An-Najah National University

Faculty of Graduate Studies

Management and Complications of Thalassemic Patients in Palestine: Retrospective Study

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Dedication

To my Great Parents who have always been there for me, for their endless love, support, and encouragement to accomplish this thesis

To my beloved Brothers and Sisters

Special dedication to my ever love, my husband (Muhammad-Abu alfalaieh)

For his Continuous motivation and patience

To my beloved daughter Donia, and my little son Sa'ed

And to all Thalassemic patients in Palestine

To all of them I dedicate this work with respect and love

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Finally, my special deep and sincere gratitude is extended to my **great parents**, **husband**, **sons**, **relatives and friends**.

الإقرار

أناالموقعة أدناه، مقدمة الرسالة التي تحمل العنوان:

Management and Complications of Thalassemic Patients in Palestine: Retrospective Study

أقر بأن ما اشتملت عليه هذه الرسالة إنما هونتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة كاملة، أو أي جزء منها لم يقدم من قبل لنيل أي درجة أو لقب علمي أو بحث لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: **اسم الطالبة:** نداء منقال مصطفى دراغمة

Signature: **التوقيع:**

Date: **التاريخ:** 7/12/2016

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Abstract

In Palestine little data concerning current thalassemia management and complication is available. This study aimed to evaluate the existing management protocol administrated to the registered β-thalassemia major (BTM) patients in comparison with internationally used ones. In addition, to identify commonly developed complications and their association with iron overload.

This cross-sectional study was conducted retrospectively from January 2012 to September 2014. The study included all reported BTM patients admitted for treatment and care at Al-Wattani thalassemic ward. Medical files were reviewed and data was collected in a specially designed questionnaire. Missing data was obtained by direct interview with patients or their guardians. Statistical analysis was performed using the IBM SPSS v.21. Conformation of diagnosis was carried out for five cases. A total of 54 patients includes 26 (48.1%) males and 28(51.9%) females, with average age of 17.07 ± 1.8 ranged from 1-68 years. 84.4% of them were born to relative parents.

Excluding five miss-diagnosed patients, the main age of the rest reported BTM patients was 13.96 ± 7.75, ranged from 1-28 years.

The annual haemoglobin mean was below 7.6g/dL in three studied years (2012- 2014) indicating a sever anaemic state among the study group. Hepatitis infection (one with B the other with C) were reported in two cases (3.7%). Splenectomy was carried out for 29(53.7%) patients. Serum ferritin mean was 2721±2204.1ng/ml in 2012 and 2069.6± 1770.6 ng/ml in 2014 with significant decrease (P-value= 0.001). In 2014, 73.5% of patients were with levels above the internationally recommended cut off value, ≤1000ng/ml, reflecting iron overload.

Biochemical characteristics strongly indicate deteriorated liver, kidney and endocrine functions. Elevated ALT and AST levels were reported among 43.4% to 93.5% of tested patients over the study period. Comparative analysis of the levels of these enzymes showed an increased level with increased age for both enzymes. Variations in the levels of ALT were of no significant value, however variations in AST levels were statistically significant (P-value= 0.001). A strong positive correlation was observed between SF and hepatic enzymes (P- value= 0.00, r_s>0.5 for both enzymes). Elevated ALP levels were reported among 32.7% to 67.5% of tested patients over the study period. Variation in the levels of this enzyme with elapsed time were significant (P-value= 0.002).

Hypercalcemia was reported among 58.7% and 17.3% of the tested patients in 2012 and 2013, respectively with significant decrease in calcium level between those years (P-value= 0.001). Kidney function test showed low creatinine levels among 84.3% of tested patients in 2012 and in 59.2%

for those tested in 2014, with significant decrease (P-value= 0.024) . High serum urea levels were reported among 12% patients in 2012 and among 23.6% of those tested in 2014 with significant increase in urea levels with elapsed time (P- value= 0.00).

Poor endocrine follow up was reported as testing for endocrine function only and partially started in 2014. The most common complication among the tested patients was hypogonadism in 46.7%, hypothyroidism was reported in 13.3%, and hypercortisolism was reported in 5.5%.

Poor monitoring for bone profile and cardiovascular function were also reported. Cardiac complications were reported in 9.3% of the patients.

This study is the first to address a detailed profile of the health situation of β-thalassemia major patients. The observed impaired biochemical and hormonal levels reflect a deteriorated clinical picture. Such findings emphasize the need for reinforcement and importance of adopting effective strategic policy for management and care thalassemic patients in Palestine.

Chapter One

Introduction

1.1 Overview

1.1.1 Human hemoglobin's

Human hemoglobins are tetrameric in structure, made up of two different pairs of globin polypeptide chains that are packed inside the red blood cells (RBCs). Each globin chain is attached to heme molecule (1-5). Hemoglobins are heterogeneous proteins and different forms are synthesized during the developmental stages of human life (2, 4, and 6).

After birth, fetal hemoglobin (HbF, α2 γ2) is switched to adult hemoglobin: HbA (α2 β2) and HbA2 (α2 δ2). This switch occurs at about the time of birth and ends 6 months later as illustrated in Figure 1. Hemoglobin in adult composed from 97% of HbA, approximately 2% of HbA2 and small amount (< 2%) of HbF is also found in adult blood (6, 7).

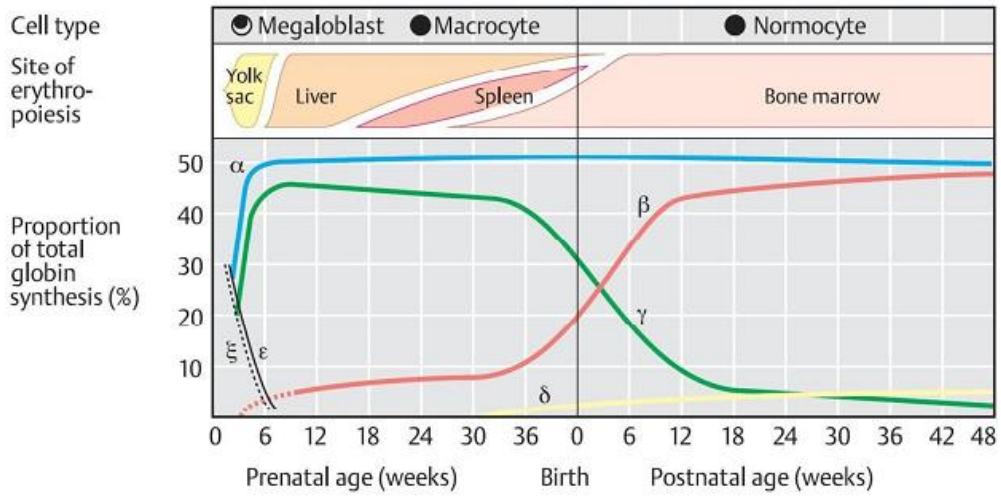

Figure 1. Developmental changes in human globin chain production (7)

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1.1.2 Inherited hemoglobin disorders

Inherited hemoglobin disorders, are commonly known as hemoglobinopathies, it is considered as one of the major public health problems in many countries (1, 3, 5). Mutations in the genes controlling human hemoglobin can be classified in three categories. The first is quantitative, result from defect in the rate of production of one or more of the globin chains. The second is qualitative, result in the production of different abnormal hemoglobin molecules. Hereditary persistence of fetal hemoglobin (HPFH) is a third type of hemoglobin genetic disorders, in which the pattern of hemoglobin production of the unborn child (HbF) continues into adult life. However, no deleterious effects are apparent, even when 100% of the hemoglobin synthesized is HbF. The quantitative abnormalities are known as thalassemias, while the qualitative abnormalities are known as structural hemoglobin variants (6, 7, and 8). The most common and medically important abnormal hemoglobins among those variants are: Haemoglobin S (HbS), hemoglobin C (HbC) and hemoglobin E (HbE). Patients affected with these structural variants in association with β-thalassemia trait showed severe anemic conditions similar to that of β-thalassemia major, a condition might complicate diagnosis of such cases (8).

1.1.3 Thalassemia

Thalassemias are common autosomal recessive disorders, which result from a defect in the rate of hemoglobin production (1, 2). The first

case was described in North America in 1925, based on observations of Italian children with characteristic of anemia and bone deformities (3, 4). The disease is well recognized in the Mediterranean region, so it was termed as “thalassa anemia" anemia by the sea" (5). Thalassemia is found in about 60 countries worldwide, with the highest prevalence in the Mediterranean region, so it considered as increasing public health issue (3, 5). Worldwide, carriers of the globin variant are estimated around 5% (2).

Figure 2. Geographical distribution of β-thalassemia around the world. Black areas indicate the countries where thalassemia is prevalent (8)

1.2 Types of thalassemia disorders

Alpha and beta thalassemia are considered as the two most important common forms of these diseases that result from a defect in alpha and beta globlin chains in the hemoglobin of erythrocytes (6). Alpha thalassemia is the most common inherited disorder of hemoglobin (Hb) synthesis in the world that is mainly due to deletion of one or both alpha globin genes in

each locus on chromosome number 16 leading to an excess of beta globin chain amount (7). To cause a clinical impact, mutations in three or total four alpha globin alleles are needed (8). On the other hand, beta thalassemia is the most severe form due to the presence of one gene loci (6, 8). It is an autosomal recessive monogenic defect that results from a point mutation of beta globlin gene on chromosome number 11, mainly substitution that cause absence of beta chains, a condition known as (β^0 thalassemia) or reduction in the synthesis of beta globulin chains which is known as (β+ thalassemia). Deletion of the whole gene also occurs, however, its occurrence is rare (6, 9). In case of beta thalassemia, alpha chain production will continue to occur. This increased synthesis of alpha chains makes the developing erythrocytes more fragile leading to early damage, ineffective erythropioses and anemia (2).

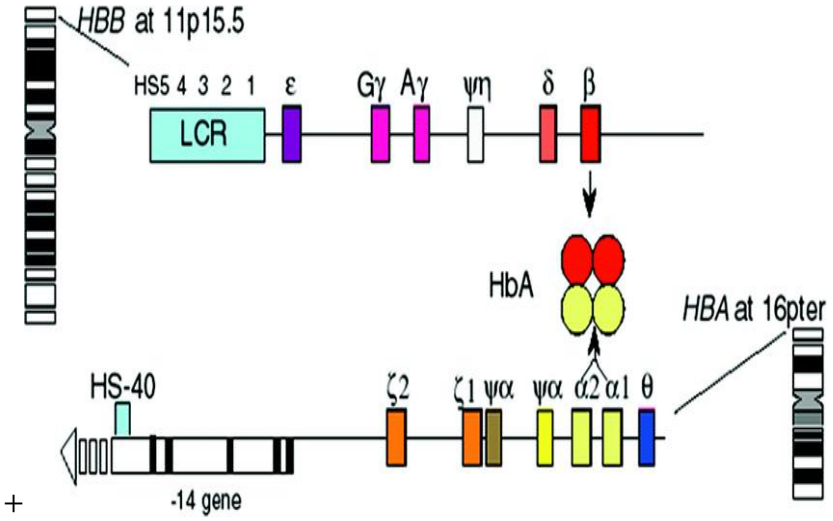

Figure 3. Chromosomal localization and structure of alpha and beta globin gene clusters (9)

1.2.1 β-Thalassemia forms

Depending on the severity of disease symptoms of anemia, three forms of beta thalassemia were recognized (1, 6, and 9).

1. Beta thalassemia minor: is the most common form which results from the inheritance of one mutant beta globin allele. This condition is asymptomatic, silent and the carrier state requires no medical management.
2. Beta thalassemia intermedia: result from a reduction in the rate of synthesis of beta chains and patients have moderate anemia and rarely need blood transfusion.
3. Beta thalassemia major: also called Cooley's anemia and considered as the most severe form. It is a transfusion dependent anemia where a patient requires regular blood transfusion to survive. This occurs as a result of inheritance of two beta globin gene mutations either in homozygous or compound heterozygous states.

1.3 Control and management of β- thalassemia major

Focus on control and management of β-thalassemia is centered on cases with sever clinical symptoms associated with the major type. Blood transfusion therapy is the most commonly used procedure for control and management of disease state among BTM patients. This is usually done through regular blood transfusion to maintain nearly normal hemoglobin level. This was found to improve patient's growth and development. It

was also reported to reduce complications associated with bone deformities, liver and spleen complications. Thus, such regimen reduces the need for hepato-splenomegaly surgery (2).

Bone marrow transplantation and gene therapy are the only potentially curative treatments (10). Bone marrow transplantation has successfully treated thousands of patients. However, the problem of this treatment lies in the cost, finding a suitable and it is association with post-transplantation complications (6, 10, and 11). Gene therapy using stem cells for permanent correction of the gene defects of hematopoietic system was also reported with high rate of success however, identification of all sequences required for stable, high-level expression of the genes and the development of more effective and safe vectors for the transfer of genes still a research challenge (6).

1.4 Complication of β-thalassemia major

Lifelong blood transfusion combined with chelation therapy are cornerstones in the disease management. They were found to increase patient survival and decrease disease complications that include anemia and bone expansion, which are prevalent in untreated patients. On the other hand, frequent transfusion can lead to serious secondary complications such as hepatitis infections (12, 13). This is clear from previous studies were about 70% of children's in developing countries who receive blood may acquire infection as hepatitis B and C during transfusion (9). Hyper bilirubinemia and a propensity to gallstone formation are also considered as

common complications due to rapid breakdown of red blood cells (13). In addition, increased iron overload along with increased iron absorption lead to iron accumulation in vital tissues and cause severe secondary complications (12, 13) including bone disease, osteoporosis and osteopenia (14). On the other hand, cardiac complications are still the main cause of mortality due to iron deposition in heart, even in the presence of chelation therapy in transfusion dependent patients (15). Endocrine complications were also reported, including disruption in hormonal secretion that arises in the form of severe clinical problems. Acquired hypothyroidism, hypogonadism, short stature, glucose intolerance and diabetes mellitus are the most reported endocrine complications (16). Otherwise, because liver is the main depot of iron containing about 70% or more of body iron content; liver disease is increasingly become a serious cause of death. Excessive hemosiderosis, cirrhosis and hepato cellular carcinoma are the most frequent consequences of this severe iron overload along with chronic hepatitis. Liver disease in these patients also manifest by aspartate and alanine transaminase activities combined with high alkaline phosphatase activity (6, 8, and 9).

1.5 Management of transfusion therapy

The aim of transfusion therapy is correction of anemia, suppression of erythropoiesis and gastrointestinal iron absorption inhibition (17, 18, and 19). Individuals with thalassemia major usually come to medical attention within the first two years of life and require regular RBC

transfusions to survive (18). Poor growth along with facial or bone abnormalities and/or hemoglobin levels below 7g/dL for more than two weeks are indications to administrate blood transfusion (17, 18). If Hb level dropped to less than 7g/dL, then regular monthly transfusion regimen must begin with the aim to maintain pre-transfusion hemoglobin levels above 9-10g/dL and post-transfusion levels to 13 to 14g/dL. Such Hb levels were found to be effective in preventing growth impairment, organ damage, bone deformities and allowing normal activity and quality of life (18). Transfusion is usually administrated monthly in infancy and subsequently in 2-4 week interval, volume of transfused blood is around 8 to 15 ml of packed RBC/Kg of body weight and should not exceed 20 ml RBC/Kg. RBCs are usually infused over a span of 1-2 hour at a maximum rate of 5ml/kg/hour at each event in clinically stable patients to avoid fast increase in blood. In patients with heart failure and/or if Hb level was below 5g/dL, a smaller RBC dose must be administrated (5ml/ Kg) to avoid volume overload and then Hb level should be increased gradually to 9g/dL (17, 18).

Before the first transfusion patients RBCs are usually typed for: Rh, ABO and other red blood cell antigens in order to prevent allo-immunisation; a common complication of chronic transfusion therapy. The standard protocols recommend the use of washed, leukocyte-depleted RBCs for patients to reduce allergic reactions as well as virus contamination. Also vaccination for hepatitis B should be performed, whereas hepatitis A vaccination is carried at appropriate age (17, 19).

To evaluate the effectiveness of transfusion therapy patients must be monitored at each transfusion event for weight, volume of transfused blood, pre and post transfusion Hb levels and records of all transfusion events should be monitored annually to identify hypersplenism (17).

1.5.1 Standard management of the complications

Patients who undergo regular transfusion regimen are expected to develop iron overload complications. Thus, it is important to asses iron levels in order to evaluate its clinical relevance, need of treatment, timing and monitoring chelating therapy. Serial measurement of serum ferritin is the most commonly used method to evaluate iron overload and efficacy of chelation therapy. Testing liver iron concentration using liver biopsy is the gold standard for the evaluation of iron overload, but because it is an invasive technique its use is very limited. Other techniques such as nuclear magnetic resonance imaging (MRI) are recently used for more accurate value (2, 18).

Monitoring of cardiac function is also important and carried out at the age of 7 to 8years. This can be carried out using electrocardiogram, echocardiogram and cardiac MRI. These tests in addition to chest X-ray are usually carried out to detect preclinical cardiac iron complications (17).

Since iron over load induces damage to the endocrine system therefore, annual endocrine evaluation is recommended mainly for thyroid and parathyroid gonadal functions as well as monitoring of bone health with nutritional counseling. Annual testing is also recommended between 8 to 10 years for luteinizing hormone (LH), follicular stimulating hormone

(FSH), insulin-like growth factor and insulin-like growth factor binding protein-3. Testing these factors is important for decisions on the use of hormone replacement therapy before puberty (17, 18).

To detect developmental changes Tanner staging is usually performed every 6 months. Before puberty children are usually tested annually for normal bone growth using bone age films to assess skeletal maturation, bone marrow density along with evaluation of calcium, vitamin D3 metabolism, thyroid and parathyroid functions (17).

Hemolysis among this group of patients may result in progressive over activity of the spleen and lead to the development of hypersplenism leading to an increase in transfusion requirement. Splenectomy is recommended if annual transfusion is above 220ml RBCs and the patient usually undergoes splenectomy by the age of 10 or 12 years. In this case, patients are vaccinated for pneumococcal and meningococcal infections before surgery and treated with penicillin after splenectomy (17).

Glucose tolerance testing is also recommended annually for early detection of insulin resistance caused by pancreas destruction (18).

1.5.2 Use of serum ferritin as a marker for iron overload

In general ferritin is a protein found in all organisms. Cellular ferritin found in the cell cytoplasm sequesters iron in a liganded form for storage. Cellular ferritin is at the center of managing body iron balance, especially during transfusion therapies (20, 21, and 22). Serum ferritin

(SF) reflects tissue ferritin under normal conditions and serves as indirect marker for tissue iron overload. SF test is used as indirect, non-invasive marker for iron overload and is the most accessible method currently available at many sites around the world for general increase and decrease in body iron store (20, 23). On the other hand, it is less accurate during iron overload because SF assay measure ferritin protein using antibodies which do not distinguish ferritin with varying iron content and the second reason is that antibody of SF assay is prepared against ferritin from few tissues as the composition of ferritin protein is not the same for all tissues (20, 22). Therefore, more precise measurement of iron load and better evaluation of the hemosiderosis status among thalassemia patients is strongly needed, especially for liver and heart mainly by using noninvasive MRI T2* (20, 24).

1.5.3 Serum ferritin as product of damaged cells

Despite that SF secretion are not completely understood and need further characterization (25), it was found that it originates from damaged cells (26) as described in Figure 4. Liganded iron can break down, losing most of its iron on the way and leave that iron in un-liganded forms which have exponential catalytic activity. This unbound iron has the ability to participate in reactions that create hydroxyl radicals. Hence, this describes the correlation between SF with serum markers of hydroxyl radicals formation that lead, of course, to oxidative stress causing growth failure as well as liver, cardiovascular, endocrine and neurological complications in

β-thalassemia major. In turn high SF has negative impact on the health (20, 27, and 28).

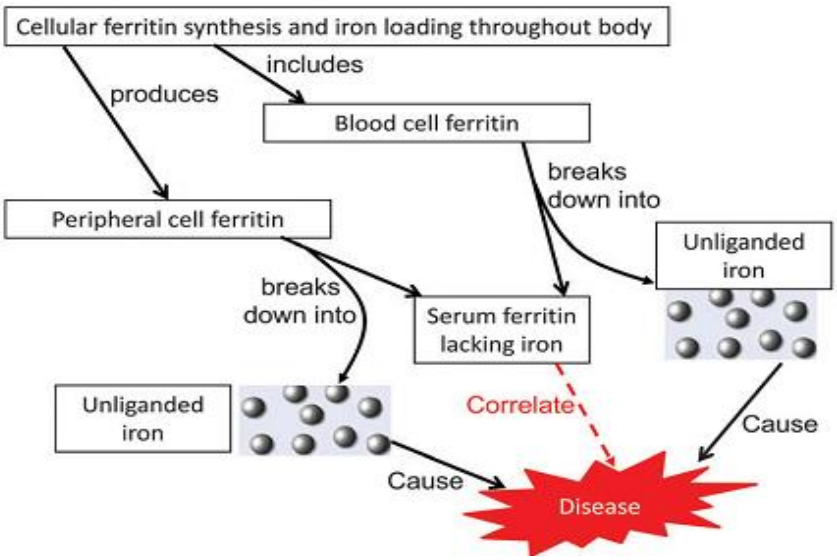

Figure 4. Cellular ferritin and its excretion by damaged cells (20)

In similar view different studies found correlation between SF with serum aspartate amino-transferase and serum alanine amino-transferase, which implies that SF originates from cellular damage consistent with the view that SF is indeed a marker of damaged cells (29, 30, and 31).

1.5.4 Management of chelation therapy

Patients on regular transfusion regimen usually take about 200mg of iron with each RBC unit, therefore the cumulative iron burden is an inevitable consequence (17, 23). This group progressively develops clinical manifestations of iron overload. Since human body has no effective means for removing iron, the use of iron binders (chelators) is

expected to solve iron overload problem through direct binding and passage of iron through urine and stool (18, 32). Iron chelators are usually administrated starting from the age 2 to 4 years of age, once they have had 10-20 transfusions or when ferritin levels rise above 1000ng/ml (17, 18).

To date, there are 3 major classes of iron chelators. Hexadentate (Desferal®) was the first available chelator that is derived from the fungus Streptomyces pilosus. The drug has very short half-life of 8-10 minutes and is administrated by intravenous or subcutaneous parenteral administration for 8 to 12 hours nightly infusion (18, 19). The initial recommended dose of Desferal® (DFO) is 23-40 mg/kg/day for regularly transfused patients and the dose may increase to 50-60mg/kg/day in high iron overload risk patients. Ascorbate administration with a daily dose not exceeding 100-150mg was found to enhance iron removal using Desferal® (19). The major drawback of DFO chelation therapy is low compliance resulting from complications of its way of administration and local inflammatory reactions at the site of infusion (18). In addition, it was found that administration of DFO in the presence of low iron burden cause auditory toxicity, growth retardation and other problems (19). Thus, regular monitoring of DFO toxicity is very important.

The bidentate (Deferiprone®) has binding ability more than DFO. It is a synthetic compound that can be absorbed by the gastrointestinal tract and has a plasma half-life of 1.5-4 hours (17). The usual recommended daily dose of Deferiprone® (DFP) is 75 mg/kg/day and can be increased to

100mg/kg/day depending on patient's ferritin levels (17). Several studies reported that DFP penetrate cell membrane more rapidly than DFO and can remove intracellular iron from heart tissue preventing iron-induced cardiac disease and improving cardiac function (17, 18). Agranulocytosis and neutropenia are the most serious side effects, necessitating weekly follow-up with complete blood count. In addition, gastrointestinal symptoms, arthropathy, zinc deficiency are also common (18, 19).

Tridentate (Deferasirox- Exjade®) was approved in 2005 as an orally ingested drug. The drug can be absorbed by the GI tract and has a half-life of 12-18 hours (17). The recommended dose of Exjade® (DFX) for most patients is 20mg/kg/day, although this can be modified to 10 or 23mg/kg/day depending on the number of transfusions and therapeutic goal (18). Some patients who take this chelator shows unexplained symptoms of increased transaminases, serum creatinine and progressive gastrointestinal symptomatology, thus monthly monitoring of serum ferritin and creatinine level and liver function must be performed (17, 18). Recent studies reported that the combination of DFO and DFX drugs seems to increase levels of iron execration (17, 18).

1.6 Retrospective studies on disease management and complications

Results obtained from previous studies in the region reveals that this disease is still a public health problem that requires strict policies to decrease morbidity and mortality rates as a consequence of the disease.

Poor disease management, lack of awareness about disease symptoms and lack of knowledge of the role of chelating agents was reported in Morocco (33). Hypothyroidism, hypoparathyroidism, diabetes mellitus and delayed puberty were reported as the most common endocrine complications (33, 34, and 35). Poor endocrine follow up was reported in Pakistan (36). Delayed puberty was reported as the most frequent complication of the disease in some studies (33). Heart failure due to iron overload was recorded to be the main cause of death in China, Tunisia and Saudi Arabia (34, 35, and 37). Death due to infections was also reported in Tunisia, Greece and Italy (35). Despite these complications increased life span among these patients was reported (37).

1.7 β- thalassemia major in Palestine

Although Palestine is one of the Mediterranean basin countries, in which beta thalassemia major are prevalent, few studies were carried out on incidence of disease, biochemical and molecular diagnosis (8, 38). The percent of thalassemia trait is 4% with more than 150,000 carriers of this mutation in the Palestinian territory (38, 39). Around 60% of the cases in the West-Bank were residence of the northern districts (40). Increased life span among Palestinian thalassemic patients from 7-8 years in 1996 to 18-19 years in 2013 was reported (39). Reports released by Thalassemia Patients Friend Society (TPFS) in the country in 2013 reported that beta thalassemia major patients in the West Bank area were 436 patients and the number of patients in Gaza strip was 232 (40). Issuing legislation of

compulsory pre-marital test in 2000 was an effective tool in reducing new thalassemia cases from 40 cases/ year before 2004 to less than 10 registered cases annually since 2004 (39). In addition, limited cases with BTM were reported after 2013 (40).

Thalassemic patients, especially with the BTM, require a special care. The ideal management for these patients involves a multidisciplinary therapeutic team approach and usually carried in inclusive thalassemia care centers (11). Adopting such ideal management strategies that include regular follow-up visits are considered as a heavy financial burden on the health services (41).

Being a developing country with a poor economy and due to the prevailing political situation in the area, one should expect lack of ideal management for this group of patients. The only choice under these conditions is the management of the disease through blood transfusion aiming at hemoglobin levels in the normal range, associated with the use iron chelating therapy to preclude early death (2, 17). The current study is the first to address a detailed profile about the health situation of registered thalassemia patients at AL- Wattani hospital in Nablus city. The data is expected to reflect the current practice with respect to management, which might be used for improving strategic treatment protocol for these patients.

1.8 Aims of the study

The current study aimed at evaluating the existing management protocol for β-thalassemia major patients in the area in comparison with internationally used protocols in this regard. In addition, we aim to identify commonly developed complications and their association with serum ferritin levels.

Chapter Two

Materials and Methods

2.1 Study design

The current study is a cross sectional descriptive study that was carried out retrospectively starting from January 2012 till September 2014. The study protocol depends on collecting a naturalistic observation that is reported in patient's medical records after routine clinical procedure, including the hematologists and endocrinologists clinical review as well as the biochemical laboratory checkup.

2.2 Study population

A total of 54 reported β-thalassaemia major patients who are under treatment and care at thalassemia ward at AL-Wattani hospital in Nablus city were enrolled in this study. Thalassemic patients who underwent successful bone marrow transplantation and those reported with β-thalassaemia intermedia or combined sickle-thalassemia were excluded. Data presented in (Table1) provides a description of this group.

Table 1. **General characteristics of study population**

Variable	No. (%)
Gender	Male: 26 (48.1%)
	Female: 28 (51.9%)
Age groups (year)	1-10: 21(38.9%)
	10-20: 17(31.5%)
	20-30: 11(20.4%)
	30-40: 3(5.6%)
	40-50:0
	50-60:0
	60-70:2 (3.7%)
Age at diagnosis and entered transfusion regimen	Fetal stage: 4(7.4%)
	Before the second year: 31(57.4%)
	Within the second year: 10(18.5%)
	Within the third and fourth year: 4(7.4%)
	Between 5-24 years: 5(9.3%)
Distribution/districts	Nablus: 48(88.9%)
	Tubas: 1(1.86%)
	Salfeet: 5(9.3%)
Parents consanguinity	1st degree: 36(66.7%)
	2nd degree: 9(17.7%)
	Non relatives: 9 (17.7%)

2.3 Ethical consideration

An official approval was obtained from the General Directorate of Higher and Continuing Education from the Ministry of Health– Nablus to facilitate reviewing patient's records (Annex 1). Purpose and objectives of the study were explained to the patients, or guardian(s) for all participants (consent form, Appendix 1). Patients or their representatives were asked to answer a set of questions, through direct interview with the researcher, and

then data was recorded in a specially designed questionnaire prepared for the study purpose.

2.4 Data collection

The data for this study was collected via a specially designed questionnaire. All data and regular follow up that performed and recorded in the patient medical files from January 2012 till September 2014 were recorded in their related sections in the questionnaire. All subjects were interviewed by the researcher to complete missing data in patient's files at day of blood transfusion and recorded in each questionnaire. Body mass index (BMI) of the latest record of height and weight was calculated by the researcher using the following formula; BMI= weight (kg)/height^2 (m). BMI of 18.5–24.9 was considered as normal, BMI< 18.5 were considered as underweight and overweight BMI was 25–29 (42).

2.4.1 Questionnaire form

The questionnaire (see appendix 2) included three major sets of questions. These included:

1. Socio-demographic and general characteristics of the study subjects.
2. Medical history and clinical data
3. Regular checkup tests

2.5 Hemoglobin analysis

According to the Palestinian Ministry of Health, all patients were diagnosed using standard gel electrophoresis for the detection of changes in hemoglobin variants associated with β-thalassemia major; mainly HbA2. Reviewing patient's medical files raised some concern and doubt about the correct diagnosis of some of the patients who were reported as β-thalassemia major patients; especially those enrolled in the transfusion regimen at late ages as indicated in Table 1. To confirm diagnosis, blood samples were taken before blood transfusion and analyzed (by a specialized lab) for the presence of HbA, HbA2, and HbF using High Performance Liquid Chromatography (HPLC).

2.6 Statistical analysis

Collected data was tabulated encoded and statistically analyzed using the IBM SPSS Statistics v. 21. Repeated values were presented as the total mean of each year. Means, Medians, frequencies and percentages along with corresponding 95% confidence interval were computed. The statistical analysis evaluated the percentage of patients receiving a regular evaluation and the percentage of those with abnormalities. Differences of medians for continuous measures were tested by the non parametric tests (Friedman's 2-Way Anova by Ranks Test, Wilcoxon Matched- Pair Signed-Rank Test and 2 Independent Sample Mann-Whitney U Test). Testing the correlation between continuous data was carried out using (Spearman's Rank Correlation) and Binomial test for proportion comparison. These statistical

tests were performed and computed at corresponding 95% confidence interval and P-value≤ 0.05 was considered statistically significant. The above tests were carried out with the aim to describe and identify significant relationship, correlations and differences between the study items, variables and parameters. Patients follow up results were considered low or high according to the reference range reported in Palestinian Clinical Laboratory Guide (43).

Chapter Three

Results

3.1 Patient's general characteristics

The current study included all previously reported β-thalassemia major patients (54) who are currently managed and transfused for the clinical symptoms and manifestations of the disease. Out of 54 previously diagnosed thalassemic patients included in this study; 26(48.1%) were males and 28(51.9%) were females (Table 1). Based on morphological and clinical picture; 49(90.74%) patients were β-thalassemia major and 5(9.26%) were either with thalassemia trait or combined with other hemoglobinopathies (see Table 2 and appendix 3). Therefore, diagnosis for such cases needs to be confirmed with genetic tests and globin chain analysis.

Table 2. Hemoglobin variants levels for the re-tested miss-diagnosed Patients

Patient No.	Age (Year)	Hb A (> 94.0%)	Hb A2 (1.3-3.5%)	Hb F (<2%)
1	33	88.2%	4.6%	7.2%
2	34	89.6%	2.4%	8%
3	39	84.4%	2.6%	12.6%
4	64	93.0%	5.1%	1.9%
5	68	94.6%	4.3%	1.1%

The average age of the study group was 17.07 ± 1.8 and age ranged from 1-68 years. Excluding those miss-diagnosed patients, the average age among BTM patients was 13.96 ± 7.75 (median=14 years) and ranged from 1-28 years. No significant difference was found between the age of males and females (P-value= 0. 965). The majority 21/49(42.86%) of BTM patients were ≤10 years old, 17/49(34.7%) were in the age group 10-20 years, and 11/49(22.45%) were in the age group 20-23 years. The five

miss- diagnosed patients were in the age between 32- 68. Three of them were within the age group 23-40 and the rest tow patients were within the age group 60-70 as illustrated in Figure 5.

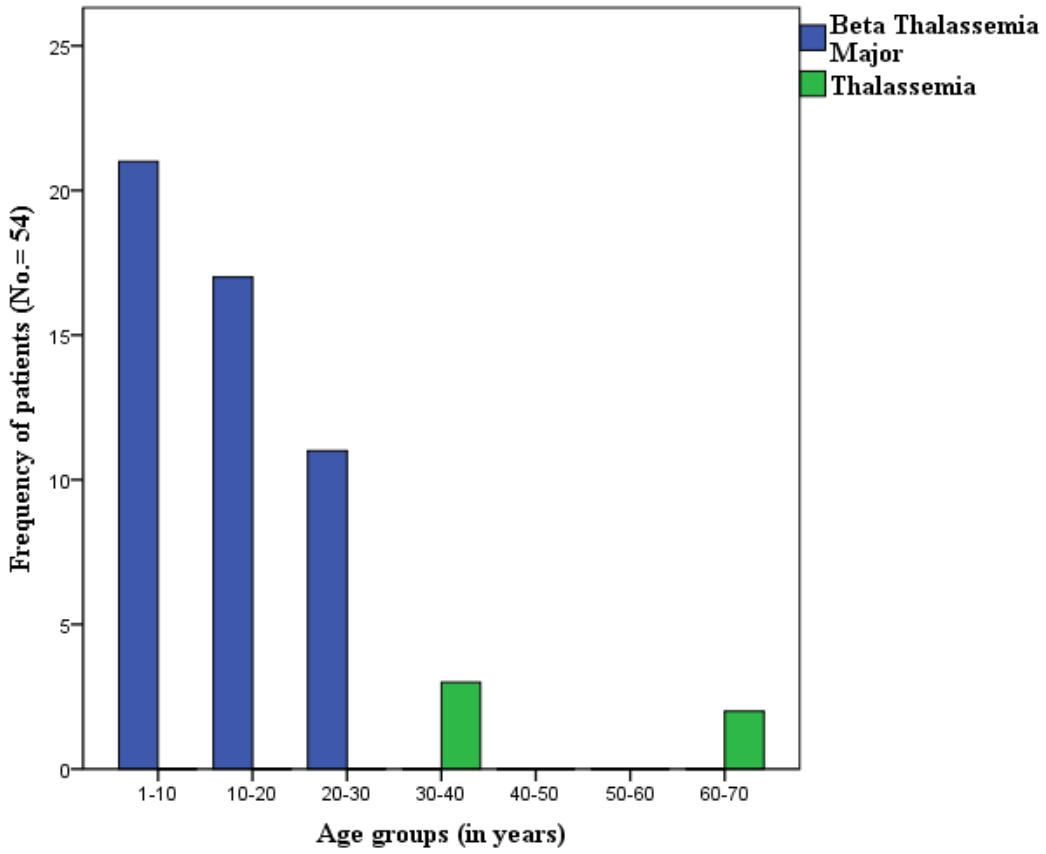

Figure 5. Bar chart representation of age groups for the study group

With respect of consanguinity (Table1); 36(66.7%) of the patients were born to parents with a first relative degree, 9(17.7%) patients were born to parents with a second relative degree and the rest 9(17.7%) patients were born to non-relative parents.

3.2 Patient's clinical picture

Hemoglobin levels of all patients admitted to the thalassemia ward were measured in order to evaluate their need of blood transfusion. Data on hemoglobin levels for the last three years (2012 to 2014) was obtained from patient's files, tabulated and the hemoglobin mean/year for all patients was calculated using SPSS statistical program (Table 3.1). Statistical testing using 2 related sample test reported significant increase in the mean of hemoglobin level in 2014 compared to 2012. The Mean and standard deviation (SD) of pre transfusion hemoglobin level were 7.33± 0.46 g/dL, 7.45± 0.62 g/dL, 7.58± 0.58 g/dL in the preceding three years, respectively. Independent samples test (Mann Whitny U test) showed no significant differences between male and female ferritin levels. The range of pre transfusion hemoglobin level in 2014 was 6.47g/dL to 8.87 g/dL (Table 3.2).

To safeguard the health of the transfusion recipient, blood usually obtained from carefully selected volunteers, processed, stored and distributed to care centers according to international protocols. Based on Hb levels, the assigned physicians decide the duration of blood transfusion. All patients were given packed red blood cells and blood transfusion usually administered with bedside filters. Data presented in (Table 3.3) showed that patients were categorized into three groups with respect to blood transfusion. Most of the patients 38(70.48%) were reported to receive blood at an interval of 4-5 weeks, 11(20.4%) were transfused at an

interval of 2-3 weeks and the remaining 5(9.3%) patients receive blood once every two months.

Reviewing the medical files of the studied patients revealed that 33(61.1%) of them developed transfusion reactions during blood transfusion secessions. In such situation, blood transfusion stopped and immediate evaluation of the clinical state of the patient is conducted. In such cases a dose of intravenous hydrocortisone is given to patients each time before blood transfusion event.

All patients were subjected to annual testing for hepatitis B and C (Table 3.3). Transfusion acquired hepatitis infections were found in 2 females (3.7%); one was infected with HBVand the other with HCV.

Ferritin levels for patients from 2012 to 2014 were obtained (Table 3.1). A significant gradual decline in 2014 compared to 2012 was observed. No significant differences between male and female ferritin levels (Table 3.1). Ferritin levels ranged between 77.95µg/L and 7584µg/L for patients checked in the year 2014 (Table 3.2). Out of 49 cases, 36(73.5%) were with high ferritin levels (over 1000ng/dL), nearly half of the patients 24(49%) reported a moderate ferritin level 1000-2500µg/L, 13(26.5%) with a mild ferritin level <1000µg/L and sever ferritin level of >2500µg/L was found in 12(24.5%) of the patients (Figure 6). Data shown in (Figure 7) represent a diagrammatic representation of SF in relation to age. A significant positive correlation (P- value: 0.002, Spearman correlation coefficient (r_s):0.416) was found; it is clear that SF levels increases with increased age and consequently with transfusion duration.

For the patients within the age group 23-40 years, who were not classified as thalassemia major, SF levels were the highest; indicating over transfusion and inadequate chelation therapy.

Iron chelating therapy showed that the majority of patients 48(88.9%) use iron chelating agents. At present 41(75.9%) of the patients use Exjade® and were using Desferal® at earlier dates, 7(12.9%) reported the use of Desferal® (Table 3.3). The rest of the patients 6(11.1%) were reported to have no chelation therapy; three of these patients had SF within reference range. Another two patients of this group have SF levels <1000ng/ml who stopped Exjade® and receive blood once every two months. The sixth is a 4 years old child who didn't start the chelation therapy and his SF level was 1357.00 ng/ml.

With respect to splenectomy, 29(53.7%) of the patients were subjected to splenectomy at mean age of 13.83±2.474 (Table 2.3).

The use of vitamins and other supplements showed that folic acid was prescribed to all patients. Folic acid in combination with calcium or vitamin alpha D3 was prescribed for 12(22.3%) patients.

Table 3.1 Patient's clinical picture including Hb and SF levels during the study period

Variable	Year	No. of tested	Mean		P-value/ gender*	Total Mean ± SD	P-value/ 2012vs 2014**
			Male	Female			
Hb (g/dL)	2012	51	7.28	7.38	0.221	7.33±0.46	0.001
	2013	53	7.38	7.51	0.378	7.45± 0.62	
	2014	54	7.47	7.67	0.174	7.58±0.58	
SF(ng/ml)	2012	51	2987.6	2464.6	0.547	2721±2204.1	0.001
	2013	54	2317	2369	0.653	2343.7±2053.7	
	2014	49	2106	2035	0.704	2069.6± 1770.6	

*Significance of difference in Hb comparing males and females.

** Significance of difference in SF comparing 2012 with 2014.

Table 3.2 Pre-transfusion Hb and ferritin levels range reported in 2014

Variable	Range	Frequency (%)
Hb (g/dL)	6-7	8(14.8%)
	7-8	34(62.9%)
	8-9	12(22.2%)
SF (ng/ml)	≤1000	13(26.53%)
	1000-2500	24(48.97%)
	>2500	12(24. 49%)

Table 3.3 Patient's clinical picture

Variable		No. of patients (%)
Transfusion interval	2-3weekes	11(20.4%)
	4-5weekes	38(70.48%)
	Every 2 months	5(9.3%)
Acquired Hepatitis infection	Positive HBV	1(1.85%)
	Positive HCV	1(1.85%)
Iron chelators	Exjade	41(75.9%)
	Desferal	7(12.9%)
	Non	6(11.1%)
Splenectomy	Yes	29(53.7%)
	No	25(46.3%)
Supplements	Folic acid	54(100%)
	Others	12(22.3%)

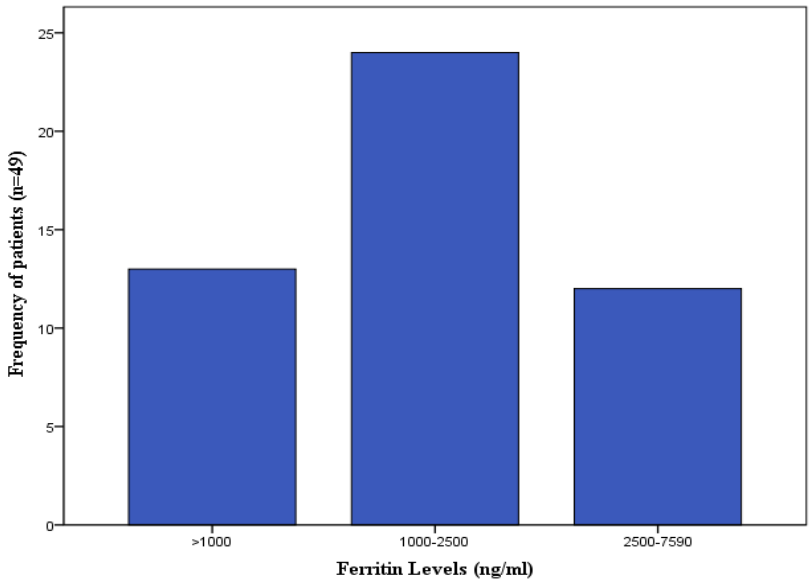

Figure 6. Serum ferritin levels recorded in 2014 for the patient group

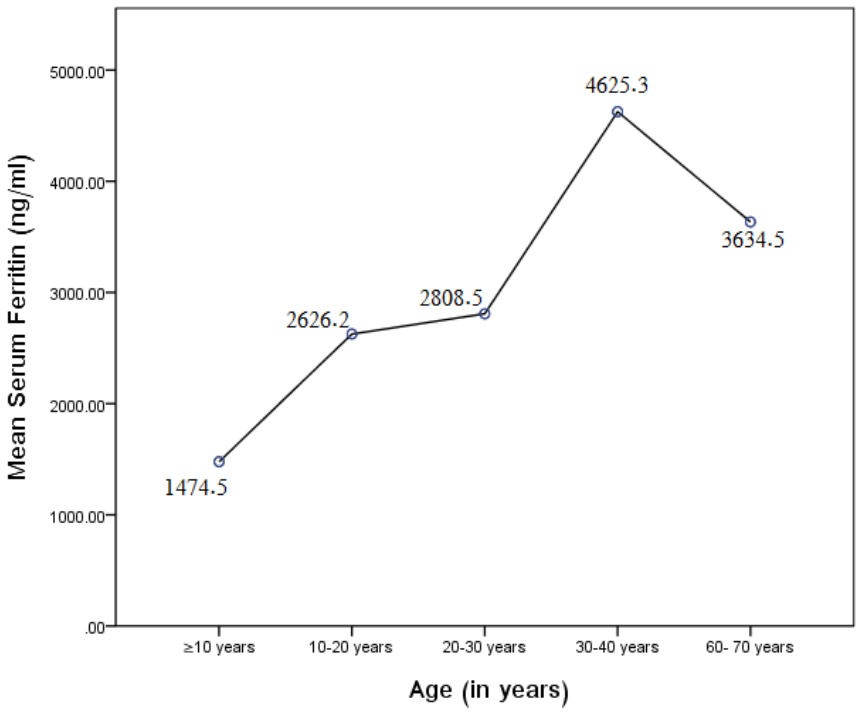

Figure 7. Relationship between age and serum ferritin levels

3.3 Patient's body mass index

Last record measurements of height and weight were extracted for 47/54 (87%) patients. The mean body weight and height for those patients were 33.61±17 kg and 1.37±0.24cm, respectively. BMI mean for female=19.14 and for male=18.15, statistical analysis showed no significant differences in the distribution of BMI across males and females (P-value= 0.141).

Underweight was found in 22(46.8%) patients, overweight was found in only one case (2.31%), while 24/47(51.06%) reported normal BMI (Figure 8). No significant difference in the distribution of SF between normal and under-weight patients; 2508.8 ng/ml±1878.6 vs.1967.4 ng/ml ±1686.1, (P-value= 0.270).

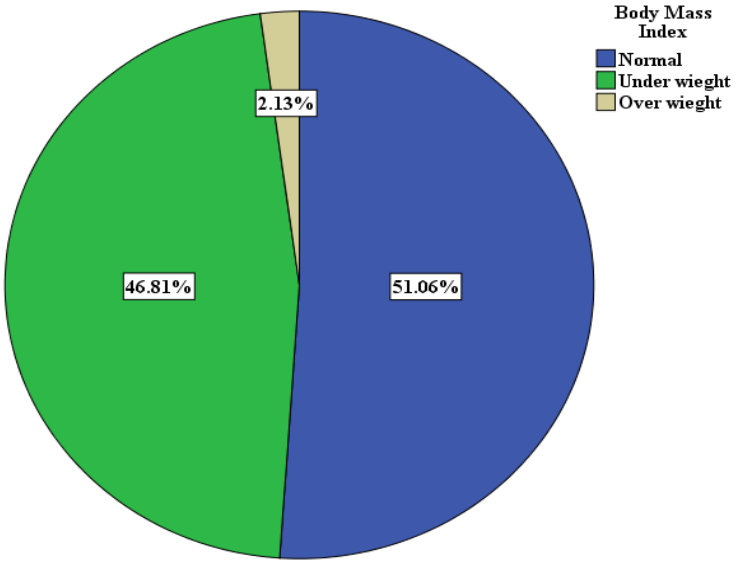

Figure 8. Body mass index of the study group

3.4 Patient's liver enzymes levels

Tested liver enzymes included: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and alkaline phosphatase (ALP). The average mean of ALT levels during the study period 2012-2014 were 51.54U/L, 41.35U/L and 58.23U/L, respectively. Elevated enzyme levels were observed in 50.98% in 2012, 43.39% in the year 2013 and 63.83% in 2014. A comparison between ALT levels in the last year of the study (2014) with first year of the study (2012) revealed no significant differences among those years. However, a noticeable decline in ALT level was reported in 2013 in comparison with the previous year, raised ALT level in comparison to the next year, 2014 in comparison with 2013; these differences were statistically significant ≤ 0.05). This fluctuation indicates a non-stable ALT level among thalassemic patients in the current study (Table 4).

The mean of AST levels among patients in the study period was 56.68U/L, 49.78 ± 22.3U/L and 74.42U/L, respectively. Elevated enzyme levels above the reference range were reported in the three successive years (76.47%, 86.79% and 93.48%, respectively). A comparison between AST levels in 2014 in comparison with the previous two years showed raised enzyme level with increased elapsed time (P-values<0.05).

The mean levels of ALP in the last three years were 341.13 U/L, 237.56 U/L and 370.54 U/L, respectively. Abnormal findings were reported in 32.65%, 33.33%, 67.5% of the tested patients. Statistical analysis using 2 Related samples test showed a significant increase ALP levels enzyme 2014 in comparison to 2012 (Table 4), also performing the test in comparison with 2013 showed a significant increase (P-value≤ 0.05), while a detected significant decrease in the mean of ALP enzyme in 2013 compared to the previous year (P-value ≤0.05). Statistical results seem to reflect a non stable ALP levels among the studied patients.

Table 4. Liver enzymes levels during the study period

Variable	Year	No. of tested patient	Mean± SD	No of elevated –finding (%)	P-value/ 2012vs2014 **
ALT (U/L)	2012	51	51.54± 29.3	26(51%)	0.391
	2013	53	41.35± 17.5	23(43.4%)	
	2014	47	58.23± 33.3	30(63.8%)	
AST (U/L)	2012	51	56.68 ± 36.4	39(76.5%)	0.001
	2013	53	49.78 ± 22.3	46(86.8%)	
	2014	46	74.42 ± 59.8	43(93.5%)	
ALP (U/L)	2012	49	341.1±124.4	16(32.7%)	0.002
	2013	51	307.6±114	17(33.3%)	
	2014	37	370.5±142.3	25(67.5%)	

**Significance of difference in enzyme level comparing 2012 with 2014.

Spearman rank-order correlation was performed to find out if there is any correlation between SF and liver enzyme levels. The correlation was carried out between the mean of SF levels reported among the three years and the mean of liver enzyme levels reported in the same period (Table 5). A significant, strong positive correlation was observed between SF with ALT level; (P-value= 0.00, r_s=0.542) as showed in (Table 5, Figure 9), also with AST level (P-value= 0.00, r_s= 0.518) as showed in (Table 5, Figure 10). In contrast insignificant, weak negative correlations were observed between SF and ALP (P-value= 0.993, r_s= -0.001) as showed in (Table 5, Figure11).

Table 5. Correlation between SF and liver enzyme levels during the study period

Variable	Spearman's correlation coefficient(r_S)	P- value
Mean ALT (U/L)	0.542	0.000
Mean AST (U/L)	0.518	0.000
Mean ALP (U/L)	-0.001	0.993

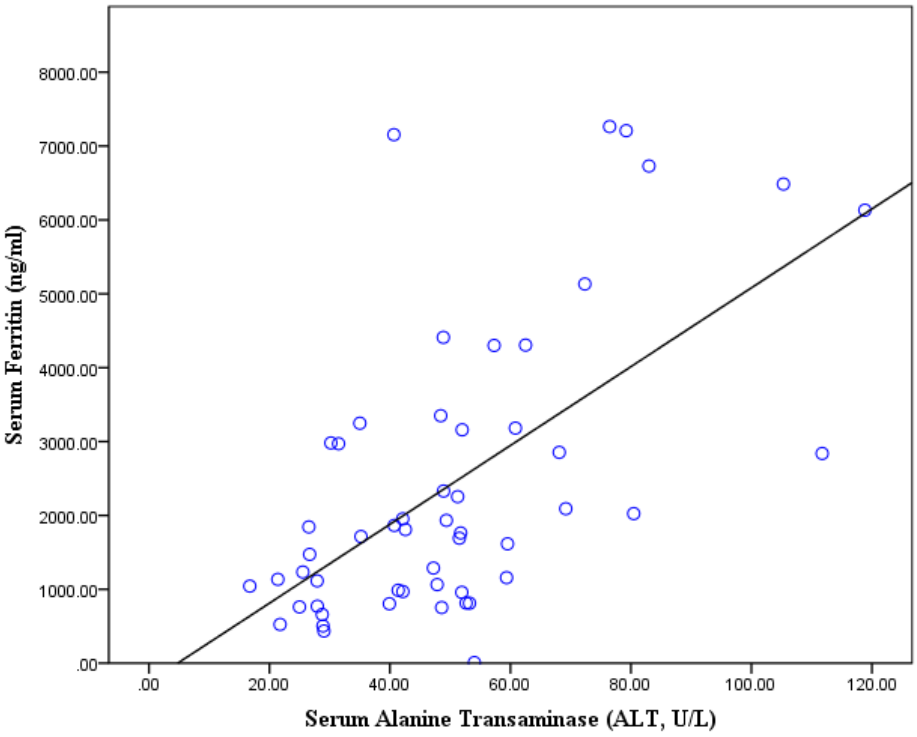

Figure 9. A scatter plot demonstrating a significant a strong positive correlation between SF and ALT levels

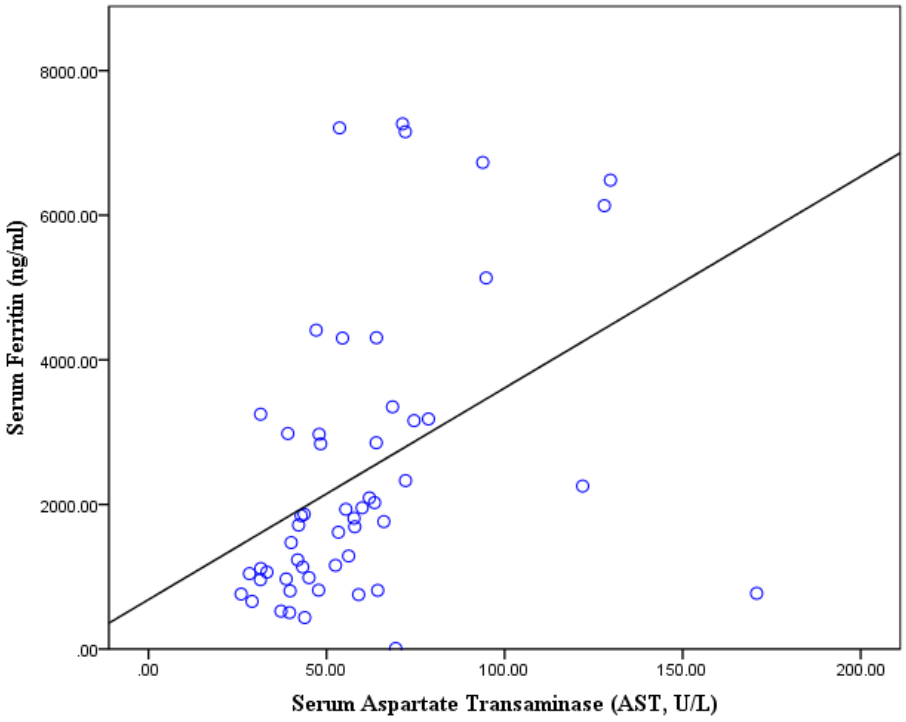

Figure 10. A scatter plot demonstrating a significant strong positive correlation between SF and AST levels

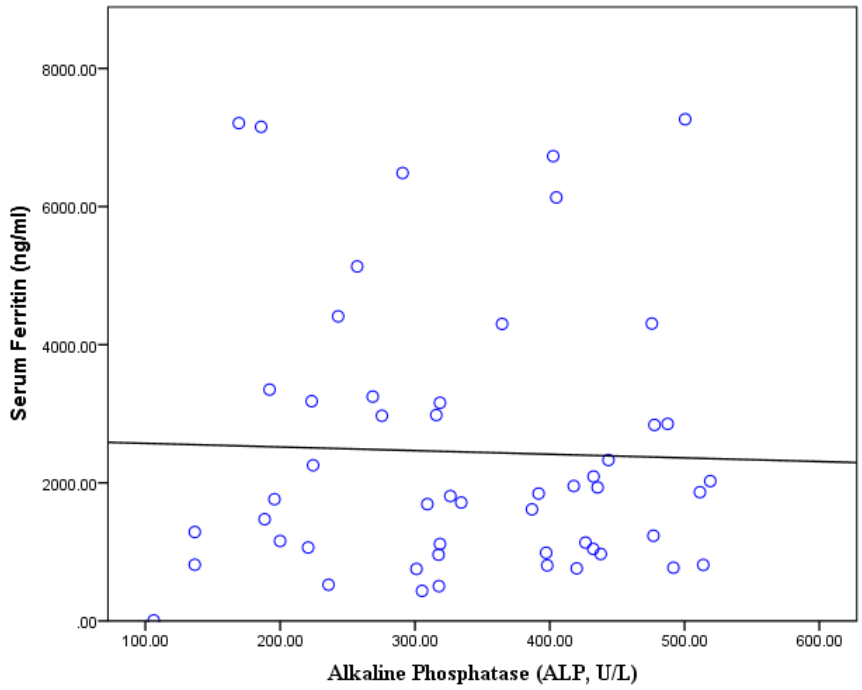

Figure 11. A scatter plot demonstrating insignificant weak negative correlation between SF and ALP levels

3.5 Patient's calcium levels

Data presented in Table 6 clearly indicates a significant decrease in calcium level in 2013 compared to 2012 (P-value≤ 0.05). The means and SD of calcium levels were 10.58±1.3mg/dL and 9.68±0.97mg/dL in 2012 and 2013, respectively. For unknown reasons, the majority of the patients were not subjected to analysis of calcium levels in 2014. In addition, few cases were reported to be examined for phosphorus levels during the study period.

Table 6. **Calcium levels among patients during the study period**

Variable	Year	No. of tested	mean± SD	No. of abnormal – finding (%)			P-value/ 2012vs 2013**
				Below	Above	Total (%)	
Calcium (mg/dL)	2012	46	10.45 ± 1.1	2(4.3%)	27(58.7%)	63%	0.000
	2013	46	9.68± 0.97	5(10.9%)	8(17.3%)	28.3%	

**Significance of difference in calcium levels comparing 2012 with 2014.

3.6 Patient's kidney function

Both creatinine and urea usually tested for evaluation of kidney function. Reviewing medical files showed that the means of creatinine levels during the study period 2012-2014 were 0.27±0.23mg/dL, 0.32±0.25mg/dL and 0.34±0.2mg/dL, respectively. Abnormal findings were observed in 86.3%, 79.2% and 59.2% of the checked patients. Comparing creatinine levels in 2014 with 2012 (Table 7) and with 2013 showed significant increase (P-values≤ 0.05).

With respect to urea levels, the results showed that the mean levels of urea were 23.16±8.4mg/dL, 33.81±10.3mg/dL and 23.82±13mg/dL, respectively. Higher abnormal levels were observed in 20%, 43.4% and 32.7% among patients during the study period, respectively. Differences in urea levels during 2013 (P-value≤ 0.05) and 2014 (Table 7) were statistically significant compared to that observed in the year 2012.

Table 7. Creatinine and urea levels among patients during the study period

Variable	Year	No. of tested patient	Mean± SD	No. of abnormal –finding (%)			P-value/ 2012vs14 **
				Below	Above	Total	
Creatinine (mg/dL)	2012	51	0.27± 0.23	43 (84.3%)	1(2%)	44 (86.3%)	0.024
	2013	53	0.32±0.25	41(77.4%)	1 (2%)	42 (79 %)	
	2014	49	0.34±0.2	29(59.2%)	0	29 (59.2)	
Urea (mg/dL)	2012	50	23.2± 8.4	4(8%)	6 (12%)	10 (20%)	0.000
	2013	53	33.8±10.3	0	23(43.4%)	23(43.4%)	
	2014	49	30.8±13	1(2%)	15 (30.6%)	16 (32.7%)	

**Significance of difference in creatinine and urea levels comparing 2012 with 2014.

3.7 Patient's hormonal levels

Data presented in Table 8 shows hormonal tests that were conducted during the study period. Primary analysis of the data revealed that not all patients were tested for these hormones, especially during 2012 and 2013. In 2014, more hormonal tests were added, including total testosterone, parathyroid stimulating hormone (PTH) and cortisol. However, other important recommended tests were not performed like growth hormone test.

Levels of LH were checked for 19 males and 23 females, pre-pubertal basal LH was found in 73.7% and 73.9%, respectively. FSH test was checked for 20 males and 25 females, pre-pubertal basal FSH was found in 40% and 32%, respectively.

The most prevalent endocrine dysfunction was male hypogonadism (88.9%). Eight out of 9 tested males were reported with hypogonadism and their age ranged between 10-26 years. All male patients with hypogonadism showed low testosterone levels; 3 of them were with low FSH and LH levels, 3 were with low LH and normal FSH indicating the clinical condition of central hypogonadism. The rest 2 patients were reported with normal LH and FSH indicating the clinical condition of primary hypogonadism.

With respect of testosterone levels in female patients; 21 females were checked, 9 of them reported with elevated testosterone levels as showed in (Table 8). Low estradiol levels were used as an indication for female hypogonadism. Low E2 level< 18pg/mL was reported in 6 out of

21 tested females (28.6%). The age of these females ranged between 14 and 26 years including; 2 females with low levels of both LH and FSH, 3 females were reported with low LH but normal FSH indicating central hypogonadism and the last female reported both normal LH and FSH indicating primary gonadal failure with high testosterone level.

Tests for thyroid stimulating hormone (TSH), total thyroxin hormone (T4) were performed in 16 and 14 patients in 2012, respectively. All tested patients in 2012 were with normal TSH and T4 levels.

In 2013, 50 and 48 patients were tested for the two hormones, respectively. Disrupted hormonal levels were found in 2(4%); they showed elevated TSH but normal T4 level with age 22 and 33 years, indicating mild thyroid dysfunction called subclinical primary hypothyroidism. Three (6.25%) patients showed low T4 level with age rang 6-13 years, including normal TSH indicating a clinical condition called secondary hypothyroidism.

In 2014, TSH test was carried out to 49 patients and T4 test was carried out to 29 patients. Disrupted hormonal levels were found in 1(2.04%), who showed high TSH with age 33 years (T4 was not tested). Three (10.3%) showed low T4 and normal TSH with age between 7-20 years old, indicating secondary hypothyroidism. Parathyroid hormone (PTH) test was performed for only four patients in 2014 and showed normal results.

Testing for cortisol hormone was performed only in 2014 for 36 patients, 2 patients were reported with high hormonal levels (20 years old female and 68 years old male).

Table 8. Endocrine levels among patients during the study period

Variable		Year	No. of tested-patients	Mean± SD	No. of abnormal –finding (%)		
					Below	Above	Total
LH (mIU/mL)	Male	2014	19	2.6±6.05	13 (68.4%)	1 (5.3%)	14 (73.7%)
	Female	2014	23	1.62±1.89	17 (73.9%)	0	17 (73.9%)
FSH (mIU/mL)	Male	2014	20	1.63±2.9	8 (40%)	0	8 (40%)
	Female	2014	25	2.49±2.1	7 (28%)	1 (4%)	8 (32%)
T. Testosterone (ng/mL)	Male	2014	9	0.59±0.27	8 (88.9%)	1 (11.1%)	9 (100%)
	Female	2014	13	0.85±1.2	0	9 (69.2%)	9 (69.2%)
E2 (pg/mL)	Male	2014	19	18.2±18	0	1 (5.3%)	1 (5.3%)
	Female	2014	21	30.4±41.8	6 (28.6%)	0	6 (28.6%)
TSH(µIU/mL)		2012	16	2.87±1.20	0	0	0
		2013	50	2.91±1.28	0	2 (4%)	2 (4%)
		2014	49	2.76±1.2	0	1 (2.04%)	1 (2.04%)
T4 total (µg/dL)		2012	14	7.50±1.06	0	0	0
		2013	48	7.39±1.4	3 (6.25%)	0	3 (6.25%)
		2014	29	7.03±1.47	3 (10.3%)	0	3 (10.3%)
PTH (pg/mL)		2014	4	36.75±21.6	0	0	0
Cortisol (ng/mL)		2014	36	14.78±20.9	0	2 (5.5%)	2 (5.5%)

Random glucose test (reference range: 65-165mg/dL) was used as an indication of normal blood glucose level. In 2012 and 2013, all patients showed normal glucose levels. In 2014, 2/51(3.9%) patients showed high glucose levels above 165mg/dL. One of them (1.96 %) is 26 years old male who showed an elevated glucose levels in two from total four occasions however, Fast blood sugar test (FBS) was not done to confirm diagnosis of diabetes mellitus. The second is 7 years old female, a known case of diabetes since early age. Glucose levels were significantly higher in 2014 compared to 2012 (P-value$\leq$ 0.05). As reported in Table 12; the

total mean of glucose level in 2014 was 109.7±27.5mg/dL and in 2013 was110. 51±26.7mg/dL versus 96.8±17.5 mg/dL in 2012.

Table 9. **Patient's random glucose levels during the study period**

Variable	Year	No. of tested patients	Mean± SD	No. of elevated–finding (%)	P-value/ 2012vs14*
Glucose (mg/dL)	2012	50	96.8±17.5	0	0.00
	2013	53	107.5±15.1	0	
	2014	51	109.7±27.5	2(3.9%)	

**Significance of difference in glucose level comparing 2012 with 2014

The highest prevalence of endocrinopathy was hypogonadism (46.7%), the second was hypothyroidism (13.3%), the third was hypercortisolism in 5.5% (Table 10). No significant differences were found between patients with or without endocrinopathy according to age (Table11)

Table 10. Prevalence of endocrinopathies among the included patients

Endocrinopathy	**Year**	**No. (M/F)**		**Prevalence**	**Age range (year)**
		Tested	**Affected**		
Hypogonadism	2014	30(9/21)	14(8/6)	46.7% (88.9%, 28.6%)	10-26
Hypothyroidism	2012	14	0	0	-
	2013	48(24/24)	5(2/3)	10.4 (8.3%, 12.5%)	6-33
	2014	30(16/14)	4(2/2)	13.3% (12.5%, 14.3%)	7-33
Hypoparathyroidism	2014	4	0	0	-
Hypercortisolism	2014	36(16/20)	2(1/1)	5.5(6.25%, 5%)	20-68
Diabetes mellitus	2012	50(25/25)	0	0%	-
	2013	53(26/27)	0	0%	-
	2014	51(25/26)	0	0%	-

Table 11. Comparison between patients without vs. with endocrinopathy

variable	Without-endocrinopathy	With-endocrinopathy	P- value***
No. (%)	4 (7.84%)	47 (92.15%)	0.00
Age in year (mean ±SD)	17.8 ± 14.6	17.5 ± 13.1	0.99
Age range	4-34	4-68	-
SF ng/ml (mean± SD)	2763.3 ± 2615.8	2007.9 ± 1704.7	0.765

***Significance difference in SF level comparing patients without vs. with endocrinopathy

3.8 Monitoring of cardiac function

Reviewing medical files revealed that not all patients were subjected to annual Echocardiogram (echo) and Electrocardiogram (ECG) over the study period. In 2012, only 5/53 (9.4%) patients were evaluated; 24/54 (44.4%) were evaluated in the same test in 2013 and only 6 (11.1%) in 2014. It is obvious that these patients received poor annual monitoring of cardiac function. Only 5(9.3%) were on cardiac medication; one female (1.9%) of 34 years old and four males (7.4%) with an age range between 24-68 years old. Two of these patients were known cases of heart failure condition.

Chapter Four

Discussion

This is the first study to date concerning BTM management used protocol and defines the prevalence of disease complications among Palestinian patients. The study was conducted in one of the main demographic focal region for thalassemia in the northern parts of the West Bank region of Palestine. This study provides a reliable data on the current status of survival for these patients, including the prevalence of the developed complications that could be useful for management and treatment protocol policy in the country and can be used for other comparative studies.

Dramatic improvements in the clinical management of BTM over the last two decades have led to an impressive increase in the lifespan and quality of life of patients who can access, afford and comply with treatment regimens (44). Currently, there are many recommended guidelines and standard protocols for care and treatment for these patients (17, 19, 45, and 46).

4.1 Patient's general characteristics

The age range of the study patients in the current study was 1-68 years. The average age was 17.07±1.8. Considering miss-diagnosed thalassemic patients (Table 2) average age was 13.96 ± 7.75 (median 14 years old), ranged from 1-28 years. Similar mean age was reported in Tunisia with average of 10.7 years (35), in Pakistan it was12±8 years (36), and in Saudi Arabia the median age was 12.5 years, ranged from 1-32 years (37) and in Egypt the average age was 10.2 ± 6.6, ranged from 10 months-

31 years (47). On the other hand, improved life expectancy was reported in Turkey with an average age of 18.66 ± 6.48 (48). In France a median age of 19.3 years, with age range 6 months-52 years was reported (49). In North America the median age of 20 years, ranging from1-51 years old (50). The increase in life expectancy among patients in these countries was reported as a reflection of good management (49, 50).

Worldwide consanguineous marriage is prevalent in many parts of the world, especially in the Mediterranean and Islamic countries due to educational level, local traditions, socioeconomic status and demography (51, 52). In Palestine a high degree of consanguinity is still persistent; the rates of total consanguinity reach 45% and could reach in some urban areas up to 60% of the marriages (53). To limit the role of consanguinity as a risk factor for development of thalassemia, the country adopted a prevention program of obligatory premarital testing in 2000 for beta-thalassaemia (54).

In the current study, 45 (84.4%) of the thalassemic patient were born to parents with first and second cousin marriages. Similar findings were reported by previous studies in the area (8, 33, 55 and 56). Such findings are in agreement with the role of Consanguinity; as it is known to play an important role in hereditary diseases, particularly in the inheritance autosomal recessive traits as in thalassemias (56).

4.2 Patient's clinical picture

4.2.1 Hemoglobin level

In the year 2014, the mean and SD of pre-transfusion hemoglobin level in our cohort was 7.58g/dL±0. 58.9 with a range of pre-transfusion hemoglobin level between 6.47g/dL-8.87g/dL (Table 3.1). This level is less than the lower Hb level (9g/dL) recommended by Thalassemia International Federation (TIF) and also lower than median baseline of 10.0g/dL reported by previous studies (57, 58). Our findings in this respect, reflects a severe anemic presentation of the study group. Similar findings in the region were reported by Ragab *et al.*, among Egyptian thalassemia patients with an average of 5.7 ± 1.16 g/ld. and among Iraqi patients with an average of 8.7 ±1. 4 (47, 59). Similar low hemoglobin levels among thalassemia patients were also reported in Jordan and Saudi Arabia (60, 61).

Table 3.2 shows pre-transfusional Hb levels. From the total tested 54 patients, 14.8% of them had Hb levels less than 7g/dL, the majority (62.9%) had Hb levels between 7g/dL-8g/dL and only 22% of the patients maintained Hb levels between 8-9 g/dL. The finding of low hemoglobin concentration level among the study patients reflects improper blood transfusion policy which might be in part due to; insufficient administration of transfused blood for some clinical hazards and/or low patients adherence to transfusion regimen, in addition to limited donated blood for some rare blood groups. We also found that all patients receive the same packed RBCs dose (1 unit= 450 ml) regardless of their body mass index, a practice

that does not agree with internationally recommended standard and guidelines were a volume of 8-15ml RBCs/Kg of body weight is recommended (17,46). Such practice is expected to complicate the anemic state of the patients and might result in further complications as a consequence of such practice. Higher pre-transfusion Hb levels were maintained among thalassemic patients in developed countries as a result of good transfusion policy (50).

In general, allergic (urticarial) reactions were reported to complicate the transfusion process in approximately 1%-3% of all blood transfusions. In the current study, 33/54(61.1%) patients developed allergic reactions during blood transfusion process. In Egypt, allergic reactions were reported to occur in 72% of blood transfused thalassemic patients (47). In North America, allergic transfusion reactions were reported in 17% of transfused patients and only 7.7% showed severe allergic symptoms (62). Such differences in the incidence rate in developed countries were attributed to the fact that these countries follow specifications and standards for leuko-reduction that reduce the incidence of acute non hemolytic febrile reactions (63, 64). In addition to the use of pre-stored filtered RBCs that showed easier quality control rather than blood filtration at the bedside. The significant use of pre-stored filtered washed leuko-depleted blood component is to prevent accumulation of cytokines, which are synthesized by WBCs during storage and abrogates the deleterious effect of degenerating white cells on the stored red cells (64). The use of packed un-washed RBCs with bedside filters, in limited cases in which

these filters is not always available, is most likely to be the reason behind the finding of high incidence rate of allergic reaction in our study group. It is also important to note that different filters types were used and variations in the effectiveness of such filter, ranging from low performance to high performance filters, were reported (64). It was also found that most of the allergic reactions were seen among those patients who had splenectomy. Being a major lymph node, removal of the spleen might be in part involved in such allergic reactions (65).

Transfusion-transmitted infection is difficult to avoid completely, because of impaired immune system in patients as a result of repeated blood transfusion or iron overload (34). In the current study, two female (3.7%) acquired hepatitis infection as a result of transfused contaminated blood. Internationally, transfusion transmitted infections as complications of repeated blood transfusion were also widely reported around the world by previous studies (34, 35, 37, 50, and 66).

4.2.2 Serum ferritin level

The average of SF level for patients in the current study was 2069.6 ng/ml±1770.7 (Tables 3.1) and the majority 36/49(73.5%) had SF ≥1000ng/ml. This is much higher than the cutoff point recommended for blood transfusion dependent patients ≤ 1000ng/ml (67). Such increase in SF level is well known to put these patients at a great risk of developing cardiac injury (58, 68). Iron overload complications, reflected by high SF levels ≥ 1500 ng/ml, are not restricted to heart complications as they are known for their effect on other organ functions (12-14 and 17). According

to Thalassemia International Federation TIF guidelines, SF cut off limit must be kept below 1000ng/ml (67). Finding of high SF level among thalassemic patients indicate either irregular or inadequate chelation therapeutic practices (47). This could be the reason behind the observed high SF levels among our study patients. On the other hand, variable response, availability and low compliance to chelators might play a significant role in this regard (47, 69).

4.2.3 Chelation therapy

Iron chelation treatment has changed dramatically over the last few years with the introduction of Exjade® in 2005. Desferal® was and still one of the most frequently used chelator however, due to the associated pain during administration and dose related toxicity, the drug has low compliance rate (8, 47). In the recent years, Desferal® was progressively replaced by the oral chelator Exjade® which improves the compliance to chelation (70). At present nearly 80% of our patients are on Exjade®. It is worth noting that the drug availability is a problem due to its high cost and usually donated as free gift from other countries like United Arabs Emirates (71). Recently, experimental studies proved that a combination of DFX with DFO chelation results in additive iron excretion, especially with high over load patients, without any significant complication. This approach can be useful especially in poor countries with limited resources (17, 49, and 72). Comparing serum ferritin levels over the last three years revealed a gradual significant decline (P= 0.001). Replacement of the parenteral administrated drug DFO with the more effective chelator DFX

(70), for most of the patients could be the reason for this decline. In this context, previous finding reported by TPFS reported mean SF of 5000ng/dL in 2009 in thalassemic Palestinian patients in northern West–bank using DFO when DFX was not yet introduced in the chelation therapy (73). This assumption might also explain the reason behind the finding of much higher SF levels (3231.0±1560.5µg/L) among Palestinian thalassemic patients in Gaza strip where Desferal still being used as the main chelator drug (8).

4.2.4 Splenectomy

Spleen makes an important contribution to the cellular and humoral immune response, in addition to its filter, catabolic and reservoir functions. As a filter organ, it strains defective red blood cells out of the circulating blood. Its main function is to removes cellular inclusions without destruction of erythrocytes. The operation performed most often on the spleen for hematological disorders is splenectomy (74).

In the current study, splenomegaly was reported to be a major complication among thalassemic patients. Splenectomy was done for more than half of the patient 29/54 with prevalence of 53.7%. In France and North America splenectomy was carried out to 54% and 55%, respectively (49, 50). Study in Iran reported that splenectomy was performed to 44% of patients (75). Previous Chinese study reported that 37% of them underwent the surgery (34). Another retrospective study in Saudi Arabia recorded that 25% cases had undergone splenectomy (37). Our finding in this respect is in agreement with most studies around the world indicating

that splenomegaly is a major complication among thalassemic patients and is carried out aiming at reducing transfusion requirements (49).

Splenectomy adds an additional risk factor for infections among those who had the surgery. A previous study in Saudi Arabia, reported that 10% of patients subjected to splenectomy died from infections (37). Preoperative pneumococcal vaccine is considered as a powerful prophylactic tool against overwhelming post splenectomy infection (37). In the current study preoperative, vaccination practice was reported among most patients who underwent splenectomy. In countries with limited health resources, partial rather than total splenectomy could offer an alternative measure to avoid this fatal complication (76).

4.2.5 Folic acid, calcium and vitamin D

Folic acid is a coenzyme for many important biochemical reactions including synthesis of purines, pyrimidines and nucleoproteins (77). Folic acid deficiency has been reported in both thalassemias major and minor as a consequence of increased folate use caused by increased erythropoiesis. Daily folate supplementation is advised for patients with hemoglobinpathy (77, 78). In addition, recent studies found that folic acid plays important role in preventing progression of arteriosclerosis (77). Folic acid was prescribed to all patients (Table 3.3) however, low compliance was reported. In addition to folic acid, calcium and alpha D3 were also prescribed to12 patients with percent of 22.2% as shown in Table 3.3. Vitamin alpha D3 is considered as the main support of hypoparathyroidism

(HPT) treatment, since vitamin D (VD) is critical for calcium homeostasis and for skeletal mineralization (79). VD deficiency and insufficiency is reported to be high among thalassemic patients in many countries, despite the presence of good sunshine and routine prescription (80). The status of VD among our thalassemic patients is poorly characterized as no regular evaluation for the level of this vitamin was conducted.

4.3 Body mass index

BMI is one of the most preferred methods to asses underweight and obesity (81). Regarding body weight and BMI in our study population; underweight was reported in 22 (46.8%) patients and overweight in one (2.13%) patient as depicted in Figure 8. Previous studies also reported low BMI in thalassemic patients; Fahim *et al*., reported low BMI in 43 % (42). Hashemi *et al*., reported underweight in 45.71% compared to control and low BMI in 18.6% of their patients with BTM, also they found that mean SF level was significantly higher in the patients with low weight and BMI compared to those with normal final weight and BMI (82). In our patients group no clear association between BMI and SF was observed (P-value=0.270).

Low BMI in beta thalassemia patients, especially who are older than 10 years old, has many etiologies. Multiple endocrinopathies especially hypogonadism seems to be the most important etiological factors. The findings regarding BMI among our study group is in agreement with such assumption as shown in Table 9. As mentioned before, multiple endocrinopathies are usually the result of overload consequences and

possibly side effects of chelating therapy (81, 83). Thus, more attention is required for BMI in evaluating and management of health status of these patients in this age, through regular monitoring of growth (81).

4.4 Patient's liver enzyme levels

Abnormal liver function among thalassemic patients appears to be related to the high ferritin levels and to transfusion frequency. Iron-induced liver disease is often aggravated by viral infections. Hepatic hemosiderosis, portal fibrosis and cirrhosis may develop despite iron chelation therapy (70).

Alanine aminotransferase (ALT) is an enzyme, produced mainly in the liver which belong to the transaminases family where it catalysis the transfer of amino group between L-alanine and glutamate. Elevated levels of this enzyme were reported in association with hepatic injury, even before the appearance of hepatic diseases (57, 60, and 84-86). Elevated levels of ALT and bilirubin are used largely as markers for liver damaged and impaired liver functions (59). Previous studies reported high elevated levels of ALT among thalassemic patients in association with multiple blood transfusions (18, 59, and 84). The findings of our study were in agreement in this respect as about 64% of tested study patients in 2014 were with elevated ALT (Table 4).

Aspartate amino transaminase (AST) is another enzyme of the transaminase family that is typically expressed in the liver and sometimes in heart (84). Records on AST levels among our study group in 2014

showed that around 93.5 % of the tested patients were with elevated AST levels (Table 4). Our findings of elevated levels of liver enzymes mainly ALT and AST are consistent with most reports reflecting the role of iron overload on impaired liver functions (60, 75, and 87)

Alkaline phosphatase (ALP) is an enzyme that expressed in many tissues and organs including liver, bone, intestine, placenta, kidney and leukocytes (88, 89). Elevation of serum ALP levels were reported in many bone diseases where it serves as a marker for normal bone tissue development (88, 89). The finding of high levels of alkaline phosphatase of 33% in 2012 and 68% in 2014 (Table 4) in our patients and its association with osteomalacia these could be in part behind the observed morphological characteristics of bone deformities among patients (90). Unfortunately, data on regular bone density is lacking and few patients were reported to be tested for bone density. The exact mechanism of ALP is not clear and further investigations are needed to elucidate its role (84, 91, and 92).

Iron overload, indicated by high SF levels, is known as a cause of liver dysfunction which is reflected by elevated ALT and AST levels. A statistically significant positive correlation between SF levels and ALT, and AST levels (Table 5, Figures 10 and 11). Our findings regarding SF levels and liver enzymes were in agreement with previous reports from Iraq and Iran (59, 93).

Comparing liver enzyme levels among patients during the study period showed a non stable fluctuated pattern of these enzymes. However,

the observed high increased levels among tested patients in 2014 compared to previous years (Table 4) might be attributed to the toxic effect of iron overload, that causes liver damage as mentioned previously, and or the use of Exjade® toxicity as unexplained symptoms of increased levels of transaminase were reported in association with the use of this chelator (17, 18). Finally, one might not ignore the fact that the lack of quality control in our laboratory tests and used diagnostic kits may contribute partially to miss leading results.

The findings on SF and liver enzymes, mainly, ALT and AST indicates the need for re-evaluation of the used policy for blood transfusion and use of chelators and their doses (93). It is also important to point out that one must not rely only on SF levels as an indicator for iron overloaded and in this situation measurement of liver iron concentration by MRI once a year allows real quantification of iron accumulation and reflects the effectiveness of the used chelation therapy (68, 90, and 94).

4.5 Patient's Calcium levels

In humans, calcium plays a major role in skeletal mineralization, as well as a wide range of biologic functions (95). Calcium, inorganic phosphorous and alkaline phosphatases are considered as essential bio materials of human bones and teeth development (96). Disturbance of calcium homeostasis is also known as a complication among thalassaemic patients due to hypoparathyroidism, vitamin D deficiency, bone marrow expansion or chronic liver involvement (97). Few of the current study patients showed decreased calcium levels below the reference normal

range, a clinical condition called hypocalcaemia. Among these patients 2 (4.3%) were reported in 2012 and five patients (10.86%) were reported in 2013. No data was found for calcium among thalassemic patients in 2014 (Table 6).

Hypocalcaemia and hyper-phosphatemia seems to be related to hypoparathyroidism, a well-known clinical condition associated with thalassemia major as a consequence of iron deposition in the parathyroid gland (90, 98) however PTH was not performed in 2012 and 2013 to our patients, so it is difficult for us to ensure this association.

On the other hand, results revealed that 58.7% in 2012 and 17.3% of the patients in 2013 showed elevated serum calcium level, a clinical condition called Hypercalcemia (Table 6). According to Sanctis *et al.*, hypercalcemia is considered mild if the serum calcium level is between 10.5 and 12 mg /dL and only patients with serum calcium level above 14 mg/dL need treatment (99). In this regard, cases of high calcium levels in the study group are considered as mild hypercalcemia and none of them exceed 14mg/dL. This unexpected increased in calcium level was also reported by some previous studies (100), who were unable to find any satisfactory explanation. In another study by Sanctis *et al.*, they reported that elevated calcium level among thalassemic patients could be due to excessive intake of VD or active analogues that lead to increased calcium absorption from the gut and mobilization of calcium from the bone (99).

Based on previous observations on calcium levels it seems to be essential to monitor of calcium levels and the description of vitamins

recommended for our patients. This also emphasizes the importance of adopting a strategic policy for treatment and health care for these patients.

4.6 Patient's kidney function

Transfusion-dependent thalassemia patients are prone to develop renal dysfunction due to iron overload, chronic anemia and/or chelation therapy (101). Determination of biochemical indices of renal function might help prevention of serious kidney damage before any clinical symptom observed (101, 102). A study conducted by Jalali *et al.*, showed that kidney dysfunction in thalassemic patients is associated with increasing blood transfusion frequency and hypercalciuria (103). This might explain our findings were hypercalcemia was observed in 58.7% and 17.3% of the tested patients in 2012 and 2013, respectively as showed in Table 6. This again emphasizes the importance of regular testing of calcium levels among thalassemic patients being a risk factor for kidney dysfunction (103).

Creatinine and urea are waste products of protein digestion that serves as vehicles for ridding the body of nitrogen. These products are normally filtered from the blood and excreted with the urine (102). Estimation of the concentration of creatinine and urea in serum is widely used and accepted as a measure of renal function in clinical medicine (104, 105).

Results on kidney function among our patients showed significant decrease in serum creatinine as well as a significant increase in serum urea

for most patients over the study period. Such significant differences were observed when comparing tested patient in 2014 with those tested in 2012(P-value≤ 0.0.5) as showed in Table 7. In a study conducted by Jafari, similar findings were reported, however, no evidence of renal tubular and glomerular damage was reported (104). Other researchers referred the reason to disturbance of kidney function reflected by low creatinine and or/ low body mass index, due to growth retardation and lower muscle mass, usually encountered in β-thalassemia patients (106, 107). In addition to the side effect of chelators mainly DFX (18, 17, and 101). In our study 17 (77%) out of 22 under-weight patients as depicted in Figure 8 were reported with low creatinine levels. This finding is in agreement with the previously mentioned study which confirms the association between low creatinine level and low body mass index. On the other hand, evaluation of Kidney function among our patients requires more investigation at the anatomical level of kidney tissue (103). In contrast, other previous studies carried out in Bangladesh and Jordan reported significant increase in serum creatinine level in BTM (105, 108). It was also reported that the severity of renal abnormalities was correlated with anemia degree irrespective to creatinine levels (109).

Elevated serum urea in our study was in agreement with results obtained by Mansi *et al.*, who explained both increased creatinine and urea levels among their Jordanian patient group to high iron deposition in kidneys. The authors believe that shortened red cell life span and excess

iron could be the reason behind the physiological abnormalities in kidney among thalassaemic patients (105).

4.7 Patient's hormonal levels

Endocrine complications are widely reported in multi-transfused thalassemia major patients (61, 110, and 111). Disruption in the hormonal secretion of the anterior pituitary is well known, as a result of the high sensitivity of this gland to iron overload, however, the mechanism of iron effect is not known (16). In addition to iron effects, lipid peroxidation, oxidative stress and free radicals release seems to affect function of this gland (112).

Data presented in Table 8 summarizes the hormonal status of patients group in the current study and indicated by number and percentage of abnormal findings. Hypogonadism was the most prevalent endocrinopathy and represented by 46.7% of the tested patients including both primary and secondary hypogonadism. Similar high incidence rate of hypogonadism among thalassemic patients were also reported in different countries like Pakistan, KSA, Oman, UAE and Romania (36, 61, 97, 113, and 114). Hypogonadotrophic (central) hypogonadism was the predominant form (36.7%) were most of the affected patients in the second or third decade of life (mean age 22.36 years, range 10-64 years). These patients were in a serious clinical condition because central hypogonadism is rarely reversible even with iron chelation therapy.

Few patients in our study group were subjected to hormonal replacement therapy. In this regard, regular follow up for all recommended

hormones, especially growth hormones, is essential and management can help these patients achieve fertility by exogenous gonadotropin therapy (115).

Thyroid dysfunction is known to occur frequently in thalassaemia major, but its prevalence and severity varies in different countries; e.g. 4% 8%, and 19.4% in Greece, Iraq and India, respectively (116, 117, and 118). Cases of hypothyroidism was reported in 10.4%, 13.3% of the tested patients in 2013 and 2014 respectively. Primary subclinical hypothyroidism was reported in few cases. Similar incidence of low primary hypothyroidism was reported among thalassemic patients from Oman (96). However, a higher percentage of primary hypothyroidism was reported among patients in Greece and Egypt (116, 119). In addition, Secondary hypothyroidism was also noticed in our cohort with percent of 6.25% and 10. 3% in 2013-2014. In contrast, secondary hypoparathyroidism was not observed in some of the previous studies (96, 116).

No cases of overt hypothyroidisms were found among our study group indicating rare occurrence of this complication among our patients. A similar situation was reported among Egyptians patients (119). However, previous studies conducted in Iran and Greece reported overt hypothyroidism among their patients (110, 116). This indicates that thyroid pituitary axis seems to be less sensitive to iron deposition damage than gonadal axis (96).

Hemosiderosis (iron over load) is reported to be responsible for parathyroid gland dysfunction in BTM patients, particularly in the form of hypoparathyroidism. In previous studies, the prevalence varies greatly from low to as high as 22.5% (120). Hyperparathyroidism was also reported in some cases (48). In this study, poor PTH follow up was observed and only four BTM patients were tested for PTH and showed levels within the reference range. As mentioned previously in this study, some of the cases had low serum calcium level. It was found that hypoparathyroidism is usually, but not always, accompanied by hypo-calcaemia (36). Thus, our result could set as an indication for parathyroid glands problem. To confirm this point view PTH level along with serum phosphorus levels should be checked regularly to all patients, especially who showed low calcium level. A study of Basha and other co-workers, emphasized on the need for testing parathyroid function periodically, particularly when other iron overloaded associated complications occur (120).

The excess iron has also the potential to disrupt the adrenal function which results in adrenal insufficiency within the hypothalamic-pituitary-adrenal axis in the form of hypocortilism (111). Several studies reported a significant prevalence of adrenal insufficiency, ranging from 18-45% (121). In our study hypocortisolism was not reported. In contrast, hypercortisolism was reported in two cases (5.5%) as shown in Table 8.

Glucose intolerance and diabetes mellitus are common consequence of transfusion therapy and could lead to toxic hemosiderosis in liver among

BTM patients (16, 111). However, the exact mechanism of iron induced diabetes is unclear (111). Scientists reported different mechanisms that involved in precipitating diabetes mellitus; the most likely one is iron overload in the pancreatic beta-cells leading to pancreatic dysfunction. Other contributing factors include insulin resistance, liver dysfunction, genetic predisposition or family history of diabetes (96).

In our study, elevated glucose levels were reported in tow cases in 2014. One of them is a known case of diabetes mellitus that diagnosed since the first years of life, so can't consider as a complication. The other is at risk of being diabetic, thus more medical attention is required and diagnosis needs to be confirmed. Similar low rate of diabetes mellitus was previously reported in KSA and the researchers referred the role in delaying the onset of diabetes to the low BMI (61). This could be also the case in our study since low BMI was reported in 46.8 % (Figure 8).

Previous researchers suggested further investigation about the relationship between glucose homeostasis and body composition (61). In contrast to our finding, high prevalence of diabetes mellitus as secondary complication of transfusion therapy is widely reported. In Morocco the prevalence was 7%, in China the prevalence was 21.7%, in France and North America the prevalence of diabetes mellitus were 6% and 10%, respectively (33, 34, 49, and 50).

In the current study, statistical analysis revealed no significant role (P- value= 0.77) of recent annual SF level, in 2014 and the development of endocrinopathy (Table 11). Contradictory results were reported in this

respect (61, 96, and 122-124). Explanation of such finding could be related to iron toxicity in early life rather than at the time of screening (61, 96, and 122). It is also known that SF ferritin levels is an indirect marker for tissue ferritin deposition and high SF level found to be associated with acute and chronic disorders particularly inflammatory and hepatic conditions, such as chronic hepatitis (125, 126). Again, this may limit the validity and effectiveness of serum ferritin in reflecting iron status in beta-thalassaemia (96, 122, 125, and 126).

In order to reduce the disease complications especially the endocrinopathy, different researchers suggested better evaluation of iron toxicity and regular monitoring of liver function, accompanied by an improvement in chelation therapy. In addition, they suggested the administration of selective antioxidants (vitamins E and C), which can protect different organs against oxidative stress and its consequences, specifically cardiac and endocrine problems (87, 127).

4. 8 Monitoring of cardiac function

Annual monitoring of heart function along with the determination of the magnitude of iron loading within the heart tissue is very essential in the disease management (6). It was also reported that cardiac failure and rhythm disturbances remain the main causes of death among young adults with thalassemia (50). It is obvious that our patients received poor regular cardiac function follows up were 9.4%, 44.4% and 11.1% of the study

group were subjected to annual Echo and or ECG testing during the last three years, respectively. In this context, the use of cardiac T2* MRI measurements and chelation regimens targeted on cardiac iron overload present the most recent progresses in TM treatment that may improve life expectancy, beside hematopoietic stem cell transplantation (49). Cardiac T2* MRI is widely performed in modern countries, as the UK and Italy, which can detect preclinical cardiac iron accumulation (17, 49). Unfortunately, MRI testing technique is not available to our patients and none of them was subjected to this test.

To limit cardiac complications, Mula-Abed *et al.* and Fung *et al.* recommended periodic assessment of bone profile in addition to vitamin D status and maintenance of normal serum calcium to avoid the risk of arrhythmias, particularly in the presence of cardiomyopathy (96, 128).

In our study group five patients (9.26%) had heart problems requiring medication; including two with heart failure. The prevalence of heart complications were also reported in many previous studies conducted in different countries; in Morocco it was 6%, in KSA it was 9%, in North America it was 10% and in Turkey it was 22.4% (33, 37, 50, , and 129).

Chapter Five

Concluding Remarks and Recommendations

5.1 Concluding remarks

Result of the current study revealed impaired clinical picture among our thalassemic patients and this is evident from the different findings and the clinical remakes reported in this study including:

1. Presence of 5 miss-diagnosed patients reported as β-thalassemia major. These patients were older than 32 years old and found to be either with β-thalassemia trait or thalassemia combined with other hemoglobinopatheis.
2. Severe anemic presentation seen in the all patients; as all of them showed hemoglobin levels≤ 9mg/dL and with mean around 7.5mg/dL over the three successive years.
3. SF annual mean levels during the three studied years were above 2070 ng/dL. Such levels were above the internationally recommended cut off value (less than 1000ng/dL) reflecting high iron over load, where its consequences are clear from frequency of splenoctomy as well as deteriorated organ functions.
4. Low BMI reflecting underweight state in nearly half of the patients
5. A significantly deteriorated biochemical picture in all retrospectively studied years for liver, kidney, bone and endocrine functions.
6. Poor follow up for hormonal levels as only few of the recommended tests were applied and not all patients were subjected to regular check-ups.
7. Poor monitoring of cardiovascular state as no evidence were seen for regular check up for all patients.

5.2 Recommendations

1. Although limited cases were reported in the country since 2013, educational and awareness programs aiming to provide the population at risk with information, regarding the risks for consanguineous marriages and disease complications are essential.
2. Suitable programs aiming to increase patients adherence to the treatment are essential.
3. Confirm diagnosis for new cases with thalassemia, if any, and reviewing molecular screening for already existent enrolled thalassemic patients especially in reported β-thalassaemia major patients, who do not show the typical clinical picture of the disease, is strongly recommended.
4. Modification of the current care policy administrated to those patients to more strategic effective one; either by adopting internationally recommended guidelines or by conducting appropriate local management protocol, in the context of available resources. This in turn can improve their clinical situation.
5. Adopting a specialized clinical multidisciplinary therapeutic team for comprehensive treatment.
6. There is a great need for regular assessment of bone profile, regular hormonal test and performing MRI technique at least once annually to accurately determine tissue iron overload, especially heart and liver, and to predict risk factors as well as to prevent development of serious secondary complications to iron over load.

7. There is a great need for assessment of transfusion frequency, use of chelators in relation to BMI and other indicators to limit further possible complications as this can lead to improvement of patient's clinical situation and better survival. It will also reduce cost which is a burden to our small country with its limited resources.

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Annex 1: Facilitation Letter

Appendix 1: Consent Form

Date:

Management and Complications of Thalassemic Patients in Palestine: Retrospective Study

Researcher: Nida Daraghmeh, Master candidate, Graduate Program, Biology, An-Najah National University

Purpose of the Research: To evaluate the cuurent management protocol that administrated to thalassemic patients in AL- Wattani Hospital-Nablus-Palestine with comparison to international one, and to determine the common disease complication in our patients group.

What you will be asked to do in the Research: To fill questionnaire that include direct questions about the disease diagnosis and first transfusion, disease complication, regular organ follow up. Also certain patients (5 patients) will asked for blood withdrawal (5-10ml) by the responsible nurse in the thalassmic ward at Al-Wattni Hospital-Nablus.

Risks and Discomforts: I do not foresee any risks or discomfort from your participation in the research. Benefits of the Research and Benefits to You: it will reflect the current clinical situation of the included thalassemic patients that could be used in near future to improve the current management protocol administrated to thalassemic patients in Palestine.

Voluntary Participation: Your participation in the study is completely voluntary and you may refuse to answer any question or choose to stop participating at any time. Your decision not to volunteer will not influence the *[treatment you may be receiving] [nature of the ongoing relationship you may have with the researcher]*.

Withdrawal from the Study: You can stop participating in the study at any time, for any reason, if you so decide. If you decide to stop participating, you will still be eligible to receive the promised pay for agreeing to be in the project. Your decision to stop participating, or to refuse to answer particular questions, will not affect your relationship with the researcher or the University. If you decide to withdraw from the study; all data generated as a consequence of your participation will be destroyed.

Confidentiality: All information you supply during the research will be held in confidence and, unless you specifically indicate your consent, your name will not appear in any report or publication of the research. Your data will be safely stored in a locked facility and only the researcher will have access to this information. Confidentiality will be provided to the fullest extent possible by law.

Questions about the Research: If you have questions about the research in general or about your role in the study, please feel free to contact Nida Daraghmeh (0597625585), MA candidate in Biology, at the Department of Biology, An-Najah National University, or by e-mail (nida.daraghmeh@gmail.com). This research has been reviewed and approved for compliance with research ethics protocols by An-Najah National University.

Legal Rights and Signatures:

I *(fill patient name)*, consent to participate in *(*Management and Complications of Thalassemic Patients in Palestine: Retrospective Study) conducted by (Nida Daraghmeh). I have understood the nature of this project and wish to participate. I am not waiving any of my legal rights by signing this form. My signature below indicates my consent.

Researcher Participant

Signature Signature

Date

Appendix 2: Questionnaire
Management and complication of thalassemic Patients in Palestine-West-bank

Section A: Demographics and personal data

Case no.: File no.:

Patient name: Patient ID no.:

Date of birth:

Telephone no.:

City: Referral center:

Gender: Male☐ Female☐

Consanguinity: First and second cousin☐ Related☐ Not-related☐

Marital status: Single☐ Married ☐

Section B: Clinical data

Section B1: Compatibility test

1- ABO typing: Type: A ☐ B☐ AB ☐ O☐

2- Rh: +ve☐ -ve☐

3- Other antigens typing: Yes☐ No☐

If yes: D☐ C☐ c☐ E☐ e☐ Kell☐

Section B2: Transfusion transmitted infection

1-Vaccination administrated before transfusion regimen:

Hepatitis B: Yes☐ No ☐ Hepatitis C: Yes ☐ No☐

2- If the patient has transfusion transmitted infections: Yes☐ No ☐
If yes, mention:

3- Any drug taken against such infection(s)? Yes☐ No ☐
If yes, mention:

4- Antigen screening for transfusion transmitted infections

Test name	Result(Positive/Negative)	Notes

Section B3: Transfusion history

1- Age at first diagnosis:

2- Age at first transfusion:

3- Average intervals between transfusions:

2-3 weeks ☐ 4-5 weeks ☐ Every two months ☐

4- Type of transfused blood:

Whole blood ☐ frozen thawed RBC cells ☐ packed RBC ☐

5- Volume of transfused blood:

6- Any reaction(s) appear during transfusion event? Yes ☐ No ☐.
If yes, Mention:

7- Any drugs taken against these reaction(s)? Yes ☐ No ☐

Section B4: regular monitoring of hemoglobin and ferritin levels before blood transfusion

1-Hemoglobin levels (g/dL)

Year / Month	1	2	3	4	5	6	7	8	9	10	11	12
2012												
2013												
2014												

2- Serum Ferritin levels (ng/ml)

Year / Month	1	2	3	4	5	6	7	8	9	10	11	12
2012												
2013												
2014												

Section C
Section C1: Bone transplantation

1- If the patient underwent bone transplantation? Yes ☐ No☐
2- If yes, at any age

Section C2: Splenectomy

1- If the patient underwent regular monitoring to detect splenomegaly?

2- If the patient underwent splenectomy? Yes ☐ No☐
3-If yes, at any age done?

4- Any vaccination administrated to the patient before splenectomy?

5- Any medication administrated to the patient after splenectomy?

Section D: Chelation therapy

1- If the patient takes chelators: Yes ☐ No☐
2- If yes, at the age:

3- Chelation drug type: Deferiprone ☐Desferal ☐ Exjade☐

4- Any complication appears from using the above drugs? Yes ☐ No☐
If yes, mention?

Section E
Section E1: Hormonal replacement

1- If the patient has any endocrine complications? Yes ☐ No☐
If yes, mention:

2- If the patient takes any hormone replacement before puberty? Yes ☐ No☐
If yes, mention:

At the age:

Section E2: Glucose monitoring

Year / Month	1	2	3	4	5	6	7	8	9	10	11	12	Notes
2012													
2013													
2014													

1- Regular glucose monitoring (mg/dL)

2-If the patient has diabetes mellitus? Yes ☐ No ☐

3- If the patient has other complications: Yes ☐ No ☐

4- If yes, mention?

Section F: Vitamins and other supplements

1- If the patient takes any supplement? Yes ☐ No ☐

If yes, Mention:

2- If the patient takes these supplements? Yes ☐ No ☐

Section G: Regular follow up

1- Monitoring of Growth

Year	2012			2013			2014			Notes
Weight(kg)										
Height (cm)										

2-Monitoring of cardiac function

1-If the patient underwent regular monitoring of cardiac function?

3- If the patient suffers from any heart complication(s)?

If yes, mention?

3-Monitoring of liver function

Test name / Year	Liver tests									
	2012			2013			2014			Notes
ALT U/L										
AST U/L										
ALP U/L										
Bilirubin mg/dL										

4- Monitoring of kidney function

Test name / Year	Kidney test									
	2012			2013			2014			Notes
Urea mg/dL										
Creatinine mg/dL										
Calcium mg/dL										

5- Monitoring Endocrine function

Test Name / Year	Hormonal test									
	2012			2013			2014			Notes
T4 Free ng/dL										
T4 total ng/dL										
T3 total ng/dL										
TSH mIU/ml										
Testosterone Total ng/ml										
LH mIU/ml										
FSH mIU/ml										
PTH mIU/ml										
E2 pg/ml										
Cortisol ng/dL										

جامعة النجاح الوطنية

كلية الدراسات العليا

معالجة و تعقيدات مرضى الثلاسيميا في فلسطين: دراسة رجعية

إعداد

نداء مثقال دراغمة

إشراف

د. نائل صدقي أبو حسن

د. مراد أبو الحسن

قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في العلوم الحياتية بكلية الدراسات العليا في جامعة النجاح الوطنية، نابلس– فلسطين.

2016

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الملخص

في فلسطين, هنالك قلة في البيانات المتعلقة بالإدارة الحالية لهذا المرض وما ينتج عنه من مضاعفات. تهدف هذه الدراسة إلى تقييم البروتوكول العلاجي المقدم لمرضى البيتا ثلاسيميا العظمى المسجلين من خلال مقارنته مع ذلك المستخدم دوليا. بالإضافة إلى ذلك، تهدف هذه الدراسة إلى تحديد المضاعفات الشائعة و ارتباط ظهورها بمستوى الحديد الزائد.

لقد أجريت هذه الدراسة المسحية رجعيا من كانون ثاني 2012 إلى أيلول 2014. وشملت الدراسة على جميع المرضى المسجلين كبيتا ثلاسميا عظمى, الذين يتلقون العلاج والرعاية في وحدة الثلاسيميا- المستشفى الوطني- نابلس. تمت عملية جمع المعلومات من خلال مراجعة الملفات الطبية لهؤلاء المرضى وتسجيلها في استبيان مصمم خصيصا لهذا الغرض. تم الحصول على البيانات غير المسجلة في الملفات الطبية من خلال مقابلة مباشرة مع المرضى أو أولياء أمورهم. كما أنه تم التأكد من التشخيص الطبي لخمس حالات. أجري التحليل الإحصائي باستخدام برنامج IBM SPSS v.21. شملت هذه الدراسة على54 مريض، منهم 26 (48.1٪) من الذكور و 28 (51.9٪) من الإناث، تراوحت أعمارهم بين 1-68 سنة. كان منهم 84.4٪ ممن ولدوا لآباء أقرباء.

باستثناء خمس مرضى مشخصون بشكل خاطئ بلغ متوسط أعمار المرضى الباقين المسجلين كبيتا ثلاسيميا عظمى 13.96 ± 7.75 تراوحت أعمارهم بين 1-28 سنة. كان متوسط المعدل السنوي لمستوى الهيموجلوبين أقل من 7.6 ± مغ/دل خلال الثلاث سنوات

(2014,2013,2012) مما يعكس حالة فقر دم شديد بين مجموعة المرضى. سجلت حالتين بعدوى التهاب الكبد(حالة بالنوع ب والأخرى بالنوع ج) بنسبة3.7٪.

وجرى استئصال الطحال ل29(53.7٪) من المرضى. كان معدل SF 2721±2204.1نانوغرام/مل في عام 2012 و 2096.6 ± 1770.6 نانوغرام/مل في عام 2014 بانخفاض بقيمة ذات دلالة إحصائية (P-value= 0.001). سجل 73.5٪ من المرضى مستويات SF أعلى من الحد المسموح به عالميا (≥1000 نانوغرام/مل), مما يعكس حالة عالية من ترسب الحديد.

الخصائص البيوكيميائية تشير بقوة إلى تدهور في وظائف الكبد والكلى إضافة إلى الغدد الصماء. تم تسجيل مستويات مرتفعة من ALT وAST بين 43.4٪ إلى93.5٪ من المرضى الذين تم اختبارهم في فترة الدراسة. أظهر تحليل مقارنة مستويات هذه الأنزيمات زيادة في مستويات كلا الأنزيمين مع زيادة العمر. كانت قيمة الاختلافات في مستويات ALT بدون دلالة إحصائية, بينما كانت قيمة الاختلافات في مستويات AST ذات دلالة إحصائية (P =0.001). ولوحظ وجود علاقة إيجابية قوية بين مستوىSF ومستويات الإنزيمات الكبدية (P = 0.00، r_s>0.05 لكلا الأنزيمين). تم تسجيل مستويات مرتفعة من أنزيم ALP بين 32.7٪ إلى 67.5٪ من المرضى الذين تم اختبارهم في عامي 2012 و 2014. وكانت الاختلافات في مستوى الأنزيم بين تلك السنوات بقيمة ذات دلالة إحصائية بزيادة مضطردة مع الوقت (P =0.002)

بلغت النسبة في حالة فرط كالسيوم الدم في 58.7٪ و 17.3٪ من المرضى الذين تم اختبارهم في عامي2012 و 2013 على التوالي و كان الانخفاض في مستوى الكالسيوم بين هاتين السنتين بقيمة ذات دلالة إحصائية (P =0.001).

كما أظهر اختبار وظيفة الكلى مستويات منخفضة من الكرياتينين في84.4٪، 77.4٪، 59.2٪ بالنسبة للمرضى الذين تم اختبارهم في عام 2012 و 2013 و 2014، على التوالي مع انخفاض بفروقات ذات دلالة إحصائية (P =0.024). وتم تسجيل ارتفاع في مستويات اليوريا بين 12٪ و23.6٪ من المرضى الذين تم اختبارهم في عامي 2012و 2014, مع زيادة بقيمة ذات دلالة إحصائية في مستويات اليوريا على مدار السنوات المنصرمة (P =0.00).

تم تسجيل ضعف في متابعة إجراء اختبارات وظائف الغدد الصماء, والتي بدأت تسجل بشكل جزئي في عام 2014. كان قصور الغدد التناسلية أكثر المضاعفات شيوعا بنسبة 46.7٪, كما سجل انخفاض بنشاط الغدة الدرقية بين 13.3 ٪ من المرضى الذين تم اختبارهم. كما سجل داء فرط إنتاج الكورتيزول بين 5.5% بين المرضى المفحوصين.

تم تسجيل ضعف لرصد حالة العظام و وظيفة القلب. سجلت المضاعفات القلبية بين 9.3% من المرضى.

تعتبر هذه الدراسة الأولى من نوعها والتي تقدم نبذة مفصلة عن الحالة الصحية لمرضى البيت ثلاسيميا العظمى. إن الخلل في المستويات البيوكيميائية و الهرمونية يعكس صورة صحية متدهورة ويعزز ويؤكد الحاجة الماسة إلى أهمية اعتماد سياسة إستراتيجية فعالة لرعاية مرضى الثلاسيميا في فلسطين.