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Investigating β-Thalassemia Major Cases Emerged After Obligatory Premarital Testing in Palestine

Mais Khader Yousef Al Shatleh

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Prepared by:

Mais Khader Yousef Al Shatleh

B.Sc. in Medical Laboratory Sciences / Al-Quds University / Palestine

Supervisor: Dr. Rania Abu Seir

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Prepared By: Mais Khader Yousef Al Shatleh

Registration No.: 21910081

Supervisor: Dr. Rania Abu Seir

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

Head of Committee	**Dr. Rania Abu Seir**	**Signature……**
Internal Examiner	**Dr. Khalid Younis**	**Signature……**
External Examiner	**Dr. Akram Karma**	**Signature……**

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Dedication

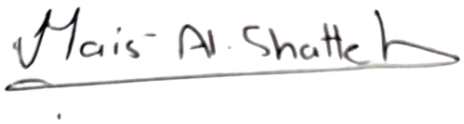
I dedicate this thesis to my loving family, whose unwavering support and encouragement have been my guiding light throughout this academic journey. Their belief in me has been a constant source of motivation, and I am forever grateful for their sacrifices and presence in my life.

To my mentors, I extend my heartfelt appreciation for their invaluable guidance and mentorship. Their wisdom, expertise, and unwavering dedication have shaped my scholarly growth and inspired me to reach new heights. I am indebted to their insights and grateful for the opportunities they have provided me. This thesis is dedicated to the collective community of scholars, researchers, and thinkers whose contributions have paved the way for this work. Their groundbreaking research and intellectual pursuits have shaped my understanding and ignited my passion for knowledge. I am honored to contribute to the ongoing scholarly conversation they have fostered.

Mais Khader Al Shatleh

Declaration:

I certify that this thesis submitted for the degree of Master, is the result of my own research, except where otherwise acknowledged, and that this study (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signed: 

Mais Khader Al Shatleh

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Mais Khader Al Shatleh

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Abstract

Background

β-Thalassemia major is a prevalent autosomal recessive disorder worldwide, affecting approximately 3-4% of the Palestinian population. Management of this condition poses significant challenges, particularly in resource-constrained settings. This study aims to investigate the factors contributing to the emergence of new cases of β-thalassemia major after the implementation of obligatory premarital screening, thereby informing strategies for prevention and intervention.

Methods

We conducted a cross-sectional case-series study that included all patients diagnosed with β-thalassemia born in or after 2010 in the West Bank. 69 eligible patients from 62 families were included in the study. The data used in this study were collected through a comprehensive questionnaire covering the demographic and medical information of each thalassemic child born after 2010 in the family, familial sociodemographic characteristics, background characteristics of the parents (fathers and mothers), premarital screening, and the knowledge, attitudes, and practices (KAP) of parents (mothers and fathers). In addition, we collected blood samples from 68 children and 62 parents for hematological assessment (complete blood count and hemoglobin electrophoresis).

Results

The largest proportion of emerging thalassemia cases were from the northern region and resided in rural localities. 71% of the cases were from families married before 2010. 56.5% of the parents reported undergoing premarital screening tests. The proportion of parents who underwent premarital screening differed significantly by type of locality, year of marriage, and age at marriage. In addition, investigating the self-reported results of the premarital screening tests of each couple, we have found that among 24 partners who did not get tested, 22 were married before 2010 and 19 had children with β-thalassemia major. Furthermore, among 12 couples who reported that the two partners were tested and were non-carriers, 4 couples had children with β-thalassemia major, 4 had children with sickle cell thalassemia, and 4 had children with thalassemia intermedia. Overall, the proportion of children with thalassemia major was lower by 20% among parents who married in/after 2010. On the other hand, comparison between the self-reported results of premarital screening and the results of the hematological assessment (mean capsular volume), a total of 24 out of 32 parents had discrepancies in their results. the hematological assessment also showed that 3 out of 62

parents had normal MCV, all of which had high HbS and were parents of children with sickle cell thalassemia. Also, 8 parents had both low MCV and high HbS and one parent had low MCV and high HbC and was a parent of two children with hemoglobin SC disease. Assessment of knowledge showed that all parents had adequate knowledge about thalassemia, 51.7% of the parents had poor overall attitudes, and 76.3% had poor attitude scores towards the termination of pregnancy. Furthermore, 47.4% of the parents had good overall practice scores. Knowledge scores were positively correlated with attitude scores (r=300; p-value=0.01) but not with practice scores (r=0.058; p-value=0.543). Attitude score towards prenatal diagnosis and overall practice scores were also positively correlated (r=0.271; p-value=0.003).

Conclusions

This study highlights the pressing need for proactive measures to address the prevalence of hemoglobinopathies in Palestine. Findings highlight the crucial role of Health Education and Awareness Programs, aimed at disseminating information about thalassemia, its inheritance patterns, and preventive measures. Additionally, the screening criteria should be revised to include screening for other hemoglobinopathies, and the screening must be performed in Designated Receptions Centers where trained staff perform and interpret the results in accordance with standard laboratory diagnostic protocols. Strengthening pre-marital counseling and screening services is imperative to ensure comprehensive coverage. Early detection, accurate carrier identification, and informed decision-making regarding marriage and family planning should be central in preventive efforts. Continuous training for healthcare professionals and community workers is crucial to effectively prevent and manage thalassemia.

العوامل المساهمة في ظهور حالات جديدة من مرض الثلاسيميا بعد تطبيق قانون الفحص الإجباري قبل الزواج في فلسطين

إعداد: ميس خضر الشتلة

إشراف: د. رانية أبو سير

ملخص

مقدمة

تعدّ الثلاسيميا مرضًا وراثيًا سائدًا على مستوى العالم، حيث يصيب حوالي 3-4٪ من السكان الفلسطينيين. إدارة هذا المرض تمثل تحديات كبيرة، خاصة في البيئات ذات الموارد المحدودة. تهدف هذه الدراسة إلى التحقيق في العوامل التي تسهم في ظهور حالات جديدة من الثلاسيميا بعد تنفيذ القانون الإلزامي لفحص ما قبل الزواج للكشف عن صفة الثلاسيميا، وبالتالي تقديم استراتيجيات للوقاية والتدخل.

طرق البحث

تم إجراء دراسة مقطعية شملت جميع المرضى الذين تم تشخيصهم بالثلاسيميا الذين ولدوا في أو بعد عام 2010 في الضفة الغربية. شملت الدراسة 69 مريضًا من 62 عائلة. تم جمع البيانات المستخدمة في هذه الدراسة من خلال استبيان شامل يغطي المعلومات الديموغرافية والطبية لكل طفل مصاب بالثلاسيميا ولد في عام 2010 أو بعد ذلك، الخصائص الاجتماعية والديموغرافية للعائلة، وخصائص الوالدين (الآباء والأمهات)، والفحص الإلزامي ما قبل الزواج، ومعرفة وتوجهات وممارسات الوالدين (الأمهات والآباء) نحو الثلاسيميا. بالإضافة لذلك، تم جمع عينات دم من 68 طفلاً و62 أب وأم لتحليل الدم (تعداد الدم والفصل الكهربائي للهيموغلوبين).

النتائج

كانت أكبر نسبة من الحالات الجديدة من الثلاسيميا من شمال الضفة الغربية ومن سكان المناطق الريفية. 71٪ من الحالات ولدت من عائلات تزوجت قبل عام 2010. أفاد 56.5٪ من الوالدين بأنهم خضعوا للفحص الإلزامي ما قبل الزواج. اختلفت نسبة الآباء الذين خضعوا لاختبارات الزواج الإجبارية بشكل ملحوظ بحسب نوع المنطقة وعام الزواج والعمر عند الزواج. علاوة على ذلك، من بين 12 زوجًا أبلغوا أن الشريكين تم اختبارهما وأنهما غير حاملين، تبين أن 4 أزواج كان لديهم أطفال مصابين بالبيتا ثلاسيميا الكبرى، 4 أزواج لديهم أطفال مصابين بالثلاسيميا المنجلية، و4 أزواج لديهم أطفال مصابين بالثلاسيميا الوسطى. وبصفة عامة، كانت نسبة الأطفال المصابين بالثلاسيميا الكبرى أقل بنسبة 20٪ بين الأزواج الذين تزوجوا في/بعد عام 2010. ومن ناحية أخرى، بالمقارنة بين النتائج المبلغ عنها ذاتياً لفحص ما قبل الزواج ونتائج تحليل الدم (متوسط حجم الكريات)، كان لدى مجموعة مكونة من 24 أب وأم تناقضات في النتائج بين الفحصين. كما أظهرت نتائج تعداد الدم أن 3 من 62 أب وأم كانت لديهم قيم MCV طبيعية، وأظهرت نتائج الفصل الكهربائي للهيموغلوبين يحملون نسب عالية من HbS وكانوا والدين لأطفال مصابين بالثلاسيميا المنجلية. إضافة إلى ذلك، كانت لدى 8 آباء قيم MCV منخفضة ونسب عالية من HbS، كما كان لدى واحد من الآباء قيمة MCV منخفضة ونسب عالية من HbC ولديه طفلين مصابين بمرض الهيموجلوبين SC. على صعيد آخر، بينت نتائج تقييم المستوى المعرفي للآباء أن جميع الآباء كانت لديهم معرفة كافية عن الثلاسيميا، بالمقابل، فقد بلغت نسبة الآباء الذين لديهم

توجهات سيئة بشكل عام نحو الثلاسيميا 51.7% و76.3٪ من الآباء أظهروا توجهات سيئة تجاه الإجهاض. وعلاوة على ذلك، كان لدى 47.4٪ من الآباء درجات ممارسة جيدة بشكل عام. بينت النتائج كذلك وجود علاقة إيجابية بين مستوى المعرفة حول الثلاسيميا والتوجهات نحو الثلاسيميا بشكل عام (r=300؛ قيمة p=0.01) ولكن ليس بمستوى الممارسة (r=0.058؛ قيمة p=0.543). كما تبين وجود علاقة موجبة بين التوجه نحو التشخيص السابق للولادة ودرجات الممارسة (r=0.271؛ قيمة p=0.003).

الخلاصة

تسلط هذه الدراسة الضوء على الحاجة الملحة لاتخاذ تدابير نشطة لمعالجة ظاهرة انتشار الثلاسيميا في فلسطين. تُظهر النتائج الدور الحاسم لبرامج التثقيف الصحي والتوعية، التي تهدف إلى نشر معلومات حول الثلاسيميا وأنماط نقلها الوراثي، والتدابير الوقائية. بالإضافة إلى ذلك، فإن من الضروري مراجعة معايير الفحص لتشمل البحث عن أمراض الدم الأخرى مثل الأنيميا المنجلية والهيموغلوبين C، ويجب أن يتم الفحص في مراكز استقبال مخصصة حيث يقوم الموظفون المدربون بإجراء الفحوصات وتفسير النتائج وفقًا لبروتوكولات التشخيص المعمول بها في المختبرات القياسية. كما أنه من الضروري تعزيز الخدمات الاستشارية والفحص قبل الزواج لضمان تغطية شاملة. كما يجب أن يكون الكشف المبكر وتحديد الناقلين بدقة واتخاذ قرار مستنير بشأن الزواج وتخطيط الأسرة في صميم الجهود الوقائية. بالإضافة لذلك، فإن التدريب المستمر للمحترفين الصحيين والعاملين الاجتماعيين أمر ضروري لمنع وإدارة الثلاسيميا بشكل فعال.

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List of Abbreviations

TIF	Thalassemia International Federation
TDT	Transfusion Dependent Thalassemia
NTDT	Non-transfusion-dependent thalassemia
TPFS	Thalassemia Patients Friends Society
LCR	locus control region
KAP	Knowledge, Attitude, & Practices
Hb	Hemoglobin
HH	hypogonadotropic hypogonadism
WHO	World Health Organization
IF	Inefficient erythropoiesis
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
IVS	Intervening sequence
MCV	Mean cell volume
MCH	Mean cell hemoglobin
FL	Femtolitre
Pg	Pictogram
dL	Deciliter
HPLC	High Performance Liquid Chromatography
PCR	Polymerase chain reaction
UTR	Untranslated Region
UAE	United Arab Emirates
MENA	Middle East and North Africa
MCHC	Mean cell hemoglobin concentration
RDW	Red cell distribution width
PLT	Platelets
ID	Identity number
EDTA	Ethylene diamine tetra-acetic acid
HCV	Hepatitis C virus
HBV	Hepatitis B virus
PMCG	Premarital screening and genetic counselling
HSCT	hemopoietic stem cell transplantation
QOF	Quality of life
COVID-19	Corona virus international disease-19
ICT	Iron Chelation Therapy
SD	Standard Deviation
IQR	Interquartile range

Chapter One

Introduction

1.1 Background

Thalassemia is one of the most common hereditary disorders in the world, affecting quantitatively the synthesis of human hemoglobin (Swee Lay Thein, 2004). It was first described in a patient of Italian origin by pediatrician Thomas Benton Cooley in 1925, at that time, it was designated as Cooley's anemia (AL-Zwaini, 2018). Later on, the term thalassemia was coined by George Hoyt Whipple of the University of Rochester, New York. The term was derived from the ancient Greek word for sea, thalassa, since the ancients thought it was a "disease of the sea“, and the suffix -aima (aíma), which means blood denoting the high occurrence of this hematologic disorder among populations of Greek, Italian, Cypriot, Turkish, or Sicilian origins, all of whom are from the Mediterranean and Black Seas (Siddiqui, Steensma, & Kyle, 2017).

Thalassemia has been found in ethnicities from almost every geographic location on the globe, but they are most frequent in people from Africa and Asia's tropics and subtropics, as well as the Mediterranean basin (Weatherall & Clegg, 2001) and the great majority being born in the developing world (Renzo Galanello & Origa, 2010). However, the epidemiology of thalassemia has been changing over the past few decades due to migration (A. Kattamis, Forni, Aydinok, & Viprakasit, 2020). Annually, between 60,000 and 70,000 children are born with severe thalassemia, affecting approximately 4.4 out of every 10,000 live births worldwide (Muncie & Campbell, 2009), most of whom are affected by β-thalassemia major.

Depending on the reduction of the globin chain, thalassemia is classified as alpha-thalassemia, β-thalassemia, gamma-thalassemia, delta-thalassemia, and delta-beta-thalassemia (Viprakasit & Ekwattanakit, 2018). Although α and β thalassemia are the most common types of thalassemia, the most important genetic variety and the most common widespread type is β-thalassemia, causing severe anemia in both homozygous and compound heterozygous individuals (Raffaella Origa, 2000).

Thalassemia syndromes are among the few genetic disease paradigms in which early mortality has been replaced by promising chronicity (Farmakis, Giakoumis, Angastiniotis, & Eleftheriou, 2020). Thalassemia is a chronic condition with a variety of clinical and physiological complications, it has a wide range of clinical manifestations, ranging from transfusion-dependent anemia to a clinically asymptomatic characteristic that is usually identified accidentally (Tarazi, Al Najjar, Lulu, & Sirdah, 2007). In addition, physical deformities, underdevelopment, and delayed puberty can all result from its effects on health (Sanctis, 2006). β-thalassemia has a wide spectrum ranging from mild to clinically manifested diseases including β-thalassemia major and β-thalassemia intermedia (Traivaree, Monsereenusorn, Rujkijyanont, Prasertsin, & Boonyawat, 2018). The clinical complications of β-thalassemia disease includes chronic hemolytic anemia, hepatosplenomegaly, failure to thrive, and other consequences of β-thalassemia that can be fatal in children if not treated properly (Renzo Galanello & Origa, 2010).

The Thalassemia International Federation (TIF) published the 2021 Guidelines for the Management of Transfusion-Dependent Thalassemia (TDT), which classifies β-thalassemia based on clinical severity and transfusion requirement into two primary groups: transfusion-dependent thalassemia (TDT), which is a condition that requires regular blood transfusions throughout life, and non- transfusion-dependent (NTDT), which is characterized by anemia but is not severe enough to demand regular blood transfusions (Cappellini, Cohen, Porter, Taher, & Viprakasit, 2014).

Almost 300 alleles of the β-thalassemia gene have now been identified (Renzo Galanello & Origa, 2010). Identifying the factors that cause the wide range of clinical presentations has clinical implications, and the reason for this is that the diversity of mutations (Sankaran, Lettre, Orkin, & Hirschhorn, 2010) and the degree of imbalance between α- and non-α globin chains determines the severity of β-thalassemia (Hassan, Badr, El Safy, Hesham, Sherief, & Zakaria, 2016). Unlike the deletions that cause the majority of α-thalassemia syndromes, β-thalassemia is caused by point mutations in the β-globin gene functionally important regions (Traivaree et al., 2018). Furthermore, the variability of clinical severity can be attributed to the inheritance of other blood disorders such as α-thalassemia and sickle cell disease and complex forms of thalassemia resulting from defective production of two to four different globin chains (δβ-, γδβ-, and εγδβ-thalassemia) (Cao & Kan, 2013).

Due to a high carrier rate and a cultural preference for consanguineous marriages, the prevalence of β-thalassemia has traditionally been high in the Middle East (A. Kattamis et al., 2020). Arab countries have some of the highest rates of consanguinity in the world, ranging from 20% to 50% (Tadmouri, Nair, Obeid, Al Ali, Al Khaja, & Hamamy, 2009). The most important factors that contribute to the high prevalence of consanguineous marriages in these regions are cultural and racial factors, as well as a lack of knowledge about the negative outcomes of consanguineous marriages (Karimzaei, Masoudi, Shahrakipour, Navidiyan, Jamalzae, & Zoraqi Bamri, 2015).

1.2 Problem Statement

β-Thalassemia is one of the most common autosomal recessive disorders worldwide. Similar to other Mediterranean populations, β-thalassemia is common among Palestinians (Darwish, El-Khatib, & Ayesh, 2005). Approximately 3-4% of the Palestinian population are carriers of β-thalassemia (Al Sabbah, Khan, Hamadna, Abu Ghazaleh, Dudin, & Karmi, 2017; Darwish, El-Khatib, & Ayesh, 2005) and the prevalence of sickle cell trait in the West Bank is estimated to be 1.2% (Samarah, Srour, Yaseen, & Dumaidi, 2018). According to the Thalassemia Patients Friends Society (TPFS), there were 864 β-thalassemia patients in 2021 (557 in the West Bank and 307 in Gaza Strip) (TPFS, 2021). The cure for this disease is dependent on successful bone marrow or cord stem cell transplantation, both of which are out of reach for the majority of families in our country. As a result, Palestinian β-thalassemia major patients rely solely on supportive care, resulting in serious medical, financial, social, and psychological burdens, in addition to the complexity and high price associated with the management of thalassemia that burden the national medical services, which are considered insufficient and understaffed to meet these costs.

Prevention is considered the best strategy to maintain the ability of the national healthcare system to provide the best care and support for thalassemia patients and their families in light of the available resources. Several countries have set up comprehensive national

prevention programs, which include population screening, mass awareness programs, antenatal screening and counseling, premarital carrier screening, cascade screening, and student screening (Cao & Kan, 2013; LIPKIN Jr, FISHER, ROWLEY, LOADER, & IKER, 1986).

The Thalassemia Patients' Friends Society (TPFS), a nonprofit Palestinian non-governmental organization, was established in 1996 by a group of thalassemia patients, their families, and professionals interested in the disease. Since its establishment, the TPFS has continuously worked to improve the national awareness towards thalassemia through campaigns targeting all classes of the Palestinian community, especially youth. Furthermore, from September 2000 and up until 2008, the TPFS had implemented a prevention program that provided premarital screening services for couples who are about to get married. In 2010, the superior legitimate judge adopted the implementation of premarital tests for β-thalassemia as an obligatory step before any proposed couple can be issued with a marriage certificate. The screening program was then integrated into the national healthcare system by the Palestinian Ministry of Health (MoH) and premarital screening can be performed for free in any of the Health Directories of the Palestinian MoH (Al Sabbah et al., 2017; Tarazi et al., 2007; TPFS). This program has successfully reduced the number of new thalassemia cases in Palestine. Additionally, the prevention of β-thalassemia is advanced by the introduction of prenatal diagnosis via chorionic villus sampling (CVS) and amniocentesis. Prenatal diagnosis determines if an unborn child has thalassemia major, to provide the couple with the option of aborting the baby to prevent the birth of an affected child if the fetus appeared to be affected (Ayesh, Al-Sharef, Nassar, Thawabteh, & Abu-Libdeh, 2005; Tarazi et al., 2007).

Although more than a decade has passed since the obligatory premarital screening program was put in place, new cases of thalassemia are still born. Several factors that could influence the outcomes of the thalassemia prevention program. Multiple social factors, beliefs, and personal decisions in addition to certain genetic abnormalities that lead to failure in detecting carriers could potentially explain why new cases of thalassemia major continue to be born (LIPKIN Jr et al., 1986). For instance, consanguineous marriages, low literacy rates, and low socioeconomic status are all common sociodemographic factors in developing countries that contribute significantly to the continuing emergence of new β-thalassemia major cases (Al Sabbah et al., 2017). Moreover, genetic factors, such as a mutation in the promoter region of β-globin gene or the locus control region (LCR), result in no detectable increase in HbA2 levels and near-normal CBC findings. These mutations have the tendency to give false negative results, making β-thalassemia carriers difficult to identify (Swee Lay Thein & Rees, 2015). Understanding these factors is important to understand how to further prevent thalassemia.

1.3 Study Justification

The obligatory premarital screening test has been used in Palestine for about ten years and has reduced the incidence of the disease (Tarazi et al., 2007). However, new cases of β-thalassemia are still born each year. Based on data from the TPFS, between 2010 and 2022, 77 children have been born with thalassemia in Palestine. A previous study investigated the factors that influence the choice of parents to give birth to a fetus affected by thalassemia in the West Bank. The study showed that religious beliefs were the most reported reason and that mothers with lower educational level were more likely keep an affected fetus (Al Sabbah

et al., 2017). Another study that assessed the attitudes of couples toward prenatal diagnosis and its outcomes as a preventive method. The study reported good acceptability for prenatal diagnosis in β-thalassemia afflicted families and that prenatal diagnosis was effective as all couples with affected fetuses opted for abortion (Ayesh et al., 2005). To our knowledge, there have been no studies that have investigated the factors other than prenatal diagnosis that could contribute to the continuing emergence of new β-thalassemia major cases even after more than a decade of implementing the obligatory premarital screening law.

1.4 Study Aim and Objectives

The goal of this study is to investigate factors associated with the emerging of β-thalassemia major cases after the implementation of obligatory premarital testing in Palestine including:

- Factors related to premarital screening tests.
- Sociodemographic and cultural factors.
- Beliefs and personal decisions.

Chapter Two

Literature Review

In this chapter, we provide an extensive literature review that explains the pathogenesis, molecular basis, diagnostic criteria, prevention strategies, treatment and the factors associated with emerging of β-thalassemia.

2.1 Human Hemoglobin

Human hemoglobins are a diverse group of proteins found within red blood cells that transport oxygen from the lungs to tissues and organs (H. W. Kim & Greenburg, 2004). This heterogeneity manifests itself at all stages of development, when different types of hemoglobin are synthesized (Williams & Weatherall, 2012).

2.1.1 Hemoglobin Structure

All human hemoglobins are tetrameric in structure, consisting of two different pairs of globin polypeptide chains (2 alpha-like, 2 beta-like). Each globin chain is linked to one heme molecule. However, different types of hemoglobins are synthesized during the stages of human development as shown in Table (2.1) (Juul & Christensen, 2018).

Table (2.1): Hemoglobin types in the different developmental stages of human life.(Source:(Al Haddad, Yassin, & Sirdah, 2012))

Hemoglobin Type	Structure	Developmental Stage
Hb Gower 1	$\zeta_2\varepsilon_2$	Embryo
Hb Gower 2	$\alpha_2\varepsilon_2$	Embryo
Hb Portland	$\zeta_2\gamma_2$	Embryo
HbF	$\alpha_2\gamma_2$	Fetal and adult
HbA2	$\alpha_2\delta_2$	Adult
HbA	$\alpha_2\beta_2$	Adult

2.1.2 Fetal to Adult Hemoglobin Switch

Following birth, the adult β and δ globin chains gradually replace the γ-globin chain. This causes a significant shift in hemoglobin synthesis from HbF ($\alpha_2\gamma_2$) to adult hemoglobin HbA ($\alpha_2\beta_2$) synthesis, which begins around the time of birth and lasts 6 months (Liu, 2021). Adult hemoglobin (HbA) accounts for 96–98% of all hemoglobin that functions in adults. Fetal hemoglobin (Hb F) is abundant during fetal life, but declines after birth to less than 1% by the first year of life (Bain, 2011). Figure (2.1) shows gene expression of hemoglobin before and after birth, in addition to the cell's types and organs where different subunits are being produced over time.

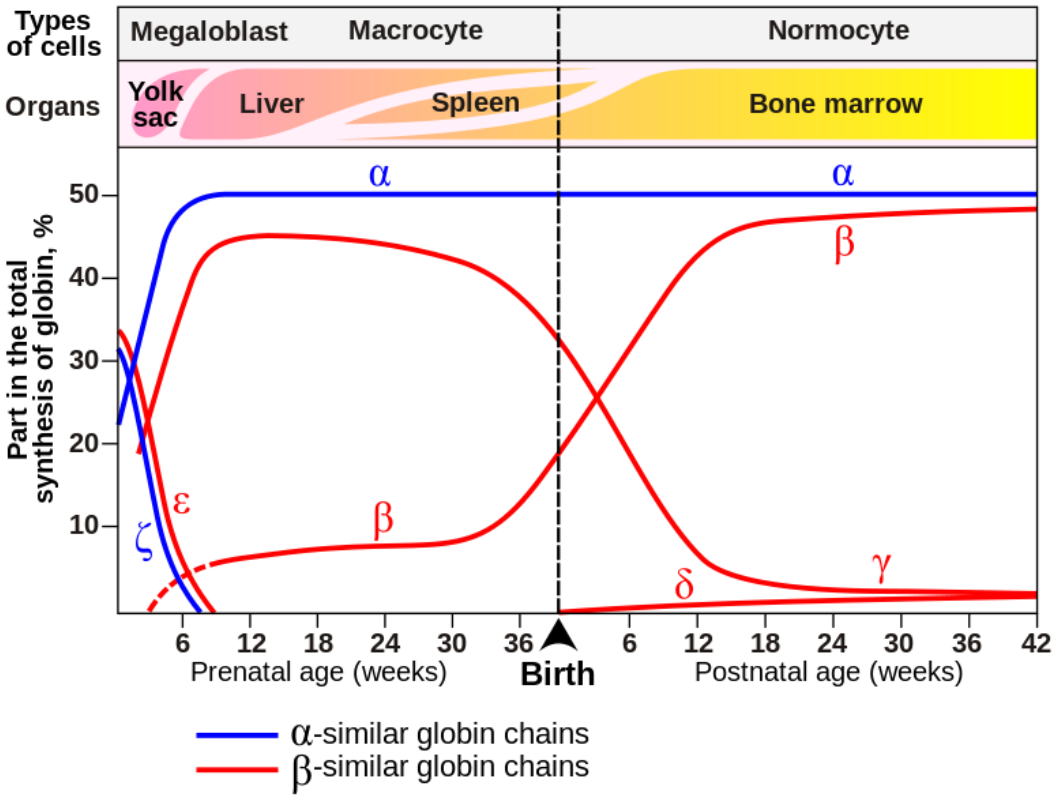

Figure (2.1): Gene expression of hemoglobin before and after birth. (Source:(Wood, 1976))

2.2 Thalassemia

Thalassemias are a group of inherited hematologic disorders caused by defects in the synthesis of one or more hemoglobin chains, imbalances of globin chains results in hemolysis and impair erythropoiesis, which can lead to a variety of health issues Thalassemia (Muncie & Campbell, 2009). Thalassemias results in a quantitative defect in hemoglobin synthesis, this contrasts with hemoglobinopathies, such as sickle cell disease, which are structural or qualitative hemoglobin defects (Needs, Gonzalez-Mosquera, & Lynch, 2018). There are two main types of thalassemia, alpha thalassemia and β-thalassemia. The type of thalassemia is determined by the number of gene mutations and the section of the hemoglobin molecule – alpha or beta – that is affected.

2.2.1 Alpha Thalassemia

Alpha-thalassemia is caused by insufficient or absent alpha globin chain synthesis, resulting in an excess of beta globin chains. Two genes on each chromosome 16 control the production of alpha globin chains (Chui & Waye, 1998). α -thalassemia is typically a milder form of the disease; this is due to the fact that there are four α-globin genes, which necessitate multiple mutations to have a clinical impact. Furthermore, when compared to unpaired α-globin chains in β-thalassemia, unpaired β-globin chains in α-thalassemia are intrinsically less prone to precipitation (Rund & Rachmilewitz, 2005).

2.2.2 Beta-Thalassemia

β-thalassemia is an autosomal recessive inherited blood disorder characterized by decreased or missing beta globin chain production, with phenotypes ranging from severe anemia to clinically asymptomatic patients (Raffaella Origa, 2000). It is estimate that about 1.5 % of the world's population (80 to 90 million people) are β-thalassemia carriers (Renzo Galanello & Origa, 2010) and the annual incidence of symptomatic patients with β-thalassemia is estimated to be 1 in 100,000 worldwide (Malakar, Kour, Malviya, & Dangi, 2016).

β-thalassemia has historically been classified based on the zygosity of the beta-gene mutation into three categories: β-thalassemia minor (trait), intermedia, and major (Bajwa & Basit, 2019). When a defective gene is unable to produce any β-globin, it is designated as "β0," resulting in the more severe type of β-thalassemia. If the mutant gene can still fulfill some functions, it is classified as "β+" (Wahed & Dasgupta, 2015).

β-thalassemia could be associated with other hemoglobinopathies (Renzo Galanello & Origa, 2010). Beta-thalassemia with associated Hb anomalies includes:

- HbC/Beta-thalassemia
- HbE/Beta-thalassemia
- HbS/Beta-thalassemia (clinical condition more similar to sickle cell disease than to thalassemia major or intermedia)
- Hereditary persistence of fetal Hb and β-thalassemia
- Autosomal dominant forms
- Beta-thalassemia associated with other manifestations.
- Beta-thalassemia-tricothiodystrophy
- X-linked thrombocytopenia with thalassemia

2.3 Clinical Classification of β-Thalassemia

Thalassemia classification can vary depending on specific factors. Thalassemia is primarily categorized into two major types: Transfusion-Dependent Thalassemia (TDT) and Non-Transfusion-Dependent Thalassemia (NTDT).

1. **Transfusion-Dependent Thalassemia (TDT)**:

This variant of thalassemia is characterized by its more severe nature. Patients typically necessitate regular blood transfusions for managing their condition. Frequent transfusions may lead to iron overload, requiring chelation therapy to eliminate excess iron from the body. TDT is often associated with mutations that result in a significant reduction or absence of one of the globin chains essential for hemoglobin production.

2. **Non-Transfusion-Dependent Thalassemia (NTDT)**:

NTDT, in contrast, exhibits milder symptoms compared to TDT. Patients may not require regular blood transfusions, or they may only need them intermittently, such as during illness or pregnancy. NTDT is typically linked to genetic mutations that reduce, but do not entirely halt, the production of one of the globin chains. Despite being less severe, NTDT still

presents certain risks, including iron overload (even without transfusions), bone deformities, and an elevated risk of blood clot formation.

These classifications offer insights into the diverse manifestations and severity levels of thalassemia. Alternatively, thalassemia can also be classified into three types based on clinical characteristics:

2.3.1 β-Thalassemia Major (Cooley's Anemia)

β-thalassemia major is caused by a homozygous mutation in the beta-globin gene (beta-zero thalassemia), which results in the complete absence of beta chains (Bajwa & Basit, 2019). β-thalassemia homozygotes can develop either thalassemia major or thalassemia intermedia (Cao & Galanello, 2010). It is the most severe form of thalassemia and manifests itself between the ages of 6 and 24 months. Thalassemia major is a severe transfusion-dependent anemia, Growth retardation and sexual maturation complications, as well as those seen in adults with HFE-associated hereditary hemochromatosis (HH), are all consequences of iron overload (Cao & Galanello, 2010). Affected infants fail to thrive and become progressively pale, feeding difficulties, diarrhea, irritability, recurring spells of fever, and gradual abdominal enlargement due to spleen and liver enlargement are all possible symptoms. Deformities in the long bones of the legs, as well as usual craniofacial changes, are skeletal modifications and tendency to a mongoloid slant of the eye(Renzo Galanello & Origa, 2010).

2.3.2 β-thalassemia Intermedia

β-Thalassemia intermedia is a clinical condition that falls in between asymptomatic carrier β-thalassemia minor and transfusion-dependent severe anemia β -thalassemia major (Asadov, Alimirzoeva, Mammadova, Aliyeva, Gafarova, & Mammadov, 2018).Patients with thalassemia intermedia have a wildly heterogeneous clinical profile, they present later than those with thalassemia major, have milder anemia, and they do not need or only need transfusions occasionally (Kesse-Adu, 2013). Patients between the ages of 2- and 6-years old present at the severe end of the clinical spectrum, and while they are capable of surviving without regular blood transfusions, their growth and development are limited. Patients who are asymptomatic until adulthood with only mild anemia are on the other end of the range (Renzo Galanello & Origa, 2010).

2.3.3 β-thalassemia Minor

Carriers or people with thalassemia minor are people who have the thalassemia trait in one gene. When both parents are carriers there is a 25% risk at each pregnancy of having children with homozygous thalassemia(Renzo Galanello & Origa, 2010). Thalassemia minor causes carriers to become anemic or mildly anemic (Steven E. McKenzie MD, 2011). Hemoglobin electrophoresis with an increased HbA2 is a hallmark of β-thalassemia minor (Silbermins, 2012).

2.4 Epidemiology of β-thalassemia

Approximately 1.5% of the world's population is β-thalassemia carriers. β-thalassemia is most common in South Asia, the Middle East, North Africa, and Southern Europe (De Sanctis et al., 2017; A. Kattamis et al., 2020).

According to studies, the Maldives has one of the highest thalassemia carrier rates in the world and the highest in Asia, with 16–18% of Maldivians being β-thalassemia carriers (Mustafa et al., 2020). Cyprus is an Eastern Mediterranean Island where a high prevalence of thalassemia poses a major public health concern with carriage rate of around 12% (Kountouris et al., 2016). furthermore, Sardinia has a carriage rate of 12.6 % (Cao et al., 1978).

In the Arab region, the carrier frequency ranges from 1% to 11% (Khan, Al-Sulaiti, Younes, Yassin, & Zayed, 2021). For example, ß-thalassemia is one of Jordan's most common genetic ailments, with a carrier prevalence of 2-4 % attributed to high consanguinity marriages, with studies estimating that first cousin marriages account for 20-30% of all marriages (Hinda, Qubbaj Waffa, Mohammed, Saleh, & Mohammed). In Egypt, β-thalassemia is the most frequent kind of chronic hemolytic anemia (85.1%) with a carriage rate of 9-10.2% (A El-Beshlawy et al., 1999). Moreover, in Gaza Strip, β-thalassemia is considered a major public health issue, which is exacerbated by the high occurrence of consanguinity that accounts 40.5- 47.5% of all marriages. The overall prevalence of β-thalassemia in Gaza Strip was 4.3% (M. Sirdah, 2008).

Although thalassemia used to be a problem mainly in certain regions of the world, migration is increasing the prevalence of β-thalassemia and other major haemoglobinopathies in various European nations, and major haemoglobinopathies have now become Europe's most frequent genetic uncommon disease (A. Kattamis et al., 2020). Due to the increasing in the adoption rate of children with thalassemia from China and other Asian nations to the United States in recent decades, the incidence and prevalence of β-thalassemia have significantly increased (E. P. Vichinsky, MacKlin, Waye, Lorey, & Olivieri, 2005). The COVID-19 pandemic has greatly reduced migration, although the long-term effects are still undetermined (A. Kattamis et al., 2020). Table (2.2) shows the distribution of β-thalassemia carriers among different populations.

Table (2.2): Distribution of β-thalassemia carriers among different populations.

Country	**Prevalence (%)**	**References**
Maldives	16–18%	(Mustafa et al., 2020)
Sardinia	12.6%	(Cao et al., 1978)
Cyprus	12%	(Kountouris et al., 2016)
Egypt	9-10.2%	(A El-Beshlawy et al., 1999)
United Arab Emirates	8.5%	(S. Kim & Tridane, 2017)
Greece	7.4%	(Malamos, Fessas, & Stamatoyannopoulos, 1962)
Gaza Strip	4.3%	(M. Sirdah, 2008)
Palestine	4%	(Al Sabbah et al., 2017)
Jordan	2-4%	(Hinda et al.)

2.4.1 Spectrum of β-thalassemia Carriage in the Mediterranean Belt and Arabic Countries

According to the study held at 22 different Arab countries in 2020, the majority of HBB gene mutations were found in Saudi Arabia followed by Syria, while Palestine ranked the highest fourth Arab countries (Khan et al., 2021). The number of mutations found in each population varies based on its origin and interaction with other populations, and the methods employed to characterize it (H. A. Hamamy & Al-Allawi, 2013).

IVS-1-110 (G>A) is the most prevalent and widespread mutation among Arabs with a rate range between 12%–38%. The latter mutation is most common in Cyprus and Greece, suggesting that it has originated in Greece. It is also the most frequent thalassemia allele in the Turkish population (De Sanctis et al., 2017). The most common and widespread mutations are most probably the oldest is codon 39 (C>T). All Arab countries, without exception, share this mutation; it is believed to be of Roman origin (Zahed, 2001).

According to Khan et al, the following HBB gene mutations had the highest prevalence across Arab countries among the 99 shared mutations with other ethnic groups: c.93–21 G > A, c.118 C > T, c.92 + 1 G > A, c.92 + 6 T > C, c.92 + 5 G > C, c.315 + 1 G > A, and c.27dupG (Khan et al., 2021).

On the other hand, in the Mediterranean belt, the most prevalent thalassemic genes in the Po River Delta region are β°39 (nonsense mutation at codon 39) and β+ IVS-1-110 (G→ A) mutation (Del Senno et al., 1985). The 0-39 (C→ T) mutation, on the other hand, is almost exclusively prevalent in Sardinia (Cao & Kan, 2013), It's also common in continental Italy, Spain, as well as Portugal and Tunisia (De Sanctis et al., 2017).

Recent research has revealed that the majority of β-thalassemia cases in Palestine are caused by a small number of mutations in the β-globin gene, and that similar mutations are also widespread in the surrounding area (Reading, Sirdah, Tarazi, & Prchal, 2014; Zahed, 2001). [Cd5 delCT, Cd6 delA, Cd27 G→ T, IVS-I-1 G→ A, IVS-I-6 T→ C, IVS-I-110 G→ A, Cd37 G→ A, Cd 39 C→ T, and IVS-II-1 G→ A] are the most common mutations found among Palestinians (Reading et al., 2014).The IVS-I-6 (T→ C) represents the most common one. The IVS-I-110 (G→ A) and codon 37 (G→ A) mutations, combined with the IVS-I-6 (T→ C) mutation, account for more than 50% of the total alleles discovered., according to the study were held in West Bank in 2010 (Darwish, El-Khatib, & Ayesh, 2005).

2.4.2 β-thalassemia among Palestinians

In 1996, the World Health Organization (WHO), in collaboration with The Thalassemia Patients' Friends Society (TPFS), conducted a survey that revealed a 4% prevalence of thalassemia carriers (Al Sabbah et al., 2017). According to Thalassemia Patients' Friends Society (TPFS) data, the prevalence of thalassemia was 17.4 per 100,000 people in 2018, with 847 symptomatic thalassemia patients across the West Bank and Gaza Strip. Furthermore, records show that two-thirds of Palestinian patients were from the West Bank, the northern governorates had the highest proportion of patients (47%) in the West Bank, while the central governorates had only 9% and the southern governorates had 12% (Aldwaik et al., 2021). In Gaza Strip, the prevalence of thalassemia is 0.02% (Tarazi et al., 2007).

As of 2021, the number of β-thalassemia patients in Palestine is 864 patients, 557 of them were from West Bank, and 307 were from Gaza Strip (TPFS, 2021). The relatively high prevalence of Thalassemia gene carriers in Palestinian increases the likelihood of new cases in the future; this is due to mutations being passed down from carrier parents to their offspring.

Pre-marital β-thalassemia testing was introduced in Palestine in September 2000, resulting in a decrease in the number of new β-thalassemia births. In addition, to supplement the screening program for carriers married before the year 2000, prenatal diagnoses via chorionic villus sampling (CVS) and amniocentesis were introduced (Tarazi et al., 2007).

According to the Thalassemia Patients' Friends Society (TPFS), the prevalence of thalassemia was 17.4 per 100,000 in 2018, with a total of 847 symptomatic thalassemia patients across the West Bank and Gaza Strip (Aldwaik et al., 2021). More than 300 β-thalassemia patients are currently being treated in Gaza Strip government hospitals through blood transfusions and iron chelation (Ayyash & Sirdah, 2018).

The focus of β-thalassemia control and care is on those with severe clinical symptoms associated with the main type. The most prevalent procedure for controlling and managing disease conditions in BTM patients is blood transfusion treatment (Daraghmeh, 2016). Patients with β thalassemia need blood transfusions to survive. The primary purpose of blood transfusion is to treat anemia, and the decision to begin transfusion therapy should be based mostly on Hb levels (Renzo Galanello & Origa, 2010). The average age of patients in Palestine has increased from 7–8 years in 1996 to 19–20 years in 2015 (Al Sabbah et al., 2017).

Although blood transfusions save lives in individuals with β-thalassemia, they also burden the body with extra iron, which leads to hemosiderosis and other comorbidities, as well as irreversible biological damage such as cirrhosis, liver fibrosis, heart disease, and endocrine abnormalities (Ayyash & Sirdah, 2018) and, most critically, the heart. If effective iron chelating is not used, death occurs in TM due to heart failure or arrhythmia (Hoffbrand, Vyas, Campo, Haferlach, & Gomez, 2019).

The current accepted mean Hb is 12 g/dl, with a post-transfusion Hb of 14–15 g/dl and a pre-transfusion Hb of 9–10.5 g/dl, according to TIF recommendations (Cappellini et al., 2014). Anemic presentation of Palestinians thalassemia patients in West Bank showed a mean Hb of 8.4±1.4 g/dl during the last 2-year follow-up (Aldwaik et al., 2021). These low Hb levels suggest inadequate transfusion treatment regimen management, a lack of adherence to international standards, and maybe a failure to ensure patient adherence to therapy on a regular basis.

According to the study were held in Gaza Strip in 2018, β-thalassemia were experiencing a worsening clinical situation as seen by their hematological and biochemical test results, which is mostly owing to their severe anemia and iron overload caused by a prolonged blood transfusion routine combined with insufficient chelation (Ayyash & Sirdah, 2018).

Endocrinopathies are the most negative side effects of these β-thalassemia syndrome and iron overload, accounting for 44.7 % of cases (Aldwaik et al., 2021). A study was held in Palestine in 2014 has shown endocrine abnormalities of β-thalassemia patients, hypogonadotropic hypogonadism (HH) or secondary hypogonadism associated to the hypothalamus and pituitary glands was found in 47 % of cases, thalassemia patients also showed signs of growth retardation, such as short stature and underweight, which is a sign of insufficient transfusion (K Dumaidi, Al-Jawabreh, Al-Assi, & Karmi, 2015).

According to the study in Gaza Strip in 2014, splenectomy was performed in nearly 90% of the 65 transfusion-dependent thalassemic patients due to Hypersplenism (cytopenia caused by splenomegaly) or enormous splenomegaly that caused agony and discomfort for the patient (Ayyash & Sirdah, 2018).

According to the TIF Guidelines, which have been adopted and used by many countries, it is recommended to monitor body iron levels by assessing liver iron content using MRI-based approaches; however, MRI-based approaches are not yet available in the West Bank (Aldwaik et al., 2021). Serum ferritin testing may be the only way to estimate iron overload

in developing countries such as Palestine. It can detect changes in iron concentration in patients who are being closely monitored. Iron chelating therapy, when combined with blood transfusions, is the greatest option for reducing comorbidities and extending patients' live (Cianciulli, 2009).

In Palestine, the average life expectancy of a thalassemia patient is only 15 years, and many suffer from iron overload, with iron levels reaching 1,000 mg/dL (Al Sabbah et al., 2017). This has highlighted the importance of management and monitoring the patients' health status, including measuring the rise in serum iron, ferritin, liver, and cardiac iron concentrations, which results in organ damage and death.

2.5 The Changing Epidemiology of the Ageing Thalassemia Populations

The average life expectancy for individuals with β-thalassemia increased from 17 years in 1970 to 27 years in 1980 to 37 years in 1990. In 1964, only 37% of patients survived to 16.4 years of age (Engle, Erlandson, & Smith, 1964). In contrast, in 2011, 89% of patients survived to the age of 40 (Ladis et al., 2011). Recent evidence indicates that 63% of patients are expected to live until the age of 50, and 33% of these patients are expected to be disease-free at this age, indicating a significantly longer life expectancy (Weidlich, Kefalas, & Guest, 2016). The Survival of β-thalassemia patients over the past several decades, as reported by a selection of publications is outlined in Figure (2.2) (Farmakis et al., 2020)

According to a study published in Science Direct, life expectancy for patients with β-thalassemia has improved dramatically since the development of iron chelation therapy (ICT). In Palestine, the average patient age has increased from 7–8 years in 1996 to 19–20 years in 2015 (Shah, Sayani, Trompeter, Drasar, & Piga, 2019). In a study published in Frontiers, a total of 309 patients with β-thalassemia were included with an average age of 23.4±10.4 years ranging from 2 to 68 years (Aldwaik et al., 2021).

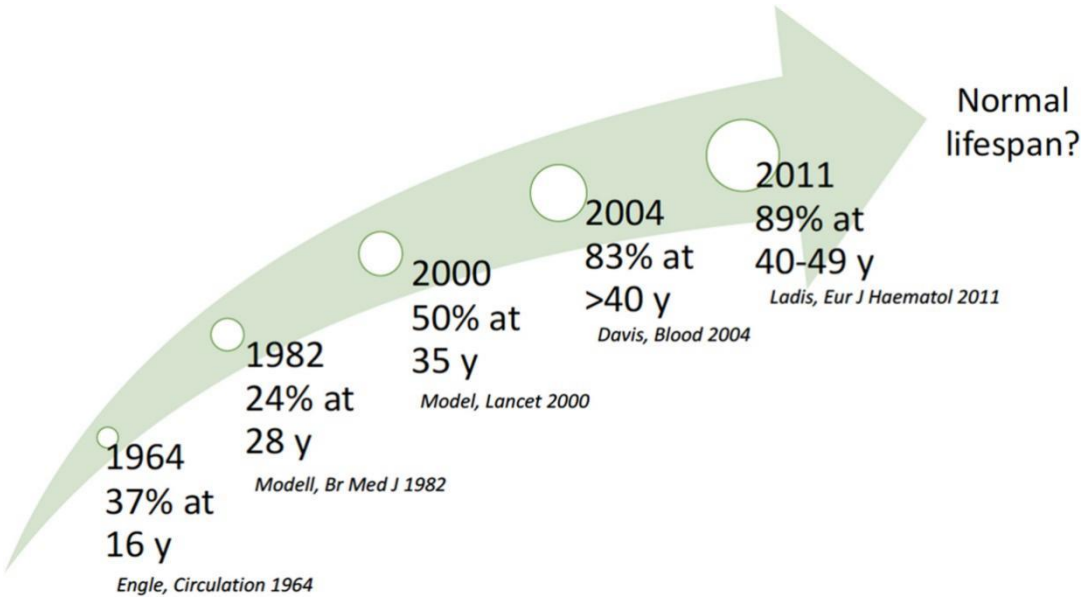

Figure (2.2): Survival of thalassemia patients over the past several decades, as reported by a selection of publications. (Source: (Farmakis et al., 2020))

2.6 Pathophysiology of β-thalassemia

β-Thalassemia is characterized by a quantitative reduction of structurally normal globin chains. They are caused by exceedingly diverse mutations that almost all damage the globin locus (Nienhuis & Nathan, 2012). Because α- and non α-chains pair at a nearly 1:1 ratio to generate normal Hb, in β-thalassemia, the molecular defects lead to absent or decreased synthesis of β-chains and because alpha chain synthesis is unaffected, globin chain production is unbalanced, resulting in an imbalance of chains, the excess mismatched chains accumulate in the cell as an unstable product, resulting in cell death in the bone marrow and extramedullary locations. This is known as inefficient erythropoiesis (IE) and it is a defining feature of β-thalassemia (Hassan et al., 2016). Moreover, excessα-chain precipitations cause structural and functional damage to the cell membrane and intracellular organelle membranes; they also stimulate the formation of reactive oxygen species, which damage the protein and lipid elements of cell membranes (Nienhuis & Nathan, 2012).

During erythropoiesis, the production of α-chain inclusions begins early and peaks in polychromatophilic erythroblasts leading to cellular apoptosis. Anemia in severe β-thalassemia reflects both inefficient erythropoiesis and shortened red cell survival (Mathias et al., 2000).

Extramedullary erythropoietic tissue, particularly in the thorax and paraspinal area, may be stimulated by increased erythropoietin production resulting in a rapid but ineffective expansion of the bone marrow (up to 25 to 30 times normal), in addition to abnormalities of the skull and face, marrow enlargement causes osteopenia(Hassan et al., 2016). In fact, rather than underproduction of hemoglobin, the degree of imbalance in α-globin vs β + γ globin biosynthetic ratio is the most important predictor of disease severity (Nienhuis & Nathan, 2012). Figure (2.3) summarizes the pathophysiology of β-thalassemia.

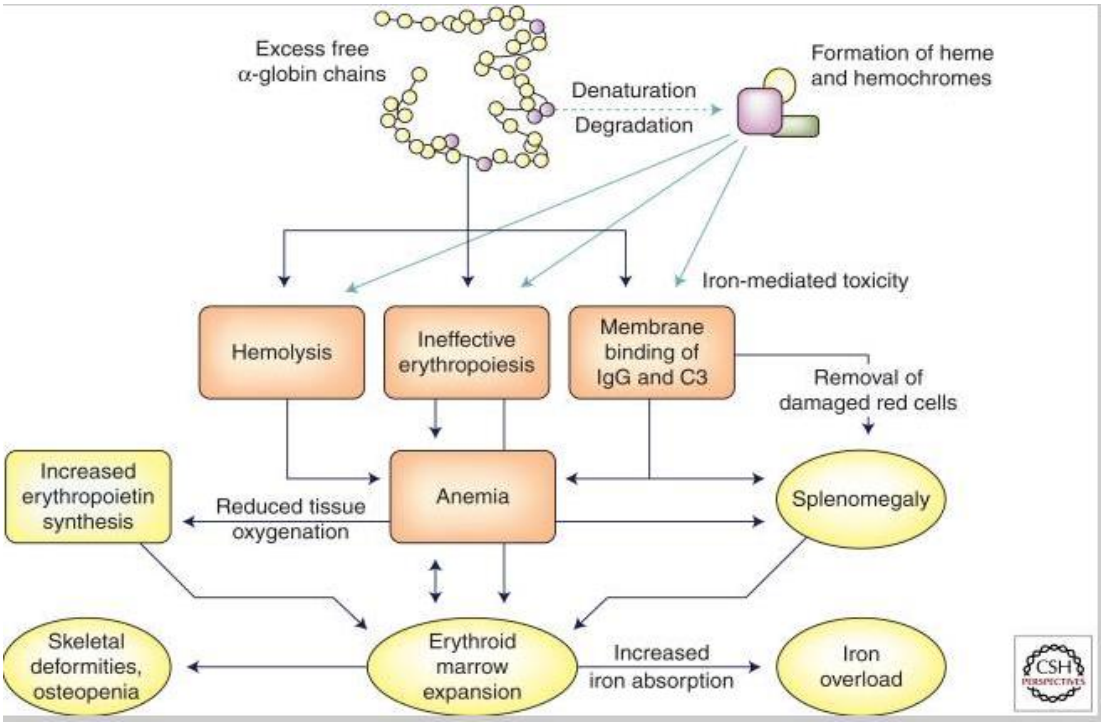

Figure (2.3): Pathophysiology of β-thalassemia. (Source: (Nienhuis & Nathan, 2012)).

2. 7 Molecular Basis of β-thalassemia

The human β-globin locus is approximately 70 kb in length and contains five genes, each with its own promoter, arranged in the following order: ε, Gγ, Aγ, δ, and β (G.T.Hesslein, 2004). β globin is encoded by a structural gene found in a cluster with the other β-like genes on chromosome 11 (11p15.15) and it is made up of three exons separated by two interconnected intron sequences known as "IVS." (Stamatoyannopoulos, 2005). β - globin gene expression is tightly regulated during development and hematopoiesis, and its expression is dependent on the locus control region (LCR), which is made up of five DNase I hypersensitive sites (HS)(Swee Lay Thein, 2013).

For developmental expression, two mechanisms have been proposed: 1) gene competition for the upstream β-LCR, with the gene nearest to the LCR gaining an advantage (Hanscombe et al., 1991), and 2) autonomous silencing (transcriptional repression) of the preceding gene (Raich, Enver, Nakamoto, Josephson, Papayannopoulou, & Stamatoyannopoulos, 1990).While the ε and γ globin genes are silenced autonomously at the proper embryonic stage, production of the adult globin gene is dependent on the absence of competition for the LCR sequences from the upstream gene (S. L. Thein, 2018).

β-thalassemia is caused by a variety of genetic abnormalities that induce a quantitative reduction in structurally normal globin chains (Farmakis, Porter, Taher, Domenica Cappellini, Angastiniotis, & Eleftheriou, 2022). Although about 300 thalassemia alleles have been identified, only roughly forty of them account for 90% or more of all thalassemia globally, this is because only a few mutations are widespread in locations where thalassemia is prevalent, probably indicating local selection owing to malaria. As a result, each of these populations has its own set of thalassemia alleles (Bajwa & Basit, 2019).

Unlike most α-thalassemia syndromes, which are caused by deletions, 95% of β-thalassemia's are due to point mutations (Sabath, 2017) that disrupt all aspects of β-globin production, including transcription, translation, and product stability or nonsense mutations that result in aberrant protein synthesis or nonsense-mediated RNA degradation (Hassan et al., 2016; Sabath, 2017).

2.7.1 Non-Deletion Form of β-Thalassemia

The majority of the β-thalassemia alleles are caused by these abnormalities. They affect practically every known stage of gene expression and include single base alterations, minor insertions, and deletions within the gene, or its immediate flanking sequences (Renzo Galanello & Origa, 2010) as shown in Figure (2.4).

2.7.1.1 Transcriptional Mutations

Patients from various ethnic groups have been shown to have point mutations in the conserved DNA sequences that make up the β-globin promoter and a stretch of 50 nucleotides in the 5′ UTR (Swee Lay Thein, 2004). They usually cause mild to minimal globin output deficiency, which reflects the relatively moderate phenotype of these β+ thalassemias (S. L. Thein, 2018). Some of these β-thalassemia alleles are so mild that heterozygotes (carriers) have nearly normal red cell indices and HbA2 levels, with the main aberration being an imbalanced globin-chain synthesis (Gonzalez-Redondo et al., 1989). Overall, "silent" β -thalassemia alleles are uncommon, with the exception of the –101 C→ T mutation, which has been seen quite commonly in the Mediterranean region, where it interacts with a range of more severe β-thalassemia mutations to produce milder types of β-thalassemia, (Maragoudaki et al., 1999). Several 5′-UTR mutations, such as CAP + 1A-C, have a 'silent' phenotype (C. Wong, Dowling, Saiki, Higuchi, Erlich, & Kazazian, 1987).

2.7.1.2 Mutations Affecting RNA Processing

A wide range of mutations obstruct the original mRNA transcript's processing. These mutations at the splice junction can damage either of the invariant dinucleotides (GT at 5′ and AG at 3′), in which case normal splicing is completely abolished, resulting in the phenotype of β ° thalassemia. Splicing efficiency is reduced to variable degrees by mutations in the consensus sequences at the splice junctions, resulting in a β+ phenotype that ranges from mild to severe (Swee Lay Thein, 2004).

Mutations at position 5 IVS1 G → C, T, or A, for example, significantly diminish splicing at the mutant donor site when compared to normal. Three "cryptic" donor sites, two in exon 1 and one in IVS1, appear to be activated by the mutations and are used preferentially to the altered donor site (Treisman, Orkin, & Maniatis, 1983). The G to A mutation in the IVS1-110 gene was the first identified base change in a β-thalassemia gene. In the Mediterranean population, it is one of the most common types of β-thalassemia (Spritz et al., 1981). Whereas this mutation is the second most prevalent mutation in our community (Darwish, El-Khatib, & Ayesh, 2005). β-thalassemia major affects 0.02% of the population in the Gaza Strip, with the IVS-I-110 mutation being the predominant mutation found in 34% of thalassemic patients in this region (M. M. Sirdah et al., 2013).

2.7.1.3 Mutations Affecting Translation of β-Globin mRNA

Mutants affecting translation of β-globin mRNA have been identified as key contributors to the pathogenesis of β-thalassemia. These mutations disrupt the normal translation process, leading to the production of abnormal β-globin proteins. Examples of such mutants include

alterations in the ribosome binding site, start codon, or stop codon of the mRNA. These mutations can result in truncated or nonfunctional β-globin chains, which subsequently lead to hemolytic anemia and ineffective erythropoiesis. Understanding the molecular mechanisms underlying these mutants is crucial for accurate diagnosis, prognosis, and the development of targeted therapeutic interventions (Jaing, Chang, Chen, Lin, Wen, & Chiu, 2021).

The clinical implications of mutants affecting translation of β-globin mRNA in β-thalassemia are significant. The resulting aberrant β-globin protein production disrupts the normal structure and function of hemoglobin (Swee Lay Thein, 2013). This disruption leads to the characteristic manifestations of β-thalassemia, including varying degrees of hemolytic anemia, ineffective erythropoiesis, and clinical phenotypes ranging from thalassemia major to intermedia. The severity of the clinical phenotype may vary depending on the specific mutation and its impact on β-globin translation. Therefore, identifying these mutants and understanding their clinical implications is crucial for appropriate management and genetic counseling of individuals affected by β-thalassemia (S. L. Thein, 2018).

The study of mutants affecting translation of β-globin mRNA has opened up avenues for potential therapeutic strategies in β-thalassemia. Researchers are exploring innovative approaches to correct or bypass the abnormal translation process and restore normal β-globin protein synthesis (S. L. Thein, 2018). These approaches include gene therapy, RNA-based therapies, and the use of small molecule compounds. For instance, gene editing techniques using CRISPR/Cas9 hold promise for targeted correction of specific mutations. By understanding the molecular basis of mutants affecting β-globin translation, scientists aim to develop effective and personalized treatments for individuals with β-thalassemia, ultimately improving their quality of life and prognosis. Continued research in this field is essential to advance our understanding and expand the repertoire of therapeutic options for β-thalassemia patients (Jaing et al., 2021).

2.7.1.3.1 Mutations Affecting the Initiation Codon

All β0- thalassemia is caused by mutations in the initiation codon (ATG)(Swee Lay Thein, 2013). However, these alternate start cordons are expected to cause premature termination, rendering mutant mRNAs nonfunctional and vulnerable to nonsense-mediated decay surveillance (Garcia-Rodriguez et al., 2020).

2.7.1.3.2 Premature Termination Codons

Approximately half of all β-thalassemia alleles are caused by the introduction of premature termination codons, either as a result of direct mutations that produce a stop codon or as a result of a change in the reading frame caused by the insertion or deletion of a single to a few nucleotides (S. L. Thein, 2018).

The frameshift and nonsense mutations that produce recessively inherited β0- thalassemia often result in premature exon 1 and 2 terminations, with minimal steady-state quantities of aberrant mRNA (Swee Lay Thein, 2013). The mutation at codon 39 (CAG to TAG) was one of the first nonsense mutations to be identified and investigated extensively (Humphries, Ley, Anagnou, Baur, & Nienhuis, 1984). This mutation is the second most prevalent cause of β-thalassemia among Mediterranean people, and it accounts for the majority of cases in Sardinia (Takeshita, 1984; Swee Lay Thein, 2013)

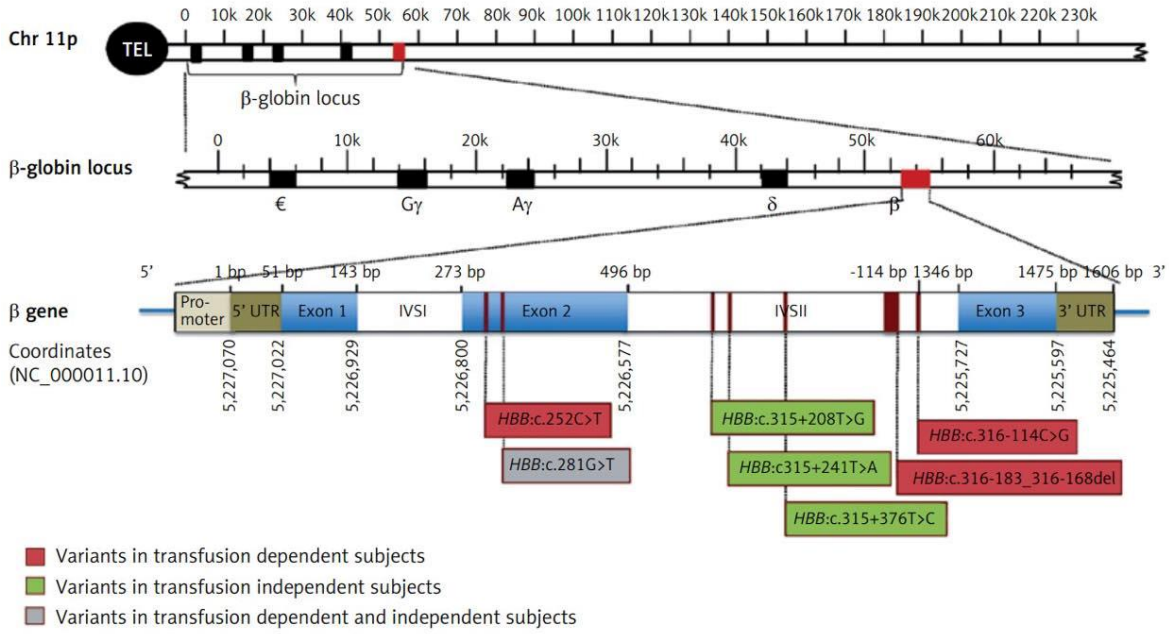

Figure (2.4): Location of mutations of the β-globin gene that result in β-thalassemia. ((Source: (Aldakeel et al., 2020)).

2.8 Genotype-Phenotype Correlation

β-thalassemia is divided into four types based on genotype-phenotype correlations: β-thalassemia major (also known as Cooley's anemia), β-thalassemia intermedia, β-thalassemia minor and silent β-thalassemia (Origa, 2017).

Silent β-thalassemia carriers are heterozygous for the β++ allele (β++/β), and their red blood cells and HbA2 levels are normal (Swee Lay Thein, 2013). Patients with a single β0 or β+ allele in the β-thalassemia minor gene are asymptomatic. Their laboratory data, on the other hand, show elevated HbA2 levels as well as decreased microcytosis and hypochromia (Khan et al., 2021).

Patients with β-thalassemia intermedia can be homozygous for the β+ allele (β+/β+) or compound heterozygous for the β0 and β+ alleles (β0/β+). The phenotypic spectrum of β-thalassemia intermedia ranges from mild to severe anemia with occasional blood transfusions (Ho, Hall, Luo, Weatherall, & Thein, 1998). The most severe form of thalassemia, referred to as β-thalassemia major, this type requires regular blood transfusions to survive. The majority of β-thalassemia major instances are homozygous with the β0 allele (β0/β0), with a lower cases having compound heterozygous (β+/β0)(Chen et al., 2010).

2.9 Diagnostic Criteria of β-thalassemia

A complete clinical history, physical examination, and laboratory results, including a complete blood count, Hb profiles, and molecular studies, are required for screening and definitive diagnosis of thalassemia and hemoglobinopathies (Ghosh et al., 2014).

2.9.1 Clinical Diagnosis

If an infant or child under the age of two years has severe microcytic anemia, mild jaundice, or hepatosplenomegaly, β-thalassemia major should be suspected (Raffaella Origa, 2000). Some major adolescents experience delayed puberty. Many people with thalassemia major experience such severe symptoms that they require blood transfusions on a regular basis to replenish their red blood cell supply (Cao & Galanello, 2010).

Patients with thalassemia intermedia present with moderate anemia later in life and do not require regular transfusions. The main clinical features of these patients are erythroid marrow hypertrophy with medullary and extramedullary hematopoiesis and its complications (osteoporosis, masses of erythropoietic tissue that primarily affect the spleen, liver, lymph nodes, chest and spine, and bone deformities and typical facial changes)(Renzo Galanello & Origa, 2010).

The β-thalassemia carrier state, which results from β-thalassemia heterozygosity, is clinically asymptomatic and is characterized by specific hematological features (Cao & Galanello, 2010).

2.9.2 Hematological Diagnosis

A diagnosis of β-thalassemia in individuals aged 12 months or older is typically based on specific hematologic findings. These include microcytic hypochromic anemia, anisopoikilocytosis with the presence of nucleated red blood cells on a peripheral blood smear, and hemoglobin analysis revealing decreased or absent hemoglobin A and increased levels of hemoglobin F (Munkongdee, 2020).

β-thalassemia carriers commonly exhibit a mean corpuscular volume (MCV) of less than 80 fL and a mean corpuscular hemoglobin (MCH) of less than 27 pg, along with elevated red blood cell (RBC) counts. In cases where there is a delay in sample analysis, MCH is considered a more reliable parameter than MCV (Sabath, 2017).

Patients diagnosed with thalassemia major present with severe microcytic and hypochromic anemia, characterized by a reduced number of red blood cells and low levels of hemoglobin (Hb) (<7 g/dl), as well as decreased MCV (50-70 fL) and MCH (12-20 pg) (Galanello & Origa, 2010).

Individuals carrying either the β° or severe β++ mutations typically exhibit a relatively high red blood cell count (RBC), while their mean corpuscular hemoglobin (MCH) levels are significantly lower (MCV: 60-70 fL; MCH: 19-23 pg). Hemoglobin levels can vary considerably, ranging from normal to a decrease of up to 2 g/dL (Brancaleoni, Di Pierro, Motta, & Cappellini, 2016). The reference ranges for RBCs indices are listed in Table (2.3).

It is important to note that these two red blood cell indices alone cannot differentiate between thalassemia trait and iron deficiency, or between α- and β-thalassemic conditions. When microcytic hypochromic parameters are present, a differential diagnosis is necessary to exclude iron-deficient anemia (Ghosh et al., 2014).

The Mentzer index, calculated as the ratio of MCV to RBC count, can aid in distinguishing thalassemia from iron deficiency anemia. In cases of iron deficiency anemia, this ratio is typically greater than 13, whereas in thalassemia, it is typically less than 13 (Wahed, 2020).

Table (2.3): RBC indices in β-thalassemia. (Source: (R. Galanello et al., 1979))

Red Blood Cell Index	Normal		Affected	Carrier
	Male	Female		
Mean corpuscular volume (MCV) (fL)	89.1±5.01	87.6±5.5	50 – 70	<79
Mean corpuscular hemoglobin (MCH) (pg)	30.9±1.9	30.2±2.1	12 – 20	<27
Hemoglobin (Hb) (g/dL)	15.9±1.0	14.0±0.9	<7	Males: 11.5–15.3 Females: 9.1–14

2.9.3 Qualitative and Quantitative Hemoglobin Analysis

Hb analysis is performed by cellulose acetate electrophoresis and high-performance liquid chromatography [HPLC] . The Hb pattern in β-thalassemia differs depending on the type of β-thalassemia. HbA and HbF levels are highly dependent on the underlying molecular defects and the degree of ineffective erythropoiesis (Brancaleoni, Di Pierro, Motta, & Cappellini, 2016).

At hemoglobin analysis, HbA is absent in the classical form of β-thalassemia major (homozygotes β°), and HbF accounts for 92–95 % hemoglobinHbA2 is 2–5% as shown in Table (2.4) (Renzo Galanello & Origa, 2010).

The Hb pattern in beta+-thalassemia homozygotes with residual variable beta globin synthesis or beta0/beta+ compound heterozygotes show HbA between 10 and 30%, HbF between 70–90%, and HbA2 between 2–5% (Cao & Galanello, 2010).

The most valuable test for detecting β-thalassemia carriers is quantitative HbA2 determination. There are several methods available: Michrochromatography, cation exchange HPLC, and capillary electrophoresis are the most accurate, fast, and simple (Lippi & Plebani, 2016). In normal individuals, the expected normal range for HbA2 is between 2.4% and 3.2%, while in typical β-thalassemia carriers, it is between 3.6% and 7%. Values between 3.2% and 3.6% are considered borderline and warrant further investigation, particularly in young subjects or at-risk couples (Brancaleoni et al., 2016).

Despite an abundance of knowledge gathered, several issues in carrier identification persist. Some β-thalassemia carriers have normal or borderline HbA2 levels but low MCV and MCH levels, these include heterozygotes for mild-to-very-mild mutations as (IVS1-6, -87, 101) as well as double heterozygotes (Paglietti et al., 2016). To distinguish these atypical β-thalassemia carriers from others, a family study or additional molecular analysis is required (Viprakasit et al., 2013).

A Saudi Arabian study found that HbA2 levels were 3.5% higher in β-thalassemia trait individuals who were confirmed to have β-thalassemia HBB gene mutations. Individuals with c.17 18het delCT, c.25 26delAA, c.218 G > C, c.281 G > T, c.370A > C, or c.431A > T, HBB mutations had HbA2 levels <3%, while those with c.[118 C > T], c.79 G > A, or c.92 5 G > C had borderline HbA2 levels (Al-Amodi et al., 2018). This was seen also in a Palestinian study, approximately 15.3 % of the microcytic and/or hypochromic cases had a CBC profile consistent with the β-thalassemia carrier state; however, the HbA2 qualitative and quantitative tests did not confirm the carrier state in those subjects. The HbA2 levels were lower than the levels of diagnosis (Tarazi et al., 2007).

As a result, based solely on the HbA2 level, these β-thalassemia trait individuals would have been misidentified as healthy, which is critical. This observation suggests that in the case

of β-thalassemia traits, the phenotype does not always reflect the genotype and that making a diagnosis solely based on phenotype can be misleading; thus, molecular tools such as HBB gene sequencing are important molecular diagnostic tools (Khan et al., 2021).

Table (2.4): Hemoglobin pattern in β-thalassemia. ((Source:(Renzo Galanello & Origa, 2010))

Hemoglobin Type	Normal	Affected		Carrier
		β^0-Thal Homozygotes	β^+-Thal Homozygotes or β^+/β^0 Compound Heterozygotes	β–Thal Minor
HbA	96% - 98%	0	10% - 30%	92% - 95%
HbF	<1%	95% - 98%	70% - 90%	0.5% - 4%
HbA2	2% - 3%	2% - 5%	2% - 5%	>.3.5%

2.9.4 Molecular Genetic Testing

Single-gene testing is the recommended molecular genetic testing method for β-thalassemia (Raffaella Origa, 2000). PCR-based procedures are used to detect commonly occurring mutations in the beta globin gene (Vrettou, Traeger-Synodinos, Tzetis, Malamis, & Kanavakis, 2003). The most common methods are reverse dot blot analysis or primer-specific amplification, which use a set of probes or primers that are complementary to the most common mutations in the population from which the affected individual was derived. If targeted mutation analysis fails to detect the mutation, beta globin gene sequence analysis can be used to detect mutations in the beta globin gene (Renzo Galanello & Origa, 2010).

DNA analysis which sequences the coding regions and introns of the beta-globin gene (HBB) in both orientations, detects hemoglobin variations that are difficult to detect with electrophoresis/HPLC and can be used to diagnose β-thalassemia (Roth, Lachover, Koren, Levin, Zalman, & Koren, 2018). Figure (2.5) shows the flow chart of diagnostic criteria of β-thalassemia.

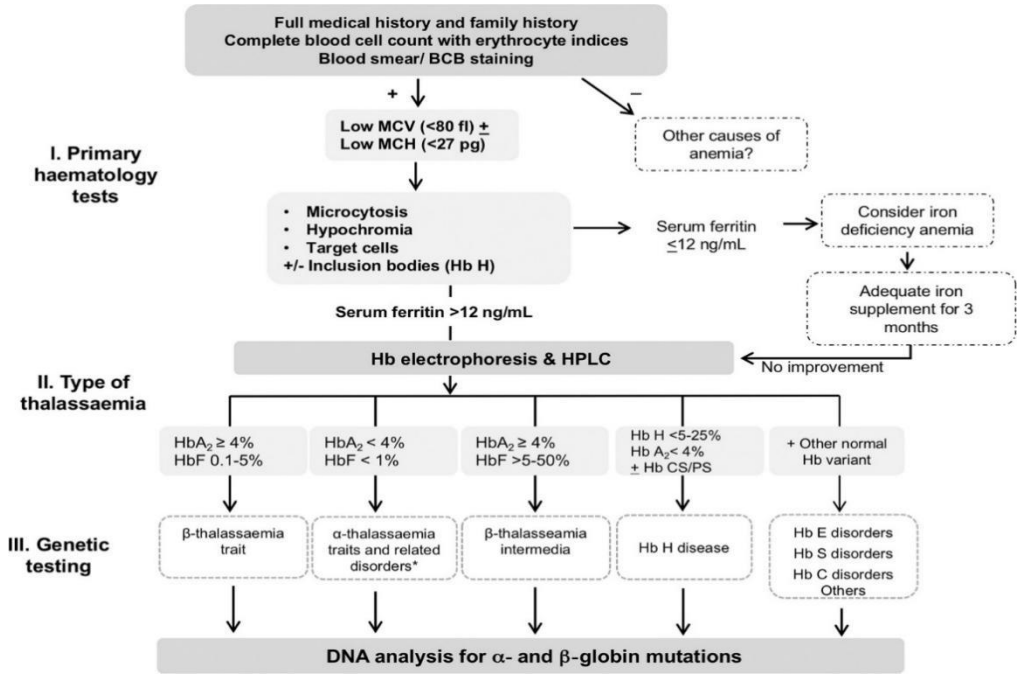

Figure (2.5): Diagnostic flow chart for identification of thalassemia carrier and thalassemia intermedia ((Source: (Brancaleoni, 2016)).

2.10 Factors Associated with Continuing Emerging of β-thalassemia

2.10.1 Sociodemographic Factors

Religious beliefs, ethnicity, culture, consanguineous marriages, and low socioeconomic level are all factors associated with continuing emerging of β-thalassemia (A. Kattamis et al., 2020). In the majority of Asia's developing countries, the prevalence of β-thalassemia is closely linked to the country's social, cultural, and religious difficulties (Chawla, Singh, Lakkakula, & Vadlamudi, 2017).

The high prevalence of β-thalassemia among endemic regions is highly attributed to high consanguineous marriages(Origa, 2017). Depending on ethnicity, religion, culture, and geography, the prevalence of consanguinity and rates of first cousin marriage vary greatly within and between people and communities. Consanguineous marriages are culturally and socially preferred in North Africa, West Asia, and South India where, β-thalassemia is endemic, accounting for 20–50% of all marriages, with first cousin unions accounting for nearly one-third of all weddings (H. Hamamy, 2012). These marriages are also high in rural, impoverished and poorly educated communities (Kumar, Arya, & Agarwal, 2015). The United Arab Emirates (UAE), with a consanguineous marriage rate of over 50%, has a high frequency of thalassemia carriers among the MENA countries (S. Kim & Tridane, 2017). Jordan's high frequency of ß-thalassemia and other genetically determined recessive diseases is partly attributable to the country's high consanguinity marriage rate, which has been estimated to be between 20-30% for first cousin marriages (Hinda et al.). Also, consanguinity is the main cause of high prevalence in Pakistan (Asif & Hassan, 2014).

Moreover, the high prevalence of β-thalassemia in Turkey's Southeast Anatolia region is linked to consanguineous marriages and high birth rates (Gunes & Gozden, 2021). A study was held at Tunisia showed that the geographic distribution of Thalassemia major revealed that it is concentrated in small towns, especially in the western part of the country, were close relative’s marriage (Bejaoui & Guirat, 2013).

2.10.2 Perceptions towards thalassemia

KAP surveys reveal misconceptions or misunderstandings that could be obstacles to the behaviors we would like to implement, as well as potential barriers to behavior change. Understanding the factors that affect thalassemia emergence and patients' quality of life is critical for developing appropriate clinical programs, providing social support, and enhancing treatment outcomes (Jeesh, Aser Adnan, & Al-Haboub, 2018).

2.10.2.1 Knowledge

Lack of understanding and awareness regarding the disorder, its consequences, as well as psychosocial and cultural difficulties, may act as barriers to thalassemia prevention, disclosure, and testing. Due to a major lack of awareness and understanding regarding thalassemia, there is a lot of confusion about the differences between thalassemia carriers and thalassemia major, inheritance patterns, and the physical and psychological effects of the condition on affected people and their families (L. P. Wong, George, & Tan, 2011).

According to the findings of the survey held at Greece, education has the biggest impact on the likelihood of being aware of thalassemia and having proper understanding of the disease's basic aspects (Politis, Richardson, & Yfantopoulos, 1991). In Jordan, 75% of families were

unaware of the disease prior to the birth of their first affected child (Sadiq, 1999). Similarly, insufficient understanding about the risk of carrying a gene that results in the birth of a thalassemic child has been found in Pakistani families in the United Kingdom (S. Ahmed, Bekker, Hewison, & Kinsey, 2002).

The most important causes contributing to the high prevalence of consanguineous marriages in Iran involve cultural and racial characteristics, as well as a lack of knowledge about the adverse effects of these marriages (Karimzaei et al., 2015).

A study was conducted at Pakistan to assess the knowledge, beliefs, and practices regarding thalassemia in an urban population, the study's most alarming finding was that most respondents were unaware of the hereditary nature of disease transmission and the role of consanguineous marriages in disease transmission (Ebrahim et al., 2019). This is supported by Ishaq et al study from Lahore (Ishaq, Abid, Kokab, Akhtar, & Mahmood, 2012).

Poor knowledge was found among college students in Bangladesh, nearly two-thirds (64.5%) of those who had heard of thalassemia were unaware that thalassemia carriers are essentially as healthy as non-carriers (M. S. Hossain et al., 2020). A similar lack of awareness regarding thalassemia carriers has been reported in Saudi Arabia (64%), India (89%) and Malaysia (79.7%) (Basu, 2015; Olwi, Merdad, & Ramadan, 2018; L. P. Wong, George, & Tan, 2011).

Despite the fact that many Indonesians claimed to have heard about thalassemia, the majority of people had little awareness of the disease (Wahidiyat, Yo, Wildani, Triatmono, & Yosia, 2021). Moreover, a study was conducted to assess how patients' and caregivers' (KAP) affected their quality of life in Thalassemia Major patients in Syria showed that patients' knowledge and skills about the disease were limited (Abo Jeesh, 2018).

The high-income, high-education, and professional and management sectors of multi-ethnic population were more aware of thalassemia. This finding suggests that education programs aimed at raising thalassemia awareness should focus more on low-acculturated communities (L. P. Wong, George, & Tan, 2011).

2.10.2.2 Attitudes and Practices

A number of studies conducted around the world found that religious convictions were linked to attitudes toward prenatal diagnosis. In general, factors influencing public acceptance are determined by cultural, social, and religious factors (Malik, Al-Shafai, & Abdallah, 2022). On religious beliefs, some Muslim couples, for example, have been reported to resist prenatal diagnostics (S. Ahmed, Atkin, Hewison, & Green, 2006; Zahed & Bou-Dames, 1997). Ahmed et al also found that religion has a powerful effect on screening decisions (N. Ahmed & Chizhevsky, 2007). The most obvious example is Termination of Pregnancy (TOP) decisions in which couples take into account the Islamic Fatwa. TOP can be considered before 120 days of gestation, i.e., before ensoulment, and only for severe conditions (NE, Bakur, Edrees, & Al-Aama, 2017).

When pregnant females in Egypt were properly counseled on religious aspects addressing Islamic views on prenatal diagnosis and TOP, 100% of females with a thalassemia fetus chose TOP, demonstrating the importance of taking the region's beliefs into account in shaping patients' attitudes toward TOP (A. El-Beshlawy et al., 2012).

The recent legalization of abortion in Iran in shariah laws has had a significant impact on people's attitudes toward prenatal diagnosis and the abortion of affected fetuses

(Akhlaghpoor, 2006). However, abortion is legal in Palestine, as it is in many other Islamic countries, if the pregnancy increases the risk to the mother's life or if the fetus exhibits anomalies that make life after birth incredibly hard; lack of a religious consensus on the acceptability of abortion one of the main reasons for the refusal to abort a fetus with β-thalassemia in Palestine (Al Sabbah et al., 2017).

In a study of Saudi families with children who had haemoglobinopathies, religious education had a significant impact on parents' attitudes on seeking prenatal diagnosis and pregnancy termination (Alkuraya & Kilani, 2001). According to Wong, termination of pregnancy is not accepted among Asians due to a complex web of moral, cultural, and traditional religious beliefs of family and community (C. Wong, Antonarakis, Goff, Orkin, Boehm, & Kazazian, 1986).

However, in an Indian study, where 29% of the fetuses were found to have β-thalassemia, parents chose to terminate the pregnancy regardless of gestational age. This emphasized the significance of prenatal diagnosis for affected children in developing countries (Agarwal, Gupta, Gupta, Sarwai, Phadke, & Agarwal, 2003).

Religious beliefs also have a significant impact on service utilization and decision making. 92.7 % of pregnant Palestinians Arab women consider themselves religious, and 40% of that group does not favor genetic services because they believe counselors will be dismissive of their religious beliefs (Sharkia, Tarabeia, Zalan, Atamany, Athamna, & Allon-Shalev, 2015).

In addition, social factors such as socioeconomic status, health insurance, and service accessibility are known to influence the use of genetic counseling services. Several factors, for example, were associated with the acceptance of prenatal counseling services among pregnant in occupied Palestine. First, due to the high cost of services, low financial affluence was associated with service rejection (Malik, Al-Shafai, & Abdallah, 2022).This finding was replicated in a cohort of Tunisian couples whose service acceptance was influenced by socioeconomic status (Chaabouni et al., 2001).

Parental awareness of various aspects of β-thalassemia is crucial. Not only for the prevention of thalassemia major in the family, but also for the effective management of thalassemic children (Meah, Choudhury, Yeamin, Das, & Sharma, 2021).

The community's literacy level has a positive impact on how parents handle the condition or use prenatal diagnosis to determine the risk of thalassemia in pregnancy (Ishaq et al., 2012). According to a study conducted in Rawalpindi, increased maternal education resulted in a considerable increase in the usage of prenatal diagnostic in pregnant women (Naseem, Ahmed, & Vahidy, 2008), however, the level of literacy among the participating mothers in the study conducted in Pakistan in 2010 was low (Ishaq et al., 2012).

A study of β-thalassemia major patients of Pakistani origin in the United Kingdom discovered that Pakistani women's attitudes toward prenatal diagnosis and termination of pregnancy were influenced by a variety of factors, and religion alone should not be interpreted as a reflection of their attitudes toward abortion (S. Ahmed, Green, & Hewison, 2006). However, in a Palestinian study the mother's educational status had a significant impact on her decision; all of the mothers who decided to continue with a pregnancy involving an affected fetus had a very low educational background. In contrast, the mothers who chose to abort the fetus were more educated than the mothers who chose to carry the pregnancy to term (Al Sabbah et al., 2017).

A survey conducted at high school in Southeastern Iran assessing the attitudes towards thalassemia showed that students' attitudes fluctuate significantly depending on the field of study. Students who studied in the experimental area possessed the highest knowledge and a favorable attitude toward thalassemia, Furthermore, the findings revealed that there is a significant relationship between attitude and ethnicity (Miri-Moghaddam, Motaharitabar, Erfannia, Dashipour, & Houshvar, 2014).

A study held among college students in Bangladesh, showed worst outcome of this dangerous combination of lack of knowledge and misperception could be that people become afraid of being identified as carriers. This was perhaps proven by the fact that 13% of respondents refused to be screened before marriage (M. S. Hossain et al., 2020).

Many respondents in the study conducted toward Mandatory Premarital Screening Among University Students in North Jordan held a pessimistic attitude, believing that diagnosing a family member as a carrier has an impact on other family members' future marriage opportunities (Alkhaldi, Khatatbeh, Berggren, & Taha, 2016)

A study was conducted among Urban Population showed that despite the lack of understanding regarding thalassemia individuals' attitudes and practices toward the disease were generally positive, 97% were willing to donate blood to transfusion-dependent thalassemia patients, with 23 % having done so previously (Ebrahim et al., 2019). Pauisri et al., Srivastava et al., and Miri-Moghaddam et al. all come to similar conclusions (Miri-Moghaddam et al., 2014; Pauisri, Saksiriwuttho, & Ratanasiri, 2011; Srivastava, Sinha, Behera, Panja, Sarkar, & Rao, 2011).

In addition, Patel et al found that positive family history and previous enrollment in thalassemia screening had a significant impact on knowledge; indicating that personal exposure to disease severity and counseling during thalassemia testing were greatly useful (Patel, Parmar, Patel, Trivedi, & Bhartiya, 2016).

Health education concerning thalassemia awareness and prevention should be implemented on a much greater scale. People will see the significance of the disease in their daily lives, thus an awareness program with community participation will be helpful (Basu, 2015). A study in Greece supports the widely held belief among many in the biomedical community that education and economic reforms are critical to the success of public health campaigns such as the thalassemia awareness program (Politis, Richardson, & Yfantopoulos, 1991).

2.11 Management of β-thalassemia

2.11.1 Prevention Strategies

The prevention of β-thalassemia is based on raising public awareness of the disease, identifying carriers, and providing genetic counseling (Peters, Heijboer, Smiers, & Giordano, 2012).

In the late 1970s, major at-risk populations in the Mediterranean [Cyprus, Sardinia, certain sections of Continental Italy (Delta Po area, Sicily), and Greece] began trial population programs aimed at preventing β-thalassemia major through carrier screening, counseling, and prenatal diagnosis (Angastiniotis & Hadjiminas, 1981; Cao, Rosatelli, & Galanello, 1996).

2.11.1.1 Carrier Screening

Screening strategies have varied from premarital to antenatal in different countries, depending on socio–cultural and religious customs in different populations. Many countries have ongoing prenatal diagnosis programs, and recognizing the distribution of mutations has aided in the establishment of successful control programs (Colah, Gorakshakar, & Nadkarni, 2010). The main preventive strategies for thalassemia include appropriate disease information and the importance of screening through awareness programs, screening and counseling of target families, screening of the general population, and premarital and prenatal screening (Asif & Hassan, 2014).

Several procedures have been proposed for β-thalassemia carrier screening. The MCV and MCH concentration is the most commonly used methodology in the preliminary identification of individuals at risk of being carriers (L. Y. Li et al., 2012) but because of the typical increase in HbA2 levels, these carriers may still be clearly diagnosed.

The initial mechanism that determines this phenotype is the presence of heterozygosity for a β-thalassemia mutation associated with a significant residual output of β-globin chains from the afflicted locus (mild thalassemia) or some promoter mutation (C. Kattamis, Metaxotou-Mavromati, Wood, Nash, & Weatherall, 1979).

2.11.1.2 Premarital Screening

Nowadays, couples before marriage, during preconception, and shortly after marriage are the primary screening populations (prospective diagnosis). Programs can be classified based on the timing of testing in relation to pregnancy, which can be either pre-pregnancy or in the early stages of pregnancy (Cousens, Gaff, Metcalfe, & Delatycki, 2010).

Premarital screening and genetic counselling which is popular in the Middle East because it is religiously and socially unacceptable to bear children outside of marriage, aims to identify β-thalassemia carriers among couples planning to marry (Saffi & Howard, 2015). High risk countries, including Iran, Saudi Arabia, the Palestinian Territories, and Cyprus, have laws in place that require premarital screening for haemoglobinopathies for all couples before marriage (Alhamdan, Almazrou, Alswaidi, & Choudhry, 2007; Cousens et al., 2010; Tarazi et al., 2007). The percentage of young unmarried couples requesting screening is increasing in both Cyprus and Italy, indicating a continuous improvement in thalassemia knowledge (Kalokairinou, 2008).

The Palestinian thalassemia prevention program resulted in a 75–80% decrease in the number of annual β-thalassemia major births, demonstrating the Palestinian prevention program's competitiveness with other prevention programs, such as those implemented in Cyprus, Greece, Italy, and Iran (Tarazi et al., 2007). The antenatal diagnosis for the fetus was excluded from the current program structure due to religious restrictions and the prohibition of abortion in the Palestinian community (Tarazi et al., 2007).

2.11.1.3 Prenatal Diagnosis

Prenatal diagnosis of β-thalassemia is becoming increasingly important in lowering the incidence of β-thalassemia. Several attempts have been made to diagnose fetal disease by analyzing fetal cells in maternal circulation (Simpson & Elias, 1993). Amniocentesis and Chorionic Villus Sampling (CVS) are the two main procedures used in the Palestinian territories for prenatal diagnosis of β-thalassemia major (Al Sabbah et al., 2017). Amniocentesis is performed between 16 and 20 weeks of gestation to diagnose chromosomal

anomalies, while CVS has the advantage of being performed earlier than amniocentesis (typically between the 10th and 12th week of gestation), allowing for earlier prenatal diagnosis (Akhlaghpoor, 2006). Moreover, a first-trimester diagnosis of β-thalassemia gives affected couples more options, especially in countries where abortion is prohibited due to religious and cultural restrictions (Ayesh et al., 2005).

2.11.1.4 Education

Intensive education aimed at both health professionals and the general public is a hallmark of all successful programs in the Mediterranean region (Cao & Kan, 2013). Population education is carried out through the use of mass media, posters, and informational booklets, which are distributed at key spots such as family planning clinics, marriage registries, and counseling rooms. It is critical to emphasize that the carrier state is easily identified at thalassemia treatment centers and is not associated with stigma, whereas couples who are both carriers have several reproductive options, including prenatal diagnosis.

According to cost–benefit studies of thalassemia programs, the total cost per case (thalassemia major) prevented was less than the cost of a single year's treatment for an individual with the disease (Ostrowsky, Lippman, & Scriver, 1985).

2.11.2 Blood Transfusion

The goal of transfusion therapy in thalassemia is to relieve anemia and reduce ineffective erythropoiesis, thereby reducing thalassemia complications. Some indices, such as pre- and post-transfusion Hb, blood unit amount and hematocrit, daily Hb fall, and transfusion interval, should be recorded at each transfusion to monitor the effectiveness of transfusion therapy. These measurements allow for the calculation of two important parameters: Red cell requirements and iron intake (Renzo Galanello & Origa, 2010).

Following presentation, patients should be closely monitored to determine their ability to maintain Hb above 7 g/dL over a two-week period. When a patient is diagnosed with thalassemia major and is unable to maintain Hb > 7 g/dL or has poor growth, regular transfusion is initiated.

A pre-transfusion Hb of 9-10 g/dL would provide adequate extramedullary suppression, allowing for a reduction in blood consumption and decreased iron absorption from the gut (Cazzola, Borgna-Pignatti, Locatelli, Ponchio, Beguin, & De Stefano, 1997; Taher, Isma'eel, & Cappellini, 2006).

Post-transfusion Hb levels should be between 13.5 and 15.5 g/dL. It should be taken at least one hour after the transfusion has been completed (Liumbruno, Bennardello, Lattanzio, Piccoli, & Rossetti, 2009).

2.11.3 Chelation Therapy

Since there is no physiologic process to remove excess iron from multiple transfusions, transfusion-dependent patients develop iron overload (Muncie & Campbell, 2009). Iron overload should be treated when serum ferritin levels exceed 1000 ng/ml, which occurs after 10 to 20 red cell transfusions, according to experts (Al Haddad, Yassin, & Sirdah, 2012). As a result, they must begin iron chelator treatment between the ages of five and eight (Fisher, Brunskill, Doree, Chowdhury, Gooding, & Roberts, 2013).Although this therapy is relatively nontoxic, it is time-consuming and costly. The Food and Drug Administration of the United States recently approved oral deferasirox (Exjade) as an alternative treatment (Al

Haddad, Yassin, & Sirdah, 2012). DFO, DFX, and DFP are the iron chelators that are now available worldwide (J. Li, Lin, Li, & Zhang, 2019).

2.11.4 Blood and Marrow Stem Cell Transplant

Thomas et al. first demonstrated the correction of this hematopoietic disorder by bone marrow transplantation in a young patient who had not undergone transfusion (Thomas et al., 1982).

The Pesaro Group's experience in the 1980s and 1990s was largely responsible for the development and acceptance of HSCT in thalassemia as a standard clinical procedure (Hematopoietic Stem Cell Transplantation in Thalassemia). Allogeneic hemopoietic stem cell transplantation (HSCT) is the only widely used therapy currently available to cure transfusion-dependent thalassemia major (D Baronciani, 2016).

2.11.5 Splenectomy

Splenectomy is usually avoidable with the current practice of optimal transfusion. Some patients with hypersplenism, however, may still require splenectomy to reduce transfusion requirements. Splenectomy can significantly reduce blood consumption (up to 30-50%) and has a long duration.

Because of the spleen's role in clearing bacteria and preventing sepsis, surgery is usually postponed until the child is at least four years old. Patients should receive the pneumococcal polysaccharide vaccine at least one month before surgery.

2.12 Complications of β-Thalassemia

Thalassemia is regarded as an exhausting, patient-suffering disease due to two factors: the disease's chronic nature, which necessitates frequent hospital visits, drug availability, drug side effects, and disease complications, and the disease's impact on patients' quality of life (QOL) in terms of physical, psychological, emotional, and social aspects (Aljeesh, 2016).

In thalassemic patients, regular blood transfusions and chelating treatment have significantly increased survival (Mohamed, 2017). Long-term transfusion therapy, on the other hand, causes iron overload in the tissues, which is the leading cause of morbidity and mortality in patients who get regular transfusions (K Dumaidi et al., 2015). Excess iron is highly toxic to all human tissues, causing severe morbidity and mortality in β-thalassemic patients as well as other iron-overload disorders such as cirrhosis, liver fibrosis, heart disease, and endocrine problems (Origa, 2017). Moreover, β-thalassemia patients are at a significant risk for getting viral infections such hepatitis B virus (HBV) and hepatitis C virus (HCV) (Kamal Dumaidi, 2018). According to the Centers for Disease Control and Prevention (CDC), complications occurred in adult patients with a median age of 31.3 years, an average age of 4.5±8.2 years at the start of transfusion, and a median length of transfusion of 18.5±12.3 years (Fianza et al., 2021).

Children, adolescents, and young adults with thalassemia major have a significant rate of endocrine problems (Karimi, 2018). Iron overload is the most common cause of endocrine problems in thalassemic patients. Between 40 and 72 percent of thalassemic patients have hypogonadotropic hypogonadism (HH) (Kurtoglu, Kurtoglu, & Temizkan, 2012).

Furthermore, liver failure due to significant iron overload and/or chronic viral infections is becoming a more common cause of death (Origa, 2017). Previous research has focused on

the negative effects of iron overload on liver function, as measured by liver enzyme activities, and have found that when serum ferritin levels exceed 1000 ng/ml and the number of transfusions exceeds 30, derangement in liver enzymes occurs in patients with transfusion-dependent -thalassemia (Suman, 2016).

Hypersplenism can be established by a transfusion volume of 225 to 250 mL/kg per year with packed red blood cells (hematocrit of 75 %). If the average hematocrit is less than 75%, the volume calculation should be adjusted (E. Vichinsky & Levine, 2015).

However, thalassemia patients who have been repeatedly transfused, red blood cell transfusion has significantly reduced mortality and morbidity, this can lead to autoantibodies and alloantibodies being produced in red blood cells, which can cause major consequences such a delayed hemolytic transfusion reaction (Yaseen, 2018).

In the previous few decades, the survival rate of Thalassemia major patients has progressively increased, with only 2% dying before 10 years after diagnosis. Improved surveillance in some parts of the world, improved treatment of cardiac complications, better accessibility to blood donors, blood screening for pathogens, treatment of infections, and treatment with deferoxamine are some of the factors that have an impact on this improving picture of TM survival (Zamani, 2015). According to the Thalassemia International Federation, only about 200,000 patients with thalassemia major are alive, registered, and receiving regular treatment worldwide (Renzo Galanello & Origa, 2010).

Chapter Three

Study Framework

This chapter presents the conceptual framework for our study, which aims to investigate the factors associated with the emergence of new cases of β-thalassemia major after 2010 in the West Bank. The study framework provides a comprehensive understanding of the variables involved in the study, helping to guide the research design and analysis.

3.1 Theoretical Framework

β-Thalassemia is one of the most common autosomal recessive disorders worldwide with a carriage rate ranging between 3-4% (Al Sabbah et al., 2017; Darwish, El-Khatib, & Ayesh, 2005) and he prevalence of sickle cell trait in the West Bank is estimated to be 1.2% (Samarah et al., 2018). Thalassemia requires complex and expensive management and the expense of preventing thalassemia is cheaper than the expense of treatment.

The main preventive strategies for thalassemia include public awareness and mass education about the disease, population screening, antenatal screening and counseling, premarital carrier screening, cascade screening, and student screening (Cao & Kan, 2013; LIPKIN Jr et al., 1986).

The choice of prevention strategy must be driven by cultural and religious acceptance as well as other factors. For instance, consanguineous marriages, low literacy rates, and low socioeconomic status are all common sociodemographic factors in developing countries that contribute significantly to the continuing emergence of new β-thalassemia major cases. Moreover, genetic factors, such as a mutation in the promoter region of β-globin gene or the locus control region (LCR), result in no detectable increase in HbA2 levels and near-normal CBC findings. These mutations have the tendency to give false negative results, making β-thalassemia carriers difficult to identify. Understanding these factors is important to understand how to further prevent thalassemia. The theoretical framework of our study is shown in Figure 3.1.

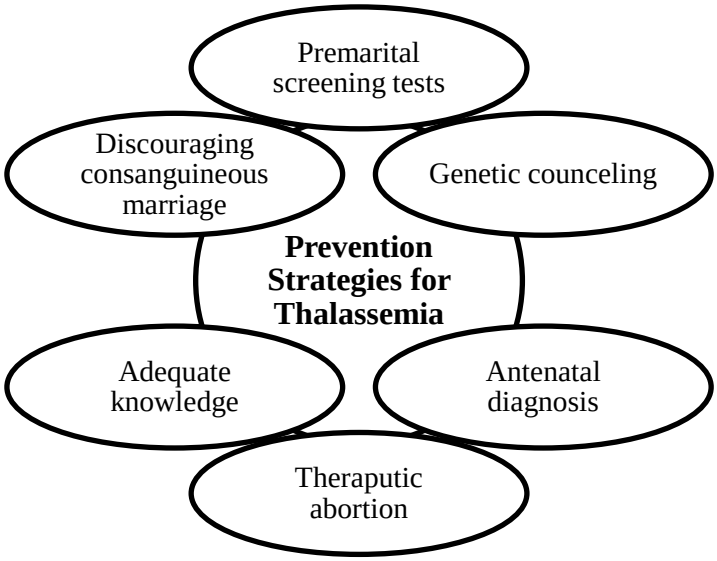

Figure (3.1): Prevention strategies for thalassemia. (Source: (Yousuf, Akter, Wasek, Sinha, Ahmad, & Haque, 2022)).

3.2 Conceptual Framework and Study Variables

The conceptual framework of our study is illustrated in Figure (3.2), and represent the connections and dependencies between these variables, providing a roadmap for the study's research objectives. The key variables that will be examined in the study are defined in Table 3.1.

Thalassemia is a genetic disease that poses a significant public health concern, especially in developing nations. Efforts have been made in Palestine by organizations like the Thalassemia Patient's Friend Society (TPFS) and the Ministry of Health (MOH) to prevent new cases through pre-marriage laws and educational programs. The aim of this study is to evaluate the levels of knowledge, attitudes, and practices (KAP) among parents of thalassemia patients and identify factors contributing to the emergence of new cases, over ten years after the implementation of the law.

Sociodemographic Factors including education level, occupation, socioeconomic status, consanguinity, family history, and age at marriage. For instance, younger couples tend to marry without awareness of the chronicity of inherited diseases, while older couples tend to be more mature and informed when making decisions. On the other hand, consanguineous marriages, which are common in the Arab world, pose a risk for genetic diseases due to the inheritance of genetic material from common ancestors.

Awareness regarding Thalassemia: the knowledge of the parents about thalassemia reflects the understanding of the disease and might alter their beliefs, cultural perceptions, and healthcare-seeking behaviors. Lack of knowledge, limited access to health facilities, and cultural and psychological issues can hinder the effectivity of prevention programs.

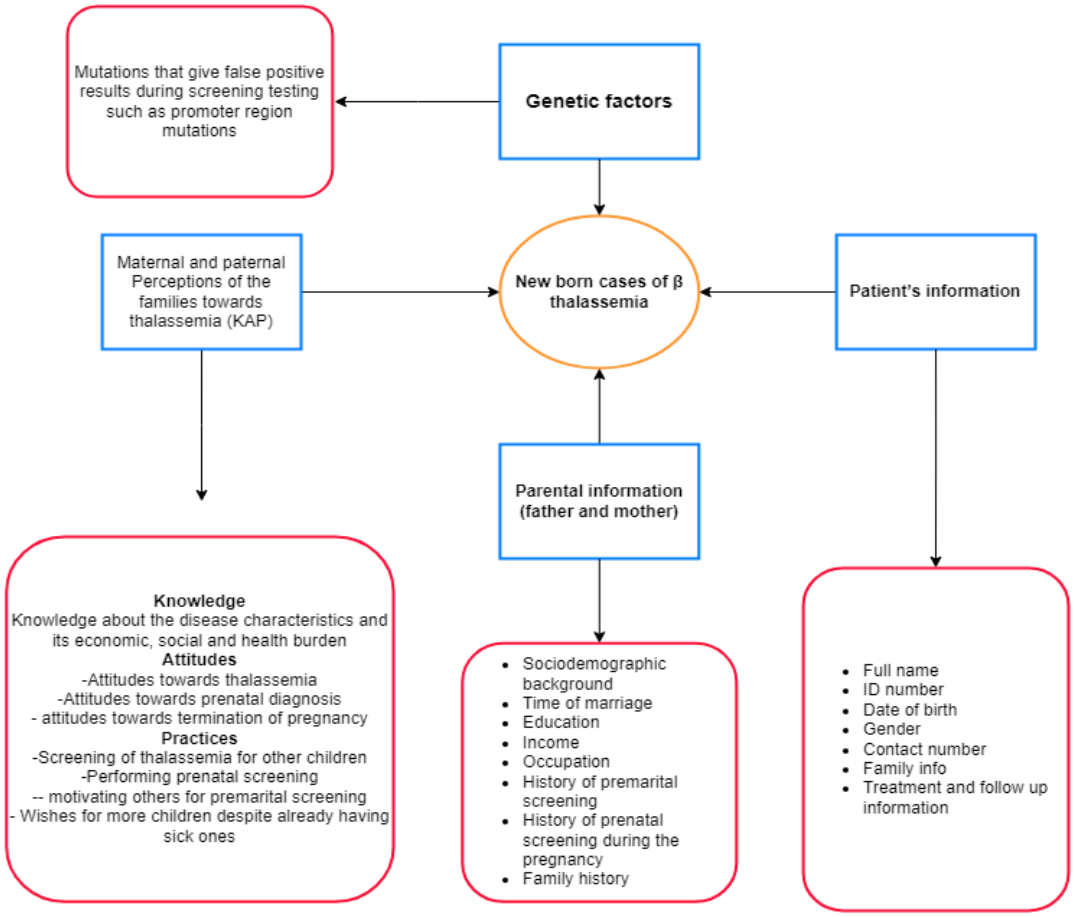

Figure (3.2): Conceptual framework of the study.

Attitudes towards Thalassemia: The attitude of an individual is primarily determined by social, cultural, and religious factors, particularly in traditional societies and rural areas. Sociocultural factors influence how people respond to and conduct in various situations, contexts, brands, and policies. Awareness and attitude towards diagnostic practices depend on the level of education and awareness among disease-affected families. Adequate public knowledge and awareness, along with a positive social attitude, are important in combating the spread of thalassemia. Religious beliefs, such as those in Islam, should not be blamed for the treatment of chronic illnesses like thalassemia. Parental practices, such as prenatal diagnosis and promoting premarital testing, can play a significant role in preventing the emergence of thalassemia.

Genetic Factors: including the genetic factors associated with the emergence of β-thalassemia major cases such as Mutations in the promoter region of the β-globin gene or the locus control region (LCR) which can produce false negative results and pose challenges in identifying thalassemia carriers.

Chapter Four

Methodology

This chapter provides a comprehensive overview of our research methodology, including the study design, setting, population, data collection methods, laboratory procedures, data analysis, and ethical considerations.

In this work, we conducted a cross-sectional case-series study that included β-thalassemia patients who were born in or after 2010 and their parents throughout the West Bank. The main goal was to determine factors associated with the continuing emergence of thalassemia in Palestine after the implementation of the obligatory premarital screening program. Furthermore, we assessed the knowledge, attitudes, and practices of parents towards thalassemia.

4.1 Study Design

This cross-sectional case-series study included all patients diagnosed with β-thalassemia born after 2010 in the West Bank and their parents. Patients with a confirmed diagnosis of β-thalassemia who were born after 2010 were identified and recruited through the Thalassemia Patients' Friends Society (TPFS). A total of 77 patients that met the inclusion criteria were identified through the TPFS. The study included 69 children from 62 families who accepted to participate in the study.

Our investigation was carried out on two levels. The first one included the utilization of a questionnaire that collected data regarding the characteristics and history of patients, and parents. The second level of investigation utilized laboratory testing for the affected children and their parents to screen and diagnose thalassemia.

4.2 Study Setting

Patients with β-thalassemia major born after 2010 in the West Bank and their parents were identified through the TPFS. The TPFS is a non-profit Palestinian civil society organization founded in 1996 by a group of patients and their families, with a related authority within the Palestinian Ministry of Health. The TPFS provides various services for all Palestinian thalassemia patients and strives to improve their quality of life and well-being. The TPFS has complete records of thalassemia patients in the West Bank and Gaza Strip.

Thalassemia daycare units located in governmental hospitals manage patients under the direction of the medical staff for the stipulation of regular, screened blood transfusions, viral markers, screening, baseline investigations, iron chelation therapy, and other medications. Patients in this study were recruited through thalassemia daycare units in seven governmental hospitals, which included Al-Watani Hospital in Nablus, Darwish Nazzal Hospital in Qalqiliya,

Thabit Thabit Hospital in Tulkarem, Khaleel Sulaiman Hospital in Jenin, Palestine Medical Complex in Ramallah, Beit Jala Governmental Hospital in Bethlehem, and Alia Governmental Hospital in Hebron.

4.3 Study Population

We identified 77 patients who met the inclusion criteria, which included being a thalassemia patient born in or after 2010 in the West Bank. Out of these, the guardians of eight patients refused to participate in the study. In addition, for the other 69 children, one family left without completing the interview, two fathers refused to fill out the Paternal KAP Questionnaire, two fathers were unreachable due to the divorce between parents, and two fathers were deceased.

4.4 Data Collection

Data collection took place between August and November of 2022. Trained field workers were responsible for data collection under the supervision of the researchers. Field workers consisted of employees and volunteers from the TPFS, whose services are already utilized in thalassemia care facilities. Field workers were properly instructed on how to approach the participants, obtain consent, and fill the questionnaires. Blood samples were collected by the healthcare staff (nurses or laboratory technicians) at the thalassemia daycare units.

Once a patient and his/her accompanying parent arrived at the daycare unit, a trained field worker explained the study's goals and objectives to the patient's parent and asked for consent to participate in the study. Parents of selected children were asked to sign a written consent form (Appendix 4.1). Then, an interview-based questionnaire was completed by the field worker, and blood samples were collected by the attending medical staff at the unit (nurse or lab technician).

4.5 Study Tools

The data used in this study were collected through several methods. First, we utilized a comprehensive questionnaire to collect sociodemographic, medical, and background information about thalassemic children born after 2010 and their families. Second, we used the medical files of the patient to collect data regarding the medical characteristics of thalassemia patients. Third, we collected blood samples from the children and their parents to perform a complete blood count (CBC) and hemoglobin electrophoresis.

4.5.1 Study Questionnaire

An interview-based questionnaire specifically developed for the goal of this study based on an extensive review of the literature was utilized. The questionnaire was completed through an interview conducted by trained field workers to ensure the collected data's consistency and quality.

The questionnaire consisted of three parts. The first part was divided into two sections, the first one focused on familial sociodemographic characteristics including place of residence, type of residence, household monthly income, type of locality, consanguinity among parents, children

affected with thalassemia, and date of marriage. The second section focused on the background characteristics of the parents (fathers and mothers) including age, age at marriage, current educational level, educational level at marriage, premarital screening and screening results, reasons for not screening if screening was not performed, family history of thalassemia and sickle cell anemia, knowledge about thalassemia before the first child was diagnosed with thalassemia and receiving genetic counseling.

The second part focused on the demographic and medical information of each thalassemic child born after 2010 in the family. After obtaining parental consent, medical information was collected from parents via questionnaire. The third part assessed the knowledge, attitudes, and practices (KAP) of parents (mothers and fathers). The knowledge about thalassemia and thalassemia prevention was assessed using a nine-point scale (yes/no). The attitudes were divided into three categories: attitudes towards thalassemia (11 items), attitudes towards prenatal diagnosis (4 items), and attitudes towards pregnancy termination (5 items). The items were assessed on a 5-level Likert scale ranging from strongly agree to strongly disagree. The practices were assessed using a five-point scale (yes/no).

The questionnaire was developed in English (Appendix 4.2) and then translated into Arabic (Appendix 4.3). The translation was validated by the forward-backward method and reviewed by a panel of experts. A panel consisting of three reviewers determined the face and content validity of the questionnaire. Based on the recommendations of the panel, minor changes were made to the questionnaire. Furthermore, the KAP Questionnaire was established based on previously validated questionnaires (Naseem Ahmed, Khan, Bukhari, Khan, Sabir, & Nazir, 2020; Ebrahim et al., 2019). The reliability of the KAP Questionnaire was further established through a pilot study (n=15). The questionnaire was distributed to random subjects from different backgrounds (students at the university, parents, and healthcare staff at the thalassemia daycare units). Amendments to the questionnaire were made based on the findings of the pilot study.

4.5.2 Blood Samples

This study involved a total of 69 patients from 62 families, with 7 of these families having two siblings. Two of the 62 families refused to provide blood samples for their children and one accompanying parent refused to provide blood sample. In addition, one family provided samples for both parents. Among parents these, 9 fathers and 53 mothers provided blood samples for analysis. The total number of collected samples from children was 67 and from parents was 62. To determine the carrier status of the parents, complete blood count (CBC) and hemoglobin electrophoresis were performed.

From each participant (child or parent), three EDTA tubes (purple top) were obtained. The first sample was promptly processed at the Ministry of Health (MOH) laboratories to obtain a complete blood count. The other two samples were refrigerated at 4ºC until transportation and processing. One of the remaining samples was transported to the nearest Medicare lab branch for hemoglobin electrophoresis. The last sample was transported to the molecular laboratory at the Medical Laboratory Department in Al-Quds University for DNA purification within 48 hours of collection.

4.6 Laboratory Testing

4.6.1 Complete Blood Count (CBC)

EDTA blood samples were obtained from patients and their accompanying parents to perform a complete blood count (CBC) as a screening test for thalassemia at MOH laboratories. An electronic counter (Nihon Kohden cell counter MEK 9100) (Nihon Kohden-Japan), was used to perform the CBC. CBC indices included white blood cell count (WBC), red blood cell count (RBC), platelet count (PLT), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW). Table 4.1 presents the normal ranges of CBC indices based on manufacturer kit.

Table (4.1): Normal ranges of CBC indices based on manufacturer kit.

Indicator	Unit	Children	Females	Males
Hemoglobin	g/dL	9.5 -14	12- 16	14- 18
Hematocrit	%	29 - 44	37- 47	42 -52
RBC	10^6/ µL	3.9 - 5.2	4.2- 5.2	4.7 – 6.1
RDW	%	11.5 - 14.5	11.5- 14.5	11.5 -14.5
MCV	fL	74 – 84	76- 99	81- 95
MCH	Pg	24 – 28	27-31	27-31
MCHC	g/L	31 – 35	32-36	32-36
WBC	10^3/ µL	6 – 18	4.5- 10	4.5- 10
Platelets	10^3/ µL	150 – 400	150-400	150-400

4.6.2 Hemoglobin Electrophoresis

One of the collected samples from children and parents was sent to conduct the identification of hemoglobin (Hb) variants using high-performance liquid chromatography (HPLC) technology. In this method, different hemoglobin variants, including hemoglobin A, A2, F, C, S, and many more variants, are identified based on the retention time (measured in seconds), the peak size, and the shape. Hb electrophoresis was performed using Adams HA-8180 analyzer, a fully automated analyzer from Arkray Company (Arkray 80A kit- Japan), coupled with quality control (QC) materials made specifically for the system. Each patient received a separate report with the specific hemoglobin fractions and kinds. Table (4.2) shows the reference ranges for the various hemoglobin types within the physiological parameters of normal individuals based on manufacturer kit.

Table (4.2) Normal ranges for Hemoglobin electrophoresis results

Hemoglobin Type	Range (%)
Hemoglobin A	>95
Hemoglobin A2	<3.5
Hemoglobin F	<2
Hemoglobin S	0
Hemoglobin C	0

4.6.3 DNA Extraction for Molecular Analysis

In this study, we hypothesized that a mutation in the promoter region of the β-globin gene or the locus control region (LCR) would not result in an increase in HbA2 levels and would produce CBC results that were close to normal. Therefore, we collected peripheral venous blood samples from patients and their accompanying parents to extract genomic DNA for molecular testing. Genomic DNA was extracted with the use of the Gentra Puregene Blood kit (QIAGEN) according to manufacturer instructions. The DNA concentration and purity of each sample were measured by measuring the optical density (OD) at 260 and 280 nm with a Nanodrop spectrophotometer.

Based on the results of the screening tests (CBC and Hb electrophoresis), parents with normal findings (MCV > 80, HbA2 < 3.5) had to be referred for molecular testing. However, the hematological results revealed that among the tested parents, only there had normal MCV (MCV ≥ 80). These three parents had children with sickle cell thalassemia. Hence, molecular testing was not carried out.

4.7 Ethical Considerations

The Research Ethical Committee (REC) at Al-Quds University, as well as the Palestinian Ministry of Health (MOH), granted approval for this study. Each patient was given a code to ensure confidentiality. The parents' written consent was also obtained. In addition, participants were assured that participation was entirely voluntary, and they could leave the study at any time without repercussions.

4.8 Statistical Analysis

The data were manually coded, cleaned, and analyzed using Statistical Package for the Social Sciences (SPSS version 23.0).

Governorates were categorized into three regions: Nablus, Tulkarem, Jenin, and Qalqiliya were categorized as the North of West Bank, while Ramallah and Jerusalem were categorized as the Middle of West Bank and Bethlehem and Hebron were categorized as the South region of the West Bank.

Families were categorized according to their reported monthly income into low-income, middle-income, and high-income. We used the classification provided by the Ministry of Finance in 2023 to define the cut-off points for income level. The ministry's categorization divided annual income into three groups: the first group consisted of families with annual income ≤75,000 New Israeli Shekels (NIS), the second group encompassed those with annual income between 75,001 and 150,000 NIS, and the third group included families with income >150,000 NIS (PIPA, 2023).

Educational level was grouped into three levels: primary level or less which consists of illiterate, can read and write, and elementary; the secondary level consists of the secondary and associated diploma; and the third level was bachelor's degree or more, which stands for bachelor and graduate studies such as Master and PhD. In Palestine's compulsory basic education system, grades 1–10 are divided into two stages: the preparatory stage (grades 1–4) as the first level of

elementary education, the stage of preparation; the second stage of Basic Education (grades 5-9) as the empowerment stage. Secondary Education (Acquisition) encompasses grades 10 to 12 and their respective academic, vocational, and technical tracks. Tertiary education consists of universities and technical colleges, all of which offer predominantly four-year or longer programs (Sabbah, 2020).

Based on the place of birth, parents were divided into two groups; those who were born in Palestine and those who were born in other countries. Other countries included Jordan, Kuwait, and Saudi Arabia.

KAP scores were computed by adding up each section's corresponding statement. Each statement with an opposite answer, such as when the correct response was (NO) or (strongly disagree), was re-coded according to the correct scale. Based on the examined literature, a modified Bloom's cut-off value of 75% was utilized to categorize participants. Knowledge, attitudes, and practices scores into two categories: poor/negative and good/positive. This threshold value was also derived from previously reported research (Wahidiyat et al., 2021).

Concerning knowledge, for each question, a correct response was given a score of one, and an incorrect response was scored as zero, with a total possible score of 0-9, with higher scores indicating better knowledge. Study population with scores of 6 (75%) and above were considered as having "adequate knowledge," and scores below 75% were interpreted as "inadequate knowledge".

For the attitude component, a total score was calculated based on responses to sentences on a five-point Likert scale (1 = strongly disagree, 2 = disagree, 3 = neutral, 4 = agree, and 5 = strongly agree). A total of three aspects of attitudes were measured: attitudes toward thalassemia with an 11-item scale, attitudes toward prenatal diagnosis with a 4-item scale, and attitudes toward abortion with a 5-item scale. Regarding practice, a total score based on responses to statements was calculated, a positive response "yes" received 1, and a negative response "no" received 0 points.

Descriptive statistics were performed and the means and standard deviations (SD), median and interquartile range) (IQR), and ranges were calculated for continuous variables and frequencies and percentages were reported for categorical variables.

Associations between sociodemographic characteristics and parental knowledge, attitudes, and practices were assessed. Discrete variables were compared using chi-square test, and continuous variables were analyzed using the independent sample t-test or one-way analysis of variance (ANOVA) as appropriate. A two-sided p-value < 0.05 was considered statistically significant. In addition, Pearson correlation coefficient was calculated to assess the strength and direction of the relationship between two continuous variables.

Chapter Five

Results

This chapter presents the findings of this study that aimed at identifying the factors contributing to the ongoing emergence of β-thalassemia major in Palestine, even after the implementation of mandatory premarital testing. These factors may be associated with the implemented screening strategies, socioeconomic and cultural aspects, and the knowledge and attitudes towards thalassemia.

To achieve our objectives, we conducted a case series and cross-sectional study that included all new cases of thalassemia major in the West Bank born after 2010. Data were collected using a specifically designed questionnaire, which consisted of three sections. The first section included information focused on familial characteristics and history such as the parental current level of education, occupation, type of locality, and housing. Additionally, information regarding the date of marriage, age at marriage, premarital education, premarital screening testing, prenatal screening during pregnancy, and family history of thalassemia were collected. The second section focused on capturing information on sociodemographic and medical characteristics of the patients, obtained from the parents and medical records. Furthermore, the knowledge, attitudes, and practices (KAP) of parents were assessed through self-administered questionnaires filled out by the parents. In addition, we collected blood samples from the children and accompanying parents for hematological assessment.

5.1 Baseline Sociodemographic and Clinical Characteristics of Thalassemia Patients Born In/After 2010

The characteristics of the thalassemia patients who participated in this study are described in Table (5.1). A total of 69 thalassemia patients from 62 families were included in this study. However, two families refused to provide blood samples. Among the patients, 33 (47.8 %) were males and 36 (52.2%) were females. The patients' mean age (±standard deviation) at recruitment was 7.8±3.3 years ranging between 0-13 years. Children between 0-4 years old accounted for 21.8% of the patients (n=15) and 42% of the children were at least 10 years old (n=29). The majority of children (n=25; 36.2%) were recruited from Al-Watani Hospital. The rest of the patients were recruited from Alia Governmental Hospital (14.6%; n=10), Jenin Governmental Hospital (14.6%; n=10), Palestine Medical Complex (PMC) (13.0%; n=9), Darwish Nazzal Hospital (10.1%; n=7), Martyr Thabit Thabit Hospital (10.1%; n=7), and one patient had his treatment at Beit Jala Hospital (1.4%). Moreover, among the patients, 37.7% were the youngest among their siblings.

Table (5.1) also presents the clinical characteristics of the study participants. β-thalassemia major accounted for 63.8% of the cases (n=44), followed by sickle cell thalassemia (21.7%; n=15), β-thalassemia intermedia (11.6%; n=8), and hemoglobin SC disease (2.9%; n=2). The mean age at diagnosis was 14.5±18.7 months with a median (IQR) of 7.5 (3.0 - 14.7) ranging between 0-96 months. The most prevalent ABO-RhD blood types among patients were O+ (30.4%; n=21), A+ (29%; n=20), and B+ (15.9%; n=11).

Our results revealed that 8.7% (n=6) of the patients had undergone splenectomy, and one patient had a bone marrow transplantation (1.4%). Most patients (92.8%; n=64) had received blood transfusions, with a mean age at the first transfusion of 14.3±15.4 months with a median (IQR) of 8.0 (4.5-18.0) ranging between 1-72 months. Most patients (66.7%; n=46) received transfusions at least once every 1 to 4 weeks. Additionally, 8.7% of the mothers had undergone chorionic villus sampling (CVS) during their pregnancies.

Table (5.1): Demographic and clinical characteristics of thalassemia cases born in/after 2010.

Variable	Category	Frequency n (%)
Age at recruitment (months)	Mean ± SD	7.8±3.3
	Median (IQR)	9.0 (5.0-10.5)
	Range	(0.0-13.0)
Age (Categorical) (years)	0-4	15 (21.8)
	5-9	25 (36.2)
	10-13	29 (42.0)
Gender	Male	33 (47.8)
	Female	36 (52.2)
Treatment care unit	Alia Governmental Hospital	10 (14.6)
	Beit Jala Governmental Hospital	1 (1.4)
	Palestine Medical Complex (PMC)	9 (13.0)
	Jenin Governmental Hospital	10 (14.6)
	Darweesh Nazzal Hospital	7 (10.1)
	Al-Watani Hospital	25 (36.2)
	Thabit Thabit Hospital	7 (10.1)
Order among siblings	Eldest	17 (24.6)
	Youngest	26 (37.7)
	Other	26 (37.7)
Medical diagnosis	β-thalassemia major	44 (63.8)
	β-thalassemia intermedia	8 (11.6)
	Sickle cell thalassemia	15 (21.7)
	Hemoglobin SC disease	2 (2.9)
Age at diagnosis (months)	Mean ± SD	14.5±18.7
	Median (IQR)	7.5 (3.0-14.7)
	Range	(0-96)
Blood type	A+	20 (29.0)
	A –	3 (4.3)
	B+	11 (15.9)
	B-	1 (1.4)
	AB+	4 (5.8)
	O+	21 (30.4)
	O –	5 (7.2)
	Unknown	4 (5.8)
Bone marrow transplantation	Yes	1 (1.4)
	No	68 (98.6)
Splenectomy	Yes	6 (8.7)
	No	63 (91.3)
Treatment with blood transfusion	Ever	64 (92.8)
	Never	5 (7.2)
Age at 1st transfusion (months)	Mean ± SD	14.3±15.4
	Median (IQR)	8 (4.5-18)
	Range	(1-72)
Frequency of blood transfusion (weeks)	Not transfused	5 (7.3)
	Irregular	11 (15.9)
	1-4	7 (10.1)

	≥5 weeks	46 (66.7)
Undergoing CVS during pregnancy	Yes	6 (8.7)
	No	61 (88.4)
	Don't know	2 (2.9)

Regarding hematologic findings, the majority of patients had low red blood cell (RBC) counts, low levels of hemoglobin (Hb), and low hematocrit (HCT). The mean values for these parameters were within the expected range for thalassemia patients. Most of the patients (80.6%; n=54) had a low count of RBCs with a mean of 3.4±0.7 $x10^6$/μL ranging from 1.9-5.3 $x10^6$/μL. Additionally, 94% of the patients (n=63) had a low level of Hb with a mean Hb of 8.3±1.1g/dL ranging between (6.4-11.7) g/dL and 88.1% (n=59) had a low HCT level± with a mean of 25.7±3.8% ranging between 18.3-35.8%.

The results showed also that 43.3% (n=29) of patients had low mean corpuscular volume (MCV) with an overall mean of 76.8±12 fL ranging between (50.3-108) fL. On the other hand, mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were within the normal range for the majority of patients and the red cell distribution width coefficient of variation (RDW.CV) was elevated in most patients (80.6%).

Table (5.2) presents the results of hemoglobin electrophoresis, showing the levels of HbA2, HbA, Hb F, Hb S, and Hb C among the patients. The majority of patients had low levels of HbA (89.6%; n=60) and elevated levels of Hb F (91.0%; n=61). A small proportion of patients had elevated levels of Hb S (25.4%; n=17) and Hb C (3.0%; n=2).

Table (5.2): CBC and hemoglobin electrophoresis profiles of thalassemia patients born in/after 2010.

Parameter		**Frequency (n=67) N (%)**	**Mean ± SD**	**Median (IQR)**	**Range**
WBC (10^3/ μL)	Low (<4.5)	4 (5.9)	9.2±5. 3	8.2 (6-10.7)	(2.9-37.7)
	Normal (4.5-15)	57 (85.1)			
	High (>15)	6 (9)			
RBC (10^6/ μL)	Low (<4)	54 (80.6)	3.4±0.7	34 (2.7-3.9)	(1.9-5.3)
	Normal (4-5.2)	12 (17.9)			
	High (>5.2)	1 (1.5)			
Hb (g/dL)	Low (<10.3)	63 (94)	8.3±1.1	8.1 (7.4-9.2)	(6.4-11.7)
	Normal (10.3-14.9)	4 (6.0)			
HCT (%)	Low (<30.0)	59 (88.1)	25.7±3.8	25.5(22.7-28.6)	(18.3-35.8)
	Normal (30-44)	8 (11.9)			
MCV (fL)	Low (<80)	42 (62.7)	76.8±12.0	77.7 (68.4-82)	(50.3- 108)
	High (≥80)	25 (37.3)			
MCH (pg)	Low (<24)	24 (35.8)	25.0±4.4	25.5(21.7-27.6)	(15.2-35.8)
	Normal (24-28)	30 (44.8)			
	High (>28)	13 (19.4)			
MCHC (g/dL)	Low (<31)	10 (14.9)	32.6±1.6	32.7(31.5-33.9)	(28-36)
	Normal (31-35)	54 (80.6)			
	High (>35)	3 (4.5)			
RDW.CV (%)	Normal (11.0-14.5)	13 (19.4)	21.3±7.1	19.1(16.2-26.8)	31.6(12.1-43.7)
	High (>14.5)	54 (80.6)			
PLT	Low (<150)	2 (3.0)	465.2±223.5	428(332.5-549)	(96-1375)

(10^3/µL)	Normal (150-400)	29 (43.3)			
	High (>400)	36 (53.7)			
HbA2 (%)	Normal (<3.5)	42 (62.7)	3.5±0.91	3.2 (3-4)	(2.2-6.6)
	High (≥3.5)	25 (37.3)			
HbA (%)	Low (<94)	60 (89.6)	67.9±31.2	84.5(46-90.5)	(0-96.5)
	Normal (≥94)	7 (10.4)			
HbF (%)	Normal (<2)	6 (9.0)	12.7±11.1	9.3 (5.1-17.6)	(0.5-51)
	High (≥2)	61 (91.0)			
HbS (%)	Normal (≤0.1)	50 (74.6)	14.2±25.9	0 (0-17.3)	(0-89.2)
	High (> 0.1)	17 (25.4)			
HbC (%)	Normal (≤0.1)	65 (97)	1.4±8.18	0 (0-0)	(0-48.3)
	High (>0.1)	2 (3.0)			

WBC= White Blood Cells, RBC= Red Blood Cell, Hb= Hemoglobin, MCV= Mean cell Volume, MCH= Mean Cell Hemoglobin, MCHC= Mean Cell Hemoglobin Concentration, RDW= Red Cell Distribution Width. Co- efficient variations, Plt= Platelets

5.2 Sociodemographic Characteristics and Family History of the Parents of Thalassemia Patients Born in/after 2010

This study included 69 thalassemia patients from 62 families. Table (5.3) provides information regarding the characteristics of the families of children born with thalassemia in/after 2010.

The majority of families (74.2%; n=46) were from the northern region of the West Bank, particularly Nablus Governorate (35.5%; n=22), and most of them lived in rural areas (59.7%; n=37). The average monthly income of the families was 3037.9±1614.7 NIS, with the majority (88.7%; n=55) falling in the middle-income level of 2000-6000 NIS. Additionally, 88.7% (n=55) of the families were homeowners.

Consanguinity was found in 69.4% (n=43) of the participating families, with 79.0% (n=34) of these marriages being among first-degree relatives (first cousins). The majority of marriages (71.0%; n=44) occurred before 2010. The average family size was 6.2±1.8 members, with a range of 3-11 members. Among families married before 2010, 40.9% (n=18) had thalassemic children who were born prior to 2010. The mean number of thalassemia carriers among children in the families was 1.3±1.3 children, with half of the families (n=31) having one to two carriers, 27.4% having none, and 11.3% having three or more children with thalassemia minor. It is worth mentioning that (11.3%; n=7) of the families reported not knowing if they had carriers of thalassemia among their children. Moreover, 19.4% (n=12) of the mothers reported a history of abortion due to a thalassemic infant.

Table (5.3): Sociodemographic characteristics and medical history of families of thalassemia patients born in 2010 and after in the West Bank.

Variable	Category	Frequency N (%)
Governorate	Ramallah	5 (8.1)
	Jenin	10 (16.1)
	Qalqilya	7 (11.3)
	Nablus	22 (35.5)
	Bethlehem	1 (1.6)
	Hebron	10 (16.1)
	Tulkarem	7 (11.3)
Region	North	46 (74.2)

	Middle	5 (8.1)
	South	11 (17.7)
Type of locality	Rural	37 (59.7)
	Urban	17 (27.4)
	Camp	8 (12.9)
Household monthly income (NIS)	Mean ± SD	3037.9±1614.7
	Median (IQR)	2500 (2000-3250)
	Range	(1000-11000)
Household monthly income (NIS) (Categorical)	Low income (<2000)	5 (8.1)
	Middle income (2000-6000)	55 (88.7)
	High income (>6000)	2 (3.2)
Type of residence	Rental	7 (11.3)
	Owned	55 (88.7)
Consanguinity	Yes	43 (69.4)
	No	19 (30.6)
Degree of consanguinity	First degree relatives	34 (54.8)
	Same family	9 (14.5)
	Not related spouses	19 (30.7)
Date of marriage	Before 2010	44 (71.0)
	After 2010	18 (29.0)
Thalassemia children been born before 2010	Yes	18 (29.0)
	No	26 (41.9)
	Married after 2010	18 (29.0)
Number of thalassemia carriers among children	Mean ± SD	1.3±1.3
	Median (IQR)	1(0-2)
	Range	(0-5)
Number of thalassemia carriers among children (categorical)	None	17 (27.4)
	1-2	31 (50.0)
	3 and more	7 (11.3)
	Unknown	7 (11.3)
History of abortion due to thalassemia	Yes	12 (19.4)
	No	50 (80.6)
Family size	Mean ± SD	6.2±1.8
	Median (IQR)	6 (5-7)
	Range	(3-11)

Table (5.4) provides a detailed profile of the parents, including their current and premarital sociodemographic characteristics, premarital testing for thalassemia, and family history of thalassemia and sickle cell anemia. The fathers had a mean age of 43.2±7.5 years at the time of the survey and a mean age of 25.4±5.3 years at marriage, while the mothers had a mean age at the time of the survey of 37.6±7.1 years and a mean age at marriage of 19.5±3.5 years. The majority of parents (85.5%; n=106) were born in Palestine, with a small percentage (14.5%; n=18) being born in Jordan and other countries such as Kuwait and Saudi Arabia.

In terms of educational level, 88.7% of the fathers (n=55) had secondary education or less, while only 11.3% (n=7) had a bachelor's degree or higher. Among mothers, 77.4% (n=48) had secondary education or less, and 22.6% (n=14) had a bachelor's degree or higher. There was no change in the premarital and current educational levels of the parents, except for one mother who obtained a bachelor's degree after marriage.

Only 35.5% of the parents reported having prior knowledge about the disease before their first child was affected. Relatives were the most common source of information for both fathers and mothers, followed by the educational system. A small proportion of parents (9.7% of fathers and 11.3% of mothers) reported receiving genetic counseling.

Family history of thalassemia major, thalassemia trait, and sickle cell anemia were also assessed among parents (Table 5.4). Among fathers, 19.3% (n=12) had a family history of thalassemia, 56.4% (n=35) had a family history of thalassemia trait, and 9.7% (n=6) had a family history of sickle cell anemia. For mothers, the percentages were 16.1%, 50.0%, and 4.9% for thalassemia, thalassemia trait, and sickle cell anemia, respectively.

Table (5.4): Background characteristics and family history of the parents of thalassemia patients born in 2010 or after.

Variable	Category	Fathers n (%)	Mothers n (%)	Overall n (%)
Age	Mean ± SD	43.2±7.5	37.6±7.1	40.4±7.7
	Median (IQR)	44 (36.5-47)	36 (32-44.2)	40 (34-47)
	Range	(29-60)	(24-50)	(24-60)
Age at Marriage	Mean ± SD	25.4±5.3	19.5±3.5	22.4±5.4
	Median (IQR)	24 (21-29)	19 (17-22)	21 (19-26)
	Range	(16-43)	(14-33)	(14-43)
Place of Birth	Palestine	54 (87.1)	52 (83.9)	106 (85.5)
	Jordan and others	8 (12.9)	10 (16.1)	18 (14.5)
Current educational level	Primary education or less	21 (33.9)	18 (29.0)	39 (31.5)
	Secondary education	34 (54.8)	30 (48.4)	64 (51.6)
	Bachelor and more	7 (11.3)	14 (22.6)	21 (16.9)
Educational level at marriage	Primary education or less	21 (33.9)	18 (29.0)	39 (31.5)
	Secondary education	34 (54.8)	31 (50.0)	65 (52.4)
	Bachelor and more	7 (11.3)	13 (21.0)	20 (16.1)
Knowledge about thalassemia before the birth of the first thalassemic child	Yes	22 (35.5)	22 (35.5)	44 (35.5)
	No	40 (64.5)	40 (64.5)	80 (64.5)
Source of knowledge about thalassemia	Educational systems	8 (12.9)	10 (16.2)	18 (14.6)
	Awareness sessions	1 (1.6)	1 (1.6)	2 (1.6)
	Relatives	11 (17.7)	9 (14.5)	20 (16.1)
	Others	2 (3.2)	2 (3.2)	4 (3.2)
	Unknowledgeable	40 (64.6)	40 (64.5)	80 (64.5)
Have you ever received genetic counseling?	Yes	6 (9.7)	7 (11.3)	13 (10.5)
	No	48 (77.4)	49 (79.0)	97 (78.2)
	Don't know	8 (12.9)	6 (9.7)	14 (11.3)
Family history of thalassemia	Yes	12 (19.3)	10 (16.1)	22 (17.7)
	No	43 (69.4)	48 (77.4)	91 (73.4)
	Don't know	7 (11.3)	4 (6.5)	11 (8.9)
Family history of thalassemia trait	Yes	35 (56.4)	31 (50.0)	66 (53.3)
	No	15 (24.2)	22 (35.5)	37 (29.8)
	Don't know	12 (19.4)	9 (14.5)	21 (16.9)
Family history of SCA	Yes	6 (9.7)	3 (4.9)	9 (7.3)
	No	46 (74.2)	50 (80.6)	96 (77.4)
	Don't know	10 (16.1)	9 (14.5)	19 (15.3)

SCA= Sickle Cell Anemia

5.3 Premarital Thalassemia Screening and Hematological Findings among Parents of Thalassemia Patients Born In/After 2010

In this study, we collected information from fathers and mothers regarding their premarital screening tests and obtained blood samples from 9 fathers and 53 mothers from 61 families representing 68 children to perform CBC and hemoglobin electrophoresis for determining their carrier status. Only one parent refused to provide a blood sample, and in one family, both parents provided samples. The history of premarital screening among parents of β-thalassemia patients born in 2010 or after is presented in Table (5.5).

Based on the information obtained from the parents, 56.5% (n=70) of the parents had undergone premarital screening for thalassemia (58.1% of the fathers and 54.8% of the mothers). Among the parents who underwent premarital screening, 55.5% of the fathers and 20.5% of the mothers self-reported being diagnosed as thalassemia carriers, resulting in an overall recognition of 38.5% of parents as carriers before their marriages.

Among parents who did not undergo premarital screening, the most commonly reported reasons for not undergoing premarital screening among fathers were that the test was not obligatory (50.0%), the test was unavailable (26.9%), or they were unaware of the test before marriage (19.2%). For mothers, the primary reasons were similar: the test was not obligatory (42.8%), unavailable (25.0%), or they were unaware of the test before marriage (17.8%).

Table (5.5): Self-reported premarital screening among parent; results and barriers.

Variable	Category	Fathers N (%)	Mothers N (%)	Overall N (%)
Have you undergone screening before getting married?	Yes	36 (58.1)	34 (54.8)	70 (56.5)
	No	26 (41.9)	28 (45.2)	54 (43.5)
Result of premarital screening test	Carrier	20 (32.3)	7 (11.3)	27 (21.8)
	Non carrier	16 (25.8)	27 (43.5)	43 (34.7)
	Not tested	26 (41.9)	28 (45.2)	54 (43.5)
Reason for not getting screened	Test was not obligatory	13 (21)	12 (19.3)	25 (20.1)
	Test was unavailable	7 (11.3)	7 (11.3)	14 (11.3)
	Didn't know about the test	5 (8.1)	5 (8.1)	10 (8.1)
	Other reasons	1 (1.6)	4 (6.5)	5 (4.0)
	Tested	36 (58.1)	34 (54.8)	70 (56.5)

5.3.1. Factors associated with screening criteria

Comparing between couples who married before 2010 with those who married in/after 2010, we have found that the proportion of parents who were not tested decreased significantly from 54.5% to 16.7%. Additionally, the most reported reasons for not getting tested before 2010 were that the test was not obligatory or not available. However, after 2010, the reasons for not being tested shifted, although they were not identified by the parents. Moreover, the proportion of children born with β-thalassemia major decreased by 20%. (Table 5.6)

Table (5.6): Comparison of self-reported premarital screening among parents by year of marriage.

Variable	Category	Year of marriage	
		Before 2010	In/after 2010
	Yes	40 (45.5)	30 (83.3)

Have you undergone screening before getting married?	No	48 (54.5)	6 (16.7)
Reason for not getting screened	Test was not obligatory	25 (28.4)	0 (0.0)
	Test was unavailable	14 (15.9)	0 (0.0)
	Didn't know about the test	8 (9.1)	2 (5.6)
	Other reasons	1 (1.1)	4 (11.1)
	Tested	40 (45.5)	30 (83.3)
Self-reported result of premarital screening test	Thalassemia carrier	15 (37.5)	12 (40.0)
	Thalassemia non carrier	25 (62.5)	18 (60.0)
Medical diagnosis of the child	β-thalassemia major	33 (70.2)	11 (50.0)
	β-thalassemia intermedia	5 (10.6)	3 (13.6)
	Sickle cell thalassemia	9 (19.1)	6 (27.3)
	Hemoglobin SC disease	0 (0.0)	2 (9.1)

We have further examined the self-reported results of premarital screening test against medical diagnosis of the children and year of marriage (Table 5.7). A total of 24 partners were not tested before marriage (38.7%), 22 (91.7%) of which were married before 2010. Nineteen families (79.2%) of these families had children with β-thalassemia major, 3 (12.5%) had children with sickle cell thalassemia, and 2 (8.3%) had children with thalassemia intermedia. In comparison, 32 partners reported being both tested, among which 6 (18.8%) reported being both carriers and 12 (37.5%) reported being both non-carriers. Among those who reported both being carriers, 4 (66.7%) were married before 2010, 4 (66.7%) had children with β-thalassemia major, and 2 (33.3%) had children with sickle cell thalassemia.

On the other hand, based on the testing strategy, if one partner tested normal (non-carrier), then the other partner is not required to undergo the test. In this study, we have identified 5 couples where one partner reported being non-carrier and the other partner was not tested. Among those, 3 couples (60%) had children with β-thalassemia major, one couple (20%) had children with sickle cell thalassemia, and one couple (20%) had children hemoglobin SC disease. In addition, 14 partners reported that both parents were tested, and the results indicated that one of them tested as carrier while the other was non-carrier. Among those 14 partners, 9 (64.3%) had children with β-thalassemia major, 3 (21.4%) had children with sickle cell thalassemia, and 2 (14.3%) had children with β-thalassemia intermedia.

Table (5.7): Results of self-reported results of premarital screening test against medical diagnosis of the children and year of marriage.

Medical diagnosis of children	Self-f-reported result of premarital screening	Before 2010	In/after 2010	Total
Beta-thalassemia major	Both carriers	2	2	4
	Both non-carriers	3	1	4
	Both not tested	17	2	19
	One carrier and one non-carrier	6	3	9
	One carrier and one not tested	0	1	1
	One partner tested non-carrier	3	0	3
Sickle cell thalassemia	Both carriers	2	0	2
	Both non-carriers	1	3	4
	Both not tested	3	0	3
	One carrier and one non-carrier	1	2	3
	One partner tested non-carrier	1	0	1
	Both non-carriers	3	1	4

Beta-thalassemia intermedia	Both not tested	2	0	2
	One carrier and one non-carrier	0	2	2
Hemoglobin C disease	One partner tested non-carrier	0	1	1
Total	Both carriers	4	2	6
	Both non-carriers	7	5	12
	Both not tested	22	2	24
	One carrier and one non-carrier	7	7	14
	One carrier and one not tested	0	1	1
	One partner tested non-carrier	4	1	5

5.3.2. Other factors associated with premarital screening

Table (5.8) presents the results of hematological analysis conducted on 62 parents (9 fathers and 53 mothers). In this study, 48.4% had high count of RBCs with the mean RBC count being 5.4±0.6 $x10^6/\mu L$ and ranging from 4.2 to $6.8x10^6/\mu L$. In terms of the Hb levels, the results showed that 69.4% of the examined parents had low Hb levels. The mean Hb was 11.6±1.5 g/dL, ranging from 8.5 to 15.7 g/dL. As for MCV, which measures the average size of RBCs, the vast majority of parents (95.2%) had low levels. The mean MCV among parents was 67.8±7.4 fL, ranging from 54.9 to 84.2 fL. Regarding MCH, which represents the average amount of hemoglobin in each RBC, 88.7% of the parents had low levels. The mean MCH was 21.5±3.1 pg, ranging from 16.6 to 29.1 pg.

Table (5.6) also presents the distribution of parents based on their hemoglobin category selection. Out of 62 parents, 82.3% had high levels of HbA2, with a mean of 4.2±0.7%, ranging from 2.8% to 5.5%. Additionally, 45.2% had low levels of HbA, 6.5% of parents (n=4) had high levels of HbF, 17.7% of parents (n=11) had high levels of HbS, and only one parent (1.6%) had high level of HbC.

Table (5.8): Hematological findings of parents of thalassemia parents born after 2010.

Parameter	Category	Frequency (n=62) N (%)	Mean ± SD	Median (IQR)	Range
WBC ($10^3/\mu L$)	Low (<4.5)	5 (8.1)	7.1±1.7	6.9 (5.6-8.4)	(4.2-10.10)
	Normal (4.5-10)	56 (90.3)			
	High (>10)	1 (1.6)			
RBC ($10^6/\mu L$)	Normal (3.76-5.70)	32 (51.6)	5.4±0.6	5.4 (5-5.9)	(4.2-6.8)
	High (>5.70)	30 (48.4)			
Hb (g/dL)	Low	43 (69.4)	11.6±1.5	11.6 (10.5-12.3)	(8.5-15.7)
	Normal	19 (30.6)			
HCT (%)	Low	36 (58.1)	36.6±4.3	36.5 (33.6-39.3)	(24-47.5)
	Normal	25 (40.3)			
	High	1 (1.6)			
MCV (fL)	Low (<80)	59 (95.2)	67.8±7.4	67.8 (61.9-72.7)	(54.9-84.2)
	Normal (≥80)	3 (4.8)			
MCH (pg)	Low (<27)	55 (88.7)	21.5±3.1	21.2 (19-23.1)	(16.6-29.1)
	Normal (27-31)	7 (11.3)			
MCHC (g/dL)	Low (<32)	40 (64.5)	31.5±1.2	31.4 (30.6-32.1)	(29-34.7)
	Normal (32-36)	22 (35.5)			
RDW.CV (%)	Low (<11.5)	2 (3.2)	13.7±1.6	13.5 (12.7-14.5)	(11.2-21.5)
	Normal (11.5-14.5)	45 (72.6)			
	High (>14.5)	15 (24.2)			
PLT ($10^3/\mu L$)	Low (<150)	1 (1.7)	284±73	269 (227-346)	(149-477)

	Normal (150-400)	58 (93.5)			
	High (>400)	3 (4.8)			
HbA2 (%)	Normal (<3.5)	11 (17.7)	4.2± 0.7	4.2 (3.6-4.8)	(2.8-5.5)
	High (≥3.5)	51 (82.3)			
HbA (%)	Low (<94)	28 (45.2)	88.1±13.6	94.5 (92.9-95.6)	(56.2-96.7)
	Normal (≥94)	34 (54.8)			
HbF (%)	Normal (<2)	58 (93.5)	0.6±0.7	0.3 (0.2-1.02)	(0.1-4.10)
	High (≥2)	4 (6.5)			
HbS (%)	Normal (≤0.1)	51 (82.3)	6.2±13.6	0 (0-0)	(0-37.9)
	High (>0.1)	11 (17.7)			
HbC (%)	Normal (≤0.1)	61 (98.4)	0.6±5.0	0 (0-0)	(0-40.1)
	High (>0.1)	1 (1.6)			

- WBC= White Blood cells, RBC= Reed Blood Cell, Hb= Hemoglobin, MCV= Mean Cell Volume, MCH= Mean Cell Hemoglobin, MCHC= Mean Cell Hemoglobin Concentration, RDW=Red Cell distribution width, PLT= Platelets
- Normal ranges: Hb (male) = (14-18), (female) = (12-16); HCT (male) = (42-52), (female) = (37-47); MCV (male) = (81-95), (female) = (76-99). We further compared the reported results of the premarital screening tests with those obtained from the hematological assessment of the parents.

Table 5.9 shows that among the 62 parents who provided samples for testing, 3 fathers and 27 mothers did not have previous results for comparison. Discrepancies between self-reported premarital screening results and results of the hematological assessment were observed in 3 fathers and 21 mothers. Among the mothers, 20 reported negative testing results for the thalassemia trait while reported being diagnosed as a thalassemia carrier based on the results of the results MCV from the hematological assessment.

Table (5.9): Discrepancies between self-reported premarital screening results and results of the hematological assessment performed among parents of thalassemic children born in/after 2010.

Parent	**MCV result**	**Self-reported Premarital Test Result**			**Total**
		Carrier	**Non-carrier**	**Not tested**	
Father	Low	2	3	2	7
	Normal	0	1	1	2
	Total	2	4	3	9
Mother	Low	5	20	27	52
	Normal	1	0	0	1
	Total	6	20	27	53

Observing the results of the hematological assessment, we have found that 3 parents (4.8%) had normal MCV results. The children of these parents had sickle cell thalassemia. In addition, these parents had high HbS based on the results of the H electrophoresis. In addition, one parent had low MCV and high HbC (Table 5.10).

Table (5.10): Results of MCV and hemoglobin electrophoresis from the hematological assessment of the parents (n=62).

Hb electrophoresis	**Category**	**MCV Result**	
		Low (<80 fL)	**Normal (≥80 fL)**
HbA2 (%)	Normal (<3.5)	8	3
	High (≥3.5)	51	0
HbA (%)	Low (<94)	25	3

	Normal (≥94)	34	0
HbF (%)	Normal (<2)	55	3
	High (≥2)	4	0
HbS (%)	Normal (≤0.1)	51	0
	High (>0.1)	8	3
HbC (%)	Normal (≤0.1)	58	3
	High (>0.1)	1	0

We have further examined other sociodemographic factors that could be related to premarital screening. Based on our analysis, the proportion of parents who reported undergoing premarital screening differed significantly by type of locality, consanguinity, degree of consanguinity, and year of marriage (Table 5.11).

Table (5.11): Socioeconomic factors related with premarital testing.

Variable	Category	Screening before marriage		P-value
		Yes	No	
Region	North	52 (74.3)	40 (74.1)	0.891
	Middle	5 (7.1)	5 (9.3)	
	South	13 (18.6)	9 (16.7)	
Type of locality	Rural	34 (48.6)	40 (74.1)	0.011
	Urban	26 (37.1)	8 (14.8)	
	Camp	10 (14.3)	6 (11.1)	
Consanguinity	Yes	39 (55.7)	47 (87.0)	<0.001
	No	31 (44.3)	7 (13.0)	
Degree of consanguinity	First cousins	27 (69.2)	41 (87.2)	0.041
	Same family	12 (30.8)	6 (12.8)	
Year of marriage	Before 2010	40 (57.1)	48 (88.9)	<0.001
	In/after 2010	30 (42.9)	6 (11.1)	
Education at marriage	Primary education	16 (22.9)	23 (42.6)	0.063
	Secondary education	41 (58.6)	24 (44.4)	
	Bachelor and graduate studies	13 (18.6)	7 (13.0)	
Knowledge about thalassemia before the first child was affected	Yes	29 (41.4)	15 (27.8)	0.115
	No	41 (58.6)	39 (72.2)	
Have you ever received genetic counseling?	Yes	11 (15.7)	2 (3.7)	0.093
	No	52 (74.3)	45 (83.3)	
	Don't know	7 (10.0)	7 (13.0)	

5.4 Assessment of Knowledge, Attitudes, and Practices (KAP) among Parents of Thalassemia Patients Born In/After 2010

In this section, we utilized a KAP questionnaire to assess the knowledge, attitudes, and practices of parents. A total of 118 parents completed the KAP questionnaire (62 mothers and 56 fathers).

5.4.1. Knowledge

Knowledge about thalassemia was assessed using a 9-item scale. the results of the knowledge assessment are resented in Table (5.12). The majority of parents (93.2%; n=110) had an adequate level of knowledge (knowledge score ≥75% about thalassemia. The mean knowledge score among parents was 8.1±0.9 out of 9, with a range of 4 to 9. The analysis showed no significant differences in knowledge levels between mothers and fathers.

Examining the responses to individual items in the knowledge assessment, a significant proportion of parents correctly identified that thalassemia is a genetic disease (94.9%) and that having both parents as carriers could result in the birth of a thalassemic child (92.4%). Furthermore, a large percentage of parents were aware that the mother of a thalassemic child has a 25% chance of having a diseased child with each pregnancy (91.5%). Moreover, a considerable number of mothers (88.7%) and fathers (80.4%) recognized that consanguineous marriage contributes to the transmission of genetic disorders. Additionally, 90.7% of parents were aware that a marriage between a healthy individual and a carrier cannot result in a thalassemic child and the majority of parents (97.5%) correctly identified that marriage between carriers can lead to children with thalassemia major, which is an encouraging finding. Furthermore, 94.4% of parents agreed that individuals with thalassemia minor should not marry each other, and 92.4% confirmed that prenatal diagnosis should be pursued if both parents are carriers. On the other hand, our results revealed that certain aspects related to thalassemia were less known among parents. For instance, only 72.9% of parents were aware that thalassemia minor cannot be cured.

Table (5.12): Distribution of parents of patients born in/after 2010 according to knowledge about Thalassemia knowledge scores.

Item		Parents with correct responses			
		Mothers (n=62)	Fathers (n=56)	P-value	Total (n=118)
		N (%)	N (%)		N (%)
76.1. Thalassemia is a genetic disease		59 (95.2)	53 (94.6)	0.898	112 (94.9)
76.2. Both parents of thalassemic children carry abnormal genes		59 (95.2)	50 (89.3)	0.230	109 (92.4)
76.3. Thalassemic child's mother has a 25% chance of bearing a diseased child with each pregnancy		57 (91.9)	51 (91.1)	0.866	108 (91.5)
76.4. Consanguineous marriage plays a role in transmission to the upcoming generation		55 (88.7)	45 (80.4)	0.208	100 (84.7)
76.5.A marriage between a healthy person and a carrier can lead to a major thalassemic child		58 (93.5)	49 (87.5)	0.259	107 (90.7)
76.6.A marriage between carriers lead to major thalassemia		60 (96.8)	55 (98.2)	0.620	115 (97.5)
76.7. If both partners are carriers, prenatal diagnosis should be made		56 (90.3)	53 (94.6)	0.377	109 (92.4)
76.8. Two people with minor thalassemia can marry each other		59 (95.2)	53 (94.6)	0.898	112 (94.4)
76.9. Minor thalassemia can be curable		45 (72.6)	41 (73.2)	0.938	86 (72.9)
Knowledge Score (Mean ± SD) (total score=9)		8.2±0.8	8.0±1.0	0.357	8.1±0.9
Knowledge level	Inadequate knowledge (< 75%)	3 (4.8)	5 (8.9)	0.378	8 (6.8)
	Adequate knowledge (≥75%)	59 (95.2)	51 (91.1)		110 (93.2)

5.4.2. Attitudes towards Thalassemia Prevention, Prenatal Testing, and Termination of Pregnancy

The attitudes of parents towards thalassemia, prenatal analysis, and abortion were assessed utilizing scales consisting of 11, 4, and 5 items, respectively. The results of the assessment of attitudes towards thalassemia, prenatal analysis, and abortion are detailed in Table 5.13. The overall attitude scores indicate positive attitudes towards thalassemia prevention, with an average score of (74.5±11.2%). There were no significant differences between the attitudes of mothers and fathers towards thalassemia, prenatal analysis, and abortion. The majority of participants recognized the importance of preventing thalassemia, with 96.7% of parents agreeing or strongly agreeing with this notion. However, there was a notable proportion (11.0%) who either agreed or were neutral to the statement "I don't mind having more thalassemic children. Additionally, there were mixed responses regarding the belief that parents with thalassemic children miss out on fun and joy, with 59.3% of parents agreeing or strongly agreeing.

Furthermore, the survey results demonstrated overwhelming support for premarital screening legislation, as all respondents strongly agreed or agreed with the need for such legislation. Similarly, the majority of participants (97.5%) agreed or strongly agreed that thalassemia carriers should not marry. However, there were a mixed perception regarding the social stigma associated with thalassemia, with 51.7% of respondents agreeing or strongly agreeing while 44.1% strongly disagreed or disagreed with the statement.

Regarding attitudes towards prenatal testing, the majority of parents expressed a desire to know if they are carrying a thalassemic baby (73.8%), and most disagreed or strongly disagreed with the statement "I don't want to know whether the infant is thalassemic" (77.1%). Similarly, a significant proportion of parents (70.3%) disagreed or strongly disagreed with the statement "Tests for thalassemia during pregnancy are not useful."

In terms of attitudes towards abortion, a considerable percentage of respondents disapproved of pregnancy termination, with 42% of mothers and 59% of fathers either agreeing or strongly agreeing. However, over half of the parents (58.5%) agreed or strongly agreed with the statement "I will not prevent the birth of a thalassemic child.

Table (5.13): Attitudes towards thalassemia, prenatal diagnosis, and abortion among families of thalassemia patients born in/after 2010.

Item	Response	Mothers (n=62)		Fathers (n=56)		P-value	Overall (n=118)	
		N (%)	Mean ± SD	N (%)	Mean ±SD		N (%)	Mean ±SD
76.1. There is a need to prevent thalassemia	Strongly Agree/ Agree	59 (95.2)	4.6±0.7	55 (98.2)	4.7±0.6	0.557	114 (96.7)	4.7±0.6
	Neutral	1 (1.6)		0 (0.0)			1 (0.8)	
	Strongly Disagree/ Disagree	2 (3.2)		1 (1.8)			3 (2.5)	
76.2.I don’t mind having more thalassemic	Strongly Agree/ Agree	4 (6.5)	4.4±0.9	4 (7.1)	4.4±0.9	0.935	8 (6.8)	4.4±0.9
	Neutral	3 (4.8)		2 (3.6)			5 (4.2)	
	Strongly Disagree/ Disagree	55 (88.7)		50 (89.3)			105 (89)	

76.3.A thalassemia's mother and father miss fun and Joy	Strongly Agree/ Agree	39 (62.9)	3.4±1.5	31 (55.4)	3.2±1.4	0.458	70 (59.3)	3.3±1.5
	Neutral	2 (3.2)		4 (7.1)			6 (5.1)	
	Strongly Disagree/ Disagree	21 (33.9)		21 (37.5)			42 (35.6)	
76.4. Thalassemia is a social and economic burden	Strongly Agree/ Agree	43 (69.3)	3.6±1.3	36 (64.3)	3.5±1.3	0.437	79 (66.9)	3.6 ±1.3
	Neutral	4 (6.5)		2 (3.6)			6 (5.1)	
	Strongly Disagree/ Disagree	15 (24.2)		18 (32.1)			33 (28)	
76.5. Taking care of children with thalassemia demands too much of me	Strongly Agree/ Agree	43 (69.3)	3.7±1.2	43 (76.7)	3.9±1.1	0.850	86 (72.9)	3.8±1.2
	Neutral	7 (11.3)		3 (5.4)			10 (8.5)	
	Strongly Disagree/ Disagree	12 (19.4)		10 (17.9)			22 (18.6)	
76.6. We have no right to prevent thalassemia	Strongly Agree/ Agree	15 (24.1)	4.0±1.3	14 (25)	3.8±1.3	0.879	29 (24.6)	3.9±1.3
	Neutral	4 (6.5)		5 (8.9)			9 (7.6)	
	Strongly Disagree/ Disagree	43 (69.4)		37 (66.1)			80 (67.8)	
76.7. Testing for thalassemia before marriage	Strongly Agree/ Agree	62 (100)	4.8±0.3	54 (96.4)	4.7±0.5	0.324	116 (98.4)	4.7±0.4
	Neutral	0 (0.0)		1 (1.8)			1 (0.8)	
	Strongly Disagree/ Disagree	0 (0.0)		1 (1.8)			1 (0.8)	
76.8. Need of legislation of premarital screening	Strongly Agree/ Agree	62 (100)	4.8±0.3	56 (100)	4.8±0.4	-	118 (100)	4.8±0.3
	Neutral	0 (0.0)		0 (0.0)			0 (0.0)	
	Strongly Disagree/ Disagree	0 (0.0)		0 (0.0)			0 (0.0)	
76.9. Thalassemia carriers should not marry	Strongly Agree/ Agree	61 (98.4)	4.8±0.4	54 (96.4)	4.6±0.5	0.5	115 (97.5)	4.7±0.4
	Neutral	1 (1.6)		2 (3.6)			3 (2.5)	
	Strongly Disagree/ Disagree	0 (0.0)		0 (0.0)			0 (0.0)	
76.10. Carrier couples should not have children	Strongly Agree/ Agree	46 (74.2)	4.1±1.1	46 (82.2)	4.3± 1.0	0.572	92 (78)	4.2±1.0
	Neutral	9 (14.5)		6 (10.7)			15 (12.7)	
	Strongly Disagree/ Disagree	7 (11.3)		4 (7.1)			11 (9.3)	
76.11. There is a social stigma associated with thalassemia	Strongly Agree/ Agree	32 (51.7)	3.0±1.6	29 (51.8)	2.8±1.6	0.914	61 (51.7)	2.9±1.6
	Neutral	3 (4.8)		2 (3.6)			5 (4.2)	
	Strongly Disagree/ Disagree	27 (43.5)		25 (44.6)			52 (44.1)	
76.1.Want to know if carrying thalassemic baby	Strongly Agree/ Agree	46 (74.1)	3.9±1.4	41 (73.2)	3.8±1.4	0.939	87 (73.8)	3.8± 1.4

	Neutral	4 (6.5)		3 (5.4)			7 (5.9)	
	Strongly Disagree/ Disagree	12 (19.4)		12 (21.4)			24 (20.3)	
76.2. Will test if test available	Strongly Agree/ Agree	50 (80.7)	4.1±1.2	48 (85.7)	4.1±1.1	0.764	98 (83.1)	4.1±1.1
	Neutral	3 (4.8)		2 (3.6)			5 (4.2)	
	Strongly Disagree/ Disagree	9 (14.5)		6 (10.7)			15 (12.7)	
76.3 you don't want to know if the baby is thalassemic	Strongly Agree/ Agree	8 (12.9)	4.1± 1.1	11 (19.6)	3.8 ±1.2	0.546	19 (16.1)	4.0±1.2
	Neutral	5 (8.1)		3 (5.4)			8 (6.8)	
	Strongly Disagree/ Disagree	49 (79)		42 (75)			91 (77.1)	
76.4. Tests for thalassemia during pregnancy are not useful	Strongly Agree/ Agree	5 (8)	4.1±1.0	8 (14.3)	4.1±1.2	0.492	13 (11)	4.1±1.1
	Neutral	13 (21)		9 (16.1)			22 (18.6)	
	Strongly Disagree/ Disagree	44 (71)		39 (69.6)			83 (70.3)	
76.1. Disapprove of pregnancy termination	Strongly Agree/ Agree	26 (42)	2.9±1.5	33 (59)	2.6±1.5	0.176	59 (50)	2.8±1.5
	Neutral	9 (14.5)		5 (8.9)			14 (11.9)	
	Strongly Disagree/ Disagree	27 (43.5)		18 (32.1)			45 (38.1)	
76.2. I'll not prevent a thalassemic from being born	Strongly Agree/ Agree	33 (53.3)	2.7± 1.4	36 (64.3)	2.5±1.4	0.476	69 (58.5)	2.6±1.4
	Neutral	10 (16.1)		7 (12.5)			17 (14.4)	
	Strongly Disagree/ Disagree	19 (30.6)		13 (23.2)			32 (27.1)	
76.3. If during the pregnancy period and before soul inspiration, you found the fetus is affected by thalassemia, would you agree with medical abortion?	Strongly Agree/ Agree	27 (43.5)	2.9±1.7	23 (41.1)	2.6±1.6	0.957	50 (42.4)	2.7±1.7
	Neutral	5 (8.1)		4 (7.1)			9 (7.6)	
	Strongly Disagree/ Disagree	30 (48.4)		29 (51.8)			59 (50)	
76.4. It is better to terminate a pregnancy than letting child suffer	Strongly Agree/ Agree	24 (38.7)	2.6±1.6	18 (32.1)	2.5±1.5	0.864	42 (35.6)	2.6±1.5
	Neutral	7 (11.3)		7 (12.5)			14 (11.9)	
	Strongly Disagree/ Disagree	31 (50)		31 (55.4)			62 (52.5)	
76.5. Abortion of thalassemic fetus is forbidden regardless of the gestational age	Strongly Agree/ Agree	40 (64.5)	2.2±1.3	40 (71.5)	1.9±1.1	0.177	80 (67.8)	2.0±1.2
	Neutral	9 (14.5)		11 (19.6)			20 (16.9)	
	Strongly Disagree/ Disagree	13 (21)		5 (8.9)			18 (15.3)	
Attitude scores towards thalassemia (total=55)	Good (≥ 42)	48 (77.4)	45.7±4.8	43 (76.8)	45.2±4.7	0.935	91 (77.1)	45.4±4.8
	Poor (< 42)	14 (22.6)		13 (23.2)			27 (22.9)	
	Good (≥15)	47 (75.8)	16.4±4.0	40 (71.4)	16.0±4.1	0.589	87 (73.7)	16.2±4.0

Attitude scores towards prenatal diagnosis (total=20)	Poor (<15)	15 (24.2)		16 (28.6)			31 (26.3)	
Attitude scores towards the termination of pregnancy (total=25)	Good (≥ 19)	18 (28.0)	13.5±6.4	10 (17.9)	12.2±5.8	0.154	28 (23.7)	12.9±6.1
	Poor (<19)	44 (71.0)		46 (82.1)			90 (76.3)	
Overall Attitude Score (total=100)	Good (≥75)	34 (54.8)	75.6±11.9	23 (41.1)	73.4±10.3	0.135	57 (48.3)	74.5±11.2
	Poor (<75)	28 (45.2)		33 (58.9)			61 (51.7)	

5.4.3. Practices towards Thalassemia

Parental practices toward thalassemia were assessed using a 5-item scale, as presented in Table (5.11). The overall practice score, combining the responses of both mothers and fathers, was found to be 3.3/5±1.0. The mean practice score for mothers was 3.2±1.0, while for fathers it was 3.3±1.0. There was no significant difference observed between the practice scores of fathers and mothers toward thalassemia, as shown in Table (5.14).

The first item of the practice sale examined whether the other children in the family had been screened for thalassemia, with responses categorized as "Yes" or "No." The survey results indicated that the majority of respondents (57.8%) had their other children screened.

Regarding prenatal screening for thalassemia, the majority of respondents (71.6%) reported not having performed such screening. Only 28.4% of respondents indicated that they did undergo prenatal screening. Furthermore, when asked if they had encouraged others to undergo premarital screening, the majority of parents (90.5%) reported that they had motivated others to undergo premarital screening.

The fourth item inquired whether the family desired to have more children despite already having sick children. The majority of parents (67.2%) expressed that they did not wish to have more children. However, a significant proportion (32.8%) still desired to have more children despite already having a child with thalassemia.

Regarding the disclosure of their child's thalassemia to relatives, the majority of parents (88.8%) reported that their relatives were aware of their child's condition. However, it is important to note that a significant percentage (11.2%) of parents had not informed their relatives about their child's thalassemia.

Table (5.14): Distribution of parents according to practices and practice scores towards thalassemia.

Item		**Mothers (n=61)**		**Fathers (n=55)**		**P-value**	**Overall (n=116)**	
		N (%)	**Mean± SD**	**N (%)**	**Mean± SD**		**N (%)**	**Mean± SD**
76.1. Are your other children screened for thalassemia?	Yes	35 (57.4)	0.5±0.4	32 (58.2)	0.5±0.4	0.930	67 (57.8)	0.5±0.4
	No	26 (42.6)		23 (41.8)			49 (42.2)	
76.2. Do you perform prenatal screening?	Yes	15 (24.6)	0.2±0.4	18 (32.7)	0.3±0.4	0.332	33 (28.4)	0.2±0.4
	No	46 (75.4)		37 (67.3)			83 (71.6)	
76.3. Have you motivated	Yes	54 (88.5)	0.8±0.3	51 (92.7)	0.9±0.2	0.440	105 (90.5)	0.9±0.3
	No	7 (11.5)		4 (7.3)			11 (9.5)	

anyone for premarital screening?								
76.4. Do you wish for more children despite already having sick ones?	Yes	19 (31.1)	0.6±0.4	19 (34.5)	0.6±0.4	0.697	38 (32.8)	0.6±0.4
	No	42 (68.9)		36 (65.5)			78 (67.2)	
76.5. Is your child's disease known to your relatives?	Yes	54 (88.5)	0.8±0.3	49 (89.1)	0.8±0.3	0.923	103 (88.8)	0.8±0.3
	No	7 (11.5)		6 (10.9)			13 (11.2)	
Overall Practice Score (total score=5)	Bad (<3)	34 (55.7)	3.2±1.0	27 (49.1)	3.3±1.0	0.474	61 (52.6)	3.3±1.0
	Good (≥3)	27 (44.3)		28 (50.9)			55 (47.4)	

5.4.4. Correlation between Knowledge, Attitudes, and Practices

Investigating the correlation between the KAP scores (Table 5.15), we have found that knowledge score had a significant positive correlation with the overall attitude score, attitude score towards thalassemia, and attitude score towards the termination of pregnancy. On the other hand, practice scores were only significantly correlated with the overall attitude score and the attitude score towards the termination of pregnancy.

Table (5.15): Correlation between KAP scores.

Correlated factors		**Pearson correlation coefficient (r)**	**P-value**
Knowledge score	Attitude score towards thalassemia	0.330	<0.001
Knowledge score	Attitude score towards prenatal diagnosis	0.096	0.302
Knowledge score	Attitude score towards the termination of pregnancy	0.228	0.013
Knowledge score	Overall attitude score	0.300	0.01
Knowledge score	Overall practice score	0.058	0.534
Attitude score towards thalassemia	Overall practice score	0.174	0.062
Attitude score towards prenatal diagnosis	Overall practice score	0.007	0.943
Attitude score towards the termination of pregnancy	Overall practice score	0.271	0.003
Overall attitude score	Overall practice score	0.225	0.015

5.4.5. Knowledge, Attitudes, and Practices and Premarital Screening

Examining the differences between KAP and undergoing premarital screening, we have found that the mean attitude scores toward termination of pregnancy among those who reported not undergoing the premarital screening test were lower compared to those who reported doing the test and the difference was significant (p-value=0.018). In addition, a

significantly larger proportion of those who reported not undergoing premarital screening had poorer overall attitude scores. (p-value=0.008).

Table (5.16): Association between knowledge attitudes and practices, and self-reported premarital testing.

Variable		Premarital testing		P-value
		Yes	No	
		N (%)	N (%)	
Knowledge score	Inadequate (<75%)	3 (4.5)	5 (9.6)	0.277
	Adequate (≥75%)	63 (95.5)	47 (90.4)	
Knowledge score (continuous)	Mean ± SD	8.18±0.91	8.04±0.95	0.406
Attitude score towards thalassemia	Poor attitudes (<75%)	18 (27.3)	9 (17.3)	0.145
	Good attitudes (≥75%)	48 (72.7)	43 (82.7)	
Attitude score towards thalassemia (continuous)	Mean ± SD	45.61±4.89	45.19±4.68	0.643
Attitude score towards prenatal diagnosis	Poor attitudes (<75%)	17 (25.8)	14 (26.9)	0.886
	Good attitudes (≥75%)	49 (74.2)	38 (73.1)	
Attitude score towards prenatal diagnosis (continuous)	Mean ± SD	16.15±4.25	16.29±3.73	0.641
Attitude score towards the termination of pregnancy	Poor attitudes (<75%)	46 (69.7)	44 (84.6)	0.059
	Good attitudes (≥75%)	20 (30.3)	8 (15.4)	
Attitude score towards the termination of pregnancy (continuous)	Mean ± SD	14.08±6.35	11.40±5.51	0.018
Overall attitude score	Poor attitudes (<75%)	27 (40.9)	34 (65.4)	0.008
	Good attitudes (≥75%)	39 (59.1)	18 (34.6)	
Overall attitude score (continuous)	Mean ± SD	75.83±11.61	72.88±10.49	0.156
Overall practice score	Poor practice (<75%)	30 (45.5)	31 (62.0)	0.077
	Good practice (≥75%)	36 (54.5)	19 (38.0)	
Overall practice score (continuous)	Mean ± SD	3.41±1.08	3.22±0.97	0.151

5.5 Association between Factors Contributing to the Continuing Emergence of Thalassemia in the West Bank

Table (5.17) presents the associations between the characteristics of the parents of thalassemia patients born in/after 2010 and their type of locality. There was a significant association between the type of housing and the type of locality (p-value=0.020). Families living in rural localities or refugee camps were more likely to be house owners compared to families living in urban localities. In addition, the mean household monthly income varied significantly by the type of locality being highest in families living in rural localities (3454.0±1842.51 NIS) (p-value=0.042).

The degree of consanguinity also showed a significant association with the type of locality (p-value=0.011). Families living in rural localities had the highest proportion of consanguineous marriages, especially among first cousins, followed by families living in urban localities.

Undergoing premarital testing for thalassemia was significantly associated with the type of locality (p-value=0.011). 45.9% of the parents living in rural areas had undergone premarital testing compared to 76.5% in urban areas and 62.5% in refugee camps.

The utilization of genetic counseling services also showed significant differences between different types of localities (p-value=0.045). In rural areas, only 5.4% of parents reported

receiving genetic counseling and only 2 parents in the camp locality reported receiving genetic counseling, while in urban areas, the percentage was 20.6%.

Having a family history of sickle cell anemia was significantly higher among parents living in refugee camps (12.4%) and urban localities (11.8%) compared to parents living in rural localities (p-value=0.015).

Attitude scores toward prenatal diagnosis also showed a significant association with the type of locality (p-value=0.036). Among parents, 21.1% in rural areas, 25.0% in urban areas, and 53.3% in the camp locality had poor attitudes towards prenatal diagnosis.

Table (5.17): Association between factors contributing to the continuing emergence of thalassemia and the type of locality.

Variable		Type of Locality			P-value
		Rural	Urban	Camp	
		N (%)	N (%)	N (%)	
Household monthly income (NIS)	Low income	2 (5.4)	3 (17.6)	0 (0.0)	0.349
	Middle income	33 (89.2)	14 (82.4)	8 (100)	
	High income	2 (5.4)	0 (0.0)	0 (0.0)	
Households' monthly income (NIS) (continuous)	Mean ± SD	3454±1842	2502±1027	2250±447	0.042
Type of housing	Rental	2 (5.4)	5 (29.4)	0 (0.0)	0.020
	Owned	35 (94.6)	12 (70.6)	8 (100)	
Consanguinity	Yes	28 (75.7)	11 (64.7)	4 (50.0)	0.320
	No	9 (24.3)	6 (35.3)	4 (50.0)	
Degree of consanguinity	First cousins	25 (89.3)	8 (72.2)	1 (25.0)	0.011
	Same family	3 (10.7)	3 (27.3)	5 (27.0)	
Number of thalassemia carriers among children (continuous)	Mean ± SD	1.5±1.5	0.7±0.6	0.8±0.6	0.122
Family size	Mean ± SD	6.4±1.9	5.5±1.3	6.6±1.3	0.175
Did you have any miscarriages due to thalassemia?	Yes	9 (24.3)	1 (5.9)	1 (12.5)	0.236
	No	28 (75.7)	16 (94.1)	7 (87.5)	
Reasons for not doing the test	Test was not obligatory	20 (50.0)	3 (37.5)	2 (33.3)	0.361
	Test not available	10 (25.0)	2 (250.)	2 (33.3)	
	Didn't know about the test	8 (20.0)	2 (25.0)	0 (0.0)	
	Other reasons	2 (5.0)	1 (12.5)	2 (33.3)	
Age at marriage (Years)	Mean ± SD	22.7±5.3	21.0±5.0	24.1±5.9	0.151
Educational level at marriage	Primary education or less	23 (31)	12 (35.3)	4 (24.9)	0.910
	Secondary education	38 (51.4)	18 (52.9)	9 (56.3)	
	Bachelor and more	13 (17.6)	4 (11.8)	3 (18.8)	
Knowledge about thalassemia before the first child was affected	Yes	25 (33.8)	13 (38.2)	6 (37.5)	0.889
	No	49 (66.2)	21 (61.8)	10 (62.5)	
Source of knowledge about thalassemia	Educational system	9 (36)	6 (46.2)	3 (50.0)	0.214
	Awareness sessions	0 (0)	2 (15.4)	0 (0.0)	
	Relatives	14 (56)	3 (23.1)	3 (50.0)	
	Others	2 (8.0)	2 (15.4)	0 (0.0)	
Have you ever received genetic counseling?	Yes	4 (5.4)	7 (20.6)	2 (12.5)	0.045
	No	58 (78.4)	25 (73.5)	14 (87.5)	
	Don't know	12 (16.2)	2 (5.9)	0 (0.0)	
Family history of thalassemia	Yes	14 (18.9)	6 (17.7)	2 (12.5)	0.632
	No	52 (70.3)	25 (73.5)	14 (87.5)	
	Don't know	8 (10.8)	3 (8.8)	0 (0.0)	

Family history of thalassemia trait	Yes	42 (56.7)	16 (47)	8 (49.9)	0.481
	No	23 (31.1)	9 (26.5)	5 (31.3)	
	Don't know	9 (12.2)	9 (26.5)	3 (18.8)	
Family history of SCA	Yes	3 (4.1)	4 (11.8)	2 (12.4)	0.015
	No	65 (87.8)	20 (58.8)	11 (68.8)	
	Don't know	6 (8.1)	10 (29.4)	3 (18.8)	
Knowledge score	Inadequate (<75%)	5 (7)	2 (6.2)	1 (6.7)	0.989
	Adequate (≥75%)	66 (93)	30 (93.8)	14 (93.3)	
Knowledge score (continuous)	Mean ± SD	7.9±0.8	8.4±0.8	8.0±1.2	0.070
Attitude score towards thalassemia	Poor attitudes (<75%)	18 (25.4)	8 (25.0)	1 (6.7)	0.278
	Good attitudes (≥75%)	53 (74.6)	24 (75.0)	14 (93.3)	
Attitude score towards thalassemia (continuous)	Mean ± SD	44.7±4.6	46.0±5.3	47.4±3.5	0.090
Attitude score towards prenatal diagnosis	Poor attitudes (<75%)	15 (21.1)	8 (25.0)	8 (53.3)	0.036
	Good attitudes (≥75%)	56 (78.9)	24 (75.0)	7 (46.7)	
Attitude score towards prenatal diagnosis (continuous)	Mean ± SD	16.5±3.9	16.4±3.4	14.0±5.0	0.085
Attitude score towards the termination of pregnancy	Poor attitudes (<75%)	54 (76.1)	26 (81.2)	10 (66.7)	0.548
	Good attitudes (≥75%)	17 (23.9)	6 (18.8)	5 (33.3)	
Attitude score towards the termination of pregnancy (continuous)	Mean ± SD	12.7±6.0	12.8±5.0	13.8±7.2	0.805
Overall attitude score	Poor attitudes (<75%)	41 (57.7)	14 (43.7)	6 (40.0)	0.263
	Good attitudes (≥75%)	30 (42.3)	18 (56.3)	9 (60.0)	
Overall attitude score (continuous)	Mean ± SD	73.±11.8	75.4±9.8	75.3±11.3	0.801
Overall practice score	Poor practice (<75%)	36 (52.2)	18 (56.2)	7 (46.7)	0.824
	Good practice (≥75%)	33 (47.8)	14 (43.8)	8 (53.3)	
Overall practice score (continuous)	Mean ± SD	3.3±1.0	3.1±1.0	3.4±0.8	0.631

Table (5.18) presents the associations between regions and factors that might contribute to the continuing emergence of thalassemia cases.

There was a significant difference in the household income level across regions (p-value=0.016) being highest among families from the southern region compared to the northern and middle. In addition, a significantly higher proportion of the families from the northern region were owners of their houses (p-value=0.01).

Furthermore, in the Northern region, the proportion of families that resided in rural areas was significantly higher (p-value=0.007) being 67.4% compared to 40.0% and 36.4% in the middle and southern regions, respectively.

Comparison of reasons for not undergoing premarital screening, the most commonly reported reason among parents from the northern region was that the test was not obligatory (55.0%) while the most commonly reported reason was the unavailability of the test among parents from the central and southern regions (80.0% and 66.7%, respectively) (p-value=0.001).

The presence of prior knowledge about thalassemia before the first child was affected was significantly lower among parents from the northern region (p-value=0.007). The source of information about thalassemia for parents who had prior knowledge also demonstrated a

significant association with the region (p-value < 0.001). In the North region, relatives were the most common source of information (61.5%), while in the Middle region, the educational system and awareness sessions were the most common sources of knowledge (50% each), and in the South region, it was the educational system (57.1%). Furthermore, a significantly lower proportion of parents from the northern region reported the utilization of genetic counseling (p-value <0.001). Moreover, there was a significant difference between the prevalence of family history of thalassemia and region (p-value=0.01).

Unexpectedly, the mean age at marriage among parents from the northern region was significantly higher (p-value=0.038).

Comparing KAP scores among parents across regions showed that parents from the southern region had significantly higher knowledge scores while parents from the northern region had the lowest scores (p-value <0.001). On the other hand, overall attitude scores and the mean attitude scores towards thalassemia, prenatal diagnosis, and termination of pregnancy were highest among parents from the southern region and lowest among parents from the northern region. However, the difference was not significant for attitude scores towards prenatal diagnosis. Although practice scores did not display significant differences, they were highest among parents from the southern region and lowest among parents from the northern region (p-value=0.551).

Table (5.18): Association between factors contributing to the continuing emergence of thalassemia and region.

Variable		Region			P-value
		North	Middle	South	
		N (%)	N (%)	N (%)	
Household monthly income (NIS)	Low income	1 (2.1)	2 (40.0)	2 (18.2)	0.016
	Middle income	44 (95.7)	3 (60.0)	8 (72.7)	
	High income	1 (2.2)	0 (0.0)	1 (9.1)	
Household monthly income (NIS) (continuous)	Mean ± SD	3154±1618	2200±714	2931±1779	0.449
Type of locality	Rural	31 (67.4)	2 (40.0)	4 (36.4)	0.007
	Urban	7 (15.2)	3 (60.0)	7 (63.6)	
	Camp	8 (17.4)	0 (0.0)	0 (0)	
Type of housing	Rental	2 (4.3)	2 (40.0)	3 (27.3)	0.010
	Owned	44 (95.7)	3 (60.0)	8 (72.7)	
Consanguinity	Yes	32 (69.6)	3 (60.0)	8 (72.7)	0.876
	No	14 (30.4)	2 (40.0)	3 (27.3)	
Degree of consanguinity	First cousins	26 (81.3)	2 (66.7)	6 (75)	0.798
	Same family	6 (18.8)	1 (33.3)	2 (525)	
Family size (continuous)	Mean ± SD	6.1±1.8	6.6±1.5	6.4±1.7	0.779
Did you have any miscarriages due to thalassemia?	Yes	9 (19.6)	0 (0.0)	2 (18.2)	0.553
	No	37 (80.4)	5 (100.0)	9 (81.8)	
Have you undergone screening before getting married?	Yes	52 (56.5)	5 (50.0)	13 (59.1)	0.891
	No	40 (43.5)	5 (50.0)	9 (40.9)	
Number of thalassemia carriers among children (continuous)	Mean ± SD	1.1±1.2	2±1.1	1.4±1.4	0.600
Result of premarital screening	Carrier	21 (40.4)	1 (20.0)	5 (38.5)	0.670
	Non carrier	31 (59.6)	4 (80.0)	8 (61.5)	
Reasons for not doing the test	Test was not obligatory	22 (55.0)	1 (20.0)	2 (22.2)	0.001
	Test not available	4 (10.0)	4 (80.0)	6 (66.7)	
	Didn't know about the test	10 (25.0)	0 (0.0)	0 (0)	

	Other reasons	4 (10.0)	0 (0.0)	1 (11.1)	
Age at marriage (continuous)	Mean ± SD	23.1±5.5	21.4±6.0	20±3.3	0.038
Education at marriage	Primary education or less	27 (29.3)	4 (40.0)	8 (36.4)	0.788
	secondary education	49 (53.3)	4 (40.0)	12 (54.5)	
	Bachelor and more	16 (17.4)	2 (20.0)	2 (9.1)	
Knowledge about thalassemia before the first child was affected	Yes	26 (28.3)	4 (40.0)	14 (63.6)	0.007
	No	66 (71.7)	6 (60.0)	8 (36.4)	
Source of knowledge about thalassemia	Educational system	8 (30.8)	2 (50.0)	8 (57.1)	<0.001
	Awareness sessions	0 (0.0)	2 (50.0)	0 (0.0)	
	Relatives	16 (61.5)	0 (0.0)	4 (28.6)	
	Others	2 (7.7)	0 (0.0)	2 (14.3)	
Have you ever received genetic counseling?	Yes	4 (4.3)	1 (10.0)	8 (36.4)	< 0.001
	No	77 (83.7)	8 (80.0)	12 (54.5)	
	Don't know	11 (12)	1 (10.0)	2 (9.1)	
Family history of thalassemia	Yes	16 (17.4)	1 (10.0)	5 (22.7)	0.010
	No	7+2 (78.3)	8 (80.0)	11 (50)	
	Don't know	4 (4.3)	1 (10.0)	6 (27.3)	
Family history of thalassemia trait	Yes	52 (56.6)	2 (20)	12 (54.5)	0.097
	No	28 (30.4)	5 (50)	4 (18.2)	
	Don't know	12 (13)	3 (30)	6 (27.3)	
Family history of SCA	Yes	8 (8.7)	0 (0)	1 (4.5)	0.170
	No	73 (79.3)	6 (60)	17 (77.3)	
	Don't know	11 (12)	4 (40)	4 (18.2)	
Knowledge score	Inadequate (<75%)	8 (9.3)	0 (0)	0 (0)	0.203
	Adequate (≥75%)	78 (90.7)	10 (100)	22 (100)	
Knowledge score (continuous)	Mean ± SD	7.9±0.9	8.4±0.7	8.7±0.4	<0.001
Attitude score towards thalassemia	Poor attitudes (<75%)	24 (27.9)	3 (30)	0 (0)	0.018
	Good attitudes (≥75%)	62 (72.1)	7 (70)	22 (100)	
Attitude score towards thalassemia (continuous)	Mean ± SD	44.2±4.3	44.5±5.95	50.4±1.7	<0.001
Attitude score towards prenatal diagnosis	Poor attitudes (<75%)	22 (25.6)	2 (20)	7 (31.8)	0.751
	Good attitudes (≥75%)	64 (74.4)	8 (80)	15 (68.2)	
Attitude score towards prenatal diagnosis (continuous)	Mean ± SD	16.0±4.0	16.4±3.37	16.9± 4.1	0.606
Attitude score towards the termination of pregnancy	Poor attitudes (<75%)	70 (81.4)	9 (90)	11 (50)	0.005
	Good attitudes (≥75%)	16 (18.6)	1 (10)	11 (50)	
Attitude score towards the termination of pregnancy (continuous)	Mean ± SD	12.3±5.9	10.4±4.4	16.3±6.2	0.008
Overall attitude score	Poor attitudes (<75%)	52 (60.5)	5 (50)	4 (18.2)	0.002
	Good attitudes (≥75%)	34 (39.5)	5 (50)	18 (81.8)	
Overall attitude score (continuous)	Mean ± SD	72.55±10.7	71.3±9.0	83.7±9.2	<0.001

Overall practice score	Poor practice (<75%)	44 (52.4)	7 (70.0)	10 (45.5)	0.435
	Good practice (≥75%)	40 (47.6)	3 (30.0)	12 (54.5)	
Overall practice score (continuous)	Mean ± SD	3.2±1.1	3.3±1.0	3.5±0.6	0.551

Table (5.19) presents the differences in the characteristics between couples who were married prior to 2010 and those who were married in or after 2010. The proportion of β-thalassemia major among new cases was lower. However, the difference was not significant (p-value =0.122). Furthermore, the educational level at marriage for parents was relatively better, although the difference was not significant (p-value=0.398).

Comparing of premarital screening, we found that a significantly larger proportion of parents reported undergoing premarital screening in couples married in or after 2010 (p-value <0.001) and a larger proportion reported that their test results were positive for thalassemia trait (p-value < 0.001). Furthermore, while among parents who were married prior to 2010 the most common reason for not performing the premarital screening test was that the test was not obligatory, most parents who were married in or after 2010 cited other unidentified reasons for not undergoing the test. In addition, the proportion of parents who had knowledge about thalassemia prior to having an affected child was significantly higher (p-value <0.001). the most cited source of knowledge was the educational system followed by relatives among parents married in or after 2010, while among parents who were married before 2010, relatives were the most cited source of information about thalassemia.

Comparison of the results of the KAP assessment showed that there were no significant differences in the KAP scores, although the knowledge score was slightly better among parents married in or after 2010.

Table (5.19): Association between factors contributing to the continuing emergence of thalassemia and year of marriage.

Variable		**Year of Marriage**		**P-value**
		Before 2010	**In/After 2010**	
		N (%)	**N (%)**	
Type of locality	Rural	27 (61.3)	10 (55.5)	0.839
	Urban	12 (27.3)	5 (27.8)	
	Camp	5 (11.4)	3 (16.7)	
Consanguinity	Yes	33 (75.0)	10 (55.6)	0.132
	No	11 (25.0)	8 (44.4)	
Degree of consanguinity	First cousins	27 (81.8)	7 (70.0)	0.421
	Same family	6 (18.2)	3 (30.0)	
Family size	Mean ± SD	6.5±1.91	5.5±1.32	0.062
Number of thalassemia carriers among children (continuous)	Mean ± SD	1.3±1.4	1±0.8	0.215
Did you have any miscarriages due to thalassemia?	Yes	8 (18.2)	3 (16.7)	0.887
	No	36 (81.8)	15 (83.3)	
Result of premarital screening	Carrier	15 (37.5)	12 (40)	0.832
	Non carrier	25 (62.5)	18 (60)	
Reasons for not doing the test	Test was not obligatory	25 (52.1)	0 (0.0)	<0.001
	Test not available	14 (29.2)	0 (0.0)	

	Didn't know about the test	8 (16.7)	2 (33.3)	
	Other reasons	1 (2.1)	4 (66.7)	
Age at marriage	Mean ± SD	21.6±5.3	24.4±5.1	0.008
Education at marriage	Primary education	30 (34.1)	9 (25.0)	0.398
	Secondary education	46 (52.3)	19 (52.8)	
	Bachelor and graduate studies	12 (13.6)	8 (22.2)	
Knowledge about thalassemia before the first child was affected	Yes	21 (23.9)	23 (63.9)	<0.001
	No	67 (76.1)	13 (36.1)	
Source of knowledge about thalassemia	Educational system	6 (28.6)	12 (52.2)	0.021
	Awareness sessions	2 (9.5)	0 (0.0)	
	Relatives	13 (61.9)	7 (30.4)	
	Others	0 (0)	4 (17.4)	
Have you ever received genetic counseling?	Yes	7 (8)	6 (16.6)	0.189
	No	69 (78.4)	28 (77.8)	
	Don't know	12 (13.6)	2 (5.6)	
Family history of thalassemia	Yes	15 (17)	7 (19.4)	0.310
	No	63 (71.6)	28 (77.8)	
	Don't know	10 (11.4)	1 (2.8)	
Family history of thalassemia trait	Yes	52 (59)	14 (38.9)	0.001
	No	18 (20.5)	19 (52.8)	
	Don't know	18 (20.5)	3 (8.3)	
Family history of SCA	Yes	7 (8.0)	2 (5.6)	0.376
	No	70 (79.5)	26 (72.2)	
	Don't know	11 (12.5)	8 (22.2)	

The association between the age at marriage of the parents and the other factors (Table 5.20) shows that the age of marriage was significantly lower in parents who were related to their spouses (p-value=0.021). Furthermore, parents who reported not undergoing premarital testing had significantly lower age at marriage (p-value=0.004). on the other hand, the comparison of KAP scores showed no significant differences in relation to age at marriage.

Table (5.20): Association between factors contributing to the continuing emergence of thalassemia and age at marriage.

Variable		**Age at Marriage**	**P-value**
		Mean ± SD	
Consanguinity	Yes	21.7±5.2	0.021
	No	24.1±5.3	
Number of thalassemia carriers among children (continuous)	Pearson's correlation coefficient (r)	- 0.157	0.439
Family size	Pearson's correlation coefficient (r)	-0.248	0.115
Have you undergone screening before getting married?	Yes	23.6±5.9	0.004
	No	20.9±4.1	
Result of premarital screening	Carrier	26.4±6.8	0.004
	Non carrier	21.8±4.5	
Reasons for not doing the test	Test was not obligatory	20.4±4.01	0.058
	Test not available	20.2±4.2	
	Didn't know about the test	23±4.2	
	Other reasons	21.4±4.2	
Educational level at marriage	Primary education or less	20.9±5.7	0.411
	Secondary education	22.8±4.8	

	Bachelor and more	24.3±5.9	
Knowledge about thalassemia before the first child was affected	Yes	23.1±5.2	0.283
	No	22.0±5.4	
Knowledge score	Inadequate (<75%)	25±5.4	0.081
	Adequate (≥75%)	21.8±4.8	
Knowledge score (continuous)	Pearson's correlation coefficient (r)	-0.156	0.305
Attitude score towards thalassemia	Poor attitudes (<75%)	22.8±4.9	0.352
	Good attitudes (≥75%)	21.8±4.9	
Attitude score towards thalassemia (continuous)	Pearson's correlation coefficient (r)	-0.059	0.268
Attitude score towards prenatal diagnosis	Poor attitudes (<75%)	21.5±4.5	0.461
	Good attitudes (≥75%)	22.±5.03	
Attitude score towards prenatal diagnosis (continuous)	Pearson's correlation coefficient (r)	0.063	0.487
Attitude score towards the termination of pregnancy	Poor attitudes (<75%)	22.3±4.8	0.269
	Good attitudes (≥75%)	21.1±5.2	
Attitude score towards the termination of pregnancy (continuous)	Pearson's correlation coefficient (r)	-0.020	0.262
Overall attitude score	Poor attitudes (<75%)	21.9±4.4	0.804
	Good attitudes (≥75%)	22.1±5.3	
Overall attitude score (continuous)	Pearson's correlation coefficient (r)	-0.014	0.073
Overall practice score	Poor practice (<75%)	21.9±5.0	0.771
	Good practice (≥75%)	22.2±4.8	
Overall practice score (continuous)	Pearson's correlation coefficient (r)	-0.035	0.073

Examining the association between educational level at marriage and the other factors (Table 5.21), we found that a significantly high proportion of parents who were first cousins to their spouses had low educational levels (p-value=0.036). In addition, the lowest proportion of parents who underwent premarital screening had low educational levels at marriage, although the difference was not statistically significant (p-value=0.063). furthermore, among KAP scores, only practice scores showed a significant difference in relation to educational level at marriage. Unexpectedly, parents with high educational levels had the lowest practice scores (2.9±1.1) while parents with intermediate levels of education at marriage had the best practice scores (3.6±0.9).

Table (5.21): Association between factors contributing to the continuing emergence of thalassemia and level of education at marriage.

Variable		**Education at Marriage**			**P-value**
		Primary education or less	**Secondary education**	**Bachelor degree or more**	
Consanguinity	Yes	31 (79.5)	41 (63.1)	14 (70.0)	0.213
	No	8 (20.5)	24 (36.9)	6 (30.0)	
Degree of consanguinity	First cousins	29 (93.5)	30 (73.2)	9 (64.3)	0.036
	Same family	2 (6.5)	11 (26.8)	5 (35.7)	
Family size	Mean ± SD	7±1.6	6.08±1.7	5.2±1.6	< 0.001
Number of thalassemia carriers among children (continuous)	Mean ± SD	1.5±1.3	1.1±1.3	0.9±1.0	0.142
Age at marriage	Mean ± SD	20.9±5.7	22.8±4.8	24.3±5.9	0.059
Did you have any miscarriages due to thalassemia?	Yes	7 (17.9)	12 (18.5)	4 (20.0)	0.981
	No	32 (82.1)	53 (81.5)	16 (80.0)	

Reasons for not doing the test	Test was not obligatory	12 (52.2)	10 (41.7)	3 (42.9)	0.373
	Test not available	4 (17.4)	8 (33.3)	2 (28.6)	
	Didn't know about the test	5 (21.7)	5 (20.8)	0 (0.0)	
	Other reasons	2 (8.7)	1 (4.2)	2 (28.6)	
Knowledge about thalassemia before the first child was affected	Yes	12 (30.8)	23 (35.4)	9 (45.0)	0.557
	No	27 (69.2)	42 (64.6)	11 (55.0)	
Source of knowledge about thalassemia	Educational system	3 (25)	10 (43.5)	5 (55.6)	0.336
	Awareness sessions	1 (8.3)	1 (4.3)	0 (0.0)	
	Relatives	5 (41.7)	11 (47.8)	4 (44.4)	
	Others	3 (25.0)	1 (4.3)	0 (0.0)	
Have you ever received genetic counseling?	Yes	4 (10.2)	6 (9.2)	3 (15.0)	0.755
	No	29 (74.4)	52 (80)	16 (80.0)	
	Don't know	6 (15.4)	7 (10.8)	1 (5.0)	
Knowledge score	Inadequate (<75%)	2 (5.3)	4 (6.6)	2 (10.5)	0.754
	Adequate (≥75%)	36 (94.7)	57 (93.4)	17 (89.5)	
Knowledge score (continuous)	Mean ± SD	8.2± 0.7	8.02±0.9	8.2±0.9	0.457
Attitude score towards thalassemia	Poor attitudes (<75%)	7 (18.4)	13 (21.3)	7 (36.8)	0.271
	Good attitudes (≥75%)	31 (81.6)	48 (78.7)	12 (63.2)	
Attitude score towards thalassemia (continuous)	Mean ± SD	45.6±4.0	45.6±5.0	44.2±5.3	0.485
Attitude score towards prenatal diagnosis	Poor attitudes (<75%)	11 (28.9)	16 (26.2)	4 (21.1)	0.816
	Good attitudes (≥75%)	27 (71.1)	45 (73.8)	15 (78.9)	
Attitude score towards prenatal diagnosis (continuous)	Mean ± SD	15.9±4.0	16.3±4.1	16.4±3.7	0.875
Attitude score towards the termination of pregnancy	Poor attitudes (<75%)	32 (84.2)	45 (73.8)	13 (68.4)	0.336
	Good attitudes (≥75%)	6 (15.8)	16 (26.2)	6 (31.6)	
Attitude score towards the termination of pregnancy (continuous)	Mean ± SD	11.6±5.6	13.5±6.2	13.3±6.3	0.300
Overall attitude score	Poor attitudes (<75%)	24 (63.2)	26 (42.6)	11 (57.9)	0.116
	Good attitudes (≥75%)	14 (36.8)	35 (57.4)	8 (42.1)	
Overall attitude score (continuous)	Mean ± SD	73.1±9.4	75.5±11.9	74±12.2	0.583
Overall practice score	Poor practice (<75%)	25 (67.6)	23 (38.3)	13 (68.4)	0.006
	Good practice (≥75%)	12 (32.4)	37 (61.7)	6 (31.6)	
Overall practice score (continuous)	Mean ± SD	3.0±0.9	3.6±0.9	2.9±1.1	0.004

Chapter Six

Discussion, Conclusions, Limitations and Recommendations

In this chapter, we analyze the findings of this study to offer comprehensive insights and draw meaningful conclusions from our research.

6.1 Discussion

Thalassemia, a group of hereditary blood disorders characterized by abnormal hemoglobin production, poses a significant global health challenge. The management of β-thalassemia syndrome poses a significant challenge to healthcare systems worldwide. This hereditary blood disorder necessitates lifelong care, including regular blood transfusions and iron chelation therapy, to alleviate severe anemia and iron overload. Such intensive and costly treatments place substantial burdens on healthcare resources and infrastructure. Furthermore, the management of β-thalassemia often extends beyond clinical care, involving psychological and social support for patients and their families due to the chronic nature of the disease. Coordinating these various aspects of care, ensuring accessibility to specialized treatment centers, and addressing the financial strain on healthcare budgets are just a few of the challenges that healthcare systems must confront in their efforts to provide comprehensive and effective management of β-thalassemia syndrome.

Over the years, substantial research efforts have been dedicated to understanding and preventing thalassemia, yet its prevalence continues to persist, specifically in low-resource countries, where the incidence of thalassemia is highest.

Although in the recent years the incidence of thalassemia had significantly dropped, especially after implementing the obligatory premarital screening program in Palestine in 2010, data from the TPFS showed that in the West Bank, 77 new cases have been registered between 2010 and 2022. In this study, we aim to investigate the factors contributing to the continuous emergence of thalassemia in the West Bank despite the obligatory premarital screening program and the efforts to improve awareness towards thalassemia.

Although the premarital screening program was established by the TPFS in September 2000, we have set 2010 as the starting point in our investigation, and thus included patients who were born after that. This is because at the time, of introducing the program, the test was not available in the Ministry of Health (MoH), and couples were only advised against marriage if they were both thalassemia carriers. However, in 2010, the superior legitimate judge in Palestine adopted the implementation of premarital tests for β-thalassemia as an obligatory step before any proposed couple can be issued with a marriage certificate and the MoH started to provide the test for all couples free of charge.

6.1.1. Characteristics of Thalassemia Patients Born After 2010

We have included all registered patients in the West Bank whose parents accepted to participate in the study. Out of 77 identified patients, 69 patients (89.6%) took part in the

study. These children were from 62 families. The patients' ages ranged from 0 to 13 years, with a mean of 7.8±3.3 years. The largest proportion of cases were in the oldest age group (10-13 years) indicating that the incidence of thalassemia is still decreasing. Furthermore, the majority of patients included in this study were from the northern region of the West Bank, indicating that the incidence is highest in this region.

Comparing the characteristics of patients in our study to those of the general characteristics of thalassemia patients in Palestine, we have found that the characteristics of newly emerging cases reflect the characteristics of the population of thalassemia patients; however, we were not able to include patients from Gaza Strip in this study. Based on a previous description of thalassemia patients in Palestine, the northern region, specifically Nablus governorate, was reported to have the highest prevalence of thalassemia (Aldwaik et al., 2021). Regional disparities highlight the need for targeted interventions and healthcare resources in the areas with a higher prevalence of thalassemia.

The study included patients with a variety of forms of thalassemia including β-thalassemia major, β-thalassemia intermedia, sickle cell thalassemia, and two cases of hemoglobin SC disease. Hemoglobinopathies, including thalassemia, sickle cell disease, and hemoglobin C disease are known to be common inherited illnesses in most Arab nations, with varied prevalence rates and molecular characterization. β-thalassemia is found in polymorphic frequencies in almost all Arab countries, with carrier rates ranging from 1 to 11% and various numbers of mutations (H. A. Hamamy & Al-Allawi, 2013).

Complex thalassemias resulting from defective production of different globin chains further complicate the management and prevention of the disease. In Palestine, it is estimated that approximately 3-4% of the Palestinian population are thalassemia carriers, (Al Sabbah et al., 2017; Darwish, El-Khatib, & Ayesh, 2005) and up until 2018, the Thalassemia Patients' Friends Society (TPFS) data reported a total of 847 symptomatic thalassemia patients in the West Bank and Gaza Strip (Aldwaik et al., 2021). Furthermore, it is estimated that up to 1.2% of Palestinians are carriers of the sickle cell trait (Samarah et al., 2018).

Examining the clinical characteristics of the patients, the mean age at diagnosis was 14.5±18.7 months ranging between (0-96) months. This indicates that thalassemia is being diagnosed at various ages, with some cases identified early in infancy and others not until later in childhood. Since this study included patients with β-thalassemia major, sickle cell thalassemia, β-thalassemia intermedia, and hemoglobin SC disease, the wide range of age at diagnosis could be a result of the variation in the type and severity of the mutations that cause the disease. However, the relatively high mean age at diagnosis suggests also that there could be delays or challenges in the early detection and diagnosis of thalassemia in our study population. In the management of thalassemia, early diagnosis is crucial for timely interventions and late diagnosis can lead to delayed treatment initiation, which can have negative implications for the affected individuals, including delayed access to necessary medical care and potential complications.

Compared to our study population, an Indian study reported that the mean age at presentation of un-transfused children was 13.2±9.7 months (Trehan, Sharma, Das, Bansal, & Marwaha, 2015). However, the average age at diagnosis of β-thalassemia in a Pakistani study was 5 months (S. Ahmed et al., 2021) another Indian study reported an age at diagnosis of 0-6 months in 63% of cases, 7-12 months in 27%, and up to 3 years in 10% (Singh, Mitra, Kaur, & Bhardwaj, 2019). In addition, Cao et al. reported that the mean age at presentation to be 8.4±9.1 months (Cao & Galanello, 2002). Trehan et al. also found that a minor proportion

(2.5%) were diagnosed before the age of 3 months, while 2.7% were diagnosed after the age of 2 years. However, their findings showed that the majority of infants with transfusion-dependent thalassemia were diagnosed within their first year of life (Trehan et al., 2015).

The majority of patients had O (30.4%) and A (29%) blood types, followed by B (15.9%) and only 5.8% had AB blood type. Compared to our findings, blood group analysis of the patients in a study conducted in Nablus showed that A blood type was the most prevalent blood group type (54.9%), followed by blood group O (29.2%), B (7.1%) and AB (8.8%) (Sawalha, Jodeh, Helo, & Sweileh, 2017). Furthermore, another study showed that the most frequent blood type among Palestinian thalassemia patients were A (40.5%) followed by O (37.7%), B (16.3%), and AB (5.6%) (Abu Taha et al., 2019). Understanding the distribution of blood types among thalassemia patients helps healthcare providers ensure a sufficient supply of compatible blood for transfusions and highlights the need for blood donor recruitment and education about blood compatibility in the population. Moreover, more than 90% of the patients in this study has previously been transfused and 76.8% were transfusion dependent.

Compared to healthy individuals, thalassemia patients have distinct hematological parameters, including low levels of erythrocytes and Hb and elevated ferritin levels (Alsaeed et al., 2018). Based on the results of the hematological assessment of the patients, we found that the majority of the patients (94%) had low hemoglobin levels (<10.3 g/dL). Considering that most patients are transfusion dependent, the observed levels of hemoglobin might indicate the need for better management of blood transfusion as the mainstay treatment for thalassemia patients. Transfusion therapy aims to maintain an adequate level of Hb and improve the overall well-being of patients. Close monitoring of Hb levels is essential to adjust transfusion schedules and ensure optimal disease management. Similarly, low hemoglobin levels were reported among thalassemia patients in Palestine (Aldwaik et al., 2021; Ayyash & Sirdah, 2018).

6.1.2. Factors Contributing to the Continuing Emergence of Thalassemia Cases in the West Bank

Although more than a decade has passed since the obligatory premarital screening program was put in place, new cases of thalassemia are still born. Several factors that could influence the outcomes of the thalassemia prevention program. Multiple social factors, beliefs, and personal decisions in addition to certain genetic abnormalities that lead to failure in detecting carriers could potentially explain why new cases of thalassemia major continue to be born (LIPKIN Jr et al., 1986). For instance, consanguineous marriages, low literacy rates, and low socioeconomic status are all common sociodemographic factors in developing countries that contribute significantly to the continuing emergence of new β-thalassemia major cases (Al Sabbah et al., 2017). Moreover, genetic factors, such as a mutation in the promoter region of β-globin gene or the locus control region (LCR), result in no detectable increase in HbA2 levels and near-normal CBC findings. These mutations have the tendency to give false negative results, making β-thalassemia carriers difficult to identify(Swee Lay Thein & Rees, 2015). Understanding these factors is important to understand how to further prevent thalassemia.

6.1.2.1. Factors related to premarital screening, prenatal diagnosis, and genetic counseling

β-thalassemia is a hereditary disorder that poses a significant health challenge in Palestine. The national thalassemia prevention strategy focuses of the prevention of thalassemia at two levels. The first level focuses on the prevention of marriage between carriers through premarital screening and public awareness. Screening for genetic diseases like thalassemia aims to identify individuals at higher risk and provide them with information about their own health, future health, and the potential health of their offspring (Mennuti, 2008). The second level focuses on the prevention of the birth of new cases through prenatal diagnosis and medical abortion of affected fetuses.

In our study, we have found that only 56.5% of the parents had undergone screening before marriage. Of those who had undergone testing, only 38.5% reported being diagnosed as thalassemia carriers. Furthermore, we have found that a substantial increase in the percentage of couples who underwent premarital testing among those who got married in or after 2010, rising from 45.5% before 2010 to 83.3% after 2010. Among parents who were married prior to 2010 the most common reason for not performing the premarital screening test was that the test was not obligatory, while most parents who were married in or after 2010 cited other unidentified reasons for not undergoing the test. Additionally, we have found that the proportion of β-thalassemia major among cases emerging after 2010 was lower among couples who got married in or after 2010. Parents may refuse thalassemia testing out of fear of being diagnosed and subsequently facing discrimination and stigma, which is a cause for concern. In addition, some families might ignore the legislations in favor of their cultural beliefs.

To further detect gaps in the premarital screening test as a preventive strategy, we have examined the self-reported results of premarital screening test against medical diagnosis of the children (Table 5.7). Based on the screening strategy, all couples planning to marry have to provide a blood sample in a certified laboratory. Complete blood count (CBC) including hemoglobin, red cell count, hematocrit, mean corpuscular volume (MCV), and red cell distribution width is performed. If the MCV was <80 fL, the individual will be referred for hemoglobin electrophoresis. If one of the partners performed the test and had a normal MCV, then the other partner is not required to perform the test. If both partners had abnormal results, the couple would not receive a marriage license, and the marriage cannot be registered legally.

Among 62 couples, a total of 24 partners were not tested before marriage (38.7%), 22 (91.7%) of which were married before 2010. Nineteen families (79.2%) of these families had children with β-thalassemia major, 3 (12.5%) had children with sickle cell thalassemia, and 2 (8.3%) had children with thalassemia intermedia. In comparison, 32 partners reported being both tested, among which 6 (18.8%) reported being both carriers and 12 (37.5%) reported being both non-carriers. Among those who reported both being carriers, 4 (66.7%) were married before 2010, 4 (66.7%) had children with β-thalassemia major, and 2 (33.3%) had children with sickle cell thalassemia, indicating that those parents were carriers of both traits (thalassemia and sickle cell).

On the other hand, based on the testing strategy, if one partner tested normal (non-carrier), then the other partner is not required to undergo the test. In this study, we have identified 5 couples where one partner reported being non-carrier and the other partner was not tested. Among those, 3 couples (60%) had children with β-thalassemia major, one couple (20%)

had children with sickle cell thalassemia, and one couple (20%) had children with hemoglobin SC disease. In addition, 14 partners reported that both parents were tested, and the results indicated that one of them tested as carrier while the other was non-carrier. Among those 14 partners, 9 (64.3%) had children with β-thalassemia major, 3 (21.4%) had children with sickle cell thalassemia, and 2 (14.3%) had children with β-thalassemia intermedia.

Although the numbers are small and the proportion of those who did not perform the test was quite large and might have affected the interpretation of the data, we may conclude that the testing strategy needs to be reevaluated in order to improve the efficiency of the thalassemia prevention program and achieve better results. First, the MCV should not be the only indicator to consider in the premarital testing program, especially considering the relatively high prevalence of sickle cell trait in Palestine (Samarah et al., 2018). Our analysis could further support this conclusion by the fact that the hematological assessment of 62 parents showed that 11 parents (17.7%) were carriers of the sickle cell trait based on the results of hemoglobin electrophoresis, of which 3 (27.3%) had normal MCV. Therefore, considering the limitations of MCV as a standalone indicator for diagnosing hemoglobinopathies other than thalassemia, it is recommended that both partners undergo the screening which should include other tests such as the hemoglobin electrophoresis and the peripheral blood film for red blood cell morphology. This comprehensive approach is necessary to avoid overlooking potential double heterozygous conditions, such as thalassemia combined with sickle cell disease or hemoglobin C. By conducting hemoglobin electrophoresis and assessing the levels of HbA2, a more accurate diagnosis can be achieved, leading to appropriate management and genetic counseling for the individuals involved. Quantitative determination of hemoglobin A2 (HbA2) is the most valuable test for detecting β-thalassemia carriers. Hemoglobin analysis reveals an increase in HbF and HbA2 levels in typical β-thalassemia carriers. The prevalence of HbA2 among β-thalassemia carriers ranges between 3.6% and 7%, while values between 3.2% and 3.6% are considered borderline and require further investigation, especially in young subjects or high-risk couples (Brancaleoni et al., 2016). An assessment of the Saudi Premarital Screening Program, which is a mandatory national premarital screening program that to provides testing and counseling services to all potential couples based on the results of a number of hematological procedures including complete blood count, peripheral blood film for red blood cell morphology, and sickling test and depending mainly on hemoglobin electrophoresis, showed that after two years of the initiation of the program, the program's objective of decreasing high-risk marriages was not as successful. However, the in this program, high risk couples (both members of the potential couple were found positive for sickle cell trait/disease and/or thalassemia trait/disease) had the right to marry regardless of the screening test results but they were required to visit a genetic counseling clinic and followed up (Alhamdan et al., 2007).

Second, comparison between the self-reported results of the premarital screening test and the results of the hematological assessment that we conducted for 62 parents, we have found that there were 24 discrepancies in the results of both tests for those who had both performed (total =32 parents). The accuracy and reliability of screening tests for thalassemia trait are influenced by a range of factors that must be carefully considered. First and foremost is the choice of screening test itself, as different methods may vary in sensitivity and specificity. Patient factors including certain medical conditions such as anemia of chronic disease or coexisting hemoglobinopathies, may interfere with the interpretation of screening tests. Variability in laboratory techniques and equipment can also introduce discrepancies in results. Furthermore, the genetic diversity within populations can affect the performance of screening tests, as the prevalence of specific thalassemia mutations varies in different

regions. Additionally, considering that the complete blood count can be performed in any medical laboratory, falsified reports might be easily fabricated. Therefore, a comprehensive approach to thalassemia screening must account for these factors to ensure accurate results and appropriate follow-up, including certifying designated receptions centers, where trained staff perform and interpret the results in accordance with standard laboratory diagnostic protocols, availability of lab reagents and equipment in all assigned laboratories is treated as a priority, and staff receive continuous training and supervision, and special training sessions when necessary.

These findings do not only emphasize the importance of the premarital screening programs, but also stress the need for accurate diagnostic tests such as hemoglobin electrophoresis to identify carriers and provide appropriate counseling and preventive measures. Therefore, we should take into consideration to expand the premarital screening program and revise the screening criteria to be able to detect other hemoglobinopathies such as the sickle cell trait. In addition, the criteria for screening for the β-thalassemia trait should be revised in order to reduce false negative results. Moreover, the contribution of molecular factors to the emergence of new cases of thalassemia should be thoroughly investigated.

Furthermore, although the implementation of of such a screening strategy might appear to be quite expensive, considering the high financial, social, and psychological cost of maintenance of patients with these illnesses, prenatal and neonatal screening programs for hemoglobinopathies have been considered cost-effective in populations with a high prevalence (Ahmadnezhad et al., 2012; Almeida, Henthorn, & Davies, 2001; Caughey, 2005).

Third, genetic counseling and prenatal diagnosis is crucial for individuals and couples affected by thalassemia. However, in our study, only 10.5% of parents had received genetic counseling. This finding highlights the need to increase awareness and accessibility to genetic counseling services to empower individuals with information about the disorder and their options for prevention and treatment. Furthermore, it is essential to develop culturally sensitive counseling approaches that respect individuals' cultural, religious, and ethical perspectives. To be effective, services for thalassemia must be sensitive to cultural practices and suitable for the particular social context. Counseling must also be sensitive to the individual's or couple's cultural, religious, and ethical perspectives. The success of genetic counseling is critically dependent on its educative, voluntary, and nonmandatory nature (Cohen-Kfir et al., 2020). In this study, most of the marriages resulting in new cases of thalassemia (71.0%) occurred before 2010. Furthermore, seven families had more than one thalassemic child born after 2010, three of these families were married before 2010. We have also found that among families married before 2010, 40.9% had other thalassemic children that were born prior to 2010. Moreover, undergoing prenatal diagnosis was reported only during the pregnancy of six of the 69 cases (8.7%) despite the availability of the test. Together, these data provide evidence that old marriages are the main cause of emerging cases of thalassemia. This indicates the need for a new strategy to prevent thalassemia that target these families.

In our study, a significant proportion of parents (92.4%) were aware of the importance of prenatal diagnosis when both parents are carriers. However, prenatal diagnosis was only performed in a small proportion of the cases (n=6; 8.7%) despite the test availability. In addition, 19.4% of the families had history of abortion due to thalassemia. This highlights the need for educational interventions to emphasize the significance of prenatal diagnosis in high-risk couples. Furthermore, although prenatal diagnosis is available, facilities for

premarital and prenatal diagnosis, along with genetic counseling services, play a crucial role in reducing the number of infants born with thalassemia major (Cao, Galanello, Rosatelli, Argiolu, & De Virgiliis, 1996; Cao, Saba, Galanello, & Rosatelli, 1997; George, 2001). Therefore, investigating the accessibility and capacity of these facilities is crucial.

On the contrary, most of the respondents (84%) in a Pakistani study disagreed that prenatal diagnosis should be performed in such cases (Ebrahim et al., 2019). Furthermore, a previous study investigated the factors that influence the choice of parents to give birth to a fetus affected by thalassemia in the West Bank and reported that religious beliefs were the most reported reason and that mothers with lower educational level were more likely keep an affected fetus (Al Sabbah et al., 2017). Another study that assessed the attitudes of couples toward prenatal diagnosis and its outcomes as a preventive method reported good acceptability for prenatal diagnosis in β-thalassemia afflicted families and that prenatal diagnosis was effective as all couples with affected fetuses opted for abortion (Ayesh et al., 2005). On the other hand, an overwhelming majority of parents among Pakistani study, chose not to go for CVS , and a similar number as well, did not know whether to choose abortion if the CVS test was positive (Naseem Ahmed et al., 2020).

Despite the apparent gaps in the screening strategy, we have found that the proportion of β-thalassemia major among the new cases was lower by 20% in families that married in or after 2010. In addition, the proportion of parents who underwent premarital screening was significantly higher among couples married in 2010 or after (83.3% compared to 45.5%; p-value <0.001).

6.1.2.2. Sociodemographic and cultural factors

The majority of families in this study (59.7%) lived in rural areas and were from the northern region of the West Bank. In addition, the proportion of families from the northern region who were living in rural areas was significantly higher (p-value=0.016). The northern region has the highest prevalence of thalassemia (Aldwaik et al., 2021) and the largest proportion of new cases.

Our results showed that only 45.9% of the parents living in rural areas had undergone premarital testing compared to 76.5% in urban areas and 62.5% in refugee camps. Rural populations tend to have less access to healthcare services and are more likely to be uninsured compared to urban populations. They also receive certain forms of healthcare, such as tests for chronic conditions, at a lower rate (Aljassim & Ostini, 2020). The majority of services, including office visits and consultations, imaging services, and diagnostic testing, are utilized less frequently in rural areas than in urban areas. In addition, social stigma and religious beliefs have been identified as barriers to premarital screening in some communities (Alkalbani et al., 2022; Loftus, Allen, Call, & Everson-Rose, 2018). These factors highlight the importance of considering cultural and socioeconomic factors when implementing thalassemia prevention programs and emphasize the importance of considering the rural-urban divide in the context of thalassemia management and healthcare planning, as well as in the improvement of prevention strategies to target these communities.

In line with our findings, a study conducted in Pakistan reported that a significant proportion (70%) of β-thalassemia patients' families were from rural areas (Shahzad, Rafiq, Ullah, Asad, Ahmad, & Waheed, 2017). On the contrary, a study conducted in India showed that 43% of patients were from rural areas and 57% were from urban areas (Singh et al., 2019)

while another study conducted in Bangladesh reported that 71.67% of the patients were from urban areas and 28.33% were from rural area (Mahzabin, 2022).

Variation in income levels highlight the socioeconomic factors that influence thalassemia management and access to healthcare services. It is well-known that income and factors associated with income have an influence on health outcomes (USDDS, 2020). Research has indicated that individuals from low-income areas are less likely to adhere to stay-at-home directives, potentially due to the need for continuous income, job requirements, or the inability to forgo income (Chiou & Tucker, 2020). Furthermore, income has a significant influence on perceptions of healthy behavior information and high income may provide individuals with better access to information and resources, which can positively influence attitudes and knowledge related to health issues (Fink, Zabawa, & Chopp, 2020).

The majority of families (88.7%) in our study had a middle-income level and were owners of their houses. In fact, the majority of the Palestinian society is have middle income level and reside in housing units owned by one of their members, with variations among urban areas, rural areas, and refugee camps ("Palestinian Central Bureau of Statistics," 2018). Similarly, a study conducted in India showed that the majority of parents of thalassemic patients belonged to the middle class (Singh et al., 2019). However, another Indian study reported different income distribution, with 71.0% belonging to low-income, 25.5% to middle-income, and 3.5% to high-income families (Safdar et al., 2017). Another study conducted at Pediatric OPD of Civil Hospital Karachi indicated that a significant proportion of parents of thalassemia patients had a low socioeconomic status (Arif, Fayyaz, & Hamid, 2008).

We have further found that the mean age at marriage for those who had undergone a premarital test was 23.6 years, compared to 20.9 years for those who had not. This suggests that older couples had a better understanding and awareness of the chronic nature of thalassemia, which prompted them to seek screening for the disease.

Tribalism and consanguineous marriages are prevalent in regions with a high prevalence of β-thalassemia mutations (S. Denic, Aden, Nagelkerke, & Essa, 2013). Arab countries have some of the highest rates of consanguinity in the world (Tadmouri et al., 2009). The most important factors that contribute to the high prevalence of consanguineous marriages in these regions are cultural and racial factors, as well as a lack of knowledge about the serious consequences of consanguineous marriages (Karimzaei et al., 2015).

In this study, consanguinity was found in 69.4% of families, with 79.0% being first cousins. Furthermore, rural areas in this study were found to have the highest proportion of consanguineous marriages, especially among first cousins. However, comparing the characteristics of families married before 2010 and families married in 2010 or after, we have found that the proportion of consanguinity dropped from 75.0% to 55.6%, although the difference was not significant. Also, marriage between first cousins was less common dropping from 61.4% to 38.9%.

Our results align with a Pakistani study where 72% of parents of thalassemia patients were first cousins and 10% were close or distant blood relatives (Aslamkhan et al., 2023). Similarly, in India, consanguineous marriages were found in 81.7% of the families (Ishaq et al., 2012). These findings highlight the cultural practices and genetic factors contributing to the prevalence of thalassemia in certain populations. Similar patterns of consanguinity have

been observed in other regions, such as India and Oman, where first-cousin marriages are prevalent (Islam, 2012).

In this study, we found that the mean age at marriage among fathers was 25.4±5.3 years ranging from 16-43 years and among mothers was 19.5±3.5 years ranging from 14-33 years. We have also found that the mean age at marriage for consanguineous marriages was 21.7 years, while for non-consanguineous marriages, the mean age at marriage was 24.1 years. This indicated that consanguinity is more prevalent among younger couples due to social and cultural beliefs that persist among Palestinians. Despite the laws that forbid the marriage of minors, some families exploit religious laws that allow for earlier age at marriage and arrange for their daughters to marry in religious ceremonies, postponing their official registration until they reach the legal age. In addition to being illegal, this practice deprives the bride of any legal rights to inheritance, alimony, or child support in case the spouse dies prematurely or abandons their underage bride. A previous study on age at first marriage in Palestine revealed that the age at first marriage increased in the years 1999-2003, with a median age of 19.6 years in 2003 (Abu-Rmeileh & Larsun, 2008).

Our study has also shown that there was a significant association between the degree of consanguinity and education at the time of marriage (p=0.037). This suggests that individuals with different educational levels may have varying attitudes and practices regarding consanguineous marriages.

6.1.2.3. Beliefs and personal decisions

In this study, only 35.5% of the parents reported having knowledge about thalassemia before having their first child affected. Yet, the proportion of parents who had knowledge about thalassemia prior to having an affected child was significantly higher among couples married in or after 2010 (p-value < 0.001). Moreover, the sources of this knowledge were different among those couples, the most common being the educational system. In addition, 18.5% of the parents who did not undergo premarital screening reported not knowing about the test as the main reason.

These findings show that the implementation of screening programs requires informing all engaged couples about the necessity of premarital screening and ensuring that they undergo the screening process. In addition, the educational system, coupled with the continued awareness-raising efforts regarding thalassemia, could have a positive impact on the population's understanding of chronic diseases such as thalassemia and their compliance with prevention programs.

On the other hand, assessment of the level of knowledge showed that the majority of parents (93.2%) in this study demonstrated adequate knowledge towards thalassemia with a mean score of 8.1±0.9. These knowledge scores provide valuable insights into how Palestinian parents perceive thalassemia. It is encouraging to note that the majority of parents exhibited adequate knowledge as parents who understand the nature of thalassemia are more willing to test their other children for carrier identification than those who are still unaware of the nature of the fatal disease their child is suffering from.

On the contrary, a study conducted in Bangladesh reported that only 18.2% of respondents possessed adequate knowledge, indicating the need for further awareness efforts (Alam et al., 2022). Similarly, an Indian study found that 57.9% of participants had adequate knowledge of thalassemia (Basu, 2015). On the other hand, studies conducted in Iran showed

that only 12.17% and 14.7% of Iranians had adequate knowledge about the disorder (Pauisri, Saksiriwuttho, & Ratanasiri, 2011; Seyam & Assemi, 2010).

Furthermore, no statistically significant differences were observed in knowledge between mothers and fathers. Similarly, a study conducted at a thalassemia center in Karachi (Maheen, Malik, Siddique, & Qidwai, 2015).

This study showed that most of the respondents were aware of the role of consanguineous marriages in the transmission of genetic disorders to the upcoming generation. Consanguineous marriages are encouraged by culture. Scientific evidence indicates that such unions result in genetic abnormalities in the offspring (Srdjan Denic, Agarwal, & Nagelkerke, 2012). In comparison, about half of the participants in a study conducted in Pakistan's urban areas were reported to be aware of the significance of consanguineous marriage (Ebrahim et al., 2019) and According to another study conducted at the thalassemia center of Kerachi, Pakistan, 71% knew that intermarriage was a significant risk factor (Maheen et al., 2015).

While the majority of parents demonstrated adequate knowledge about thalassemia, there were certain aspects that were less familiar to our participants, specifically information pertaining to the curability of thalassemia. Therefore, efforts should be made to address these gaps in knowledge and provide accurate information about the management and treatment options for thalassemia. Similar findings were reported in a study conducted in the urban population of Karachi, Pakistan, where a significant proportion of unknown information was related to curability (28.89%) (Ebrahim et al., 2019). Another study conducted at a tertiary care hospital in Kolkata revealed that the majority of unknown information also pertained to curability (Basu, 2015).

Despite the majority of parents demonstrating adequate knowledge about thalassemia, only 48.3% exhibited overall positive attitudes towards the condition, with a mean score of 74.5±11.1%. Regardless, the majority of our respondents (77.1%) displayed good attitudes towards thalassemia prevention. For instance, most parents (96.7%) agreed or strongly agreed that there is a need to prevent thalassemia. Similarly, several studies conducted in India among general population, in Myanmar among mothers of patients at Children's Hospital and in Iran among high school reported positive attitudes towards thalassemia (Han, Han, & Myint, 1992; Miri-Moghaddam et al., 2014). Furthermore, an Indonesian study found that 83.3% of students in a university in West Java, Indonesia exhibited positive attitudes towards thalassemia (Wahidiyat et al., 2021). Another study conducted in Myanmar reported that 67% to 99% of respondents were in favor of limiting thalassemic offspring (Han, Han, & Myint, 1992).

Our findings showed also that a high percentage of parents (98.4%) strongly agreed or agreed on the importance of premarital testing before marriage. This finding aligns with an Indian study where all participants were aware of the importance of premarital counseling (Han, Han, & Myint, 1992; Kalra, Kaur, Sodhi, & Kaur, 2019). Similarly, a study conducted in Pakistan indicated that respondents were well-informed about premarital screening(Shahzad et al., 2017). Another Malaysian study also revealed that the majority of participants (90.6%) believed that premarital screening for thalassemia is essential for the general population (L. P. Wong, George, & Tan, 2011). Similarly, a study conducted at a thalassemia center in Karachi found that the majority of parents (66.2%) were aware of premarital screening, while a smaller proportion (36%) were aware of prenatal diagnosis (Naseem Ahmed et al., 2020; Kosaryan, Vahidshahi, Karami, Forootan, & Ahangari, 2007).

Implementing mandatory national premarital screening programs and screening young and unmarried women for carrier detection have been shown to significantly reduce the incidence of infants born with thalassemia in various countries (Charafeddine et al., 2008; Memish & Saeedi, 2011; Rahman, Naznin, Giti, Islam, & Khatun, 2014; Toman, Nasir, Hassan, & Hassan, 2011; Zlotogora, 2009). An encouraging finding in this study was that all participants strongly agreed or agreed on the need for legislation mandating premarital testing. Similarly, a substantial proportion of respondents in a study conducted at a thalassemia center in Karachi (53.7% agreeing and 31.8% strongly agreeing) supported the use of legislation to promote the use of premarital screening (Naseem Ahmed et al., 2020). Pakistani families also held a favorable view of the implementation of premarital screening legislation(Shahzad et al., 2017). However, despite the positive attitude towards premarital screening legislation, it is important to note that the participants of this study are parents of thalassemic children. A large proportion of these parents (29%) have married despite a legislation being in place and 16.7% of which reported not undergoing premarital testing despite the legislation in 2010.

Social stigma and religious beliefs are known to influence screening decisions and attitudes towards thalassemia (S. Ahmed et al., 2006). In our study, 51.7% of respondents strongly agreed or agreed that there is a social stigma associated with thalassemia. This finding holds significance in our society, where consanguineous marriages are culturally preferred. Despite efforts to eradicate these practices deeply rooted in our culture, the risk of asymptomatic carriers and the birth of affected children remains significant in families with thalassemia patients. Promoting awareness and encouraging premarital screening can play a significant role in preventing the spread of the disease.

The significance of prenatal diagnosis of β-thalassemia in reducing the incidence of β-thalassemia is growing rapidly. Research has demonstrated the significance of offering prenatal diagnosis to women/couples at risk of having a fetus with clinically significant thalassemia or other hemoglobinopathies (Agarwal et al., 2003). In our study, a majority of parents (83.1%) expressed willingness to undergo prenatal diagnosis if accessible. Several studies reported wide support of prenatal diagnosis among participants (Basu, 2015; Han, Han, & Myint, 1992; Miri-Moghaddam et al., 2014). However, religious beliefs and societal factors may make it challenging for individuals to pursue therapeutic abortion following a positive prenatal diagnosis, despite such services being available (S. Ahmed, Green, & Hewison, 2006). Therefore, some couples might opt to skip the test in this case. Our study showed that only 6 mothers (8.7%) reported undergoing chorionic villous sampling (CVS) during their pregnancy with the child.

Regarding attitudes towards termination of pregnancy, approximately half of the participants strongly agreed or agreed with the idea of termination. This finding aligns with the majority of other studies, including those conducted in Thailand (88%), Myanmar (70%), and Malaysia (36%), where participants believed that abortion was preferable to a child's lifelong suffering (Ishaq et al., 2012; L. P. Wong, George, & Tan, 2011). However, a study conducted by Ebrahim et al. found that only 2.3% of participants were in favor of terminating a pregnancy if the fetus was known to have thalassemia major (Ebrahim et al., 2019; Han, Han, & Myint, 1992). In contrast, an Indian study found that 92% of parents were willing to terminate a thalassemia-affected pregnancy (Singh et al., 2019).

Although knowledge plays an essential role in the success of prevention programs, it is important to consider the beliefs and preferences of individuals to predict behavior. Considering the diversity of religious perspectives on the termination of fetuses, we can

conclude that cultural beliefs and misinterpretation of religious commands against termination of pregnancies with thalassemia contribute to the prevalence of thalassemia in Palestine. Thalassemia prevention efforts should consider these factors and work towards addressing cultural beliefs and disseminating accurate information in order to effectively combat the disease (S. Ahmed, Atkin, et al., 2006).

In this study, the mean score for overall parental practices was 3.3±1.0, with only 47.4% of the parents demonstrating good practice scores. Similarly, an Indonesian study reported that 45.6% of the parents of thalassemic children exhibited good practice (Wahidiyat et al., 2021). Moreover, in our study, more than half of the participants (57.8%) had their other children screened, which is an encouraging finding. Similarly, a Pakistani study also found that nearly half of the parents of thalassemic children screened their other offspring, highlighting the overwhelming majority of parents who were encouraging their children to undergo screening (Naseem Ahmed et al., 2020).

On the contrary, in the present study, only 28.4% of parents opted for prenatal diagnosis. Religious restrictions on abortion, which is the only available option for pregnant women carrying an affected fetus, may influence the decision of the parents and prevent them from undergoing prenatal testing. Furthermore, the high cost of chorionic villus sampling (CVS) testing may be a contributing factor, especially for families belonging to low and middle socioeconomic groups. Similar to our finding, a study conducted in Lahore, reported that among participants who were aware of prenatal diagnosis, only 39.1% of mothers underwent the test during their pregnancies, while others did not utilize this service (Ishaq et al., 2012).

The perceived severity of thalassemia has been found to be the most influential factor in a woman's decision about prenatal diagnosis. Therefore, effective physician-patient communication should emphasize the severity of the disorder, its treatment options, and the necessity of prenatal screening (S. Ahmed, Green, & Hewison, 2006).

In this study, the vast majority of parents (67.2%) expressed not wishing for having more children after having a sick child. These results suggest that parents who understand the nature of thalassemia could be more hesitant to expand their families, as they become more aware and concerned about the possibility of their future child being affected as well (Mat, Yaacob, & Zakaria, 2020). However, the analysis showed that seven families had two thalassemic children that were born after 2010 and 18 families had at least one thalassemic child that was born before 2010. This means that more than one third of the families have at least two thalassemic children. literature suggests that South Asian families are typically large, and parents often aspire to have more male children to attain dominance within their relatives, which might explain why families continue to grow despite having an affected infant. It has also been suggested that families may choose to terminate family expansion if the infant is born later after they have healthy children (Z. Hossain & Eisberg, 2020). However, we were not able to test this theory as we did not have data regarding the birth order of the thalassemic children that were born prior to 2010. Han et al. reported that 82% of parents did not intend to have additional children due to the fear of recurrence and the severity of these conditions (Han, Han, & Myint, 1992).

Knowledge of the disease alone may not necessarily translate into correct practices. We have found that the knowledge score and practice score were not correlated. However, it has been observed that understanding the basic concept of thalassemia positively influences attitudes, and that attitudes were significantly correlated with good practices, specifically, attitudes towards the termination of pregnancy.

This shows that awareness campaigns need to be improved and customized so they could illicit changes to the attitudes and practices of the population.

Our study revealed an unexpected finding in regard to practice scores. We have found significant differences across groups of educational level at marriage in regard to practice scores being lowest for parents with the highest and lowest levels of education.

In theory, the influence of education on thalassemia prevention behaviors should have been positive. However, more educated people in Palestine tend to be more aware of their public image, which could be affected by the social stigma associated with thalassemia. As a result, they may refuse thalassemia testing out of fear of being diagnosed and they may hide their children's illness from others out of fear of facing discrimination and stigma and the effect they may have on their image.

6.2 Limitations of the Study

This cross-sectional case-series study aims to determine the factors associated with the continuing emergence of β-thalassemia major in Palestine after the implementation of the obligatory premarital screening program, in addition to assessing the knowledge, attitudes, and practices of parents towards thalassemia. Up to our knowledge, this study is the first to date on this topic in Palestine.

The findings of the study may be influenced by specific cultural, social, and contextual factors that are unique to our population. These factors may not be applicable or generalizable to other cultural or geographical contexts. In this study, we have included most thalassemia patients who were born since 2010 in the West Bank. However, we were not able to include Gaza Strip.

The study focused primarily on sociodemographic factors, perceptions, and practices related to thalassemia as we were unable to obtain blood samples for a complete hematological profile for both parents in each of the families (only one out of the 62 families provided blood for both parents). Therefore, we were not able to derive accurate conclusions regarding the limitations of the criteria to perform thalassemia screening and we were not able to establish if genetic variants in the promoter region of the β-globin gene could contribute to the continuing emergence of thalassemia despite the obligatory premarital testing.

The study relied on self-reported data from participants, which may introduce recall bias. Additionally, participants may have provided socially desirable responses that they believed were expected or socially acceptable which might introduce Social Desirability Bias and could affect the reliability and validity of the results.

The study also utilized a cross-sectional design, which only provides a snapshot of the participants' KAP at a specific point in time when the study was conducted. However, it does not allow for the accurate assessment of the factors that may influence the decisions of individuals at the time of occurrence or changes in KAP over time.

While our study provides valuable insights regarding the factors that contribute to the continuing emergence of thalassemia in Palestine, the study may lack the statistical power required to detect subtle or less common effects or associations due to the limited number of

participants. Therefore, caution should be exercised when extrapolating these results to larger and more diverse populations.

Future research endeavors may help mitigate these limitations and provide a more comprehensive understanding of thalassemia prevention.

6.3 Recommendations

The prevention of thalassemia should continue to be a top priority in fighting against inherited diseases due to the impact it causes on the healthcare system and the patients' families. Based on the findings and limitations of this study, the following recommendations are proposed to enhance thalassemia prevention and management strategies:

Health Education and Awareness Programs: Develop and implement comprehensive health education and awareness programs targeting individuals, families, and communities at risk of thalassemia. These programs should focus on increasing knowledge about thalassemia, its inheritance patterns, and prevention methods. Special attention should be given to promoting awareness among younger couples who may lack knowledge about the chronic nature of the disease.

Pre-marital Counseling and Screening: Strengthen and expand pre-marital counseling and screening services to ensure that all engaged couples undergo screening for thalassemia and receive genetic counseling. Emphasize the importance of early detection, carrier identification, and informed decision-making regarding marriage and family planning. Electrophoresis testing should be considered mandatory to achieve more accurate results and screen for the sickle cell trait. However, research should be carried out to determine the criteria for testing and the most efficient and cost-effective strategy to integrate the test into the obligatory screening program.

Identifying Designated Receptions Centers: The premarital screening test should be performed in Designated Receptions Centers where trained staff perform and interpret the results in accordance with standard laboratory diagnostic protocols, availability of lab reagents and equipment in all assigned laboratories is treated as a priority, and staff receive continuous training and supervision, and special training sessions when necessary.

Collaboration with Religious Leaders and Institutions: Engage religious leaders and institutions to raise awareness and promote the importance of pre-marital testing and counseling for thalassemia. Develop partnerships to integrate thalassemia prevention messages into religious teachings and community gatherings, leveraging the influence of religious leaders in shaping attitudes and behaviors.

Genetic Testing and Counseling Services: Enhance access to genetic testing and counseling services, particularly for partners known to be thalassemia carriers and those who have had children affected with thalassemia previously, which helps to decrease the number of new patients. This includes providing training to healthcare professionals to deliver accurate and culturally sensitive genetic counseling services, ensuring that individuals and families receive appropriate information and support.

School-based Education Programs: Integrate thalassemia education into school curricula to reach young individuals and promote awareness from an early age. Collaborate with

education authorities to develop age-appropriate educational materials, conduct awareness sessions, and encourage participation in school health programs.

Community Support Networks: Establish and strengthen community support networks for thalassemia patients and their families. These networks can provide emotional support, share experiences, and disseminate information about available resources, including medical facilities, financial assistance, and social services.

Research and Surveillance: Continue conducting research to monitor the prevalence and trends of thalassemia, identify emerging risk factors, and evaluate the effectiveness of prevention programs. Longitudinal studies are needed to assess the long-term impact of interventions and track changes in knowledge, attitudes, and practices over time.

Multidisciplinary Collaboration: Foster collaboration among healthcare providers, researchers, policymakers, community organizations, and patient advocacy groups to develop a comprehensive and coordinated approach to thalassemia prevention and management. This collaboration can facilitate the exchange of knowledge, resources, and best practices, leading to improved outcomes for individuals and families affected by thalassemia.

Continuous Training and Capacity Building: Provide ongoing training and capacity building initiatives for healthcare professionals, educators, and community workers involved in thalassemia prevention and management. This includes updating their knowledge on advancements in thalassemia research, genetic counseling techniques, and effective educational strategies.

Empowerment of Thalassemia Patients and Families: Empower thalassemia patients and their families to actively participate in their own care and decision-making processes. Offer support services that promote self-management skills, address psychosocial needs, and enhance overall quality of life for individuals living with thalassemia. By implementing these recommendations, it is hoped that the incidence of new thalassemia cases can be reduced, individuals and families can make informed decisions regarding thalassemia prevention, and the overall quality of care and support for thalassemia patients can be improved.

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Appendix 4.1 - Consent form

العوامل المؤدية لولادة حالات جديدة من الثلاسيميا الكبرى
في فلسطين بعد تطبيق قانون الفحص الإجباري
للمقدمين على الزواج

نموذج موافقة على المشاركة في دراسة بحثية

عزيزي/ عزيزتي المشارك/ة:

هذه الدراسة تقوم بها مجموعة بحثية في جامعة القدس تحت إشراف د. رانية أبو سير في دائرة العلوم الطبية المخبرية بالتعاون مع جمعية أصدقاء مرضى الثلاسيميا. تهدف هذه الدراسة للتعرف على العوامل التي تؤدي إلى استمرار ظهور حالات جديدة من مرضى الثلاسيميا الكبرى بالرغم من تطبيق البرنامج الإلزامي لفحص ما قبل الزواج.

حول المشاركة في الدراسة

هذه الدراسة تضم الأطفال المصابين بالثلاسيميا الكبرى والذين ولدوا في عام 2010 فما بعد. تتضمن هذه الدراسة قسمين، القسم الأول عبارة عن استمارة مقسمة على أربعة أجزاء وتشمل:

معلومات حول الثلاسيميا في أسرة المريض وبيانات حول الوالدين (الأب والأم)، معلومات الطفل المريض الشخصية والطبية، والقسمين الأخيرين من الاستبيان يهدفان لقياس معرفة الأهل (الأب والأم) واتجاهاتهم نحو الوقاية من مرض الثلاسيميا.

القسم الثاني من الدراسة يشمل سحب عينات دم كالآتي:

- سحب 3 عينات دم من كل من الأب والأم وكل طفل مصاب بالثلاسيميا ولد في عام 2010 أو بعد ذلك
- العينات ستستخدم لعمل فحص تعداد الدم الكامل (CBC) واختبار الرحلاء الكهربائي للخضاب (Hb electrophoresis) إضافة إلى عمل فحص جيني للكشف عن اعتلالات وراثية محددة في الجينات المسؤولة عن مرض الثلاسيميا.

معلومات تتعلق بالمشاركة في هذه الدراسة

- المشاركة في الدراسة تطوعية وللمشاركين الخيار للمشاركة في الدراسة.
- المعلومات التي يتم جمعها ضمن هذه الدراسة سرية ولن تتم مشاركة أية معلومات شخصية للمشاركين خارج طاقم البحث حيث سيتم استبدالها بأرقام وحفظها في مكان آمن.
- المشاركة في البحث اختيارية ويمكنك الانسحاب في أي وقت من المشاركة في الدراسة.
- لقد تمت مراجعة الإجراءات البحثية والأخلاقية للدراسة من قبل لجنة الأخلاق البحثية التابعة لجامعة القدس والموافقة عليها.
- ستتم مشاركة المعرفة التي نحصل عليها من إجراء هذا البحث من خلال المنشورات العلمية ولن يتم مشاركة المعلومات السرية. ربما تكون هناك لقاءات توعوية في المستقبل تبنى على نتائج هذا البحث.
- فيما يتعلق بالعينات التي يتم جمعها للدراسة:

  - المشاركة في البحث تشمل توفير عينات دم بهدف إجراء فحوصات الثلاسيميا (CBC & Hemoglobin electrophoresis) والحصول على المادة الوراثية للتحليل الجزيئي

- العينات الخاصة بتعداد الدم الكامل واختبار الرحلان الكهربائي للخضاب سيتم استخدامها بشكل مباشر لإجراء التحليلات اللازمة وأية كمية متبقية بعد الانتهاء سيتم إتلافها.
- عينات المادة الوراثية ستحفظ في مجمد ثلاجة مع رمز فحص (كود) بدون اسم أو رقم هوية.

- سيتم إرسال عينات الدم والمادة الوراثية إلى مختبرات خاصة لإجراء فحوصات الدم والفحص الجيني.
- بعد الحصول على نتائج الفحص الجيني سيتم توفير النتائج الخاصة بك بحيث يمكنك مراجعة الباحث/الجمعية للحصول عليها.
- للباحثين صلاحية استخدام المادة الوراثية لهذا البحث فقط، إلا إذا أعطيت موافقة لاستخدامها في أبحاث أخرى مرتبطة بالمرض الذي تعاني\ن منه، أو لأبحاث في المستقبل لأي مجال.
- في حال موافقتك على استعمال العينة فقط في هذا البحث، يتوجب على الباحثين الراغبين باستخدامها لأغراض بحثية أخرى التوجه إليك مرة أخرى وطلب موافقتك الإضافية لاستعمال العينة المعرفة.
- إذا قررت لأي سبب كان الانسحاب من البحث، سيتم إتلاف العينة خاصتك هي والمعلومات المرفقة بها.
- الباحثون مخولون فقط لاستعمال المعلومات غير المعرفة التي جمعت من العينة حتى هذه المرحلة من البحث.
- يتوجب على كل بحث إضافي يطبق على العينة الحصول على الإذن من الجهات المخولة.

- إذا كانت لديك أية أسئلة أو استفسارات بإمكانك توجيهها شخصا للباحث أو الاتصال بالباحث الرئيسي د. رانية أبو سير في دائرة العلوم الطبية المخبرية – جامعة القدس عبر البريد الإلكتروني rabusear@staff.alquds.edu.

تصريح الموافقة على المشاركة

- ☐ لقد قمت بالاطلاع على المعلومات الخاصة بالدراسة وحظيت بالفرصة للاستفسار وطرح الاستفسارات وتمت الإجابة على استفساراتي بشكل مرضٍ. بناءً على ذلك، أمنح موافقتي على الاشتراك في هذا البحث.
- ☐ وفق التعليمات التي تلقيتها حول الاستعمالات الأخرى لعينة الدم الخاصة بك، الرجاء التوقيع في الفراغ الملائم لواحدة من الخيارات التي توافق عليها:
  - أوافق على استخدام عينات ال DNAخاصتي في هذا البحث فقط._____.
  - أوافق على استخدام عينات الDNA خاصتي لكل بحث مطبق حسب القانون_____.
- ☐ تم تزويدي بنسخة من الاتفاقية.

اسم المشارك:_____
التوقيع: _____
التاريخ: _____
(اليوم/الشهر/السنة)

تصريح الباحث الذي قام بأخذ الموافقة

- ☐ لقد قمت بشرح المعلومات المتعلقة بالدراسة للمشارك وحاولت التأكد بأقصى استطاعتي من فهمه لما سيتم القيام به ويشمل:
  1. تعبئة استمارة الدراسة مع الباحث.
  2. سحب عينات دم.
- ☐ أؤكد أن المشارك حظي بالفرصة لطرح الأسئلة عن الدراسة وأنه تمت الإجابة عن هذه الأسئلة. كما أؤكد أنه لم يتم التلاعب بالمشارك للموافقة على المشاركة وقد قام بالموافقة بشكل طوعي اختياري.
- ☐ تم تزويد المشارك بنسخة من الاتفاقية.

اسم الباحث: _____

توقيع الباحث: _____

التاريخ: _____
(اليوم/الشهر/السنة)

Appendix 4.2 - Study questionnaires in English

Investigating β-Thalassemia Major Cases Emerged after Obligatory Premarital Testing in Palestine

Questionnaire

Section I: Families Information				
1.	Place of residence (Governorate\residence area)			
2.	Type of locality	☐ 1- Rural	☐ 2- Urban	☐ 3-Camp
3.	Monthly income	☐☐☐☐☐		
4.	Type of housing	☐ 1- Rental	☐ 2- Owned	
5.	Consanguinity Between parents (Mother and Father)?	☐ 1- Yes	☐ 2- No	
6.	If you answered yes, what is the degree of consanguinity between parents (Mother and Father)?	☐ 1- First degree relatives (First Cousins)	☐ 2- Same family with different grandfather/ grandmother	
7.	Number of thalassemia children in the family			
8.	Date of marriage (day/month/year)	☐☐	☐☐	☐☐☐☐
9.	If you were married before 2010, did you have any thalassemia children before 2010?	☐ 1- Yes	☐ 2- No	
10.	As far as you know, were any of your children was diagnosed with thalassemia minor?	☐ 1- Yes	☐ 2- No	☐ Don't Know
11.	If yes, how many of your children were diagnosed with thalassemia minor?	☐☐		
12.	Did you have any miscarriages due to thalassemia?	☐ 1- Yes	☐ 2- No	

Section II: Parental Information			
Father		**Mother**	
13. Full Name:		14. Full Name:	
15. ID Number:		16. ID Number:	
17. Date of birth (day/month/year)	☐☐ ☐☐ ☐☐☐☐	18. Date of birth (day/month/year)	☐☐ ☐☐ ☐☐☐☐
19. Educational level for Father (Current):	☐ 1) Illiterate ☐ 2) Can read & write ☐ 3) Elementary ☐ 4) Secondary ☐ 5) Associate diploma ☐ 6) Bachelor ☐ 7) High diploma ☐ 8) Master ☐ Ph.D.	20. Educational level for Mother (Current):	☐ 1) Illiterate ☐ 2) Can read & write ☐ 3) Elementary ☐ 4) Secondary ☐ 5) Associate diploma ☐ 6) Bachelor ☐ 7) High diploma ☐ 8) Maste ☐ Ph.D.
21. Father occupation:		22. Mother occupation:	
23. Place of Birth (City/ State):	----------/----------	24. Place of Birth (City/ State):	----------/----------

Investigating β-Thalassemia Major Cases Emerged after Obligatory Premarital Testing in Palestine

Questionnaire

25. Age at marriage (years):	☐☐	26. Age at marriage (years):	☐☐
27. Educational level at marriage	☐ 1) Illiterate ☐ 2) Can read & write ☐ 3) Elementary ☐ 4) Secondary ☐ 5) Associate diploma ☐ 6) Bachelor ☐ 7) High diploma ☐ 8) Master ☐ 9) Ph.D.	28. Educational level at marriage	☐ 1) Illiterate ☐ 2) Can read & write ☐ 3) Elementary ☐ 4) Secondary ☐ 5) Associate diploma ☐ 6) Bachelor ☐ 7) High diploma ☐ 8) Master ☐ 9) Ph.D.
29. Have you been examined before getting married	☐ 1) Yes ☐ 2) No	30. Have you been examined before getting married	☐ 1) Yes ☐ 2) No
31. If yes, what was the name of the laboratory where you performed the test:	----------/----------	32. If yes, what was the name of the laboratory where you performed the test:	----------/----------
33. Result	☐ 1) Carrier ☐ 2) Non-carrier	34. Result	☐ 1) Carrier ☐ 2) Non-carrier
35. Type of test (you can choose more than one option)	☐ CBC ☐ Electrophoresis ☐ DNA analysis ☐ Don't know	36. Type of test (you can choose more than one option)	☐ CBC ☐ Electrophoresis ☐ DNA analysis ☐ Don't know
37. CBC results of the pre-marital screening test if available:	1- Hb 2- MCV 3- MCH	38. CBC results of the pre-marital screening test if available:	1- Hb 2- MCV 3- MCH
39. If no, what were the reasons of not doing the test (you can specify more than one reason)	☐ Lack of knowledge ☐ Poverty ☐ Religious beliefs ☐ Family influence/ the eldest ☐ Lack of diagnostic features ☐ Other: _____	40. If no, what were the reasons of not doing the test (you can specify more than one reason)	☐ Lack of knowledge ☐ Poverty ☐ Religious beliefs ☐ Family influence/ the eldest ☐ Lack of diagnostic features ☐ Other: _____
41. Have any of your family members been diagnosed with Thalassemia (parents, siblings, grandparents, uncles/aunts)?	☐ 1) Yes ☐ 2) No ☐ 3) Don't know	42. Have any of your family members been diagnosed with Thalassemia (parents, siblings, grandparents, uncles/aunts)?	☐ 1) Yes ☐ 2) No ☐ 3) Don't know

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Questionnaire

43. Have any of your family members been diagnosed as Thalassemia carriers (parents, siblings, grandparents, uncles/ aunts)?	☐ 1) Yes ☐ 2) No ☐ 3) Don't know	44. Have any of your family members been identified as thalassemia carriers (parents, siblings, grandparents, uncles/ aunts)?	☐ 1) Yes ☐ 2) No ☐ 3) Don't know
45. Have any of your family members been diagnosed with sickle cell disease (parents, siblings, grandparents, uncles/ aunts)?	☐ 1) Yes ☐ 2) No ☐ 3) Don't know	46. Have any of your family members been identified with sickle cell disease (parents, siblings, grandparents, uncles/ aunts)?	☐ 1) Yes ☐ 2) No ☐ 3) Don't know
47. Did you have any information about Thalassemia before your first child was affected?	☐ 1) Yes ☐ 2) No	48. Did you have any information about Thalassemia before your first child was affected?	☐ 1) Yes ☐ 2) No
49. If yes, what is the source of your information about Thalassemia?	☐ Awareness session ☐ Relatives ☐ Media ☐ Other:	50. If yes, what is the source of information about Thalassemia?	☐ Awareness session ☐ Relatives ☐ Media ☐ Other:
51. Have you ever received genetic counselling?	☐ 1) Yes ☐ 2) No ☐ 3) Don't know	52. Have you ever received genetic counselling?	☐ 1) Yes ☐ 2) No ☐ 3) Don't know

Investigating β-Thalassemia Major Cases Emerged after Obligatory Premarital Testing in Palestine

Questionnaire

Part II: Patients Information			
53.	Full Name:		
54.	ID Number:		
55.	Date of birth (day/month/year)	☐☐ ☐☐ ☐☐☐☐	
56.	Gender:	☐ 1- Male	☐ 2- Female
57.	Father Full Name:		
58.	Father ID Number:		
59.	Mother Full Name:		
60.	Mother ID Number:		
61.	Family Phone Number:		
62.	Treatment care unit:		
63.	Total number of siblings including patient:		
64.	Birth order among siblings:	☐ eldest. ☐ youngest. ☐ Others: ----------	
65.	Completed years of education:		
66.	Blood type	1) A+	2) A-
		3) B+	4) B-
		5) AB+	6) AB-
		7) O+	8) O-
67.	Underwent a bone marrow transplantation	☐ 1) Yes	☐ 2) No
68.	Underwent splenectomy	☐ 1) Yes	☐ 2) No
69.	Medical diagnosis	☐ 1) β-Thalassemia major	
		☐ 2) Sickle cell-thalassemia	
		☐ 3) Other:	
70.	Age at diagnosis (years)		
71.	Treatment with blood transfusion	☐ 1) Transfusion-Dependent Thalassemia (TDT)	
		☐ 2) Non-Transfusion-Dependent thalassemia (NTDT)	
		☐ 3) Other: _____	
72.	Age at first transfusion (years)		
73.	Were other children diagnosed (patient's siblings) with Thalassemia?	☐ 1) Yes ☐ 2) No	
74.	If the answer is yes, how many?		
75.	Patient's blood transfusion	☐ 1- Irregular	☐ 2- Regular
76.	How often does the patient transfuse? (Times between transfusions) in weeks		
77.	While mother being pregnant; have she performed a CVS test during this pregnancy?	☐ 1) Yes ☐ 2) No ☐ 3) Don't know	

Investigating β-Thalassemia Major Cases Emerged after Obligatory Premarital Testing in Palestine

Questionnaire

Section III: Maternal Perceptions Towards Thalassemia

79. Knowledge about Thalassemia		
Statement	**Yes**	**No**
58.1. Is Thalassemia a genetic disease		
58.2. Do both parents of Thalassemia children carry abnormal genes		
58.3. Does a Thalassemia child's mother have a 25% chance in each pregnancy to bear a diseased child		
58.4. Does consanguineous marriage play a role in the transmission of the disease to the upcoming generation		
58.5. Does a marriage between a healthy person and a carrier lead to a major Thalassemia child		
58.6. Does a marriage between carriers lead to a major Thalassemia		
58.7. Should a prenatal diagnosis be performed if both partners are Thalassemia carriers		
58.8. Can a couple with minor Thalassemia marry each other		
58.9. Can minor Thalassemia be curable		

Statement	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
80. Attitudes towards Thalassemia					
59.1. Is there a need to prevent Thalassemia					
59.2. I don't mind if there is more Thalassemia					
59.3. Do a Thalassemia mother and father miss fun and Joy					
59.4. Thalassemia is a social and economic burden					
59.5. Taking care of children with Thalassemia demands too much of me					
59.6. We have no right to prevent Thalassemia					
59.7. Testing for Thalassemia before marriage					
59.8. The need for legislations for premarital screening					
59.9. Thalassemia carriers should not get married					
59.10. It's not preferable that Thalassemia carriers can have children					
59.11. Thalassemia is a social stigma					
60. Attitudes towards prenatal diagnosis					
60.1. Is it preferable to know if you are carrying a Thalassemia baby					
60.2. Would you do the test if it is available					
60.3. You don't want to know if the baby is Thalassemic					
60.4. Tests for Thalassemia during pregnancy are					

not useful					
61. Attitudes towards the termination of pregnancy					
61.1.Disapprove of pregnancy termination					
61.2.I will not prevent a Thalassemia from being born					
61.3.If during pregnancy period and before the soul inspiration you found out that the fetus is affected by Thalassemia, would you agree on medical abortion?					
61.4.It is better to terminate a pregnancy than allowing a child to suffer					
61.5.Abortion of Thalassemia fetus is forbidden regardless of the gestational age					

62. Practices towards Thalassemia	**Yes**	**No**
62.1.Were your other children screened for Thalassemia?		
62.2.Do you perform prenatal screening?		
62.3.Have you motivated anyone to do premarital screening?		
62.4.Do you wish to have more children despite having sick ones already?		
62.5.Is your child's disease known to your relatives?		

Appendix 3 - Study questionnaire in Arabic

العوامل المؤدية لولادة حالات جديدة من الثلاسيميا الكبرى
في فلسطين بعد تطبيق قانون الفحص الإجباري
للمقدمين على الزواج

استمارة 1: معلومات وتاريخ الأسرة

اسم العائلة (اسم العائلة/ اسم الأب الأول/اسم الأم الأول)				
رقم العائلة				

القسم الأول: معلومات العائلة				
.1	مكان السكن (المحافظة/التجمع السكاني):			
.2	نوع التجمع السكاني:	□ 1- قرية	□ 2- مدينة	□ 3- مخيم
.3	الدخل الشهري للأسرة (شيكل):			
.4	نوع المسكن	□ 1- إيجار	□ 2- ملك	
.5	هل يوجد صلة قرابة بين الوالدين (الأم والأب)؟	□ 1- نعم	□ 2- لا	
.6	إذا أجبت بنعم، فما درجة القرابة بين الوالدين (الأب والأم)؟	□ 1- أقارب درجة أولى (أبناء عم/خال)	□ 2- ينتمون لنفس العائلة ولكن لا يتشاركان جد/جدة	
.7	كم عدد الأطفال الذين تم تشخيصهم بمرض الثلاسيميا؟			
.8	تاريخ الزواج (اليوم/الشهر/السنة):			
.9	إذا كنت قد تزوجت قبل عام 2010، هل ولد لديكم أطفال مصابين بالثلاسيميا قبل عام 2010؟	□ 1- نعم	□ 2- لا	
.10	حسب علمك، هل تم تشخيص أي من أبنائك كحامل لمرض الثلاسيميا؟	□ 1- نعم	□ 2- لا	□ 3- لا أعرف
.11	إذا كانت إجابتك بنعم، ما عدد الأطفال الذين تم تشخيصهم كحاملين لمرض الثلاسيميا؟			
.12	هل سبق وتم إجهاض جنين بسبب اكتشاف إصابته بالثلاسيميا أثناء الحمل؟	□ 1- نعم	□ 2- لا	

القسم الثاني: معلومات الأب والأم							
الأب				الأم			
.13	الاسم الكامل:			.14	الاسم الكامل:		
.15	رقم الهوية:			.16	رقم الهوية:		
.17	تاريخ الميلاد: (اليوم/الشهر/السنة)			.18	تاريخ الميلاد: (اليوم/الشهر/السنة)		

Appendix 3 - Study questionnaire in arabic (Continued)

العوامل المؤدية لولادة حالات جديدة من الثلاسيميا الكبرى
في فلسطين بعد تطبيق قانون الفحص الإجباري
للمقدمين على الزواج

استمارة 1: معلومات وتاريخ الأسرة

19. المستوى التعليمي للأب (الحالي):	□ 1- أمي □ 2 - يمكنه القراءة والكتابة □ 3- أساسي □ 4- ثانوي □ 5- دبلوم متوسط □ 6- بكالوريوس □ 7- دبلوم عالي □ 8- ماجستير □ 9- دكتوراه	20. المستوى التعليمي للأم (الحالي):	□ 1- أمي □ 2 - يمكنه القراءة والكتابة □ 3- أساسي □ 4- ثانوي □ 5- دبلوم متوسط □ 6- بكالوريوس □ 7- دبلوم عالي □ 8- ماجستير □ 9- دكتوراه
21. مهنة الأب:	_____	22. مهنة الأم:	_____
23. مكان الولادة (المدينة/ الدولة)	_____/_____ _____	24. مكان الولادة (المدينة/ الدولة)	_____/_____ _____
25. العمر عند الزواج (سنوات)		26. العمر عند الزواج (سنوات)	
27. المستوى التعليمي عند الزواج	□ 1- أمي □ 2 - يمكنه القراءة والكتابة □ 3- أساسي □ 4- ثانوي □ 5- دبلوم متوسط □ 6- بكالوريوس □ 7- دبلوم عالي □ 8- ماجستير □ 9- دكتوراه	28. المستوى التعليمي عند الزواج	□ 1- أمي □ 2 - يمكنه القراءة والكتابة □ 3- أساسي □ 4- ثانوي □ 5- دبلوم متوسط □ 6- بكالوريوس □ 7- دبلوم عالي □ 8- ماجستير □ 9- دكتوراه
29. هل خضعت للفحص الطبي لصفة الثلاسيميا قبل الزواج؟	□ 1- نعم □ 2- لا	30. هل خضعت للفحص الطبي لصفة الثلاسيميا قبل الزواج؟	□ 1- نعم □ 2- لا
31. إذا كانت الإجابة بنعم، فما هو اسم المختبر الذي أجريت فيه الاختبار (اسم المختبر/عنوان المختبر):	_____/_____ _____	32. إذا كانت الإجابة بنعم، فما اسم المختبر الذي أجريت فيه الاختبار (اسم المختبر/عنوان المختبر):	_____/_____ _____
33. النتيجة:	□ 1- حامل للثلاسيميا □ 2- غير حامل للثلاسيميا	34. النتيجة:	□ 1- حامل للثلاسيميا □ 2- غير حامل للثلاسيميا
35. نوع الفحوصات التي تم إجراؤها (يمكنك اختيار أكثر من اختبار واحد)	□ 1- تعداد الدم الكامل (CBC) □ 2- تحليل الرحلان الكهربائي للخضاب(Electrophoresis) □ 3- تحليل الحمض النووي (DNA analysis) □ 4- لا أعلم	36. نوع الفحوصات التي تم إجراؤها (يمكنك اختيار أكثر من اختبار واحد)	□ 1- تعداد الدم الكامل (CBC) □ 2- تحليل الرحلان الكهربائي للخضاب (Electrophoresis) □ 3- تحليل الحمض النووي (DNA analysis) □ 4- لا أعلم

Appendix 4.3 - Study questionnaire in arabic (Continued)

العوامل المؤدية لولادة حالات جديدة من الثلاسيميا الكبرى
في فلسطين بعد تطبيق قانون الفحص الإجباري
للمقدمين على الزواج

استمارة 1: معلومات وتاريخ الأسرة

37. نتائج CBC لاختبار الفحص ما قبل الزواج إذا كانت متوفرة:	59.1. الهيموجلوبين (Hb):	38. نتائج CBC لاختبار الفحص ما قبل الزواج إذا كانت متوفرة:	60.1. الهيموجلوبين (Hb):
	59.2. متوسط حجم كريات الدم الحمراء (MCV):		60.2. متوسط حجم كريات الدم الحمراء (MCV):
	59.3. متوسط تركيز الخضاب في الكريات الحمراء (MCH):		60.3. متوسط تركيز الخضاب في الكريات الحمراء (MCH):
39. إذا لم تقم بإجراء فحص الثلاسيميا قبل الزواج، فما هو السبب في أنك لم تحصل على الفحص الطبي (يمكنك تحديد أكثر من سبب واحد)	□ 1- لم يكن الفحص إجبارياً في تلك الفترة □ 2- الفحص غير متوفر □ 3- لم أكن أعرف بوجود فحص قبل الزواج □ 4- أسباب اقتصادية □ 5- أسباب دينية □ 6- أسباب اجتماعية (تأثير الأسرة / الأكبر سنا) □ 7- أسباب أخرى: _____	40. إذا لم تقم بإجراء فحص الثلاسيميا قبل الزواج، فما هو السبب في أنك لم تحصل على الفحص الطبي (يمكنك تحديد أكثر من سبب واحد)	□ 1- لم يكن الفحص إجبارياً في تلك الفترة □ 2- الفحص غير متوفر □ 3- لم أكن أعرف بوجود فحص قبل الزواج □ 4- أسباب اقتصادية □ 5- أسباب دينية □ 6- أسباب اجتماعية (تأثير الأسرة / الأكبر سنا) □ 7- أسباب أخرى: _____
41. هل تم تشخيص أي من أفراد عائلتك بالإصابة بمرض الثلاسيميا (الآباء والأشقاء والأجداد والأعمام/ الأخوال والعمات/ الخالات)؟	□ 1- نعم □ 2- لا □ 3- لا أعلم	42. هل تم تشخيص أي من أفراد عائلتك بالإصابة بمرض الثلاسيميا (الآباء والأشقاء والأجداد والأعمام/ الأخوال والعمات/ الخالات)؟	□ 1- نعم □ 2- لا □ 3- لا أعلم
43. هل تم تشخيص أي من أفراد عائلتك كحامل لصفة الثلاسيميا (الآباء والأشقاء والأجداد والأعمام/الأخوال والعمات/الخالات)؟	□ 1- نعم □ 2- لا □ 3- لا أعلم	44. هل تم تشخيص أي من أفراد عائلتك كحامل لصفة الثلاسيميا (الآباء والأشقاء والأجداد والأعمام/الأخوال والعمات/الخالات)؟	□ 1- نعم □ 2- لا □ 3- لا أعلم
45. هل تم تشخيص أي من أفراد عائلتك بالإصابة بمرض فقر الدم المنجلي (الآباء والأشقاء والأجداد والأعمام/ الأخوال والعمات/ الخالات)؟	□ 1- نعم □ 2- لا □ 3- لا أعلم	46. هل تم تشخيص أي من أفراد عائلتك بالإصابة بمرض فقر الدم المنجلي (الآباء والأشقاء والأجداد والأعمام/ الأخوال والعمات/ الخالات)؟	□ 1- نعم □ 2- لا □ 3- لا أعلم
47. هل كان لديك معرفة عن مرض الثلاسيميا قبل أن يتأثر طفلك الأول بالمرض؟	□ 1- نعم □ 2- لا	48. هل كان لديك معرفة عن مرض الثلاسيميا قبل أن يتأثر طفلك الأول؟	□ 1- نعم □ 2- لا

استمارة 1: معلومات وتاريخ الأسرة

49. إذا كان الجواب نعم، من أين حصلت على هذه المعرفة؟	□ 1- جهاز التعليم في المدارس/الجامعات □ 2- جلسات توعوية □ 3- من الأقارب □ 4- الإعلام □ 5- أخرى:_____	50. إذا كان الجواب نعم، من أين حصلت على هذه المعرفة؟	□ 1- جهاز التعليم في المدارس/الجامعات □ 2- جلسات توعوية □ 3- من الأقارب □ 4- الإعلام □ 5- أخرى:_____
51. هل تلقيت أي المشورة الوراثية؟	□ 1- نعم □ 2- لا □ 3- لا أعلم	52. هل تلقيت المشورة الوراثية؟	□ 1- نعم □ 2- لا □ 3- لا أعلم

استمارة 2: بيانات المريض

القسم الثالث: معلومات المريض					
53.	الاسم الرباعي للمريض:				
54.	رقم هوية المريض:				
55.	تاريخ الميلاد (اليوم/الشهر/السنة):				
56.	الجنس:	□ 1- ذكر	□ 2 – أنثى		
57.	الاسم الكامل للأب:				
58.	رقم هوية الأب:				
59.	الاسم الكامل للأم:				
60.	رقم هوية الأم:				
61.	رقم الهاتف (الأهل):				
62.	الوحدة (المستشفى) التي يتلقى بها المريض العلاج حالياً:				
63.	عدد الإخوة والأخوات بما فيهم المريض:				
64.	الترتيب بين الإخوة والأخوات:	□ 1- الأكبر □ 2- الأصغر □ 3- آخر (مع التحديد) _____			
65.	عدد سنوات الدراسة التي أكملها الطفل:				
66.	فصيلة الدم:	□ 1) A+	□ 2) A-		
		□ 3) B+	□ 4) B-		
		□ 5) AB+	□ 6) AB-		
		□ 7) O+	□ 8) O-		
67.	هل تم القيام بعملية زرع نخاع العظم عند الطفل المريض؟	□ 1- نعم	□ 2- لا		
68.	هل تم استئصال الطحال عند الطفل المريض؟	□ 1- نعم	□ 2- لا		
69.	التشخيص الطبي:	□ 1- بيتا ثلاسيميا الكبرى □ 2- أنيميا منجلية - ثلاسيميا □ 3- أخرى (مع التحديد) _____			
70.	العمر عند التشخيص (أشهر):				
71.	هل سبق وأن تلقى المريض علاج بنقل الدم؟	□ 1- نعم	□ 2- لا		
72.	إذا كانت الإجابة بنعم، كم كان عمر المريض عندما تلقى أول عملية نقل للدم (أشهر)				
73.	يتلقى المريض العلاج بنقل الدم بشكل:	□ 1- متقطع (عند الحاجة)	□ 2- منتظم (خلال فترات زمنية محددة)		
74.	إذا كان المريض يتلقى العلاج بنقل الدم بشكل منتظم، كم المدة بين عمليات نقل الدم (عدد الأسابيع بين جلسات نقل الدم)				
75.	خلال فترة حمل الأم بهذا الطفل، هل تم القيام بفحص عينة الزُّغابات المشيمائيّة /سائل المشيمة (CVS)	□ 1- نعم	□ 2- لا	□ 3- لا أعرف	

استمارة 3: اتجاهات الأم نحو الثلاسيميا

الاسم الكامل للأم										
رقم هوية الأم										

76. المعرفة تجاه مرض الثلاسيميا		
العبارة	نعم	لا
76.1. الثلاسيميا مرض وراثي		
76.2. عندما يكون لدى الزوجين طفل مصاب بالثلاسيميا فإن كلا الوالدين يحملان الصفة الوراثية للثلاسيميا		
76.3. عندما يكون لدى الزوجين طفل مصاب بالثلاسيميا فإن احتمالية إنجاب طفل مصاب بالثلاسيميا مع كل حمل تبلغ 25%		
76.4. يلعب زواج الأقارب دورا مهما في انتقال مرض الثلاسيميا إلى الجيل القادم		
76.5. الزواج بين شخص سليم وشخص حامل للثلاسيميا يمكن أن يؤدي إلى إنجاب أطفال مصابين بمرض الثلاسيميا الكبرى		
76.6. الزواج بين حاملي الثلاسيميا يؤدي إلى إنجاب أطفال مصابين بالثلاسميا الكبرى		
76.7. إذا كان الشريكان حاملين للثلاسيميا، ينبغي إجراء التشخيص الطبي قبل الولادة		
76.8. شخصين مصابين بالثلاسيميا الصغرى يمكنهم الزواج من بعضهم البعض		
76.9. الثلاسيميا الصغرى يمكن علاجها		

العبارة	أعارض بشدة	أعارض	محايد	أوافق	أوافق بشدة
77. المواقف تجاه مرض الثلاسيميا					
77.1. هناك حاجة لمنع ظهور حالات جديدة من مرض الثلاسيميا					
77.2. لن أمانع من وجود المزيد من حالات جديدة من مرض الثلاسيميا					
77.3. والدة ووالد مرضى الثلاسيميا يفتقدان المرح والفرح					
77.4. الثلاسيميا عبء اجتماعي واقتصادي					
77.5. رعاية الأطفال المصابين بالثلاسيميا تتطلب الكثير من وقتي وجهدي					
77.6. ليس لدينا الحق في منع مرض الثلاسيميا					
77.7. اختبار الثلاسيميا قبل الزواج أمر مهم لمنع حالات جديدة من الثلاسيميا					
77.8. هناك حاجة لتشريع قانون الفحص الطبي قبل الزواج					
77.9. يجب عدم الزواج بين حاملي الثلاسيميا					
77.10. لا يفضل الإنجاب بين الأزواج الحاملين للثلاسيميا					
77.11. مرض الثلاسيميا وصمة اجتماعية					

استمارة 3: اتجاهات الأم نحو الثلاسيميا

العبارة	أعارض بشدة	أعارض	محايد	أوافق	أوافق بشدة
78. المواقف تجاه التشخيص قبل الولادة					
78.1. أرغب في أن أعرف إذا كان الجنين مصابا بالثلاسيميا					
78.2. سأقوم بعمل في حال توفر الفحص الطبي قبل الإنجاب					
78.3. لا أرغب في أن أعرف إذا كان الجنين مصاب بالثلاسيميا					
78.4. اختبارات الثلاسيميا أثناء الحمل غير مفيدة					
79. المواقف نحو إنهاء الحمل (الإجهاض)					
79.1. لا أوافق على إنهاء الحمل (الإجهاض)					
79.2. لن أمنع ولادة طفل مصاب بالثلاسيميا					
79.3. إذا وجدت خلال فترة الحمل وقبل إلهام الروح أن الجنين مصاب بالثلاسيميا، هل توافق على الإجهاض الطبي؟					
79.4. من الأفضل إنهاء الحمل في حال كان الجنين مصاب بالثلاسيميا بدلا من ترك الطفل يعاني					
79.5. إجهاض الجنين المصاب بالثلاسيميا حرام بغض النظر عن عمر الجنين					

80. الممارسات المتبعة لمنع ظهور حالات جديدة من الثلاسيميا		
العبارة	نعم	لا
80.1. هل تم فحص أطفالك الآخرين للكشف عن الثلاسيميا؟		
80.2. هل تقوم بالفحص الطبي لصفة الثلاسيميا ما قبل الولادة (فحص عينة الزُّغابات المَشيمائِيَّة – CVS)؟		
80.3. هل قمت بتحفيز أي شخص للفحص الطبي قبل الزواج؟		
80.4. هل ترغب في الحصول على المزيد من الأطفال على الرغم من وجود أطفال مصابين بالثلاسيميا لديك؟		
80.5. هل أقاربك على علم بمرض طفلك؟		