالة كون المريض طفلا نرجو السماح لها أو له بالمشاركة). وتشمل المشاركة الاجابة عن الاسئلة المرفقة ادناه والتبرع بعينة دم، اضافة الى التوقيع على طلب المشاركة في الدراسة. علما ان هذه الدراسة تتم بالتعاون بين جامعة القدس وجمعية أصدقاء مرضى التلاسيميا.

ونؤكد ان المعلومات التي سوف تقدمها للدراسة وعينة الدم التي سوف تتبرع بها سوف تستعمل لأغراض البحث العلمي فقط وسوف يتم التعامل معها بسرية تامة.

الباحثة: راشيل فرعون – جامعة القدس

أسئلة الاستبانة

عنوان الدراسة: توصيف مرضى التلاسيميا المتوسطة في الضفة الغربية، فلسطين

اسم المريض	
الجنس	□**انثى** □ **ذكر**
مكان الاقامة	
عمر المريض	
تشخيص أو نوع التلاسيميا	
عمر المريض عند أول تشخيص	
تاريخ أول عملية نقل دم	
تاريخ آخر عملية نقل دم	
عدد مرات نقل الدم في السنة الأخيرة	
هل تعاني من تضخم في الطحال حاليا	
هل أجريت عملية استئصال للطحال؟ وفي أي عمر؟	
هل سبق وأجريت عمليات جراحية؟ يرجى تحديدها	
هل تعاني من أمراض مزمنة (يرجى التحيدي ان وجدت)؟	
هل تعاني من زيادة في تجلط الدم أو حدث لك مضاعفات نتيجة تجلط الدم (يرجى التحديد ان وجدت وتحديد عدد المرات وتاريخ آخر مرة):	
هل الاب والام اقارب	
هل يحمل أحد والديك صفة مرض التلاسيميا نوع بيتا	
هل تأخذ ادوية للتخلص من الحديد في الجسم (يرجى تحديد النوع Desferal أو Exjade)؟	
ومتى بدأت تناول أدوية التخلص من الحديد؟	
معدل الفريتينFerritin في الدم (ng/ml) في آخر مرة تم قياسها؟	
معدل الهيموغلوبين Hb (mg/dL)	
طول المريض (سم)؟	
وزن المريض (كغم)؟	
هل ترغب بالحصول على نتيجة التحاليل لعينة الدم التي تتبرع بها اليوم (يرجى تحديد طريقة التواصل مثل بريد الكتروني أو فاكس)؟	
هل ترغب باضافة معلومات أخرى؟	

اقرار بالمشاركة في الدراسة

انا الموقع ادناه أقر بالمشاركة (وفي حال كان المريض طفلا اسمح لها او له بالمشاركة) في الدراسة بعنوان: **توصيف مرضى التلاسيميا المتوسطة في الضفة الغربية، فلسطين.** علما انه تم اطلاعي على الهدف من الدراسة وان المعلومات التي قدمتها للدراسة وعينة الدم التي تبرعت بها سوف يتم استخدامها لأغراض البحث العلمي فقط.

اسم المريض:

التوقيع:

التاريخ:

--- انتهى الاقرار ------

التوصيف الجزيئي للبيتا ثلاسيميا المتوسطة في الضفة الغربية–فلسطين

إعداد: رشيل ابراهيم حسن فرعون

المشرف: الدكتور محمود عبد الرحمن سرور

الملخص:

الثلاسيميا هي اكثر الاضطرابات المتنحية الوراثة انتشارا في العالم كما في فلسطين. هناك أكثر من 200 طفرات وراثية مسؤولة عن انخفاض إنتاج سلسلة البيتا جلوبين. البيتا ثلاسيميا الوسطى تقع بين الثلاسيميا العظمى التي تعتمد على النقل المستمر للدم والثلاسيميا الصغرى التي لا تعتمد على نقل الدم. تظهر الثلاسيميا المتوسطة بصورة سريرية متغيرة بحد كبير تتراوح بين خفيفة إلى شديدة فقر الدم التي تتطلب نقل الدم في بعض الأحيان. وراثيا هي مجموعة غير متجانسة تعزى إلى الطفرات في البيتا جين وحدها أو الميراث المشترك لطفرات البيتا وجينات أخرى مثل جينات الألفا والجاما. قد تكون المحددات الوراثية للثلاسيميا المتوسطة مختلفة في مناطق مختلفة، ولا سيما في المجموعات العرقية المختلفة وتتأثر أنواع مختلفة من الألائل الثلاسيميا المشتركة في كل منطقة والمجموعة العرقية. الهدف من الدراسة هو تحديد طيف جينات البيتا والألفا والجاما لمرضى الثلاسيميا المتوسطة بمنطقة الضفة الغربية في فلسطين، وكذلك لتقييم الممارسات الإدارية لهؤلاء المرضى. تم تسجيل 51 حالة ثلاسيميا متوسطة, حيث تم تقييم فحص عد الدم الكامل والترحيل الكهربائي لخضاب الدم. ومن ثم تحليل المادة الوراثية لكل من جين البيتا, الألفا (-) $\alpha^{3.7}$, -$\alpha^{4.2}$, --^{MED}, $\alpha\alpha\alpha^{anti3}$α2-IVSI 1-5 nt del والجاما (*Xmn* I), بإستخدام عدة طرق للكشف عن هذه الطفرات وهي Gap-PCR, DNA sequencing, ARMS.

لوحظت سبع طفرات جينية للبيتا بين مرضى الثلاسيميا المتوسطة IVS-I -6 C>T, IVS-I-110 G>A, IVS-II-1 G>A, IVS-I-1 G>A, Codon 37 Trp>Stop, B -101 C>A. وقد لوحظت 10 أنماط وراثية ، المتماثلة منها IVS-I-6 C>T وهي الأكثر شيوعا لحالات الثلاسيميا المتوسطة بنسبة 74.5 %. النمط الوراثي الثاني المشترك بين المرضى هو IVS-I-110 G>A والذي شكل نسبة 5.8 % , والطفرة IVS-II-1 G>A التي شكلت 5.8 %. اما باقي السبع أنماط وراثية فشكلت 2 % من المرضى. طفرات الألفا شوهدت في خمس مرضى فقط بنسبة 9.8 % وهي من

انواع (-$\alpha^{3.7}$, $\alpha\alpha\alpha^{anti3}$α2-IVSI 1-5 nt del). طفرات الجاما ظهرت في اربعة مرضى, ثلاثة منهم متماثلة الوراثة وواحدة متغايرة الوراثة. وراثة العامل المتماثل المعتدل IVS-I -6 C>T هي العامل الرئيسي في النمط الظاهري للثلاسيميا المتوسطة في الضفة الغربية. طفرات الألفا والجاما ساعدت على تحفيز النمط الظاهري للثلاسيميا في عدد قليل من المرضى.

وفي الخلاصة وراثة العامل المتماثل IVS-I -6 C>T هو النمط الاكثر شيوعا بين المرضى في فلسطين. طفرة B-101 C>T المتماثلة وجدت في مريض واحد تسجل للمرة الاولى في فلسطين. نتائج الدراسة ستؤثر إيجابيا على الإدارة الصحية لمرضى الثلاسيما المتوسطة في فلسطين وتوفر المزيد من البيانات عن المنشأ والتوزيع لطفرات البيتا.