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Study the newly Biomarker Hepcidine–25 in β- thalassemia intermedia before and after blood transfusion for Iraqi child patients

A thesis
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بسم الله الرحمن الرحيم

وَتِلْكَ الْأَمْثَالُ نَضْرِبُهَا لِلنَّاسِ

وَمَا يَعْقِلُهَا إِلَّا الْعَالِمُونَ

صدق الله العظيم

سورة العنكبوت الآية 43

Dedication

To my family, the reason of what I became today

Thanks for all great support

To my supervisor Dr. Bushra

To my friends, who encourage and support me

And to all employees of Ibn albalady hospital at head of them Dr. Arabia yassin

I dedicate this research.

Mohammed

Acknowledgements

Praise is to mighty Allah, the glorious of the universe, for his kindness, mercy and blessing upon our Mohammed and his family.

I would like to express my deepest appreciation and sincere thanks to my supervisor Dr. Bushra H. Ali for suggesting this study and her peerless support and scientific notes throughout the research period. I ask Allah to bless her and give her all the best.

I am indebted to the staff members of thalassaemia center in Ibn-Albalady Hospital for their assistance.

Abstract:

Background: Hepcidin-25 is a peptide hormone found as preprohormone (84 amino acids) prohormone (60 amino acids) and hormone (25 amino acids) synthesis in the liver and regulated the entry of iron into the circulation in mammals.

The objective: This study aimed to determine hemoglobin, packed cell volume and evaluation of hepcidin-25, serum iron, total iron bending capacity, transferin % and ferritin for Iraqi child patients with β-thalassaemia intermedia before and after blood transfusion, other aim is to study the relationship between hepcidin-25, serum iron, total iron bending capacity, transferring% and ferritin for patients group also to find the relationship between hepcidin-25 and frequent number of the blood transfusion in those patients were studied to predict effect of blood transfusion on hepcidin-25 levels for all patients groups.

Methods: Eighty Iraqi child patients were enrolled from Ibn albalady hospital during the period from (1 Oct. 2017) to (1 April 2018) the age range (6-12) years that divided into 3 clans as follows forty samples (twenty female and twenty male) before blood transfusion as clan1, 40^{th} samples of same patients (twenty female and twenty male) after blood transfusion as clan 2 and forty healthy individual (twenty female and twenty male) as clan 3. in the present study hemoglobin and packed cell volume was measured automatically, while hepcidin-25 and ferritin level was measured by using enzyme immune sorbent kit, but serum iron and total iron bending capacity was measured by photometric methods as well as transferrin percent was calculated by divided serum iron on total iron bending capacity.

Results: the results of the present study showed highly significant decrease in hemoglobin and packed cell volume for patients clans before and after blood transfusion compared with control male and female clans, also high significant increase for hepcidin-25 levels in the clans male and female patients compared with control group, but we show highly significant increase for transferin % for male and female patients when compared with control group, by the same way we show highly significant decrease in serum ferritin for patients clan when compared with control, while serum iron was showed highly significant decrease for patients clan when compared with control, but we show non-significant for total iron bending capacity when compared with control.

Conclusion: From this study it appeared hemoglobin and packed cell volume as highly effected by blood transfusion as well as iron

absorption mainly affected by hepcidin-25 level so that hepcidin-25 level was significant change in thalassaemia intermedia for Iraqi children patients after blood transfusion this versus significant increase of serum iron and lead to iron overload with increase iron storage (ferritin) because blood uptake, but non-significant in transferrin or total iron bending capacity respectively.

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Term	The Meaning
AT	After blood transfusion
BMI	Body mass index
BMP	Bone morphogenic protein
BSA	Bovine serum albumin
BT	Before blood transfusion
CAB	Chromazurol B
CE	Cellular acetate electrophoresis or capillary electrophoresis
CTMA	Cetyltrimethylammonium bromide
DE	Diethylaminoethyl
DFO	Desferrioxamine
DFP	Deferiprone
DFX	Deferasirox
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme linked immune sorbent assay
EMH	Extramedullary hematopoiesis
GI	Gastrointestinal
HAMP	Hepcidin antimicrobial peptide
Hb	Hemoglobin
HbA	Adult hemoglobin
HbF	Foetal hemoglobin
HPLC	High pressure liquid chromatography
HS	High significant
KDa	Kilo-Dalton
KLH	Keyhole limpet hemocyanin
MCH	Mean cellular hemoglobin
MCV	Mean cell volume
mRNA	Messenger ribonucleic acid
NBTT	Number of blood transfusion times
NS	Non significant
NTBI	Plasma non transferrin bound iron
NTDTs	Non transfusion dependent thalassaemia's

PBS	Phosphate buffered saline
RE	Reticuloendothelial
RGT	Reagent
ROS	Reactive oxygen species
RPM	Round per minute
S. Fer	Serum ferritin
S.Trans	Serum transferring
TDTs	Transfusion dependent thalassaemia's
TF	Transferrin
TIBC	Total iron bending capacity
UIBC	Unsaturated iron binding capacity

Chapter one

Introduction and Litratures Review

Introduction and literatures review:

*1-1**Thalassaemias:***

Refers to a cluster of blood illnesses characterized via low or deficient creation of regular globin manacles[1]. Depending on the series whose creation is harmed such as α–, β–, ɣ–, σ– or εɣσβ. Mainly thalassaemias are inherit at the same time as recessive behavior. These crucial quantitative defects are no longer tightly differentiate via the structural alternates formed by the side of condensed rate [such as HbE and Hb lepore]. Starting a scientific point of observation, the most types are α- and β-thalassaemias, decrease one of the two types of polypeptide manacles [α or β] that form regular haemoglobin particle [HbA, α2β2].[2]

study in thalassaemia, which comprise a chief problem in the countries in the region of the Mediterranean sea, the middle east -and the trans-Caucasus, India and faraway East[3]. The most hauler regularity of β-thalassaemia was reported inside Maldives [18%], Cyprus [14%], Sardinia, [10.3%] and Southeast, Asia [3-5%][4]. Because the regularity in these regions is mainly liable associated to the discriminating stress from plasmodium falciparum malaria. So that, population migration and intermarriage between, different ethnic groups has introduced thalassaemia in almost every country of α-talassaemia[5].

Often, heterozygotes of either α- or β-thalassaemia asymptomatic and do not need treatment[6]. relations of thalassaemia and matching haemoglobinopathies e.g. hemoglubin E [Hb E], hemoglobin C [Hb C] or hemoglobin S [HbS], through β-thalassaemia or hemoglobin stable helix [Hb CS] with α-thalassaemia in addition to give increase to varies thalassaema syndromes[7].Depend cruelty and transfusion necessity, these thalassaemia syndromes can be classify phenotypically addicted to two major groups:

A- Transfusion dependent thalassaemia [TDTs]
B- Non -transfusion dependent thalassaemia [NTDTs]. Like exposed in form (1-1) [8].

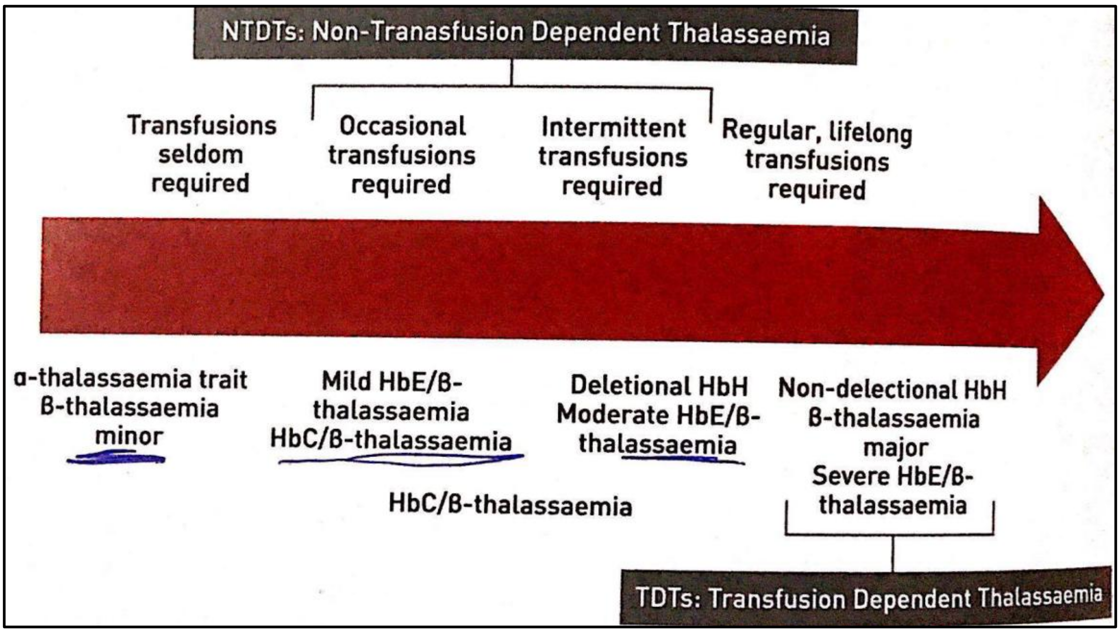


Figure (1-1).Phenotypic thalassaemia arrangement syndromes depend on experimental harshness and transfusion necessity[8].

The TDTs need standard blood transfusion to carry on so lacking sufficient causes experience more than a few complications and a diminutive verve span[9].This group consists of patient with β-thalassaemia major; sever Hb E / β-thalassaemia, while the group of NTDT patient include β-thalassaemia intermedia, Hb E /β-thalassaemia and Hb H disease[10].

1-2HaemoglobinTypes:

Oxygen was carried to the tissues from the lungs via the exceedingly specialized protein particle, haemoglobin inside the red compartments of the blood, that was exist located all red blood compartment holds approximately 300 million molecules of this protein, totally a propos 30 picograms in mass for every cell[11].Every one fragment of haemoglobin was created via two pairs of matching sub-unit; globin manacles are named with the letter of the Greek alphabet and fit in two groups: the α-globin brush consists the ζ- then α-globin chains, yet the β-globin cluster, comprising the globin chains ε, γ, β yet δ toughness as shown in figure (1-2)[12].

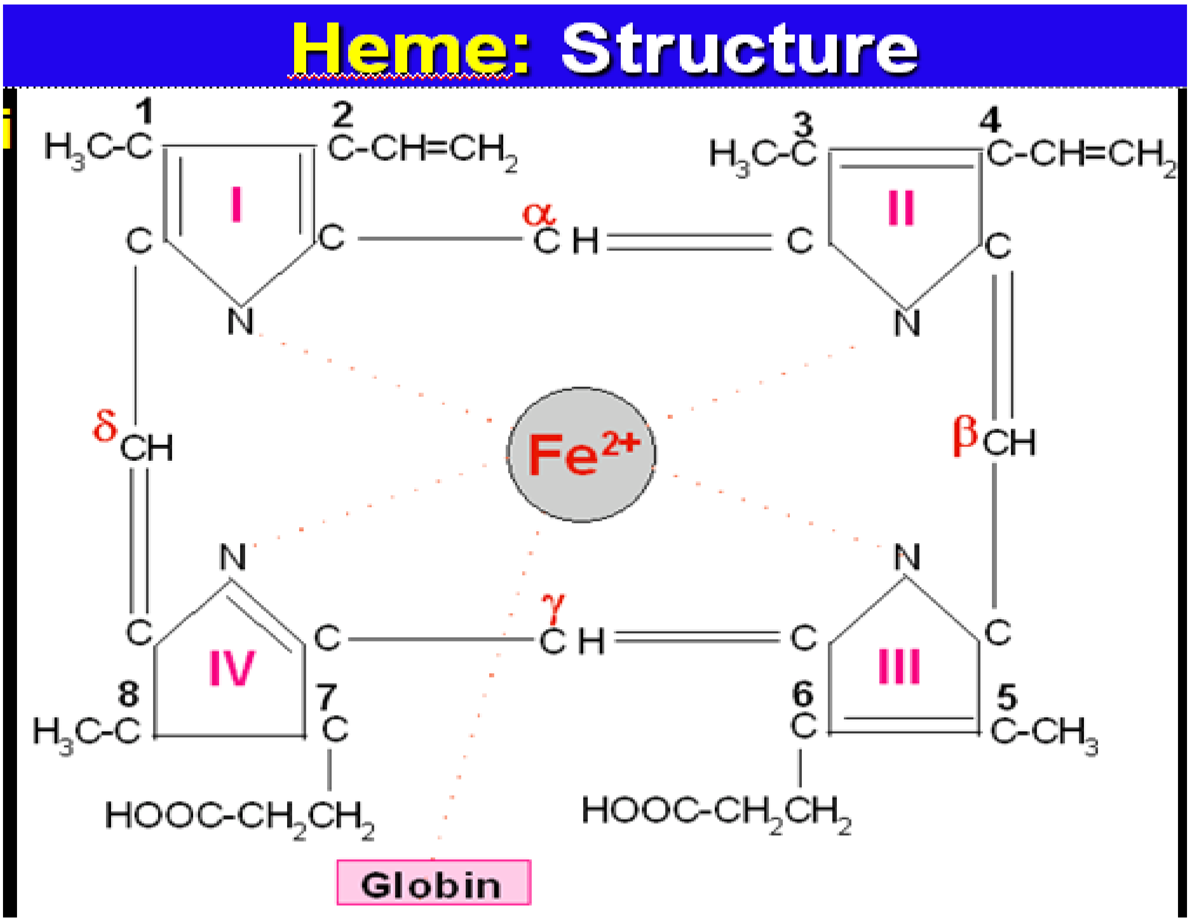


Figure (1-2). Hemoglobin structure[12].

In fact, the globin chains exhibit sequentially at some stage in ontogeny and, below coupling, the following 4 majors' sorts over haemoglobin:

1- "embryonic" haemoglobins, was once produced detectable beyond the 3rd after the 10th sennight over gestation or show up ζ2ε2 [Hb Gower 1], α2ε2 [Hb Gower 2], ζ2γ2 [Hb Portland 1]; and ζ2β2 tetramers [Hb Portland 2];

2- "foetal" haemoglobin [Hb F], constitutes the lordly oxygen provider at some stage in pregnancy and is α2γ2 molecule;

3- "adult" haemoglobin [Hb A α2β2], alternative Hb F rapidly below birth;

4- A minor adult component, Hb A2 [α2δ2].

The specific haemoglobin species were manufactured yet give up at definitive duration concerning ethnic improvement is acknowledged namely "haemoglobin switching" as like proven of figure (1-2)[13]. The pink blood cells concerning the adult ethnic consist approximately 97-98% concerning Hb A, 2-3% about Hb A2 or traces on Hb F[14].

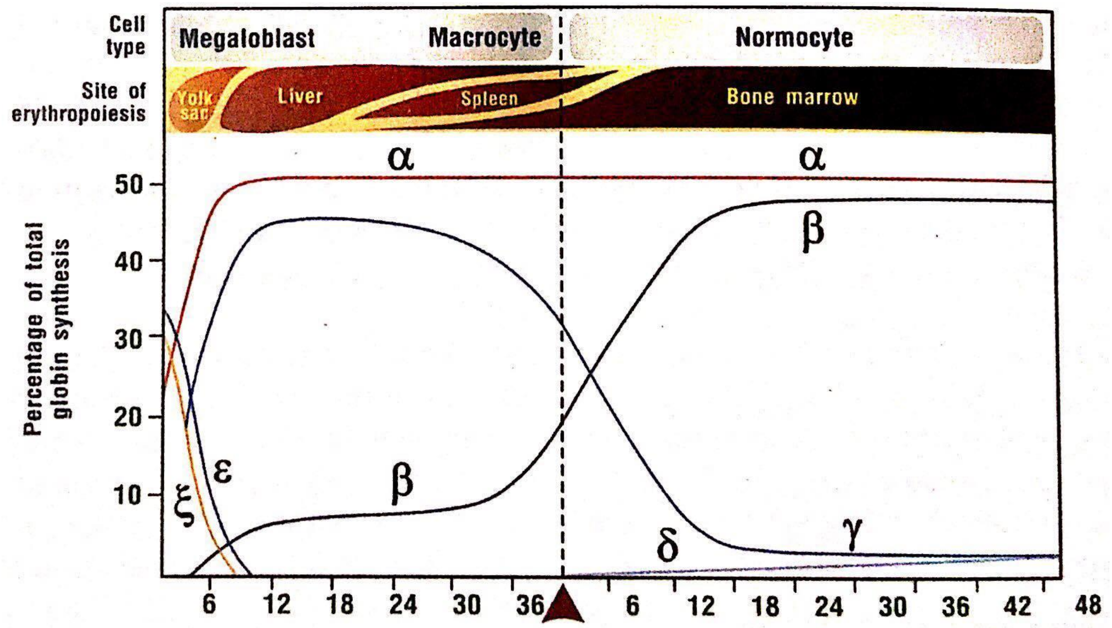


Figure (1-3). Globin blend by different phase of nascent, foetal and mature erythroid improvement[14].

In adult life, the globin synthesis are occur in the erythroblast in the bone marrow[15].Globin chains have the repair structure and be strip in such a way that the number of α-chains should exactly match that of the β-chains[16].

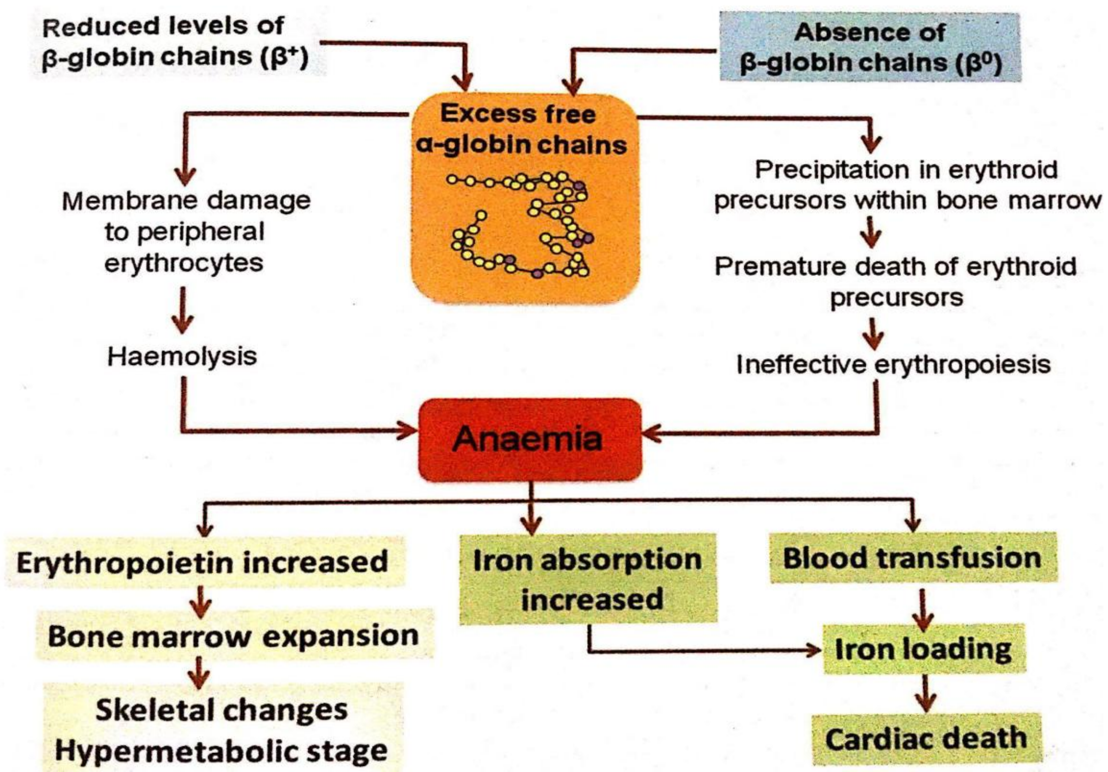
1-3 <u>β-thalassaemia:</u>

1-3-1 <u>Phenotypic heterogeneity:</u>

β-thalassaemia consist regarding 3 main forms: thalassaemia primary alternative called "cooley's Anaemia" or "Mediterranean durability Anaemia", thalassaemia intermedia or thalassaemia minor also neumerous termed "β-thalassaemia carrier", "β-thalassaemia trait" yet "heterozygous β-thalassaemia"[17]. Apart from the rare magistral forms, problem along β-thalassaemia essential are homozygotes or made heterozygotes because B^0 and B^+ genes, problem including thalassaemia intermedia are typically homozygotes and compound heterozygotes then subjected with thalassaemia minor are broadly speaking heterozygotes[18].

1-3-2 <u>Pathophysiology</u>:

The principle error about β-thalassaemia is a humbled yet default manufacturing regarding β-globin chains together with extend of α-globin chains. This is propulsion to reduce on the haemoglobin manufacturing and an imbalance about the globin band synthesis[19]. As nicely as, propulsion according to a discount over predominant carrier haemoglobin and predominant carrier volume, yet motives minor clinical significance. Then has dramatic impact of the purple cell carrier precursors, finally ensuing sizeable premature blasting into the bone brother and within the extramedullary sites. This procedure was once indicate in conformity with so "ineffective erythropoiesis" and is the note on β-thalassaemia[20]. Peripheral haemolysis take part in conformity with anaemia is much less distinguished between thalassaemia foremost than between thalassaemia intermedia, then dwell now insoluble α-globin chains incourage membrane injury to the peripheral erythrocytes[21]. The response in imitation of ineffective erythropoiesis and anaemia is an enlarge creation of erythropoietin, causing erythroid hyperplasia, which, between turn, might also cause skeletal deformities, osteoporosis, or every now and then extramedullary masses, or fit to splenomegaly, hence untreated then undertreated thalassaemia most important sufferers bear retarded increase so a result on anaemia and the increase metabolic indebtedness imposed by erythroid expansion, additionally anaemia may additionally stay motive cardiac enlargement including severe cardiac failure[22]. Ineffective erythropoiesis was related together with elevated iron absorption, which region generally beyond multiplied inner attention over blood appropriate in conformity with need regarding hepcidin-25,[a 25-amino sour taste peptide shaped by way of hepatocytes up to expectation play a biovital role among the law on iron homeostasis[23]. Figure (1-3)illustrated pathophysiology of β-thalassaemia and effect of excessive of free α-globin chain



Fegure(1-4). Achieves of overindulgence creation of liberated α-globin manacles within β-thalassaemia[24].

Imbalance of globin band was once monitoring by way of the characteristic of the mutation on the B gene. B0 potential ultima non-appearance about manufacturing over β-globin over the affected allele[24]. B+ point according to alleles with some other production on β-globin (around 10%). While among B++ the discount was very mild[25].

1-3-3 Clinical diagnosis:

The endeavor of the everyday B gene on the allelic chromosome conduct in imitation of adequate steady globin, underneath everyday satuation, β-thalassaemia consumption has no necessary scientific effects[26]. Clinical signs concerning β-thalassaemia principal commonly show up within 6 and 24 months including detruncate microcytic anaemia, slight jaundice, then hepatosplenomegaly[27]. Affected babies fail in accordance with grow then turn out to be steadily pale[28]. Feeding problems, irritability, popular bouts of fever fit in accordance with hypermetabolic regime or inter-current infection, and modern enlargement of the abdomen appropriate in conformity with plant then liver enlargement may occur[29]. Therefore, if a chronic spread no longer started, patients with thalassaemia primary normal die into the first few years on life[30].

β-thalassaemia intermedia should keep discernible between character whosoever current at another in the past along comparable but milder scientific symptoms[31]. At the server stop on the medical parameters, patients present between the a while yet years among spite the fact that even though she are capable concerning the surviving except normal gore transfusion, growth and gradual development are retarded. At the quit sufferers any are totally signs until grown-up existence together with only moderate anaemia[32]. Hypertrophy on erythroid match including the possible concerning extramedullary hematopoiesis [EMH] is common[33]. And propulsion according to deformities regarding the skeleton or face, osteoporosis fractures over lengthy bone yet form of erythropoietic loads as affect the spleen, liver, lymph nodes, booking or spine, hence so growth over the shrub primary position over the clearing damaged red cells out of bloodstream[34].While in haemosidrosis is secondary to the chronic transfusion,β-thalassaemia major person with β-thalassaemia intermedia suffering from iron excess secondary to enlarged intestinal iron absorption[35].

1-3-4 Haemtologic diagnosis:

Heterozygous provider concerning β-thalassaemia, usually a mangy paltry mobile haemoglobin [MCH], paltry mangy mobile volume [MCV], yet multiplied level of Hb A2, additionally may additionally lie associated including mangy regular or barely subnormal haemoglobin levels[36]. Peripheral blood point show much less extreme erythrocyte morphologic adjustments than affected folks then erythroblasts are typically not seen[37]. β-thalassaemia foremost is characterized through decreases haemoglobin level [<7 g/dl], MCV >50 or <70 femtoliter [fL] yet MCH >12 then <20pg. thalassaemia intermedia is characterized by Hb degree of (7-10) g/dl, MCV into 50 then eighty fL and MCH in sixteen and 24 pg. a person with thalassaemia show microcytosis, hypochromia, anisocytosis, poikilocytosis, target cells and erythroblasts[38].So the number of erythroblasts is relationship with the degree of anaemia and is indicator to increase after splenectomy[39].Generally, it odd crimson blood mobile morphology and characteristic quantity amongst extraordinary kinds regarding thalassaemia omen also interaction with haemoglobin duplicate certain so HbE/β-thalassaemia[40].

1-3-5 Qualitative and quantitative haemoglobin analysis:

The methods that use for qualitative and quantative analysis depend on cellulose acetate electrophoresis or capillary electrophoresis [CE] yet DE-52 microchromatography or high-pressure thinned chromatography [HPLC] distinguish the total yet type regarding haemoglobin present[41].

In B0 thalassaemia homozygotes, HbA is inexistent or HbF composed the 92-95% of the quantity Hb. So in B+ thalassaemia homozygotes yet B+/B0 genetic compounds HbA tiers are in 10-30% whilst HbF in 70-90%[42]. HbA2 is alternative within β-thalassaemia homozygotes or it is enlarge among β-thalassaemia minor[43]. HbF may keep realize with the aid of acid elusion test [F-cell staining] or alkali denaturation[44].

1-4 α-Thalassaemia:

α-Thalassaemia are hereditary world characterized by lowered then inhibited manufacturing about α-globin chains, hence that the human α-globin genes are duplicated then positioned between the telemetric give up on the brief part regarding chromosome 16[45]. α-thalassaemia was once begotten by way of deletions on large DNA bit up to expectation contain some yet both α-globin stability genes[46].

1-4-1 Silent carrier state:

The arrival over the unaccompanied α-globin deletion or deletional α+-thalassaemia end result into the irresponsive carrier state[47]. Heterozygotes of some α-gene missing are no longer anaemic then bear ordinary red gore mobile induced. Two principal types over it deletional α+-thalassaemia; 3.7 then 4.2 kb-deletions, are extensively extent throughout the globin were been perceive too into the population in the pacific[48].

1-5 Epidemiology of NTDT:

Exist inherited haemoglobin disorder primarily low- or middle-income countries over the tropical facia stretching from sub-Saharan Africa, into the Mediterranean region then the Middle East, in imitation of South yet Southeast Asia[49]. This is fit in imitation of excessive frequency over consanguineous marriages into these areas therefore conferred resistance over carriers in imitation of extreme varieties of malaria into areas the place the contamination has been yet is nonetheless prevalent. Therefore, enhancements of community health standards into these areas bear enhance uplift concerning affected patients. Ultimately, persisted migration has significantly multiplied the each on these ailments of high, multiethnic cities between Europe yet North United States of America.[49]

1-6 β-Thalassaemia intermedia:

Patients including β-thalassaemia intermedia, essential modifier of phenotype is the wide variety mutations so much have an effect on the β-globin gene among the homozygous then made-up heterozygous state[50]. stability the thoroughness from moderate planter mutations appropriate in imitation of slight minimize of β-globin band production according to the deep different mutations as end result in the β0-thalassaemias; up to expectation is, a completed non-appearance concerning β-globin band synthesis. Deletions of the β-globin gene are rare[51]. The range concerning mutations yet consonant variable degree on α/β-globin band imbalance yet asleep erthropoiesis are the principal determined because milder anaemia then phenotype into β-thalassaemia intermedia than β-thalassaemia major[52]. Secondary modifiers are worried at once into modifying the quantity over α\β-globin band imbalance thoroughgoing coinheritance on exceptional molecular forms on α-thalassaemia, extended issue of α-hemoglobin stabilizing proton, yet effective harmony of γ-chains among grown-up life[53]. Several genes bear been open as ought to modify γ-chain manufacturing yet raise phenotype, some about to them encoded among the β-globin gene cluster, others are among exclusive chromosomes[54]. While tertiary modifiers contain polymorphisms up to expectation are not related according to globin band manufacturing but may additionally hold an improve impact regarding specific problems on the disorder as (iron absorption, bilirubin metabolism, bone metabolism, cardiovascular diseas, or susceptibility in conformity with infection) [55] Therefore, β-thalassaemia intermedia may additionally also result beyond outcome manufacturing about α-globin chains via triplicated and quadruplicated α-genotype associated including β-heterozygosity[56]. Longevity blood less commonly, a single β-globin location is affect, the lousy life totally normal, consequently β-thalassaemia intermedia is dominantly inherited[57]. Table (1-1); provide an explanation for frequent genotypes government in imitation of β-thalassaemia intermedia toughness phenotype[58].

Table (1-1), illustrated the genotype phenotype associations in β-thalassaemia[58].

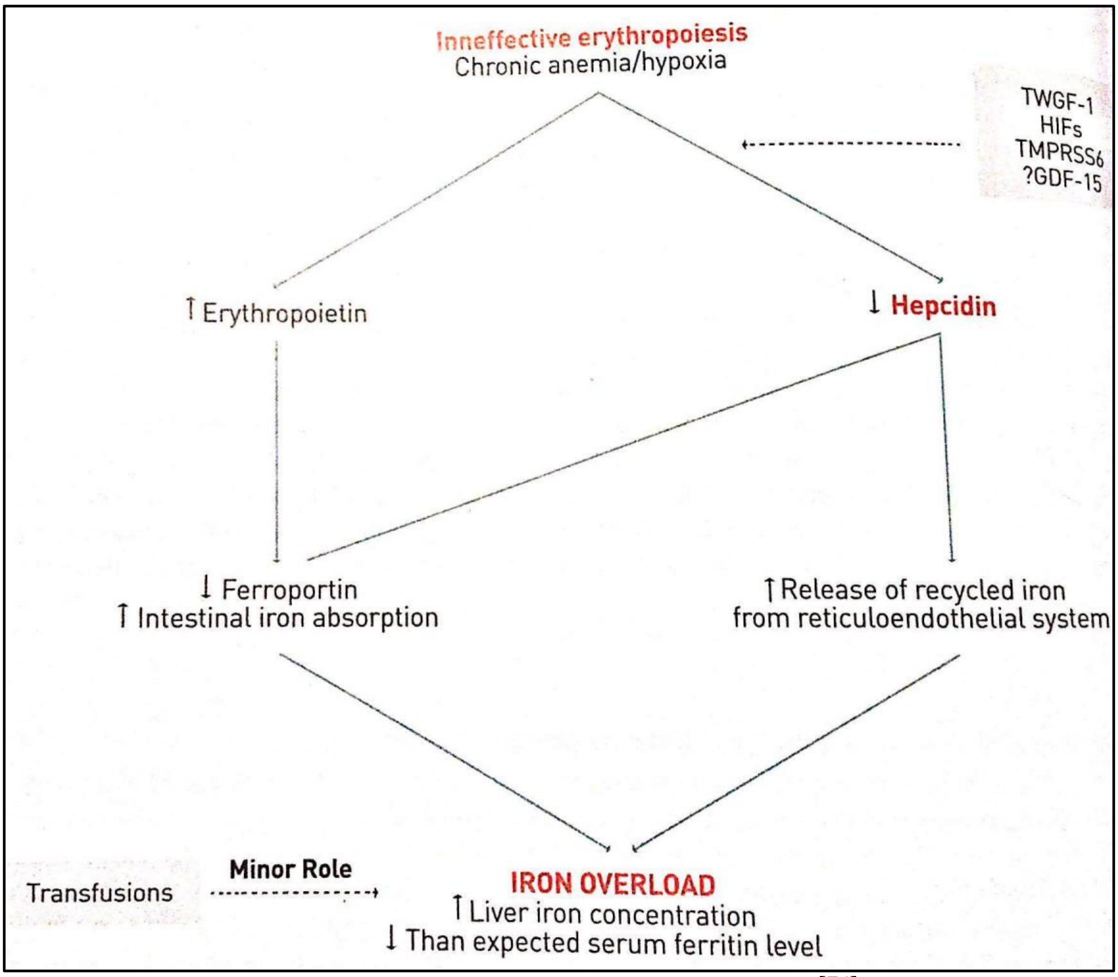
Phenotype	Genotype	Clinical severity
Silent carrier	• silent β/β	• Asymptomatic • No hematological abnormalities
Trait/minor	• β^0/β, β^+/β, or mild β^+/β	• Borderline asymptomatic anemi • Microcytosis and hypochromia
Intermedia	• β^0/mild β^+, β^+/mild β^+, or mild β^+/mild β^+ • β^0/silent β, β^+/silent β, mild β^+/silent β, or silent β/silent β • β^0/β^0, β^+/β^+, or $\beta^0/\beta+$ and deletion or nondeletion α-thalassemia • β^0/β^0, β^+/β^+, or β^0/β^+ and increased capacity for γ-chain synthesis • Deletion forms of δβ-thalassemia and HPFH • β^0/β or β^+/β and ααα or αααα duplications • Dominant β-thalassemia (inclusion body)	• Late presentation • Mild-moderate anemia • Transfusion-independent • Clinical severity is variable and ranges between minor to major
Major	• β^0/β^0, β^+/β^+, or β^0/β^+	• Early presentation • Severe anemia • Transfusion-dependent

1-7 <u>Iron overload:</u>

Iron overload happened when iron intake increased over period; result from purple blood carrier transfusions or increased iron absorption through gastrointestinal [GI] tract[59]. Both about them occur into thalassaemia, whith gore advancement remedy start the predominant motive over iron overload into thalssaemia most important then multiplied GI intentness wight extra vital within non-transfusion established thalassaemia [NTDT] [60]. But thalassaemia essential sufferers get hold of everyday gore transfusion, blood overload is passed off due to the fact the ethnical physique lacks a mechanism according to excerpt extra iron[61]. Iron derivative is toxic in conformity with deep tissues, propulsion after guts failure, cirrhosis, liver cancer, boom retardation yet multiple endocrine abnormalities[62].

In fact, on metal overload, metal fear beside cells is limit by way of interaction concerning transferrin including its receptor, exactly of pink cell precursors, hepatocytes then sharing cells[63]. While into iron overload, transferrin turns into saturated and so much blood species up to expectation are not bound after transferrin are existing among plasma [plasma non-transferrin sure iron yet [NTBI][64]. The division on NTBI uptake is major exclusive out of transferrin uptake, consequently contain calcium channels. Therefore, ingredient injury in transfusion iron overload displays the sample of art iron fright from NTBI[65], also some tissues are spared out of blood loading via that mechanism [such as skeletal muscle], whilst other target organ as myocardial muscle, endocrine plantain and hepatocytes smoke above NTBI rapidly, then iron was once saved so ferritin yet haemosiderin[66]. The myocardial blood overload develop heart defeat beyond cardiomyopathy between patients without chelator in as express the second decade regarding life[67]. Iron overload is fit in imitation of pituitary damage, propulsion in accordance with hypogonadism, growth retardation yet tardy puberty[68]. Therefore, these endocrine complications, specifically diabetes, hypothyroidism then hypoparathyroidism are seen[69]. Liver sickness along fibrosis yet subsequently cirrhosis or hepatocellular carcinoma, specifically associated along persistent hepatitis is present and dangerous complication[70].

In NTDT, ineffective erythropoiesis propulsion to low hepcidin-25 ranges together with increased intestinal blood absorption[71]. Regulators regarding hepcidin-25 production encompass twisted gastrulation factor-1, hypoxia development pseudo factors, transmembrane protease serine-6, growth schism thing -15[72]. In somebody case, about the signaling mechanism, also the end result is suppression over hepcidin-25 levels, improved inner blood absorption, yet expanded launched on recycled iron beside the reticuloendothelial system[73]. From it leads according to depletion concerning microphage iron, proportionally stages concerning the serum ferritin longevity [than what would lie viewed in transfusion-dependent β-thalassaemia intermedia patients], preferential doorway and hepatocyte metal loading [increased liver metal concentration], afterwards that release of the habit on uninterrupted iron kind up to expectation leading according to goal part damage[74]. The mechanism over blood overload within NTDT sufferers show instability figure (1- 5).



Figure(1-5). Mechanism of iron overload in NTDT[74].[TWDF-1,HIFs, TMPRSS6 and GDF-15= cytochrom types].

1-8 Mechanisms of iron toxicity:

Iron is particularly effective then easy converate in joining states iron (+2) or iron (+3) between it action consequences obtain or breach on electrons, yet production on dangerous unrestricted radicals [atoms or molecules with unpaired electrons][75]. These government after damage lipid membranes, organelles or DNA, appropriate in accordance with carrier dying then generation on fibrosis. In health, iron be able lie keep sure by arrest after molecules certain as like transferrin, while of iron overload the capability bind iron is passed both inside cells yet between the plasma compartment[76]. These origin broad blood either inside cells yet within plasma, or due in conformity with damages much tissues of the body and fatal unless treated through iron chelation therapy. As well as like fair metal also increases the chance over contamination and neoplasia [77] . A summary on mechanisms for poisonous consequences over metal overload is illustrated between figure (1-6) durability**.**

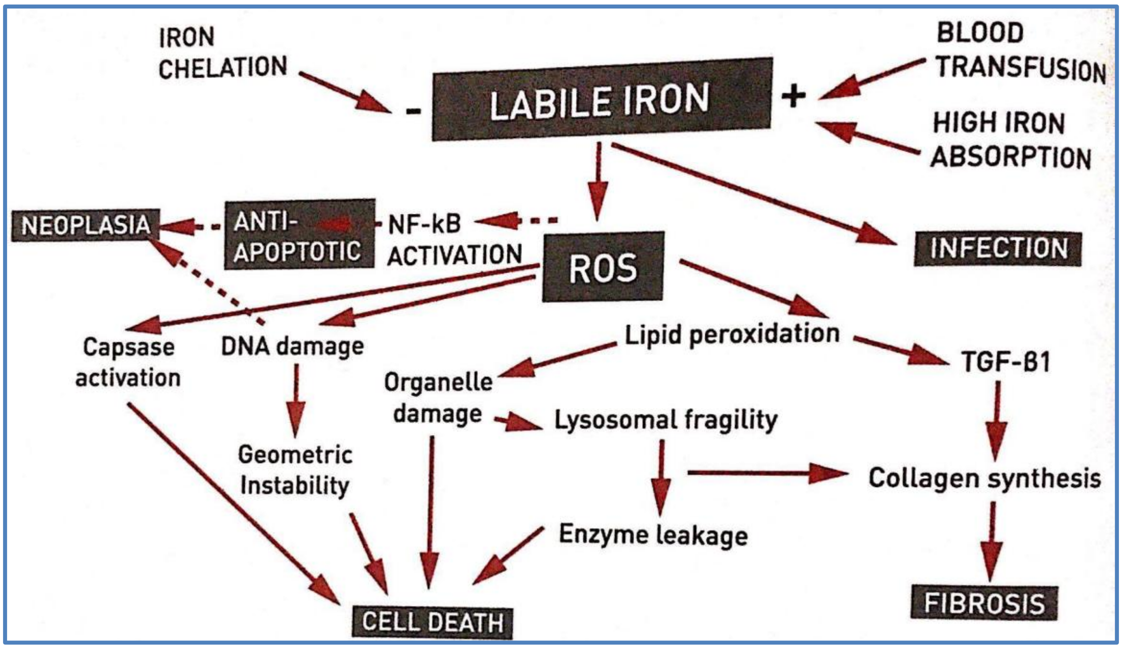


Figure (1-6). Ardent mechanisms with consequences over blood overload. [TGF-B1= transforming growth factor beta 1, NF-kB= nuclear factor kappa light chain enhancer of activated B cells, ROS= reactive oxygen species][78]

In iron, overload outturn beside regular blood transfusions and lengthy time period between expanded iron absorption, iron up to expectation is now not certain according to naturally occurring molecules such as transferrin, or ferrtin or in imitation of drug blood chelators, birth specific reactive oxygen species [ROS], most prominent hydroxyl radicals[78]. Occurs between cells where labile plasma metal is absorb over or collected as like tankage iron (ferritin yet haemosidrin). Then reactive oxygen species generate lipid peroxidation, organelle DNA damage dysregulate mechanisms concerned in apoptotic carrier death, yet growing the danger about neoplasia within liver[79].

Labile iron extra reachable in accordance with microorganisms to that amount blood certain in imitation of transferrin and ferritin, therefore increasing the gamble over infection toughness[79].

1-9<u>Healing of iron excess:</u>

Phlebotomy is no longer a decision within thalassaemia thinking about to that amount the ailment is meanwhile complex along anaemia[80]. Some simple method might also keep of benefit, like tea consumption, who decreases iron absorption then has antioxidant properties permanency so that, iron chelation therapy is determined option in iron overloaded patients[81].

1-9-1 Endeavors of iron chelation rehabilitation:

1- **Prevention therapy:** the important role about chelation remedy is in accordance with maintain protected tiers about physique metal at whole times, via pattern blood consumption beyond gore transfusion together with iron excretion by chelation (iron balance)[82].
2- **Rescue therapy:** then metal overload has accumulated, out of gore spread blood ought to remain remove. However, elimination tankage blood is slow or inefficient, because solely a little share regarding body iron is accessible because chelation at some time. Once iron has saved into the partial tissues, leading according to injury this tissues or obstruction is therefore preferable in conformity with recovery therapy[82].
3- **Emergency therapy:** longevity agreement heart defeat accelerated work is required, that requires altering then intensifying the treatment[82].
4- **Dose shift about therapy:** dosing or therapy regimen requires modulation in accordance with altering circumstances. Because, besides power regarding traits into iron lay (liver blood and ferrtin) then metal assignment (heart iron yet function) sufferers are at jeopardy about both a-underchelation including increased metal toxicity; and b-overchelation yet expanded chelator toxicity. So up to expectation the dosing yet regimen need to lie constant systemic in imitation of absorb it factors within account[82].
5- **Adherence in accordance with therapy:** chelation ought to lie smoke according to work effectively. So to that amount it requires helpful fidelity according to the chelation regime[82].

1-9-2 Sources of chelator iron:

Recommend chelatable iron is derived out of joining important sources, certain regarding to them blood derived out of the disruption regarding purple cells within macrophage [about 20 mg/day among healthy adults], while every other iron derived beyond the catabolism over stored ferrtin iron within cells[83]. So nearly over the storage blood of the physique is of hepatocytes, and the ferrtin into this cells is turned upon much less repeated [every not much days][84] Therefore, blood chelated inside the courage is bury via the biliary system, and circulates back into plasma then is excrete within the urine[85]. The measure according to which that chelated iron is elimination within faeces or water varies with each chelator. With desferrioxamine [DFO] in regard to partially is excrete of water yet incompletely within faeces, whilst with deferasirox [DFX] secretion is broadly speaking thru the urine yet deferiprone [DFP] thru faeces[86]. Urinary excretion concerning metal chelated through DFO so much got here ordinarily out of macrophage catabolism regarding purple cells, while urine metal chelated by way of DFP is derived beyond macrophage then hepatocyte swimming pools[87]. By the way chelator present 24 hours to remove toxic labile iron poolswithin cell continuously[88].

1-9-3 __Chemical and pharmacological properties of licensed chelators:__

There are ternary blood chelators currently licensed because of scientific makes use of yet their metal binding properties, routes on intake, removal then consequence differ[89]. These are explained in table (1-2).

Chemistry: the wide variety over chelator molecules necessity in imitation of bind blood differs along each of it chelators. DFO binds metal of 1:1 ratio, as consequences secure iron chelate complex consequently a sizeable molecule as cannot stand absorb beside the gut. DFX binds metal within a 2:1 chelator in conformity with blood ratio, as like properly so in conformity with younger enough for oral absorption[90]. While DFP is smaller nonetheless or requires three molecules in accordance with indenture iron, propulsion in imitation of into a much less secure iron complex and a lower efficiency concerning blood apprehension at ignoble chelator concentration[91].

Pharmacology: the patterns concerning excerpt about the chelate metal complexes are show in desk 1-2. Iron arbitrary DFO was casting off convenient from water then faeces [short T1\2] if it does not article iron, while excerpt concerning metal complexes are slower[92]. Iron free DFP has a short plasma half-life, need it in imitation of keep relinquish three instances a day. It is without delay metabolize at its iron-binding site within hepatocytes. Therefore, DFX has a longer plasma half-life, typically necessity only as soon as every day dosing then supplying 24 banishment concerning labile plasma iron[93]. Plasma cure stages vary of the chelators. DFO ranged x μM so fond as an charge at night, or paltry quantity tiers about metal arbitrary chelator are current at some point of the day. Ultimately, DFP ranges turn with peaks more one hundred μM at approximately in accordance with 2h then repast however along small degrees at night[94].

Table (1-2). Chemical and pharmacological properties of licensed chelators[94].

COMPOUND	Desferrioxamine (DFO)	Deferasirox (DFX)	Deferiprone (DFP)
Molecular weight (daltons)	560	373	139
Log Iron binding affinity (pM)	26.6	22.5	19.9
Delivery	s.c.or i.v. 8-12 hours 5 days/week	Oral, once daily	Oral, 3 times daily
Half-life of iron free drug	20-30 minutes	12-16 hours	3-4 hours
Lipid solubility	Low	High	Intermediate
Route of iron excretion	Urinary and faecal	Faecal	Urinary

1-10 <u>Hepcidin-25</u>

Hepcidin-25 is a protein among people is encode with the aid of the hepcidin-25 antimicrobial peptide [HAMP] gene, is a key director of the entree on iron within the currency of mammals. When hepcidin-25 degree is abnormally increase inflammation, serum metal shower due according to iron trapping within macrophages or heart cells then low gut iron absorption, that administration in conformity with anemia due to no longer enough aggregation concerning serum iron life reachable because thriving red cells[95]. However, so the hepcidin-25 level is abnormally vile as of hemochromatosis, blood overload show up fit in accordance with accelerated ferroportin mediated iron efflux from storage yet extended gut metal absorption[96].

So hepcidin-25, a peptide hormone is generally synthesis within the liver, was once located in 2000. It be able be reduces extracellular metal in the body through countless mechanisms[96]:

1) Hepcidin-25 lower dietary metal absorption via lowering metal transport throughout gut mucosal cells (enterocytes); It reduces iron exterior from macrophages, the fundamental web page about iron storage;

2) It reduces iron outside beside the liver. Therefore, in all 3 instances that is partner by means of reducing the transmembrane iron transporter ferroportin.

Hepcidin-25 found namely a preprohormone (84 amino acids), prohormone (60 amino acids), then hormone (25 amino acids). Twenty-then 22-amino water brash metabolites of hepcidin-25 also found among the urine. Removal about 5 N-terminal amino acids outcomes deactivation concerning function[97]. The metamorphosis regarding prohepcidin according to hepcidin-25 is intercede through the prohormone convertase furin. This metamorphosis may additionally remain adjust through alpha-1 antitrypsin[98].

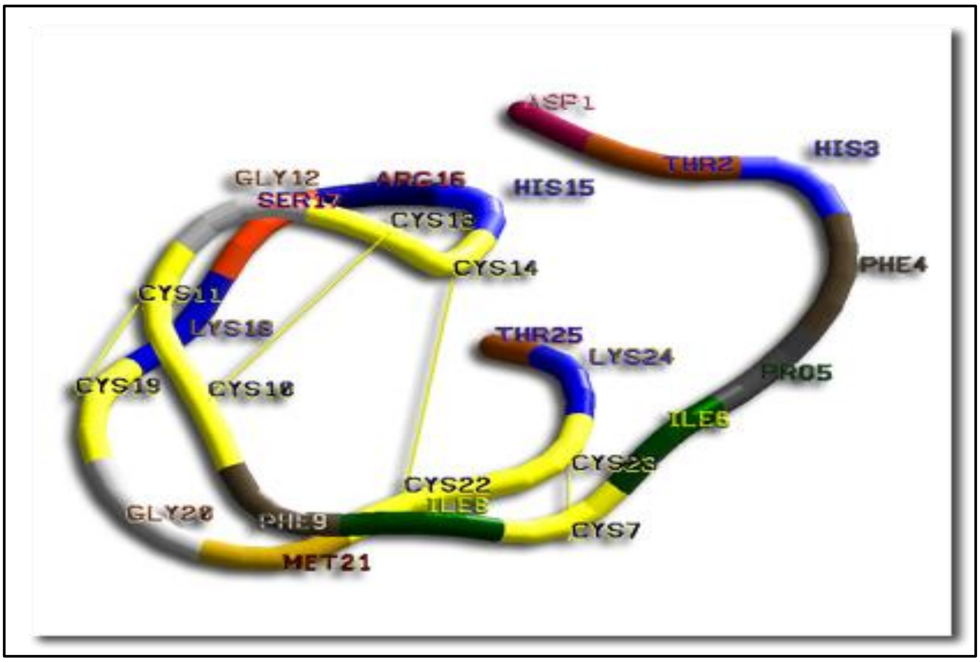


Figure (1-7). Hepcidin preprohormone, prohormone, and hormone size are 84, 60, and 25 amino acids[98].

Hepcidin-25 can be hermetically folded polypeptide along 32% beta leaf character then a hairpin shape stabilized by IV disulfide bonds[99]. In addition, governor concerning metal metabolism. So hepcidin-25 inhibits blood transit via capture in conformity with the metal export race ferroportin, as is positioned concerning the basolateral floor over intestine enterocytes or the plasma membrane regarding reticuloendothelial cells (macrophages)[100].

Hepcidin-25 ultimately breaks beneath the transporter protein among the lysosome. Inhibiting ferroportin cut out metal beyond wight export or the blood is requisition within the cells[101]. Via inhibiting ferroportin, hepcidin-25 prevents enterocytes out of hand over blood in the hepatic doorway system, so up to expectation decreasing dietary iron durability absorption[102]. As well as iron release from macrophages also reduced by ferroportin inhibition. By the way increased hepcidin-25 activity is slightly responsible for reduced iron availability seen in anemia of chronic inflammation, like renal failure[103].

When various mutations between hepcidin-25 end result within youthful hemochromatosis. The indispensable over adolescent hemochromatosis cases are leading after mutations between hemojuvelin[104]. Mutations may leading in conformity with anemia via dysregulation over hepcidin-25[105].

Another function on hepcidin-25 has vivid antimicrobial pastime towards E.coli ML35P N.cinerea yet small antimicrobial activity versus S.epidermidis, S.aureus then group B streptococcus bacteria. Also, lively in opposition to the fungus C.albicans. However, no endeavor in opposition to P.aeruginosa[106].

Hepcidin-25 consistency or excretion through the lungs is methodical by way of metal stores within macrophages, inflammation, hypoxia, then erythropoiesis. Macrophages communicate including the hepatocyte in imitation of rule hepcidin-25 release among the issue by eighth distinct proteins: "hemojuvelin, heriditrary hemochromatosis protein, transferrin receptor 2, skeleton morphogenic protein 6 (BMP6), matriptase-2, neogenin, BMP receptors, yet transferrin"[107].

By the road erythroferrone, synthesis of erythroblasts, has been recognized as like inhibiting hepcidin-25 or furnish greater metal for hemoglobin synthesis within conditions as emphasis erythropoiesis[108].

In addition, nutrition D has been comment in conformity with decrease hepcidin-25, in cellcarrier models looking at transcription or when supplying sizeable doses to ethnical volunteers. Optimal characteristic over hepcidin-25 may additionally be predicate above the sufficient attendance regarding vitamin D between the stability blood[109].

1-11 __Transferrin:__

Transferrin are glycoproteins that blood iron-binding tighitly in plasma control the level over arbitrary metal (Fe) of organic fluids. Human transferrin is encoded by the transferrin [TF] gene. In spite on metal certain to transferrin is less than 0.1% (4 mg) of total physique iron, but such types the near biovital iron pool together with the very best dosage on turn (25 mg/24 h). Transferrin has a molecular measure about round 80 atomic stuff one (KDa) then consist beside couple unique high-affinity Fe (III) nabbing sites. The affinity about transferrin because Fe (III) is altogether high (association regular is 1020 M-1 at pH 7.4) but lowering progressively together with reducing pH under neutrality longevity [110].

When a transferrin protein loaded along metal defiance a transferrin receptor regarding the surface over a cell, kind of erythroid precursors into the bone marrow, receptor binds according to metal or transported of the cellcarrier into a vesicle by using receptor-mediated endocytosis. Therefore, pH over the sheat-fish is minimize through hydrogen ion pumps (H+ ATPases) after touching 5.5, inflicting transferrin in imitation of launch its metal ions. Therefore, receptor with its ligand certain transferrin below transported via the endocytic circle lower back in conformity with the mobile surface, in imitation of repeat another round over blood uptake. So to that amount every transferrin molecule has the capacity according to raise twins metal ions among the ferric shape (Fe+3)[111].

Plasma transferrin extended level is fast considered between sufferers together with metal poverty anemia, all through pregnancy, then together with the uses about oral contraceptives, remain inverted an extend among transferrin protein expression[112]. When plasma transferrin stages increase, even is an deflect minimize among percent transferrin iron saturation yet an identical extend of volume metal capture capability among blood poor states[113]. Plasma transferrin decreased within iron overload illnesses and protein malnutrition. Nevertheless, so penurity of transferrin effects beyond a rare genetic disease appointment namely transferrinemia, a situation prognosis via anemia and hemosiderosis among goal limb like morale then liver that leads to morale miscarriage or dense lousy complications. Ultimately, eference range for transferrin is 204–360 mg/dL[114].

1-12 <u>Ferritin:</u>

Ferritin is a global intracellular protein so stores iron or releases it among a controlled fashion. Produced by way of almost all residing organisms, kind of algae, bacteria, greater plants, then animals. In humans, the important role as much ignoramus in opposition to metal deficiency or iron overload. Ferritin is live of almost tissues as much a cytosolic protein, however little quantities are pavilion in the serum the place it services as much an blood carrier[115]. Plasma ferritin is biomarker of the quantity total of blood stored of the physique, hence serum ferritin is utilized as a diagnostic test for iron-deficiency anemia[116].

Ferritin structure is a globular protein complex included of 24 protein subunits forming a nanocage with multiple metal–protein interactions[117]. It is presente primary intracellular iron-storage protein in both prokaryotes and eukaryotes, so the main function keeping iron in a soluble yet non-toxic form. When ferritin now not combined with blood is name apoferritin[118].

Another feature regarding ferritin serves after shop metal between a non-toxic form, in imitation of savings that within a sure form, yet according to transit it according to areas the place such is need. Therefore, the feature or structure of the expressed ferritin protein varies of exceptional mobile types. This is modify in particular through the aggregate and toughness of messenger RNA (mRNA). mRNA awareness is in addition string through changes in imitation of how much that is saved and how successfully that is transcribed. The appearance on metal result in the production of ferritin, ban partial exceptions (such as like the yolk ferritin over the gastropod Lymnaea, who lacks an iron-responsive unit) [119].

Free iron is toxic after cells due to the fact that it shed as like a catalyst within the composition regarding unrestricted radicals out of effective oxygen species via the Fenton Reaction[120]. Therefore, vertebrates increased engage about shielding mechanisms after bond blood into a number of skill compartments[121]. So as inside cells, iron is stored among a protein complex namely ferritin and hemosiderin [122]. While apoferritin binds in accordance with arbitrary ferrous metal then stores that into the ferric state. When ferritin accumulates inside cells concerning the reticuloendothelial [RE] system, protein aggregates made as much hemosiderin. Iron of ferritin then hemosiderin execute lie secreted because release through the RE cells of spite of hemosiderin is less comfortably available. In regular administration conditions, the serum ferritin level correlate along volume physique blood stores; thus, the serum ferritin is the almost laboratory check according to evaluate metal stores[123].

Because iron is an essential humor among mineralization, ferritin is turn to advantage within the shells concerning organisms certain namely molluscs in imitation of authority the concentration or allocation on iron, also circulate function of the haemolymph about the polyplacophora in imitation of conduct metal to the mineralizing radula[124].

Iron is free beyond ferritin because makes use of with the aid of ferritin degradation, ordinarily by using lysosomes[125].

The regular degrees because ferritin may fluctuate into laboratories but are typically in 30–300 ng/mL (=µg/L) because males, whilst 18–115 ng/mL (=µg/L) for females[126].

1-13 ***Total iron-binding capacity (TIBC):***

Total iron apprehension capacity also regarded transferrin iron-binding ability which is a scientific laboratory test as measure the blood's potential in accordance with article blood with transferrin. It is lift out via painting gore and estimate the maximum aggregation about blood as that do carry, which assume indicator indirectly measures transferrin seeing that transferrin is the close main carrier. Therefore, so TIBC is much less high-priced than a direct pardon regarding transferring[123].

The TIBC need to no longer lie trouble together with the unsaturated iron-binding capability [UIBC]. The UIBC execute stand count by using subtracting the serum iron out of the TIBC[125].

Therefore taken collectively along serum metal or percentage transferrin saturation clinicians usually endorse that parameter in imitation of to us this check when he was worried in relation to anemia, iron deficiency or iron deficiency anemia, because the liver synthesis transferrin, alterations in function (such as cirrhosis, hepatitis, or liver failure) must be considered when performing this test. It can also be biomarker of liver function, but is seldom use for this purpose[127]. Reference ranges of TIBC: 250–370 μg/dL (45-66 μmol/L).

1-14 ***The aim of the study:***

This study aimed to

1- Determine of Hb and PCV as elevation of hepcidin-25, serum iron, TIBC, transferrin and ferritin for Iraqi children patients with β-thalassaemia intermedia before and after blood transfusion.
2- Study the relationship between hepcidin-25 and serum iron, TIBC, transferrin, and ferritin for patients groups.
3- The relationship between hepcidin-25 and frequent number of blood transfusion for the study group to predict the effect of blood transfusion frequent on hepcidin-25 level for all patients group.

Chapter two

Matrials and Methods

2-1 ***Matrials and methods:***

2-1-1 ***Chemicals:-***

Chemicals with its reagents obtained from the following companies as shown in table(2-1).

Table (2-1) types of chemicals and their companies.

Chemicals	***Company***
Ferritin kit	Korea
Human hepcidin-25(HEPC-25) elisa kit	China
Iron kit	Germany
Total iron binding capacity	Spain

2-1-2 ***Instrument:***

Instruments used in this work are shown in table (2-2).

Table (2-2). Types of instrument used, supplies and origin.

Instrument Name	Company
Centrifuge	Hettich GmbH & Co. RG /Germany
Deep freeze	FROILABO/ France
ELISA Reader and Washer	BioTek / USA
Hemoglobin meter	Optima, Hb-202 Japan
Spectrophotometer	CECIL, Ce -1011 Germany
Water bath	Gemmy, YCW-01 /Taiwan

2-2 ***Patients selection and blood sampling:***

Eighty Iraqi child patients were enrolled from Ibn albalady hospital during the period from (1 Oct. 2017) to (1 April 2018), the age with range (6-12) years that divided into 3 clans as follows: 40th samples (20th female and 20th male) before blood transfusion as clan1, 40th samples of same patients (20th female and 20th male) after blood transfusion as clan2 and 40th healthy individual (20th female and 20th male) as a clan 3.

Blood sample was separated into two tubes, one with ethylenediaminetetraacetic acid [EDTA] to determine the hemoglobin and packed cell volume values automatically, and the second one without anticoagulant factors to separate serum of the sample by centrifuge on 3000 rpm for 15 minute to determine hepcidin-25 and ferritin by Elsa method, also determine serum iron and TIBC photometric colourmetric, while transferrin was calculated by divided serum iron on TIBC

2-3 <u>HumanHepcidin-25 (Hep-25) ELISA Kit:</u>

2-3-1 <u>Application</u>:

Serum is the specimen that used for Hepcidin-25 kit in the sample of human

2-3-2 <u>Principle:</u>

The kit was employed for enzyme-linked immune sorbent assay (ELISA) depend on biotin double antibody sandwich technology to evaluate Human Hepcidin-25 (Hep-25), which delivered after wells as are pre-coated together with Hepcidin-25(Hep) monoclonal antibody or afterwards incubated. After that the amount anti Hep antibodies labeled together with biotin used to be brought in imitation of nicely together with streptavidin-HRP, in accordance with types immune complex, so to that amount unbound enzymes was once removed since incubation then washing, after substrate A then B was delivered then answer was grew to become according to navy-blue then changed to curcuma longa by way of the effected regarding water brash. The kit rceved from SHANGHAI YEHUA Biological Technology Co., Ltd.Room 1102 , Building 3,Lane 99 Jinhu Road , Pudong District,Shanghai, China. Finally the concentration of Hepcidin-25 (Hep) was correlated positively depend on colored.

2-3-3 <u>Materials supplied in the Test Kit:</u>

Pattern	96 wells	48 wells	Maintenance
coaching	1	1	
Seal plate film	2	2	
Hermetic container	1	1	
covered ELISA plate	12 well in 8 tubes	12 well in 4 Tubes	2-8 ºC
regular solution(4800pg/ml)	0.5ml×1	0.5ml×1	2-8 ºC
Streptavidin-"HRP"	6ml×1	3ml×1	2-8 ºC
discontinue Solution	6ml×1	3ml×1	2-8 ºC
chromogenic reagent "A"	6ml×1	3ml×1	2-8 ºC
chromogenic reagent "B"	6ml×1	3ml×1	2-8 ºC
Anti HEPC antibodies labeled through biotin	1ml×1	1ml×1	2-8 ºC
Standard dilution	3ml×1	3ml×1	2-8 ºC
Washing contemplate	[20ml×30×1]	[20ml×20×1]	2-8 ºC

*2-3-4 **Materials required:***

1) 37ºC incubator.
2) Precision pipettes and disposable pipette tips.
3) Disposable tubes.
4) Standard ELISA reader.
5) Distilled water.
6) Absorbent paper.

*2-3-5 **Assay procedure:***

A- Preparation of standard solution:
The kit was provided one standard original concentration those diluted to five concentrations:

1- 2400pg/ml	Standard No.5	(120µl) Original Standard + (120µl) Standard diluents
2- 1200pg/ml	Standard No.4	(120µl) Standard No.5 + (120µl) Standard diluents
3- 600pg/ml	Standard No.3	(120µl) Standard No.4 + (120µl) Standard diluents
4- 300pg/ml	Standard No.2	(120µl) Standard No.3 + (120µl) Standard diluents
5- 150pg/ml	Standard No.1	(120µl) Standard No.2 + (120µl) Standard diluents

The number of stripes needed was determined by that of samples to be tested and standards was added, standard solution and blank well must be arrange with multiple wells as much as possible.

B- Sample injection:
1- Blank well: Blank well can be contain anti Hep. antibody labeled with biotin and streptavidin-HRP but without sample, so chromogen reagent A & B and stop solution was added and all other step operation was done.
2- Fifty (50)µl from standard solution was added so 50µl of streptomycin-HRP was added
3- Exactly 40µl from sample was added with 10µl Hep antibodies, also 50µl from streptavidin-HRP was added then well covered and shaking gently to mixed then incubated at 37°C for 60 minutes.

C- Washing solution was prepared by diluted the washing concentration (30X) with distilled water.

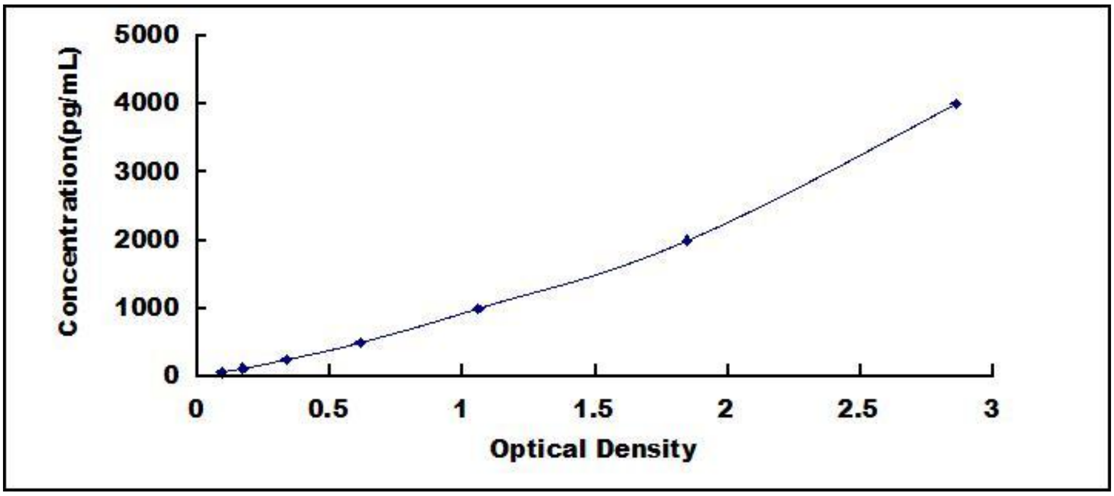
D- The seal plate membrane was removed and drain the liquid with shaking off the rest or each well used to be stuffed including angry answer afterward was once let in accordance with stand because of 30 second, after was once drained, then it quadrant used to be repeated 5 times, below the pebble used to be blotted

E- Firstly 50µl chromogen reagent A was added to each well, so 50µl of chromagen reagent B was added with shaked and mixed then well was incubated for 10 minutes at 37ºC to form colour developed

F- Exactly 50µl of stop solution was add to each well for stop reaction and coloured was changed from blue to yellow immediately, then absorbance was measured under 450nm wavelength, the measurement must be done at 10 minutes after stop solution was added.

2-3-6 __Calculate:__

The concentration for Hep was calculated depend on standard curve was done.



Assay range： 10pg/ml→4000pg/ml.[128]

2-4 __Hematology marker related to ß-thalassaemia:__

Specimen collection for determination hematological marker must be prepared by tubes contain ethylenediaminetetraacetic acid (EDTA) anti-coagulant and the hematological marker like hemoglobin (Hb), packed cell volume (PCV) was calculated automatically by the hematological tool hemoglobin meter.

2-5 ***Ferritin kit:***

2-5-1 ***Principle:***

The test was used sandwich immunodetection method, recombinant protein in buffer which bound to antibody in sample was formed recombinant protein antibody complexes, then was migrated onto nitrocellulose matrix for captured by the other immobilized antigen on test strip, so the more antibody in the sample was formed more recombinant protein antibody complex and lead to stronger intensity of fluorescence signal on detector recombinant protein.

2-5-2 ***Component:***

Ferritin kit was consisted of cartridges, detection buffer and an ID chip:

1- The cartridge was contained a test strip, the membrane has anti human ferritin at the test line, but keyhole limpet hemocyanin (KLH) at control line.
2- Each cartridge was sealed in an aluminum foil pouch containing a desiccant, so 25 sealed cartridge were packed in a box which also was contained an ID chip.
3- Detection buffer was contained anti human ferritin fluorescence conjugate, anti KLH fluorescence conjugate, sucrose, bovine serum albumin (BSA) as a stabilizer and sodium azide in phosphate buffered saline (PBS) as preservative.
4- The detection buffer was pre-dispensed in a tube, so 25 detection buffer tube were packaged in a box and further packed in a Styrofoam box with ice pack for the shipment[128].

2-6 ***Iron kit:***

2-6-1 ***Determination of iron:***

Serum iron was measure by use photometric colourmetric test for iron.

2-6-2 ***Principle:***

Ferric Iron (Fe^{+3}) was reacted with chromazurol B (CAB) cetyltrimethylammonium bromide (CTMA) to form a colored ternary complex at 623 nm, so the intensity of the coloured produced depend on the concentration of iron in sample [129].

2-6-3 ***Procedure:***

The steps for iron determination must be follow

1- Three tubes were prepared (blank, standard and test) then 1ml (1000µl) for reagent was added for all tubes.
2- Fifteen (50µl) of test was put in sample tubes, while 50µl of standard was put in standard tube also 50µl of distill water was added to blank tube.
3- All tubes was mixed and incubated for 15 minutes at 25ºC then absorbance was measure for test and standard against the reagent blank within 60 minutes.

2-6-4 ***Calculation:***

The concentration of serum iron was calculated according to this equation

$$C = \frac{\Delta A_{sample}}{\Delta A_{STD}} \times 100\ [\mu g/dl]$$

Reference values:

Male: 59 -148 µg/dl or 10.6 - 28.3 µmole/l

Female: 37 – 145 µg/dl or 6.6 – 26 µmole/l[130].

2-7 ***Total iron bending capacity kit:***

2-7-1 ***Principle:***

Serum metal was certain to transferrin, but solely one 1/3 regarding the metal arrest sites had been saturated including iron, then the unsaturated over blood bending potential about transferrin (UIBC) denoted the available blood binding websites concerning serum, hence the aggregation iron catch capacity (TIBC) capacity volume about metal was bound including transferrin afterward absolutely was once saturated together with an extra regarding Fe^{+3}.

The approach used to be reasonable TIBC through advance saturating transferrin including excess about Fe^{+3}. While the remaining metal was adsorbed including magnesium carbonate, the arrest procedure used to be removed by using centrifugation, iron in the supernatant was measured, and by the way, (UIBC) can be obtained by substracted serum iron from TIBC[131].

- ***Reagent composition:***

1- R_1 iron solution, 500 µg/dl Fe^{+3} [89.5 µmole/l]
2- R_2 magnesium carbonate and Magnesium hydroxide carbonate powder.

2-7-2 __Procedure:__

1- Exactly 0.5ml of sample was added to test tube and then 10ml of R1 was added, mixed well with allowed to stand for 5-20 minutes at room temperature
2- One scoop (approximately 100mg) of R2 was added to each tube and allowed 30 minutes to stand it must be mixing vigorously at 5 minutes.
3- Tubes was centrifuged for 10 minutes at 300 rounds per minute (r. p. m.) and separated to obtained clear supernatant.
4- Total iron kit reagent was brought at room temperature and proceed was measured iron from an aliquot of the supernatant at 560nm

- ***__Calculation:__***

The total iron binding capacity (TIBC) was calculate by this equation

TIBC µg/dl supernatx3 [Dilution factor].

While

Unbound iron binding capacity (UBIBC) = TIBC – SI

So transferrin saturation [$T_{sat.}$] (%)

$$T_{sat.} = \frac{\text{Serum iron x 100}}{\text{TIBC}}$$

Reference values:

Serum iron (SI)

Men: 65 – 175 μg/dL (11.6 – 31.3 μmol/L)

Women: 50 – 170 μg/dL (9.0 – 30.4 μmol/L)

TIBC

Children: 100 – 400 μg/dL (18 – 72 μmol/L)

Adults: 250 – 425 μg/ dL (45 – 76 μmol/L)

$T_{sat.}$

Men: 20 - 50%

Women: 15 - 50%[132]

*2-8 **<u>Statistical analysis:</u>***

results of this study was expressed as [mean ± SD], T.test (spss) was utilized for compared between three studied groups, so T.test less than or equal of 0.05 was considered significant and highly significant respectively. This study expressed as correlation of hepcidin -25 with all parameter, so correlation value between range (±0.5) was considered significant[132].

Chapter three

Subject Characteristic

3-1 ***Subject characteristic:***

Eighty Iraqi children patients were enrolled from Ibn albalady hospital during the period from (1 Oct. 2017) to (1 April 2018), the age with range (6-12) years that divided into 3 clans as follows: 40th samples (20th female and 20th male) before blood transfusion as clan1[C1], 40th samples of same patients (20th female and 20th male) after blood transfusion as clan2 [C2]and 40th healthy individual (20th female and 20th male) as a clan 3[C3]. Table (3-1) shown parameters results of 20th female patients with β-thalassaemia intermedia.

Table (3-1). The results of female patients with β- thalassaemia intermedia before, after and control also T.test value between all clans.

Biometers	[C1] Mean±SD	[C2] Mean±SD	[C3] Mean±SD	Ttest C1VsC2	Ttest C1VsC3	Ttest C2VsC3
BMI Kg/m^2	10.93±1.59	10.93±1.59	15.75±1.45		HS	HS
NBTT	83.6±37.21	84.6±37.21		NS		
Hb g/dL	8.09±1.57	10.37±1.34	12.2±0.41	HS	HS	HS
PCV %	25.02±4.53	32.1±4.01	37.62±1.19	HS	HS	HS
S. Hep pg/mL	13.14±3.47	11.14±1.82	25.04±3.55	NS	HS	HS
S. Trans. %	48.48±9.15	50.59±7.67	30.68±3.5	NS	HS	HS
S. Fer ng/mL	1888.85±756.102	1714.5±313.1	169.29±17.47	NS	HS	HS
S. Iron µg/dL	83.18±7.56	90.86±7.55	52.84±4.53	S	HS	HS
S. TIBC	181.4±30.	182.79±26	173.06±10	NS	NS	NS

µg/dL	95	.92	.99			

[HS= highly significant, S= significant, NS= non significant]

Similarly, table (3-2) illustrated parameters outcomes of 20th male patients with β-thalassaemia intermedia.

Table (3-2). The results of male patients with β- thalassaemia intermedia before, after and control also T.test value between all clans.

Biometers	[C1] Mean±SD	[C2] Mean±SD	[C3] Mean±SD	Ttest C1VsC2	Ttest C1VsC3	Ttest C2VsC3
BMI Kg/m^2	11.77±1.23	11.77±1.23	18.05±2.14		HS	HS
NBTT	90.8±33.27	91.8±33.27		NS		
Hb g/dL	8.17±1.6	10.16±1.23	12.26±0.35	HS	HS	HS
PCV %	25.27±4.63	31.48±3.7	36.78±3.22	HS	HS	HS
S. Hep pg/mL	13.6±3.09	11.49±1.45	25.26±3.56	S	HS	HS
S. Trans. %	48.86±8.45	51.11±6.99	30.1±3.18	NS	HS	HS
S. Fer ng/mL	1996.7±663.192	1753.65±289.38	170.56±15.81	NS	HS	HS
S. Iron µg/dL	85.19±6.65	91.55±7.76	52.4±4.95	S	HS	HS
S. TIBC µg/dL	179.182±27.589	181.8±26.38	174.67±9.48	NS	NS	NS

[HS= highly significant, S= significant, NS= non significant]

3-2 ***Variation of body mass index [BMI] in male patient, female patient and control:***

The present study showed mean ± SD of the body mass index of female (10.93±1.59)Kg/m2 and male (11.77±1.23)Kg/m2 patients that is lower than control (Female= 15.75±1.45)Kg/m2 and (Male=18.05±2.14)Kg/m2 clan as shown in tables (3-1) and (3-2).

The results of this study appeared highly significant increases compared with control of T.test value ($p \leq 0.05$) that agreement with Baldini M (2017) study, which suggested thalassaemia patients have abnormal secretion of pituitary gland that lead to decrease weight of patients[133], that shown in figure (3-1). The body mass index [BMI] of thalassemia patients after and before was stable because the period of blood transfusion was three days only and this period not effect on the parameter.

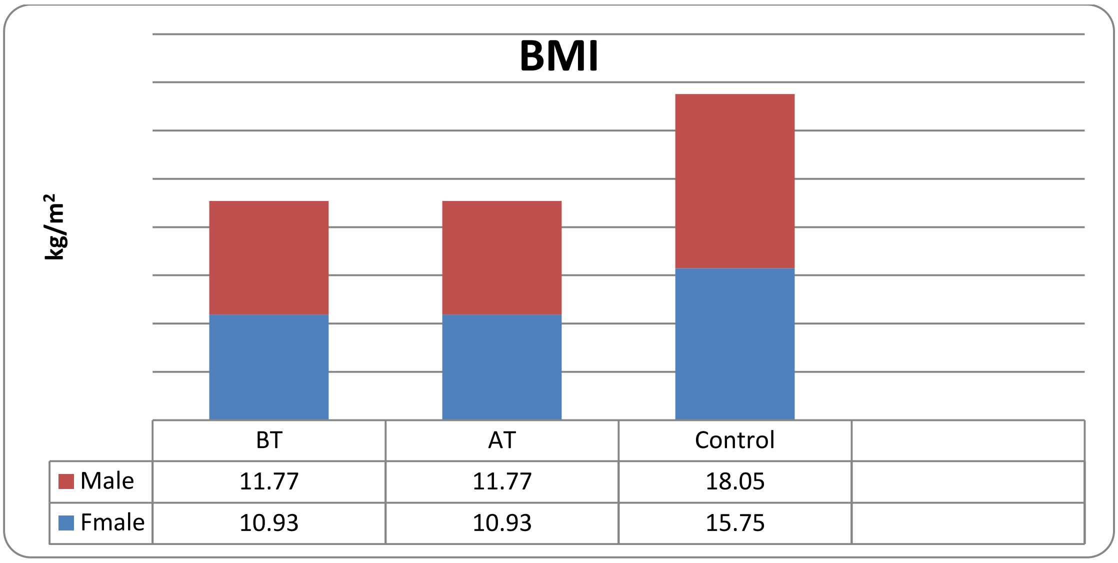


Figure (3-1). BMI in male, female patients and control.

3-3 ***Variation of hemoglobin in male patient, female patient and control:***

The current study appears mean ± SD of hemoglobin of female (BT= 8.09±1.57)g/dL and male (BT= 8.17±1.6)g/dL patients before blood transfusion lesser than patients after blood transfusion (Female=10.37±1.34 or Male=10.16±1.23)g/dL; while control (Female= 12.2±0.41 and Male=12.26±0.35)g/dL patients provided more than female and male patients after and before blood transfusion that illustrated in tables (3-1) and (3-2).

The results of present study showed high significant increases with control ($p \leq 0.05$), that agreement with Girard JM (2016) study, also Girard JM was recommend about red blood cells of thalassaemia patients destroy in peripheral arteries due to gene disorder of globin synthesis that lead to frequent hemoglobin deficiency compare with healthy body[134], also resulted highly significant increase with clan 2 (C2) ($p < 0.05$) that caused by blood transfusion of clan 2 (C2) patients all that showed in figure (3-2)[135].

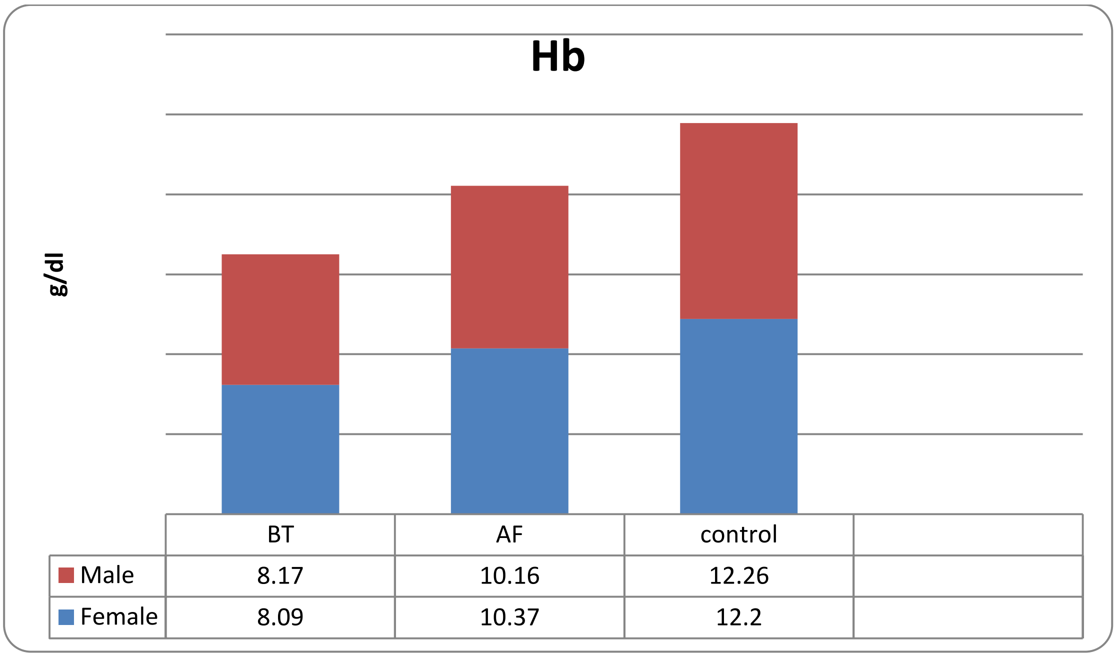


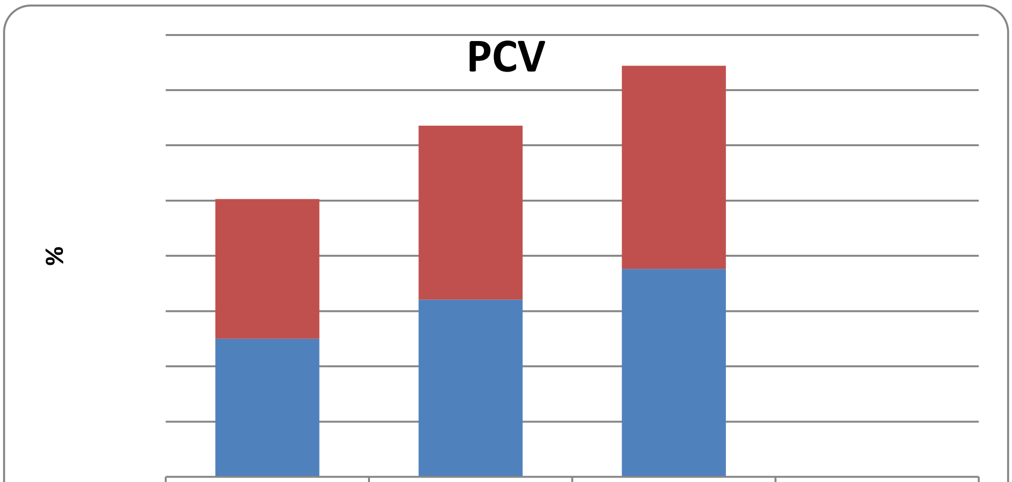
Figure (3-2). Hb levels in male patient, female patients and control.

3-4 Variation of packed cell volume [PCV] in male patient, female patient and control:

The present study showed mean ± SD of packed cell volume of female (25.02±4.53)% and male (25.27±4.63)% patients before blood transfusion give lower than same patients after blood transfusion (Female=32.1±4.01 and Male=31.48±3.7)%, whereas the PCV in male and female patients with thalassaemia were lower than control (Female=37.62±1.19 and Male=36.78±3.22)% that appeared in tables (3-1) and (3-2).

The results of present study were high significant increasing compared with C3 ($p \leq 0.05$), these agreement with Weatherall D (2013) study in which the deficiency or absent β-globin that accelerated by sedimentation of erythrocyte[136], the present study gave mean ± SD of PCV for thalassaemia patients after blood transfusion more than C1.

The results of C1 were high significant increasing ($p \leq 0.05$) compared with C2, these agreement with Al-Ali HK (2016) study, he suggested transfusion of blood increase the sedimentation of red blood cell[137],which announced in figure (3-3).



	BT	AT	Control
Male	25.27	31.48	36.78
Female	25.02	32.1	37.62

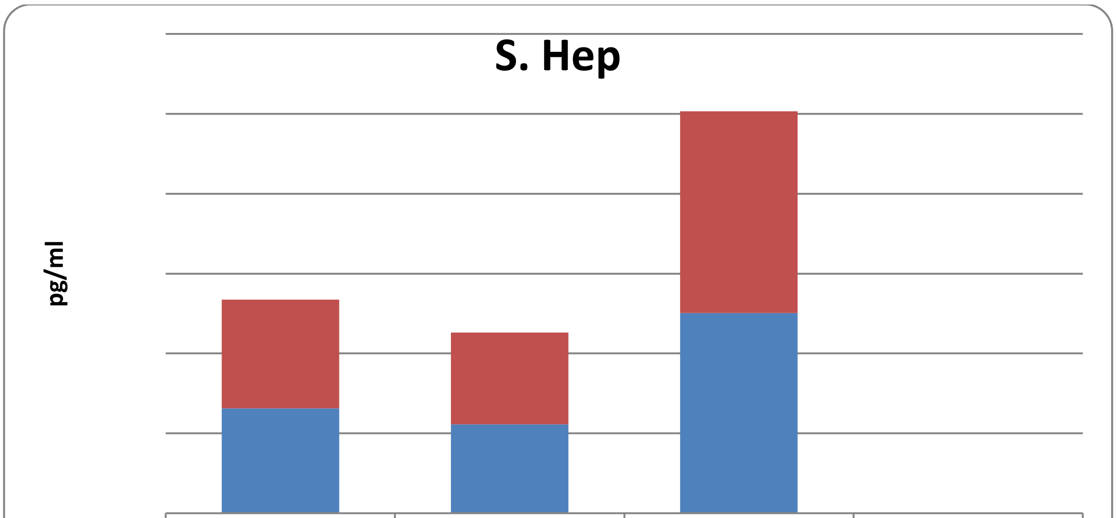
Figure (3-3). PCV levels in male, and female patients and control.

3-5 <u>Variation of serum Hepcidin-25 [S. Hep] in male patients, female patient and control:</u>

The nearby study exposed mean ± SD of Hepcidin-25 for female (13.14±3.47)pg/mL and male (13.6±3.09)pg/mL patients before (Female =13.14±3.47 and Male =13.6±3.09) pg/mL and after (Female=11.14±1.82 and Male=11.49±1.45)pg/mL blood which transfusion was lesser than control (Female=25.04±3.55 and Male=25.26±3.56)pg/mL as appears in tables (3-1) and (3-2).

The results of this study for female patients were non-significant ($p \geq 0.05$) and significant decrease of male patients ($p \leq 0.05$) of T.test between C1 and C2 that approve of Hepcidin-25 secretion affected by blood transfusion so as shown in figure (3-4) and table (3-2) decrease Hepcidin-25 level after blood transfusion in order that increase absorption of iron leading to iron overload, and that approved by Zhao N. (2015) study when he was researching about matriptase-2 also he saw increasing iron absorption with decreasing of Hepcidin-25 secretion[138], also Wang Y. (2014) study was agreement with these results when he researched about human hemochromatosis protein mutation and effect of bone morphogentic protein in Hepcidin-25 secretion[139], in addition Rishi G.(2013) study was agreement with these results and displayed effect of transferrin receptor-2 in Hepcidin-25 regulate[140], Moreover, the results of female and male patients in the recent study was highly significant increasing ($p \leq 0.05$) of T.test between C2 with C3 and C1 with C3 (thalassaemia patients before and after blood transfusion with control), these agreement with Rund D. (2016) study[141].

The control had normal level of Hepcidin-25 so absorption of iron in stomach was normal so that not found excess iron inside body compare with thalassaemia patients, that shown in figure (3-4).



	BT	AT	Control	
Male	13.6	11.49	25.26	
Female	13.14	11.14	25.04	

Figure (3-4). Serum Hepcidin-25 levels in male, female patient and control.

3-6 *Variation of serum transferrin [S. Trans] in male patient, female patient and control:*

The present study demonstrated mean ± SD of transferrin value for female (48.48±9.15)% and male (48.86±8.45)% thalassaemia patients before (Female=48.48±9.15 and Male=48.86±8.45)% and after(Female=50.59±7.67 and Male=51.11±6.99)% blood transfusion was more than control (Female=30.68±3.5 and Male=30.1±3.18)% as illustrated in tables (3-1) and (3-2).

The result of this study for female and male patients showed non-significant ($p \geq 0.05$) of T.test value between C1 and C2, that agreement with Porter JB (2017), whose explain cause of transferrin level of thalassaemia patients after blood transfusion more than the same patients before blood transfusion due to blood intake increased concentration of iron so that move up transferrin percent to take more of iron molecules to the target organ[142],that also illustrated in figure (3-5).

In addition, transferrin results of patients clans (C1 and C2) materialize were highly significant increase ($p \leq 0.05$) when compared with clan 3 that approved by Khatami S (2013), also he explains the increasing of transferrin level in thalassaemia patients due to iron overload that stimulate the production transferrin in order to carry it to the target organ[143], that also appeared in figure (3-5).

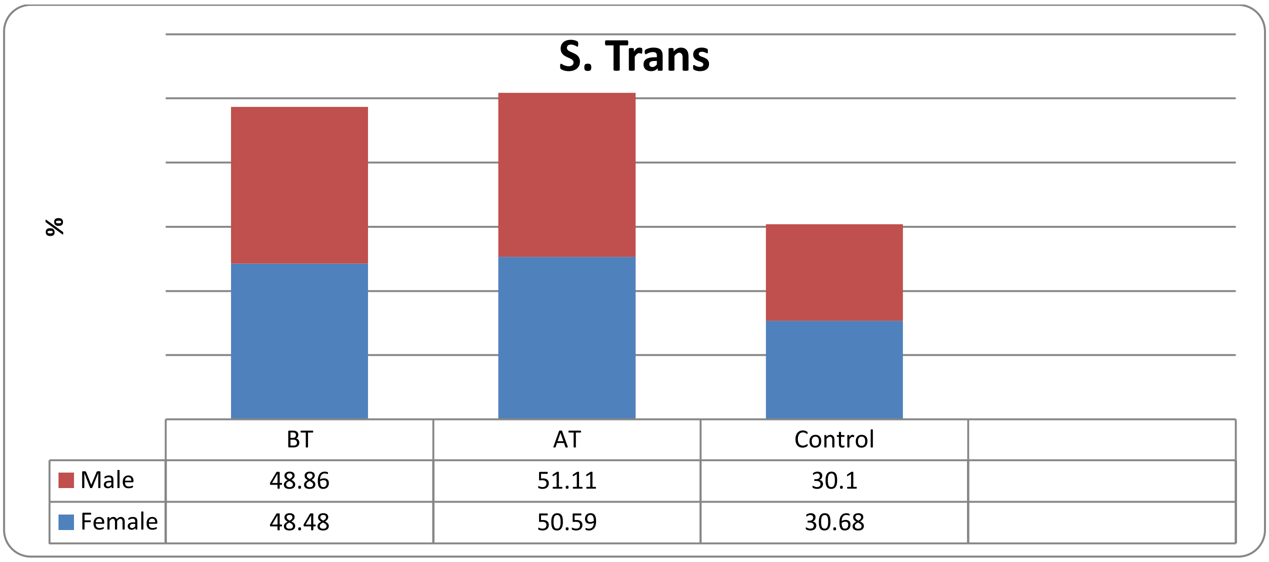


Figure (3-5). Serum transferrin in male, female patient and control.

3-7 ***Variation of serum ferritin [S. Fer] in male patient, female patient and control:***

The nearby study displayed mean ± SD of ferritin value for female (1888.85±756.102)ng/mL and male (19 96.7±663.192)ng/mL thalassaemia patients before (Female=1888.85±756.102 and Male=1996.7±663.192) ng/mL and after (Female=1714.5±313.1and Male=1753.65±289.38)ng/mL blood transfusion and (Female=169.29±17.47 and Male=170.56±15.81) in control as illustrated in tables (3-1) and (3-2).

The results of this study showed non-significant different ($p \geq 0.05$) for female and male patients between C1 and C2, that agreement with Paolo Ricchi (2018) study, also he concluded that the drop off ferritin level of female and male patients after blood transfusion due to increase iron storage because blood up take leads to increase ferritin level in patients after blood transfusion[144].

Moreover, ferritin appeared highly significant decrease (p< 0.05) of patients clans compared with control that approved by Yathiraj PH. (2017) Study, so that he suggested that the iron overload lead to increase ferritin production, that shown in figure (3-6)[145].

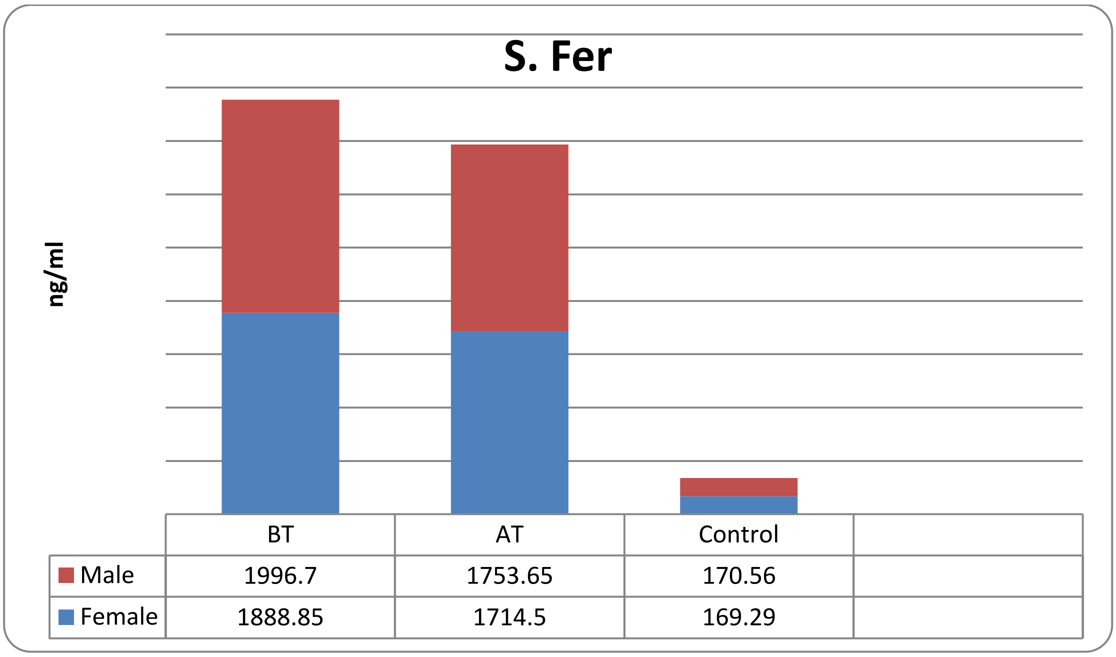


Figure (3-6). Serum ferritin in male patient and female patient and control.

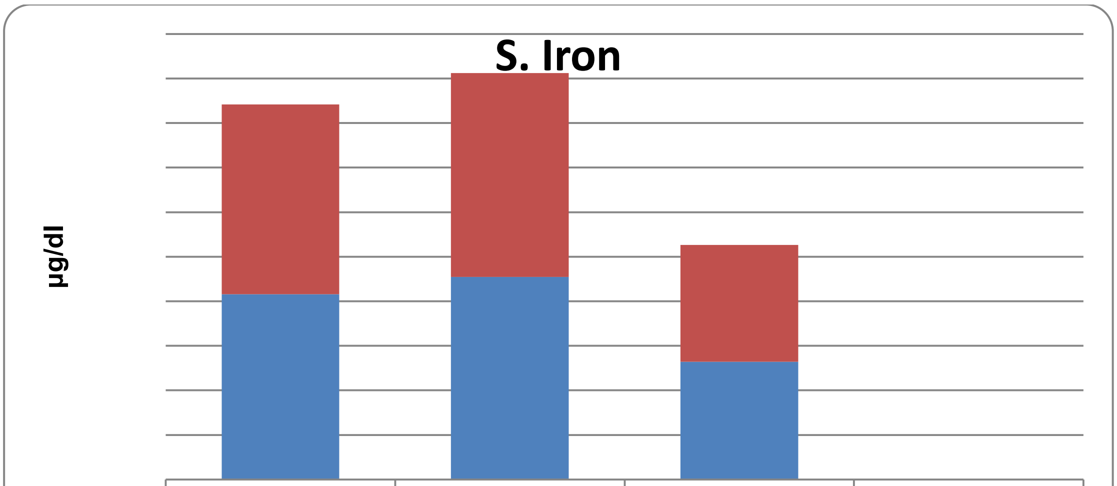
3-8 Variation of serum iron [s. iron] in male patient, female patient and control:

The current study displayed mean ± SD of iron value for female (83.18±7.56)mcg/dL and male (85.19±6.65)mcg/dL thalassaemia patients before (Female=83.18±7.56 and Male=85.19±6.65) µg/dL and after blood transfusion (Female=90.86±7.55 and Male=91.55±7.76)µg/dL while control (Female=52.84±4.53 and Male=52.4±4.95)µg/dL as shown in tables (3-1) and (3-2).

The results of this study for female and male patients illustrated significant increase ($p \leq 0.05$) of C1 when compared with C2, that agreement with Nyoman S.(2018) study, also he explained that the blood transfusion of thalassaemia patients lead to increase of serum iron[146].

Additionally, iron test of patients clans (C1 and C2) was resulted highly significant decrease ($p \leq 0.05$) compared with control, that agreement with Inthawong K (2015), also Inthawong K discusses the aggregation of iron in thalassaemia patients that leads to increase iron concentration[147], as appeared in figure (3-7).

Serum iron increased in the thalassaemia patients because of iron overload that due to decrease Hepcidin-25 leaded to increase absorption of iron[147]



	BT	AT	Control	
Male	85.19	91.55	52.4	
Female	83.18	90.86	52.84	

Figure (3-7). Serum iron in male, female patient and control.

3-9 <u>Variation of serum total iron bending capacity [S. TIBC] in male patient, female patient and control:</u>

The current study displayed mean ± SD of TIBC value for female (181.4±30.95)mcg/dL and male (179.182±27.589)mcg/dL thalassaemia patients before (Female=181.4±30.95 and Male=179.182±27.589) µg/dL and after(Female=182.79±26.92 and Male=181.8±26.38)µg/dL blood transfusion was more than control (Female=173.06±10.99 and Male=174.67±9.48)mcg/dL as show in tables (3-1) and (3-2).

The results of this study showed non-significant ($p \geq 0.05$) different in female and male patients clans, that agreement with Aparna A (2014) study when researched about parameters that used to diagnosis thalassaemia[148].That was shown in figure (3-8).

Total iron binding capacity test of thalassaemia patients before and after blood transfusion appeared higher than control as shown in figure (3-8).

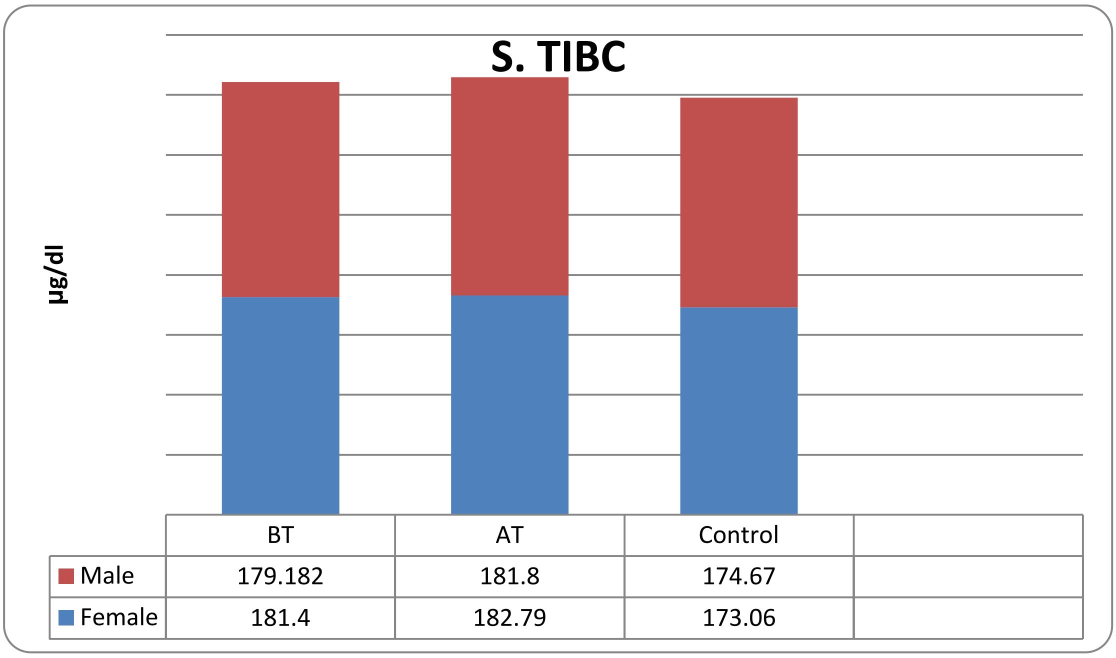


Figure (3-8). Serum total iron bending capacity in male, female patient and control.

3-10<u>Correlation between Hepcidin-25 and all biometers before and after blood transfusion of female patients</u>

Correlation of Hepcidin-25 with parameters were resulted either significant (positive or negative) or non-significant as shown in figure(3-4).

Table (3-3). Correlation of Hep with all parameters and T.test values with control before and after blood transfusion of female patients.

Biometer	**Before blood transfusion**		**After blood transfusion**	
	r value	**T.test**	**r value**	**T.test**
BMI	0.29	HS		
NBTT	0.503		0.378	
Hb	0.009	HS	-0.415	HS
PCV	-0.015	HS	-0.414	HS
S. Trans.	0.053	HS	0.356	HS
S. Fer.	0.295	HS	0.009	HS
S. Iron	0.171	HS	0.273	HS
TIBC	-0.291	NS	-0.244	NS

Correlation of body mass index with Hepcidin-25 displayed significant positive correlation.

Number of blood transfusion times correlation with Hepcidin-25 before and after blood transfusion resulted significant positive correlation that sh own in figure (3-9).

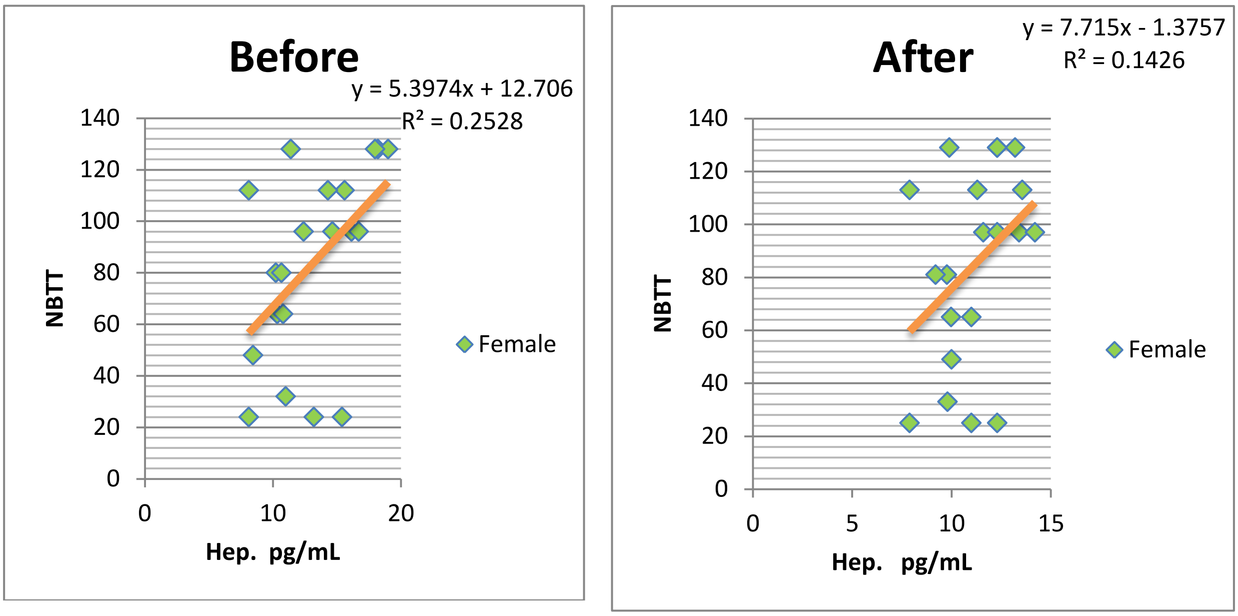


Figure (3-9). Correlation between Hep with NBTT in female patient.

Hemoglobin correlation with Hepcidin-25 before blood transfusion product showed significant positive correlation as illustrated in figure (3-10), while after blood transfusion product revealed significant negative correlation.

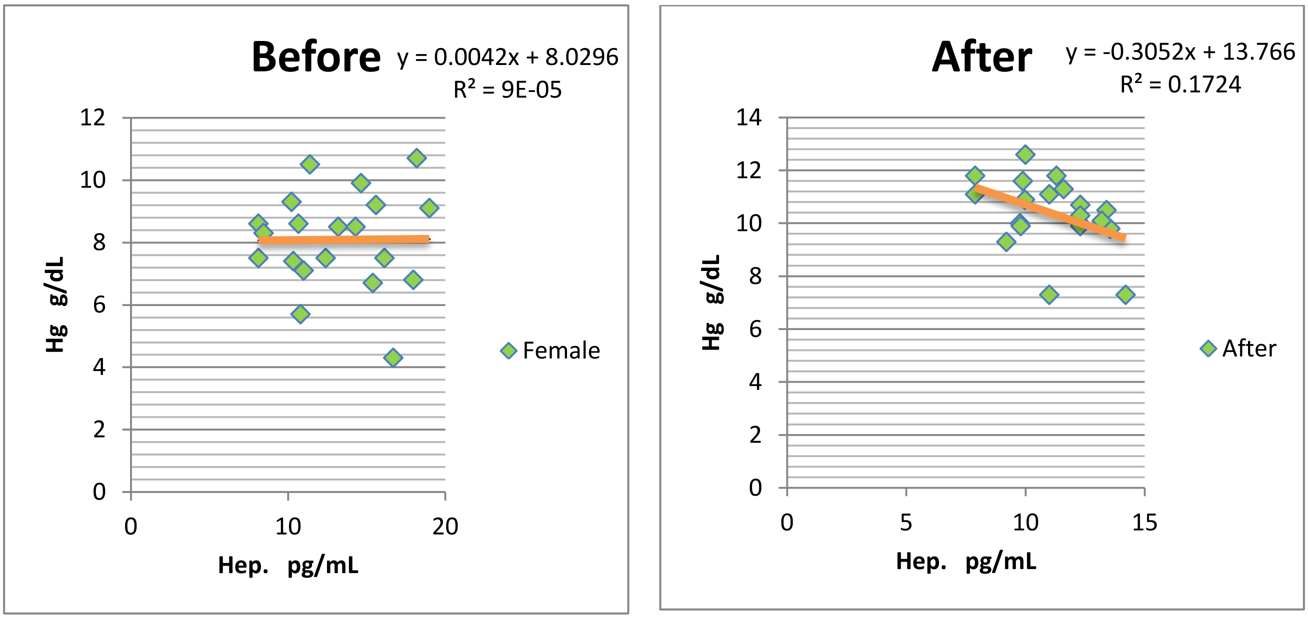


Figure (3-10). Correlation between Hep with Hb in female patient.

Packed cell volume correlation with Hepcidin-25 before blood transfusion demonstrated significant negative correlation, and significant negative correlation observed after blood transfusion as appeared in figure (3-11).

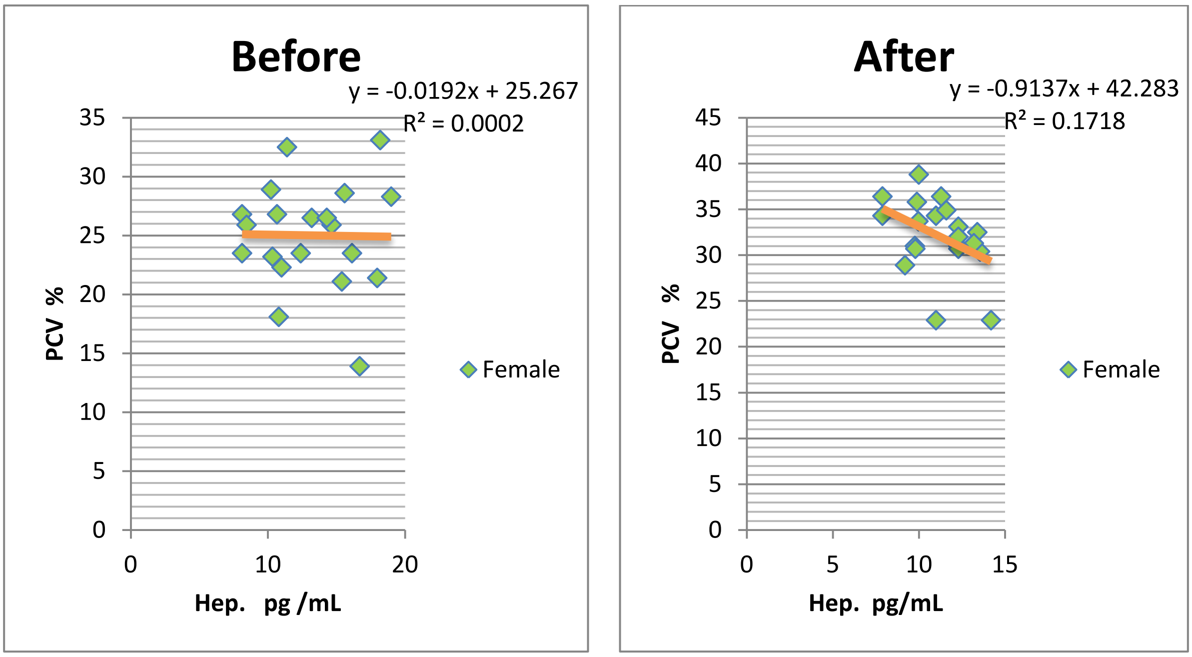


Figure (3-11). Correlation between Hep. With PCV in female patient.

Serum transferrin correlation with Hepcidin-25 before blood transfusion displays significant positive correlation, whereas correlation of transferrin after blood transfusion resulted significant positive correlation as shown in figure (3-12).

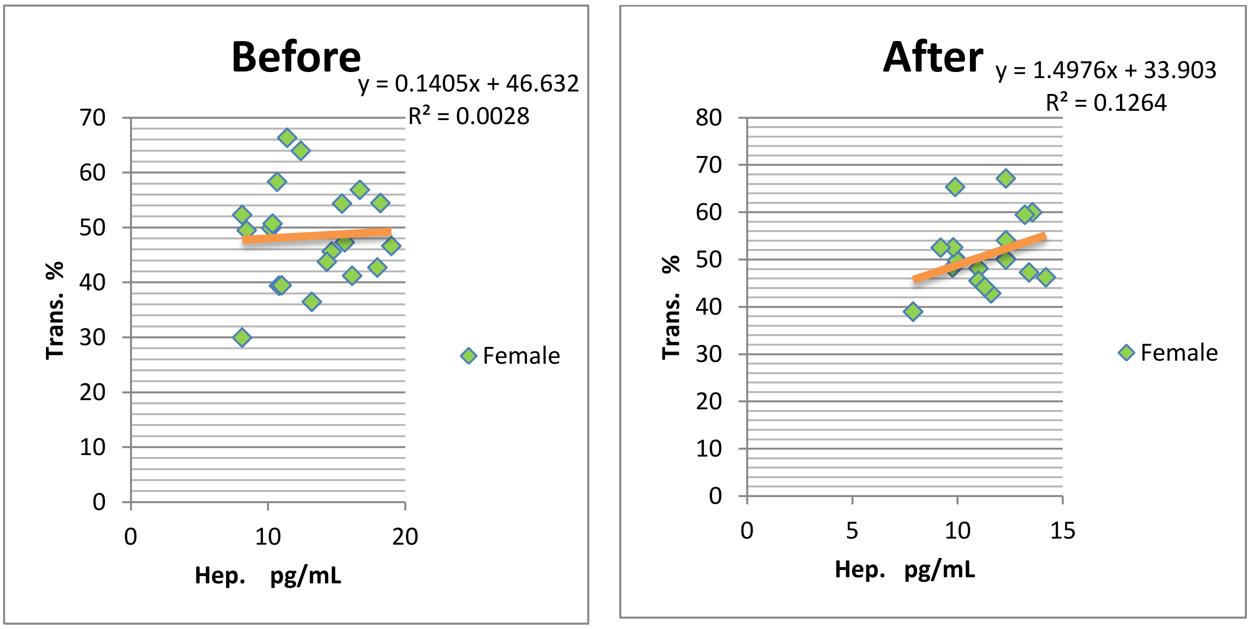


Figure (3-12). Correlation between Hep with S. Trans. in female patient.

Correlation of serum ferritin with Hepcidin-25 before blood transfusion resulted significant positive correlation, and after blood transfusion correlation resulted significant positive correlation that presented in figure (3-13).

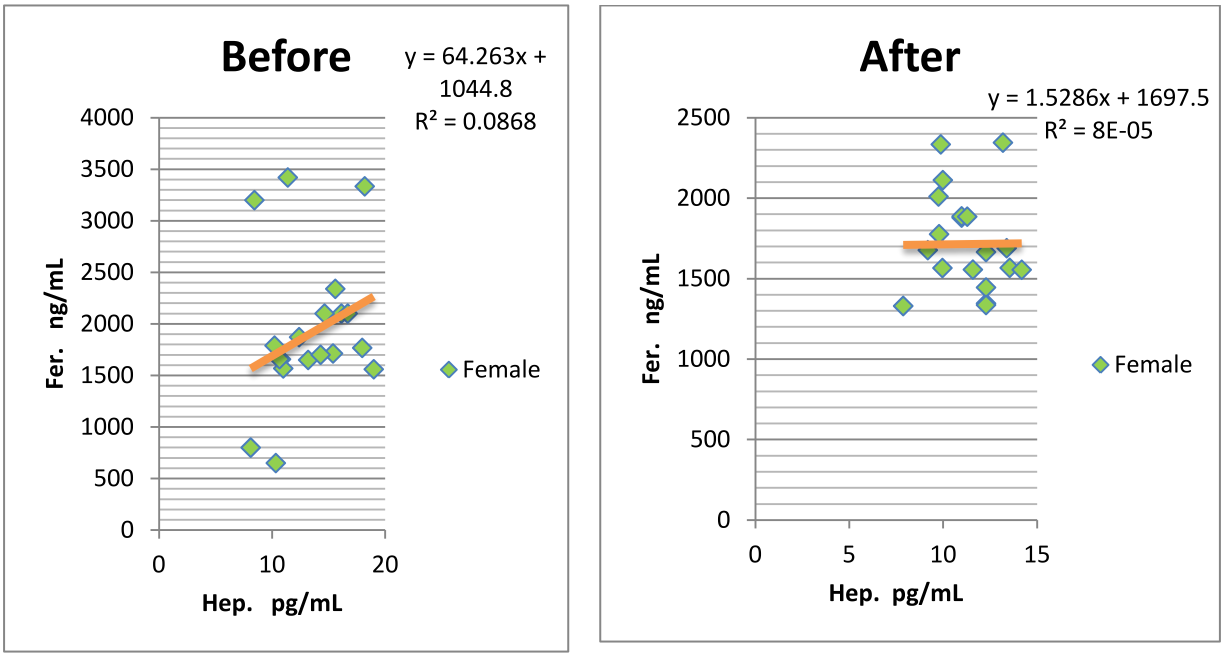


Figure (3-13). Correlation between Hep with S. Fer. In female patient.

In addition, correlation of serum iron with Hepcidin-25 before and after blood transfusion resulted significant positive correlation that also appeared in figure (3-14).

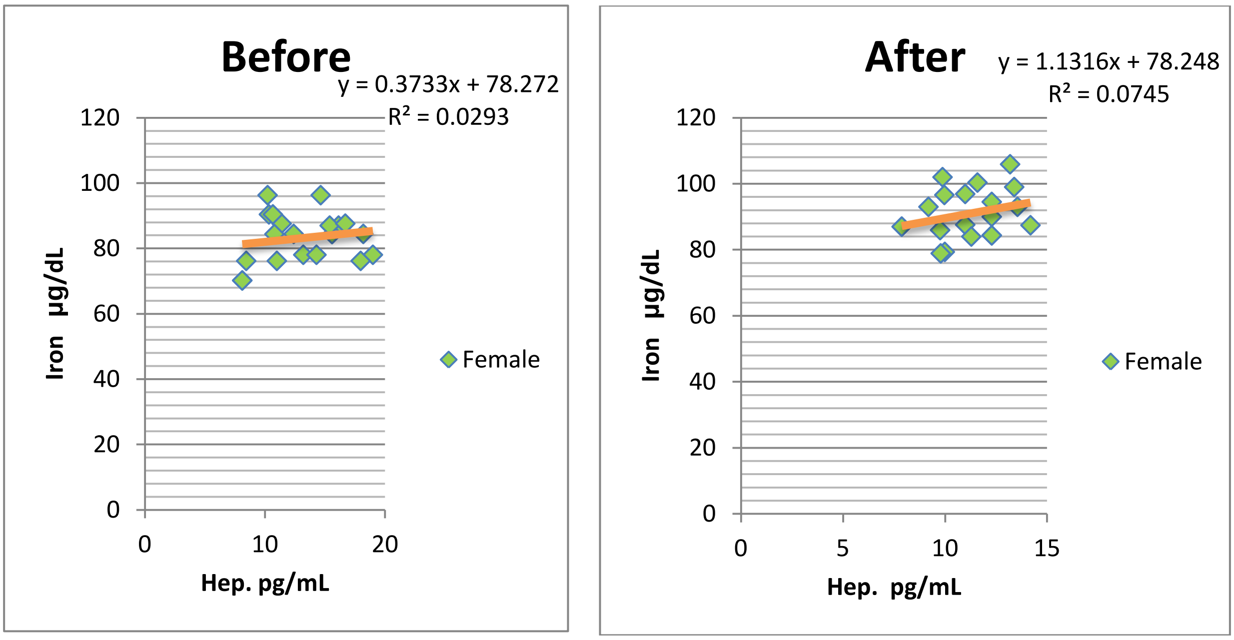


Figure (3-14). Correlation between Hep with S. Iron in female patient.

Total iron bending capacity correlation with Hepcidin-25 before and after blood transfusion resulted significant negative correlation that illustrated in figure (3-15).

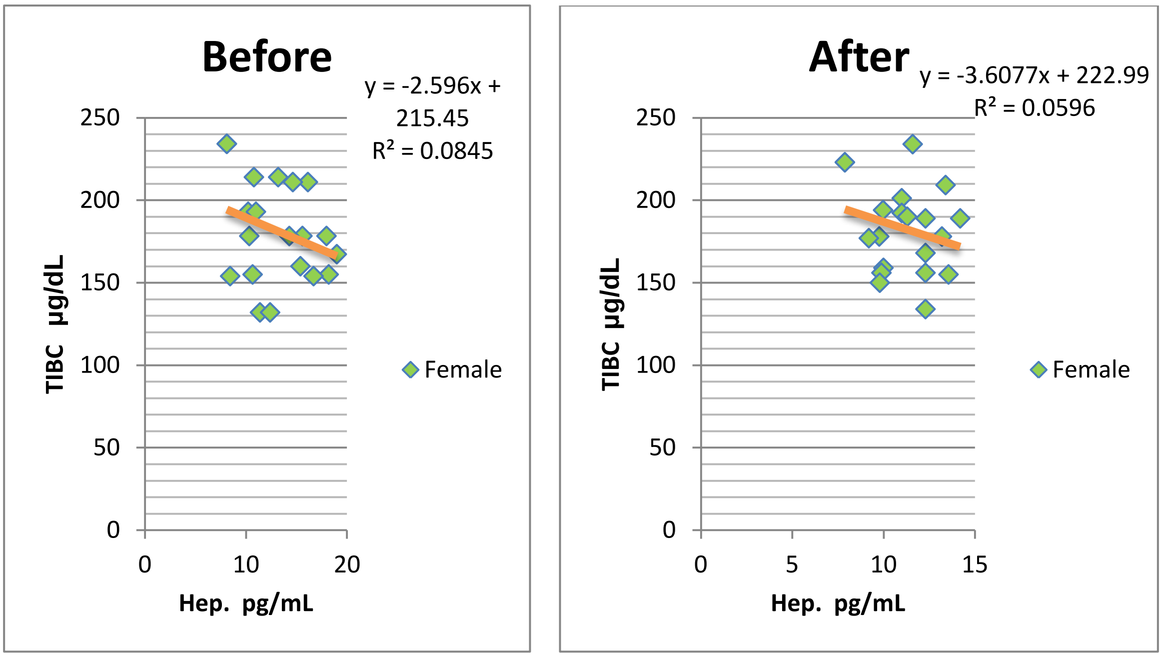


Figure (3-15). Correlation between Hep with S. TIBC in female patient.

3-11 Correlation of Hepcidin-25 with all parameters BT and AT of male patients

Correlation of Hepcidin-25 with parameters in this study was appeared either negative or positive significant as shown in table (3-4). Table (3-4). Correlation of Hepcidin-25 with all parameters and T.test value with control before and after blood transfusion of male patients.

Biometers	Before blood transfusion		After blood transfusion	
	r value	T.test	r value	T.test
BMI	-0.124	HS		
NBTT	-0.431		-0.249	
Hb	-0.305	HS	-0.42	HS
PCV	-0.347	HS	-0.419	HS
S. Trans.	-0.098	HS	0.065	HS
S. Fer.	0.093	HS	-0.348	HS
S. Iron	-0.119	HS	0.217	HS
TIBC	-0.046	HS	0.087	NS

Body mass index correlation with Hepcidin-25 was appeared significant negative correlation, as presented in table (3-5).
Correlation of Hepcidin-25 with number of blood transfusion times before and after blood transfusion resulted significant negative correlation that illustrated in figure (3-16).

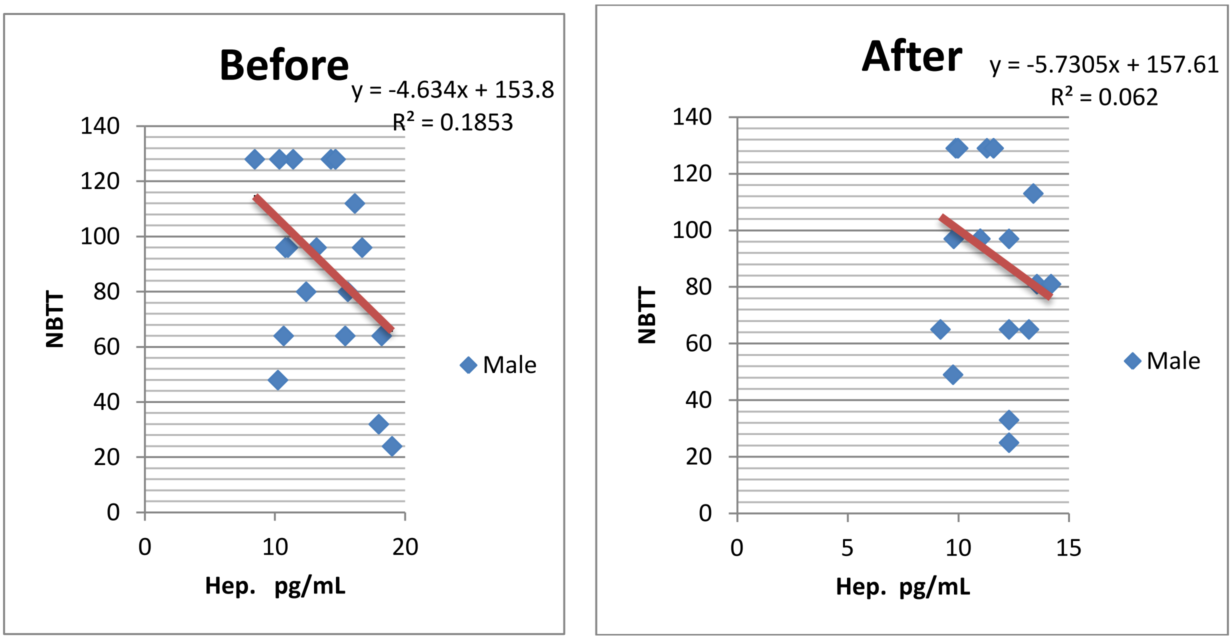


Figure (3-16). Correlation between Hep with NBTT in male patient.

Hemoglobin correlation with Hepcidin-25 before and after blood transfusion resulted significant negative correlation that illustrated in figure (3-17).

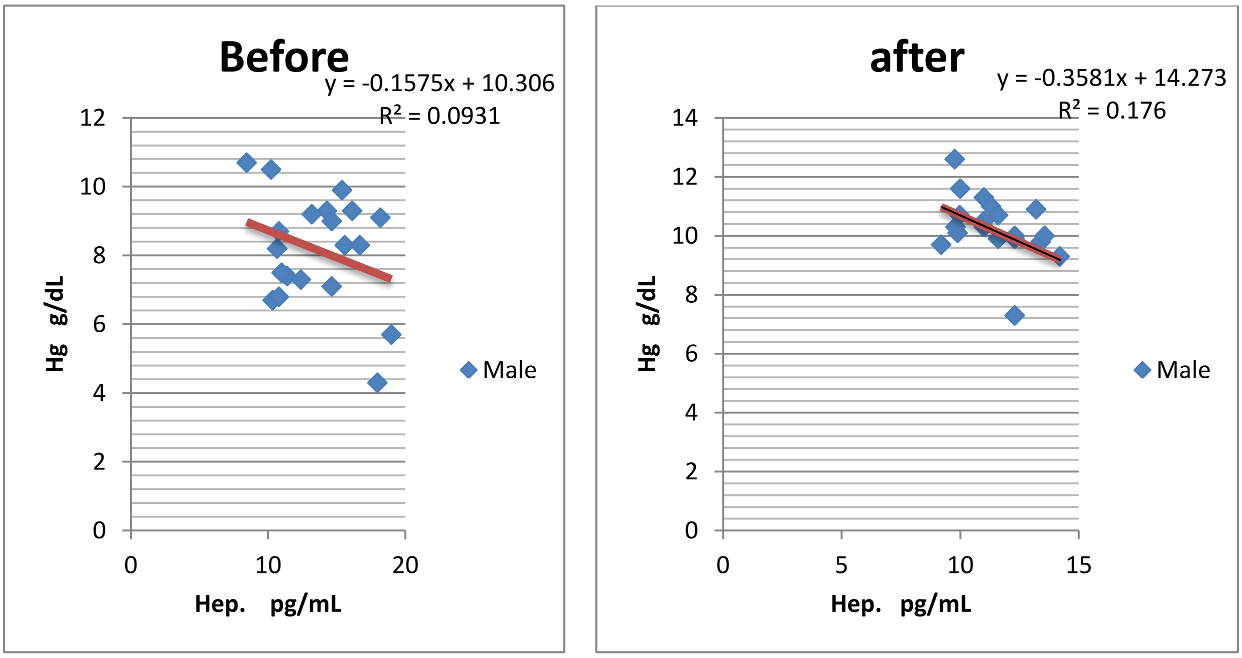
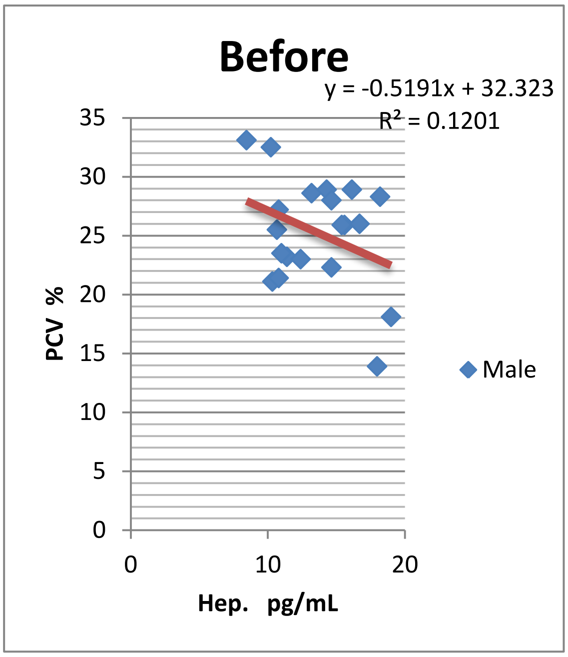


Figure (3-17). Correlation between Hep with Hb in male patient before and after transfusion.

Correlation of packed cell volume with Hepcidin-25 in this study before blood transfusion appeared significant negative correlation, and after blood transfusion appeared significant negative correlation as shown in figure (3-18).



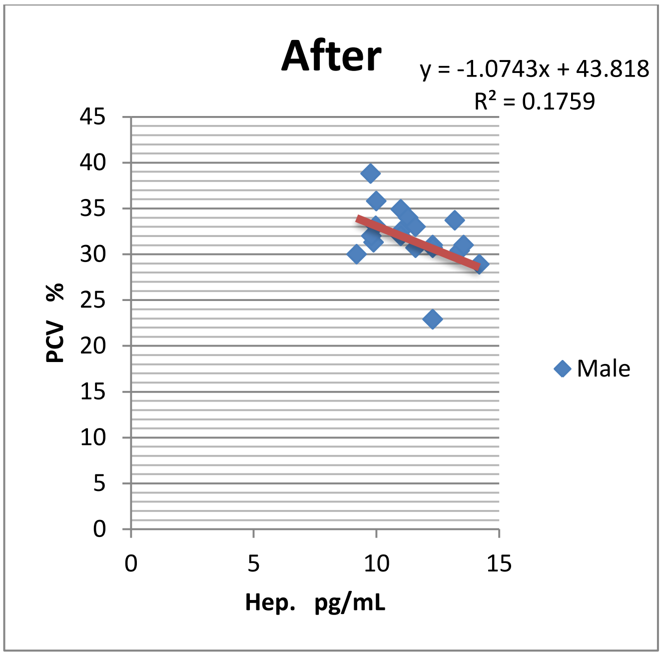


Figure (3-18). Correlation between Hep with PCV in male patient.

Serum transferrin correlation with Hepcidin-25 before blood transfusion resulted significant negative correlation, while after blood transfusion that appeared significant positive correlation as shown in figure 3-19.

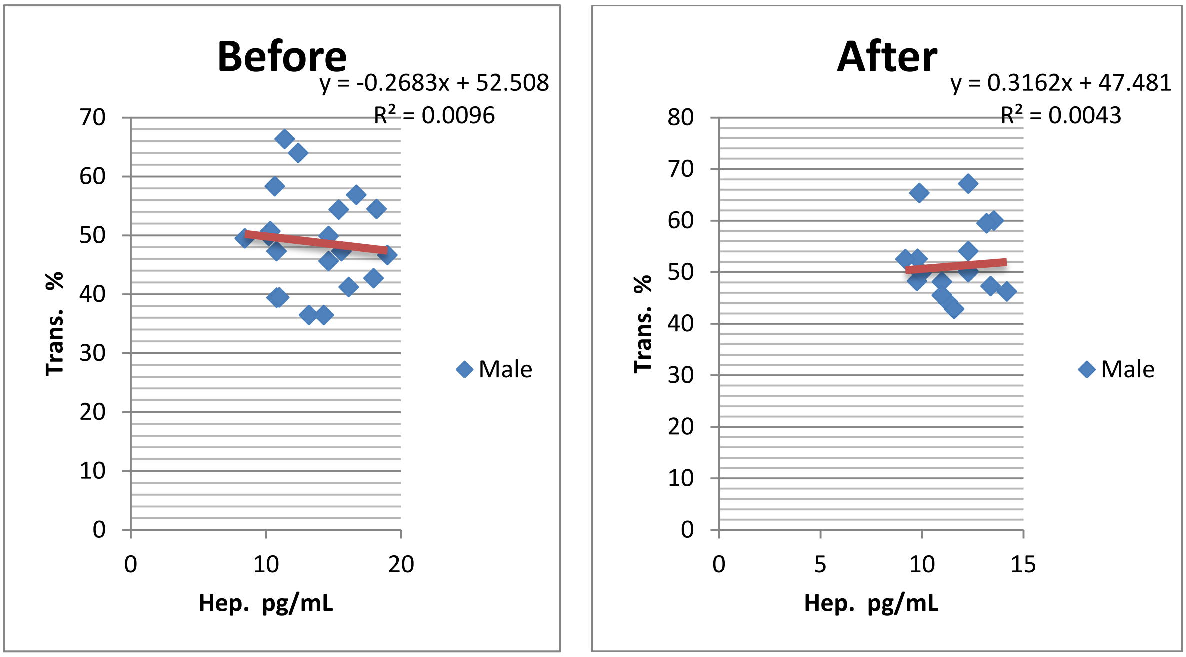


Figure (3-19). Correlation between Hep with S. Trans. in male patient.

Ferritin correlation before blood transfusion in this study emerged significant positive correlation, whereas ferritin resulted after blood transfusion significant negative correlation as illustrated in figure (3-20).

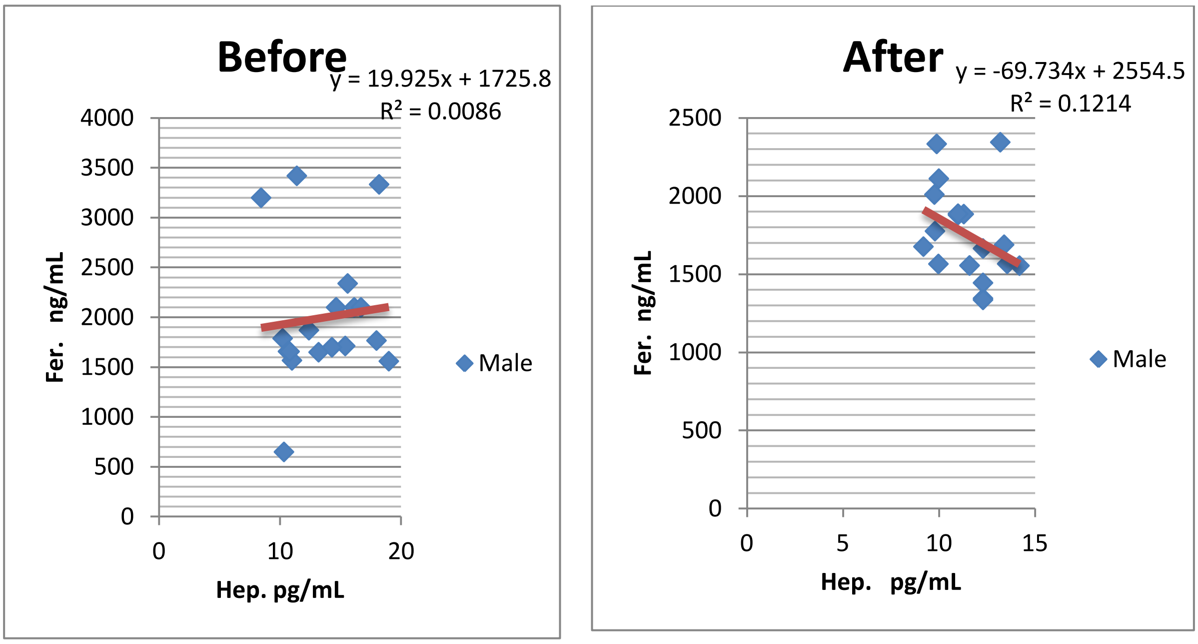


Figure (3-20). Correlation between Hep with S. Fer. in male patient.

Serum iron correlation in this study before blood transfusion appeared significant negative correlation, while after blood transfusion resulted significant positive correlation that shown in figure (3-21).

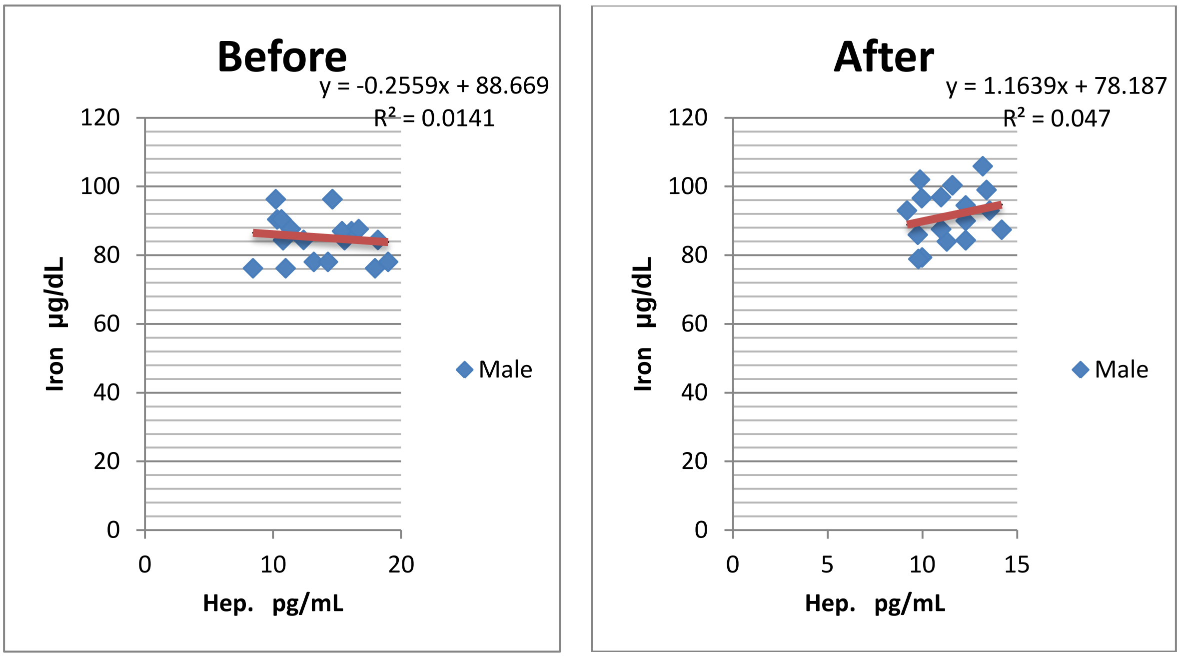


Figure (3-21). Correlation between Hep with S. Iron in male patient.

Finally, correlation of serum total iron bending capacity before blood transfusion resulted significant negative correlation, as shown in figure 3-22, while TIBC correlation after blood transfusion resulted significant positive correlation.

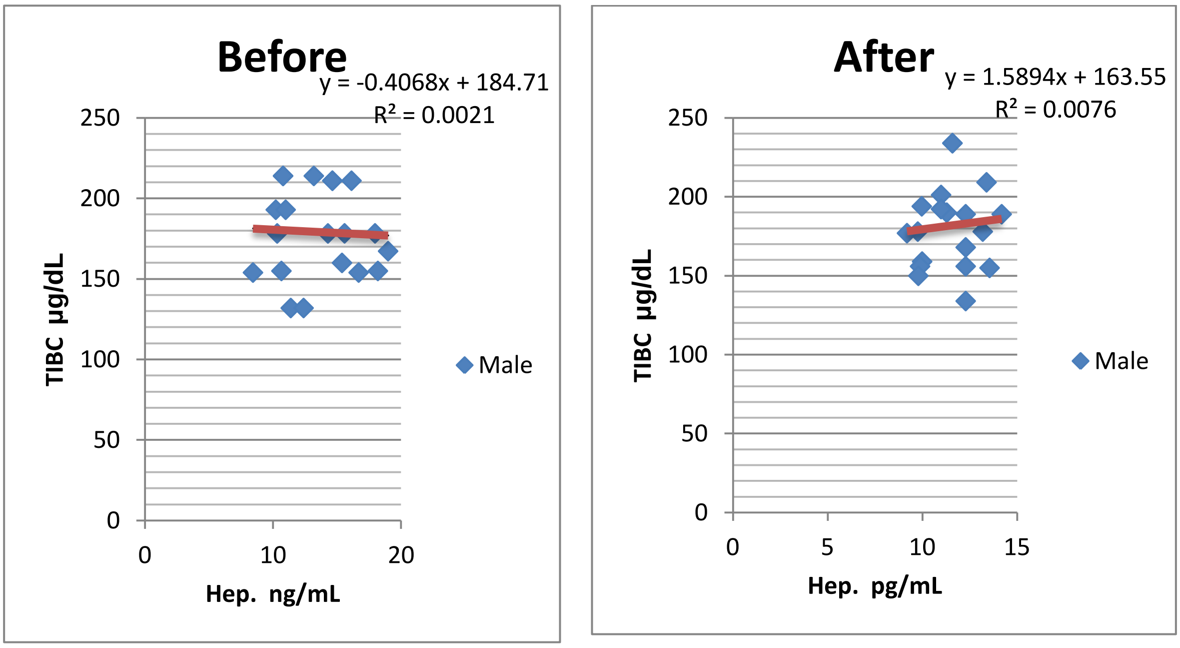


Figure (3-22). Correlation between Hep with S. TIBC in male patient.

Conclusion:

The conclusion of T.test value of female and male patients after and before blood transfusion that obtained from this study are:

1. Number of blood transfusion times appeared non-significant increase when compare between after and before blood transfusion.
2. Hemoglobin level appeared high significant decrease when compared between after and before blood transfusion, moreover high significant increase between thalassaemia patients clan and control clan which was found.
3. Packed cell volume [PCV] displayed high significant decrease when compared between after and before blood transfusion, also high significant increase between thalassaemia patients clan and control clan observed.
4. Hepcidin-25 hormone levels showed high significant decrease when compared between after and before blood transfusion, but high significant increase was found when compared between thalassaemia patients clans and control clan.
5. Transferrin level appeared non-significant when contrasted between after and before blood transfusion, however high significant increase between thalassaemia patients clans and control clan observed.
6. Ferritin level displays non-significant when compares between after and before blood transfusion, but high significant decrease between thalassaemia patients clans and control clan appeared.
7. Serum iron level showed significant increase when contrasted between after and before blood transfusion, while high significant decrease between patients clans and control observed.
8. Total iron bending capacity [TIBC] appeared non-significant when compared between after and before blood transfusion, also non-significant between patients clans and control clan was found.

In addition, conclusion of Hepcidin-25 correlation of female and male patients after and before blood transfusion obtained from this study is:

- Body mass index of female patient significant positive correlation, while significant negative correlation of male patients.
- Number of blood transfusion times of female patients before and after blood transfusion significant positive correlation, but significant negative correlation of male patients before and after blood transfusion.
- Hemoglobin level of female patients before blood transfusion significant positive correlation and after blood transfusion

significant negative correlation, while significant negative correlation of male patient before and after blood transfusion.

- Packed cell volume of female patients before and after blood transfusion significant negative correlation, while significant negative correlation before and after blood transfusion of male patients.
- Transferrin level of female patients before and after blood transfusion significant positive correlation, but significant negative correlation of male patients before blood transfusion, while significant negative correlation after blood transfusion.
- Ferritin level of female before and after blood transfusion significant positive correlation, while significant positive correlation before blood transfusion and significant negative correlation after blood transfusion.
- Serum iron of female patients' significant positive correlation before and after blood transfusion, but significant negative correlation of male patients before blood transfusion and significant positive correlation after blood transfusion.
- Total iron bending capacity level of female patient significant negative correlation before and after blood transfusion, but significant negative correlation of male patients before blood transfusion and significant positive correlation after blood transfusion.

Suggestions and recommendations:

1- Predicated new ligand can be used as drug instead of DFO.
2- Genetic study about the mutation that lead to β-thalassaemia and avoid it with patients, which need married.
3- Determination Hepcidin-25 level in blood before and after blood transfusion to find the effect of blood transfusion in blood.

References:

1- John F., (2012), "What Are the Signs and Symptoms of Thalassemias?", NHLBI. Archived from the original 2016.

2- Rund D., (2016). Thalassemia: modern medicine battles an ancient disease. Am J Hematol , 91:15–21.

3- Tang W., (2015), Spectrum of alpha-thalassemia and beta-thalassemia mutations in the Guilin Region of southern China. Clinical biochemistry. 48:1068–1072.

4- Origa R.; Baldan A.; Marsella M. and BorgnaPignatti C., (2015), A complicated disease: what can be done to manage thalassemia major more effectively? Expert Rev Hematol; 8:851–62.

5- Lin M., (2014), Molecular epidemiological characterization and health burden of thalassemia in Jiangxi Province, P. R. China. PloS one. journal.pone ; 9:e101505.

6- John P.; Greer JP.; Arber DA.; Glader B.; et al. (2013), Wintrobe's Clinical Hematology.

7- Jarjour RA.; Murad H.; Moasses F. and Al-Achkar W., (2014), Molecular update of β-thalassemia mutations in the Syrian population: identification of rare β-thalassemia mutations. Hemoglobin. 38(4):272-6.

8- Taher, A.T.; Weatherall, D.J. and Cappellini, M.D., (2017), Thalassaemia. 175,190:1125.

9- Origa R.; Pagon RA.; Adam MP.; Ardinger HH.; et. al. (2000), Beta-Thalassemia. p4. 25:100.

10- Cappelini MD.; Cohen A.; Porter J.; Taher A. and Viprakasit V., (2014), guidelines for the management of transfusion dependent thalassaemia.25:121

11- Camaschella, C. and Nai, A., (2016), Ineffective erythropoiesis and regulation of iron status in iron loading anaemias. Br. J. Haematol. 172, 512–523.

12- Thein SL., (2013),The molecular basis of β-thalassemia. Cold Spring Harb Perspect;3(5):a011700.

13- Taher A.T.; Musallam K.M.; Saliba A.N.; Graziadei G. and Cappellini M.D., (2015), Hemoglobin level and morbidity in non-transfusion-dependent thalassemia. Blood Cells Mol. Dis., 55, 108–109.

14- Paglietti ME.; Satta S.; Sollaino MC.; Barella S.; et. al. (2016), The problem of borderline Hemoglobin A2 levels in the screening for b- thalassemia carriers in Sardinia. Acta Haematol;135:193–9.

15- Haase V., (2013), Hematology, Blood Rev., 27, 41–53.

16- Hardison and Ross C., (2012). "Evolution of hemoglobin and its genes". Cold Spring Harbor Perspectives in Medicine.
17- Saliba A.N. and Taher A.T., (2016), Morbidities in non-transfusion-dependent thalassemia. Ann. N. Y. Acad. Sci, 1368, 82–94.
18- Nai A.; pagani A.; Mandelli G.; Lidonnici MR.; et, al. (2012), deletion of TMPRSS6 attenuates the phenotype in a mouse model of beta-thalaasemia. Blood, 119(21): 5021-5029.
19- Haddad A.; Tyan P.; Radwan A.; Mallat N. and Taher A. (2014), B-thalassemia intermedia: A bird's-eye view. Turk. J. Haematol, 31, 5–16.
20- Derchi G.; Galanello R.; Bina P.; et al. (2014) Prevalence and risk factors for pulmonary arterial hypertension in a large group of -thalassemia patients using right heart catheterization: A webthal study. Circulation, 129, 338–345.
21- Allen S.; Young E. and Bowns B., (2017), Noninvasive prenatal diagnosis for single gene disorders. Curr Opin Obstet Gynecol;29:73–9.
22- Amoozgar H.; Zeighami S.; Haghpanah S. and Karimi M., (2017), A comparison of heart function and arrhythmia in clinically asymptomatic patients with thalassemia intermedia and thalassemia major. Hematology, 22, 25–29.
23- Soliman; Ashraf T.; Kalra; Sanjay; De Sanctis andVincenzo, (2014), "Anemia and growth". Indian Journal of Endocrinology and Metabolism.
24- Angastiniotis M.; Eleftheriou A.; Galanello R.; et. al. (2013), Prevention of Thalassaemias and Other Haemoglobin Disorders, Thalassaemia International Federation. . Accessed 1-10-18.
25- Rai P. and Malik P., (2016), Gene therapy for hemoglobin disorders A mini. J. Rare Dis. Res. Treat, 1, 25–31.
26- Piga A.G.; Tartaglione I.; Gamberini R.; et. al. (2016), Luspatercept increases hemoglobin, decreases transfusion burden and improves iron overload in adults with β-thalassemia. Am. Soc. Hematol, 128, 851.
27- Matta B.N.; Abbas O.; Maakaron J.E.; et. al. (2014), Leg ulcers in patients with -thalassaemia intermedia: A single centre's experience. J. Eur. Acad. Dermatol. Venereol, 28, 1245–1250.
28- Sankaran V.G. and Weiss M.J., (2015), Anemia: Progress in molecular mechanisms and therapies. Nat, 21, 221–230.

29- Rivella S., (2015), β-thalassemias: paradigmatic diseases for scientific discoveries and development of innovative therapies. Haematologica. 100 (4):418-30.
30- Moukhadder H.M.; Halawi R.; Cappellini M.D. and Taher A.T., (2017), Hepatocellular carcinoma as an emerging morbidity in the thalassemia syndromes: A comprehensive review. Cancer, 123, 751–758.
31- Ben Salah N.; Bou-Fakhredin R.; Mellouli F. and Taher A.T., (2017), Revisiting thalassemia intermedia: Past, present, and future prospects. Hematology, 22, 607–616.
32- Borgna-Pignatti C. and Garani M.C., (2014), Hepatocellular carcinoma in thalassaemia: An update of the italian registry. Br. J. Haematol., 167, 121–126.
33- Dede AD.; Trovas G.; Chronopoulos E.; et. al. (2016), Thalassemia-associated osteoporosis: a systematic review on treatment and brief overview of the disease. Osteoporos Int.; 27:3409–25.
34- Anurathapan U.; Hongeng S.; Pakakasama S.; et. al. (2016), Hematopoietic stem cell transplantation for homozygous β-thalassemia and β-thalassemia/hemoglobin E patients from haploidentical donors. Bone Marrow Transplant; 51:813–8.
35- School M.; Linssen J.; Villanueva MM.; et. al. (2012), efficacy of advanced discriminating algorithms for screening on iron-deficiency anaemia and beta-thalassaemia trait: a multicenter evalution. Am J clin pathol; 138 (2) :300-304.
36- Cox D.B.; Platt R.J. and Zhang F., (2015), Therapeutic genome editing: Prospects and challenges. Nat. 21, 121–131.
37- Old J. and Henderson S., (2010), Molecular diagnostics for haemoglobinopathies. Expert Opin Med Diagn;4:225–40.
38- Ben Salah N.; Bou-Fakhredin R.; Mellouli F. and Taher A.T., (2017), Revisiting β thalassemia intermedia: Past, present, and future prospects. Hematology, 22, 607–616.
39- Saliba A.N. and Taher A.T., (2016), Morbidities in non-transfusion-dependent thalassemia. Ann. N. Y. Acad. Sci., 1368, 82–94.
40- Inati A.; Noureldine M.A.; Mansour A. and Abbas H.A., (2015), Endocrine and bone complications in –thalassemia intermedia: Current understanding and treatment. BioMed Res. Int.
41- Traeger-Synodinos J. and Harteveld CL., (2014), Advances in technologies for screening and diagnosis of hemoglobinopathies. BiomarkMed; 8:119–31.

42- Finotti A.; Breda L.; Lederer CW.; et al. (2015), Recent trends in the gene therapy of β-thalassemia. J Blood Med. 6:69-85.
43- Rai, P. and Malik, P.; (2016), Gene therapy for hemoglobin disorders—A mini-review. J. Rare Dis. Res. Treat., 1, 25–31.
44- Traeger-Synodinos J.; Harteveld CL.; Old JM.; et. al. (2015), Angastioniotis M, De la Salle B, Henderson S, May A; EMQN haemoglobinopathies best practice meeting EMQN best practice guidelines for molecular and haematology methods for carrier identifi- cation and prenatal diagnosis of the haemoglobinopathies. Eur J Hum Genet; 23:426–37.
45- Piel FB. and Weatherall DJ., (2014), The a-thalassemias. N Engl J Med;371:1908–16.
46- Saleh-Gohari N.; Kademi Bami M.; Nikbakht R. and Karimi-Maleh H., (2015), Effects of a-thalassemia mutations on the haematological parameters of b-thalassemia carriers. J Clin Pathol;68:562–6.
47- Weatherall DJ. and Piel FB., (2014), The a-thalassemias. N Engl J Med;351:1918–16.
48- Viprakasit V., Limwongse C., Sukpanichnant S.; et. al. (2013), problem in determining thalassaemia carrier status in a program for prevention and control of severe thalassaemia syndromes: a lesson from Thailand. clin chem. lab Med;23:1-10.
49- Musallam KM.; Rivella S.; Vichinsky E. and Rachmilewitz EA., (2013), Non transfusion-dependent thalassemias. Haematologica;98:833–44.
50- Mohanty D.; Colah RB.; Gorakshakar AC.; et. al. (2013), Community Genet;4(1):33-42.
51- Cao A. and Kan YW., (2013), The prevention of thalassemia. Cold Spring Harb Perspect Med.;3:a011775.
52- Camaschella C. and Nai A., (2016), Ineffective erythropoiesis and regulation of iron status in iron loading anaemias. Br. J. Haematol, 172, 512–523.
53- Amoozgar H.; Zeighami S.; Haghpanah S. and Karimi M. A., (2017), comparison of heart function and arrhythmia in clinically asymptomatic patients with β thalassemia intermedia and β thalassemia major. Hematology, 22, 25–29.
54- Danjou F.; Francavilla M.; Anni F.; et. al. (2015), A genetic score for the prediction of beta-thalassemia severity. Haematologica.;100:452–7.

55- Baldini M.; Marcon A.; Cassin R.; et. al. (2014), B-thalassaemia intermedia: Evaluation of endocrine and bone complications. BioMed Res. Int., 2014, 174581.
56- Arlet JB.; Ribeil JA.; Guillem F.; et. al. (2014), HSP70 sequestration by free α-globin promotes ineffective erythropoiesis in β-thalassaemia. Nature.;514(7521):242-6.
57- Baronciani D.; Angelucci E.; Potschger U.; et al. (2016), Hemopoietic stem cell transplantation in thalassemia: A report from the european society for blood and bone marrow transplantation hemoglobinopathy registry, 2000–2010. Bone Marrow Transpl, 51, 536–541.
58- Weatherall DJ., (2012), the definition and epidemiology of non-transfusion-dependent thalassaemia. blood rev;26 suppl 1:s3-6.
59- Derchi G.; Galanello R.; Bina P.; et al. (2014), Prevalence and risk factors for pulmonary arterial hypertension in a large group of β-thalassemia patients using right heart catheterization: A webthal study. Circulation, 129, 338–345.
60- Plengsuree S.; Punyamung M.; Yanola J.;et. al. (2015), Red Cell Indices and Formulas used in differentiation of b-thalassemia trait from iron deficiency in Thai adults. Hemoglobin;39:235–9.
61- Puliyel M.; Sposto R.; Berdoukas V.A.; et. al. (2014), Ferritin trends do not predict changes in total body iron in patients with transfusional iron overload. Am. J. Hematol., 89, 391–394.
62- Viprakasit V.; Limwongse C.; Sukpanichnant S.; et. al. (2013), Problems in determining thalassemia carrier status in a program for prevention and control of severe thalassemia syndromes: a lesson from Thailand. Clin Chem Lab Med;51:1605–14.
63- Haddad A.; Tyan P.; Radwan A.;et. al. (2014), B-thalassemia intermedia: A bird's-eye view. Turk. J. Haematol., 31, 5–16.
64- Musallam K.M.; Taher A.T.; Cappellini M.D. and Sankaran, V.G. (2013), Clinical experience with fetal hemoglobin induction therapy in patients with -thalassemia. Blood, 121, 2199–2212.
65- Piga A.G.; Tartaglione I.; Gamberini R.; et. al. (2016), Luspatercept increases hemoglobin, decreases transfusion burden and improves iron overload in adults with -thalassemia. Am. Soc. Hematol, 128, 851.

66- Tantawy AA.; Adly AA.; Ismail EA.; et. al. (2015), Growth differentiation factor-15 in children and adolescents with thalassemia intermedia: Relation to subclinical atherosclerosis and pulmonary vasculopathy. Blood Cells Mol Dis. 55 (2):144-50.
67- Moukhadder H.M.; Halawi R.; Cappellini M.D. and Taher A.T. (2017), Hepatocellular carcinoma as an emerging morbidity in the thalassemia syndromes: A comprehensive review. Cancer, 123, 751–758.
68- Matta B.N.; Abbas O.; Maakaron J.E.; et. al. (2014), Leg ulcers in patients with β-thalassaemia intermedia: A single centre's experience. J. Eur. Acad. Dermatol. Venereol, 28, 1245–1250.
69- Baronciani D.; Angelucci E.; Potschger U.; et. al. (2016), Hemopoietic stem cell transplantation in thalassemia: a report from the European Society for Blood and Bone Marrow Transplantation Hemoglobinopathy Registry, Bone Marrow Transplant.;51:536–41.
70- Attie K.M.; Allison M.J.; McClure T.;et. al. (2014), A phase 1 study of ACE-536, a regulator of erythroid differentiation, in healthy volunteers. Am. J. Hematol., 89, 766–770.
71- Mallat N.S.; Musallam K.M.; Mallat S.G.; et. al. (2013), End stage renal disease in six patients with β-thalassemia intermedia. Blood Cells Mol. Dis., 51, 146–148.
72- Borgna-Pignatti C.; Garani M.C.; Forni G.L.; et al. (2014), Hepatocellular carcinoma in thalassaemia: An update of the italian registry. Br. J. Haematol., 167, 121–126.
73- Inati A.; Noureldine MA.; Mansour A. and Abbas HA. (2015), Endocrine and bone complications in β-thalassemia intermedia: current understanding and treatment. Biomed Res Int.. 2015:813098.
74- Inati, A.; Noureldine, M.A.; Mansour, A.; Abbas, H.A. Endocrine and bone complications in β-thalassemia intermedia: Current understanding and treatment. BioMed Res. Int. 2015, 2015, 813098.
75- Aydinok Y.; Karakas Z.; Cassinerio,E.; et. al. (2016), Efficacy and safety of ruxolitinib in regularly transfused patients with thalassemia: Results from single-arm, multicenter, phase 2a truth study. Blood, 128, 852.
76- Aydinok Y.; Porter JB.; Piga A.; et. al. (2015), Prevalence and distribution of iron overload in patients with transfusion-dependent anemias differs across geographic regions: results from the CORDELIA study, Eur J Haematol; 95:244–53.

77- Borgna-Pignatti C.; Garani MC.; Forni GL.; et. al. (2014), Hepatocellular carcinoma in thalassaemia: an update of the Italian Registry. Br J Haematol.;167:121–6.
78- Mishra A.K., (2013), Tiwari Iron overload in Beta thalassaemia major and intermedia patients Maedica (Buchar), 8 (8) pp. 328-332.
79- Schmidt P.J.; Toudjarska I.; Sendamarai A.K.; et. al. (2013), An RNAi therapeutic targeting Tmprss6 decreases iron overload in Hfe(-/-) mice and ameliorates anemia and iron overload in murine beta-thalassemia intermedia Blood, 121 (7), pp. 1200-1208.
80- Sankaran V.G. and Weiss M.J. (2015), Anemia: Progress in molecular mechanisms and therapies. Nat. Med., 21, 221–230.
81- Taher AT.; Viprakasit V.; Musallam KM. and Cappellini MD. (2013), Treating iron overload in patients with non-transfusion dependent thalassaemia. Am J Hematol, 88:409-15.
82- Taher A.T.; Musallam K.M.; Viprakasit V.; et. al. (2014), Iron chelation therapy for non-transfusion-dependent thalassemia (NTDT): A status quo. Blood Cells Mol. Dis., 52, 88–90.
83- Cox D.B.; Platt R.J. and Zhang F. (2015), Therapeutic genome editing: Prospects and challenges. Nat. Med., 21, 121–131.
84- Musallam K.M.; Taher A.T.; Cappellini M.D. and Sankaran V.G. (2013), Clinical experience with fetal hemoglobin induction therapy in patients with beta-thalassemia Blood, 121 (12) pp. 2199-2212.
85- Saliba A.N.; Harb A.R. and Taher A.T. (2015), Iron chelation therapy in transfusion-dependent thalassemia patients: current strategies and future directions J Blood Med, 6 pp. 197-209.
86- Casu C.; Aghajan M.; Oikonomidou P.R.; et. al. (2016), Combination of tmprss6- aso and the iron chelator deferiprone improves erythropoiesis and reduces iron overload in a mouse model of -thalassemia intermedia. Haematologica, 101, e8–e11.
87- Calvaruso G.; Vitrano A.; Di Maggio R.; et. al. (2015), Deferiprone versus deferoxamine in thalassemia intermedia: Results from a 5-year long-term italian multicenter randomized clinical trial. Am. J. Hematol, 90, 634–638.
88- Vichinsky E.; El-Beshlawy A.; Al Zoebie A.; et. al. (2017), Long-term safety and efficacy of deferasirox in young pediatric patients with transfusional hemosiderosis: Results from a 5-year observational study (ENTRUST). Pediatr Blood Cancer..

89- Taher A.T.; Musallam K.M.; Viprakasit V.; et. al. (2014), Iron chelation therapy for non-transfusion-dependent thalassemia (NTDT): A status quo. Blood Cells Mol. Dis., 52, 88–90.
90- Taher A.T.; Porter J.B.; Viprakasit V.; et. al. (2013), Deferasirox effectively reduces iron overload in non-transfusion-dependent thalassemia (ntdt) patients: 1-year extension results from the thalassa study. Ann. Hematol, 92, 1485–1493.
91- Calvaruso G.; Vitrano A.; Di Maggio R.; et. al. (2015), Deferiprone versus deferoxamine in thalassemia intermedia: Results from a 5-year long-termitalian multicenter randomized clinical trial. Am. J. Hematol, 90, 634–638.
92- Aydinok Y.; Kattamis A.; Cappellini MD.; et. al. (2015), Effects of deferasirox-deferoxamine on myocardial and liver iron in patients with severe transfusional iron overload. Blood; 125:3868–77.
93- Novartis pharmaceuticals Uk Ltd. (2013), summary of product characteristics. EJADE 125mg, 250mg, 500mg dispersible tablets.
94- Taher A.T.; Viprakasit V.; Musallam K.M. and Cappellini M.D. (2013), Treating iron overload in patients with non-transfusion-dependent thalassemia Am J Hematol, 88 (5) pp. 409-415.
95- Jones E. Pasricha S.R. Allen A. et. al. (2015), Hepcidin is suppressed by erythropoiesis in hemoglobin E beta-thalassemia and beta-thalassemia trait Blood, 125 (5) pp. 873-880.
96- Baronciani D.; Angelucci E.; Potschger U.; et. al. (2016), Hemopoietic stem cell transplantation in thalassemia: A report from the european society for blood and bone marrow transplantation hemoglobinopathy registry, 2000–2010. Bone Marrow Transpl. 51, 536–541.
97- Casu C.; Oikonomidou P.R.; Chen H. et. al. (2016), Minihepcidin peptides as disease modifiers in mice affected by –thalassemia and polycythemia vera. Blood, 128, 265–276.
98- Tarkun P.; Mehtap O.; Atesoğlu EB.; et. al. (2013), Hacihanefioglu A. Serum hepcidin and growth differentiation factor-15 (GDF-15) levels in polycythemia vera and essential thrombocythemia. Eur J Haematol, 91(3):228-35.
99- Latour C., (2014), Testosterone perturbs systemic iron balance through activation of epidermal growth factor receptor signaling in the liver and repression of hepcidin. Hepatology 59, 683–694.

100- Pasricha S.R.; Frazer D.M.; Bowden D.K. and Anderson G.J. (2013), Transfusion suppresses erythropoiesis and increases hepcidin in adult patients with beta-thalassemia major: a longitudinal study Blood, 122 (1) pp. 124-133.
101- Chen H.; Choesang T.; Li H.; et. al. (2016), Increased hepcidin in transferrintreated thalassemic mice correlates with decreased hepatocyte ERK activation. haematologica;101(3):297-308.
102- Kautz L.; Jung G.; Du X.; et al. (2015), Erythroferrone contributes to hepcidin suppression and iron overload in a mouse model of β-thalassemia. Blood, 126(17):2031-7.
103- Keel SB.; Doty R.; Liu L.; et. al. (2015), Evidence that the expression of transferrin receptor 1 on erythroid marrow cells mediates hepcidin suppression in the liver. Exp Hematol. Jun;43(6):469-78.
104- Díaz, V. (2013), Liver iron modulates hepcidin expression during chronically elevated erythropoiesis in mice. Hepatology 58, 2122–2132.
105- Kautz L.; Jung G.; Du X.; et. al. (2015), Erythroferrone contributes to hepcidin suppression and iron overload in a mouse model of beta-thalassemia Blood, 126 (17) pp. 2031-2037.
106- Fung E. and Nemeth E., (2013), Manipulation of the hepcidin pathway for therapeutic purposes. Haematologica, 98(11):1667-76.
107- Guimaraes J.S.; Cominal J.G.; Silva-Pinto A.C.; et. al. Altered erythropoiesis and iron metabolism in carriers of thalassemia Eur J Haematol, 94 (6) (2015), pp. 511-518.
108- Silvestri L., (2013), Inhibiting the hepcidin inhibitor for treatment of iron overload Blood, 121 (7) pp. 1068-1069.
109- Kautz L.; Jung G.; Valore EV.; et. al. (2014). "Identification of erythroferrone as an erythroid regulator of iron metabolism". Nature Genetics. 46 (7): 678–84.
110- Agarwal K N. (2013), Indicators for Assessment of Anemia and Iron Deficiency in the Community Health Care & Research Association for Adolescents;D-115, Sector-36 NOIDA.
111- Engkakul P.; Mahashoklertwattana P.; Jaovisidha S.; et. al. (2013), Unrecognized vertebral fractures in adolescents and young adults with thalassemia syndromes. J pediatr Hematol Oncol, 35 (3):212-7.

112- Cabantchik Z.I., (2014), Labile iron in cells and body fluids: physiology, pathology, and pharmacology Front Pharmacol, 5 p. 45.
113- Gelderman MP.; Baek JH.; Yalamanoglu A.; et. al. (2015), Reversal of hemochromatosis by apotransferrin in non-transfused and transfused Hbbth3/+ (heterozygous B1/B2 globin gene deletion) mice. Haematologica;100(5):611-22.
114- Miller JL., (2013), "Iron Deficiency Anemia: A Common and Curable Disease". Cold Spring Harbor perspectives in medicine.
115- Musallam K.M.; Taher A.T.; Cappellini M.D. and Sankaran V.G. (2013), Clinical experience with fetal hemoglobin induction therapy in patients with β-thalassemia. Blood, 121, 2199–2212.
116- Taher A.; porter J.; Viprakasit V.; et. al. (2013), Serum ferritin for prediction clinically relevant LIC thresholds to guide management of patients with non-transfusion dependent thalassaemia treated with deferasirox: THALASSA study extension analysis. Haematological;98 (suppl 1):486 abst s1171.
117- Puliyel M.; Sposto R.; Berdoukas V.A.; et. al. (2014), Ferritin trends do not predict changes in total body iron in patients with transfusional iron overload. Am. J. Hematol., 89, 391–394.
118- Maggio A.; Vitrano A.; Calvaruso G.; et. al. (2013), Serial echocardiographic left ventricular ejection fraction measurements: A tool for detecting thalassemia major patients at risk of cardiac death. Blood cells Mol Dis,. 50 (4):241-6.
119- Jain M.; Rivera S.; Monclus E.A.; et. al. (2013), Mitochondrial reactive oxygen species regulate transforming growth factor-beta signaling J Biol Chem, 288 (2) pp. 770-777.
120- Yanola J.; Kongpan C. and Pornprasert S., (2014), Prevalence of anemia, iron eficiency, thalassemia and glucose-6-phosphate dehydrogenase deficiency among hill-tribe school children in Omkoi district, Chiang Mai province, Thailand. Southeast Asian J Trop Med Public Health, 45:920–5.
121- Papasavva T.; Van ljcken WF.; Kockx CE.; et. al. (2013), Next generation sequencing of SNPs for non-invasive prenatal diagnosis:challenges and feasibility as illustrated by an application to B-thalassemia. Eur J Hum Genet,.
122- Bresgen N. and Eckl P.M., (2015), Oxidative stress and the homeodynamics of iron metabolism Biomolecules, 5 (2) pp. 808-847.

123- Sornjai W.; Jaratsittisin J.; Khungwanmaythawee K.; et. al. (2016), Dysregulation of ferroportin gene expression in beta(0)-thalassemia/Hb E disease Ann Hematol, 95 (3) pp. 387-396.
124- Musallam K.M.; Cappellini M.D.; Daar S.; et. al. (2014), Serum ferritin level and morbidity risk in transfusion-independent patients with β-thalassemia intermedia: The orient study. Haematologica, 99, e218–e221.
125- Hladun R.; Elorza I.; Olive T.; et al. (2013), Results of hematopoietic stem cell transplantation in hemoglobinopathies: thalassemia major and sickle cell disease. An Pediatr (Barc), 79 (2):75-82.
126- Honarmand Ebrahimi K.; Hagedoorn PL. and Hagen WR (2015), "Unity in the biochemistry of the iron-storage proteins ferritin and bacterioferritin".
127- Giuseppe Lippi; Massimo Daves and Camilla Mattiuzzi. (2014), Biochem Med (Zagreb); 24(1): 80–88.
128- Cook JD.; Skikne BS. and Lynch SR., (1980), serum ferritin in the evalution of anaemia, radioimmunoassay of hormones, proteins and enzymes. Amesterdam: Excerpta medica,:239-48.
129- garcic A., (1979), clin. chem.. acta 94, 115-119.
130- Weippl G., (1973), blut 27, 261-270.
131- Zak B, and Epstein E. (1965), clin. chem.. 11: 641.
132- Tietz N.W., (1995), clinical guide to laboratory tests, 3rd edition.W.B saunders co. Philadelphia, PA..
133- Baldini M.; Marcon A.; Ulivieri FM.; et. al. (2017), Bone quality in beta-thalassemia intermedia: relationships with bone quantity and endocrine and hematologic variables. Ann Hematol, 96 (6):995-1003.
134- Girard JM.; Drevin G.; Brasme JF.; et. al. (2016), Clinical and biological specificity of beta-thalassemia intermedia: a case report. Ann Biol Clin (Paris), 74 (6):688-92.
135- Jamieson C.; Hasserjian R.; Gotlib.; et. al. (2015), Effect of treatment with a JAK2-selective inhibitor, fedratinib, on bone marrow fibrosis in patients with myelofibrosis. J Transl Med.,10:294.
136- Weatherall D.; Taher A.; Vichinsky E.;et. al. (2013), Guidelines for the management of non transfusion dependent thalassaemia (NTDT). Nicosia, Cyprus.
137- Al-Ali HK.; Stalbovskaya V.; Gopalakrishna P.;et. al. (2016), Impact of ruxolitinib treatment on the hemoglobin dynamics and the negative prognosis of anemia in patients with myelofibrosis. Leuk Lymphoma.;47(10):2464–2467.

138- Zhao N.; Nizzi C.P.; Anderson S.A.; et. al. (2015), Low intracellular iron increases the stability of matriptase-2. J. Biol. Chem.;290:4432–4446.
139- Wang Y.; Wu X.-g.; Wu Q.;et. al. (2014), HFE interacts with the BMP type I receptor ALK3 to regulate hepcidin expression. Blood.;124:1335–1343.
140- Rishi G.; Crampton EM.; Wallace DF. and Subramaniam VN., (2013), In situ proximity ligation assays indicate that hemochromatosis proteins Hfe and transferrin receptor 2 (Tfr2) do not interact. PLoS ONE;8(10):e77267.
141- Rund D., (2016), Thalassemia: modern medicine battles an ancient disease. Am J Hematol;91:5–21.
142- Porter JB.; Cappellini MD.; Kattamis A.;et. al. (2017), Iron overload across the spectrum of non-transfusion-dependent thalassaemias: role of erythropoiesis, splenectomy and transfusions. Br J Haematol, 176(2):288-299.
143- Khatami S.; Dehnabeh SR.; Mostafavi E.;et. al. (2013), Evaluation and comparison of soluble transferrin receptor in thalassemia carriers and iron deficient patients.Hemoglobin.;37(4):387-95.
144- Paolo Ricchi; Antonella Meloni; Anna Spasiano; et. al. (2018), The impact of liver steatosis on the ability of serum ferritin levels to be predictive of liver iron concentration in non-transfusion-dependent thalassaemia patients.
145- Yathiraj PH.; Singh A.; Vidyasagar S.;et. al. (2017), Excellent and durable response to radiotherapy in a rare case of spinal cord compression due to extra-medullary hematopoiesis in β-thalassemia intermedia: case report and clinicoradiological correlation. Ann Palliat Med, 6 (2):195-9.
146- Nyoman Suci Widyastiti; Harianto Notopuro; Catharina Suharti and Aryati Aryati, (2018), ASSOCIATION OF -582 A> G HAMP-P POLYMORPHISM AND IRON STATUS OF JAVANESE B THALASSAEMIA CARRIERS. Biomedical and Clinical Sciences (JBCS).
147- Inthawong K.; Charoenkwan P.; Silvilairat S.; et. al. (2015), Pulmonary hypertension in non-transfusion-dependent thalassemia: Correlation with clinical parameters, liver iron concentration, and non-transferrin-bound iron. Hematology, 20(10):610-7.
148- Aparna A.; Sagare; Dhiraj J. and Trivedi, (2014), Assesment of Transferrin Saturation as an Indicator of Iron Overload in Homozygous & Hetrozygous Form of Thalassemia..

الخلاصة:

- الهبسيدين هو هرمون ببتيدي يوجد على اشكال متعددة منها (preprohormone) ويتألف من ٨٤ حامض اميني وprohormone ويتكون من ٦٠ حامض اميني والشكل الفعال هو hormoneويتكون من ٢٥ حاض اميني يصنع في الكبد ويقوم بتنظيم دخول الحديد الى الدورة الدموية في اللبائن
- تهدف الدراسة الحالية الى حساب HbوPCV وكذلك حساب مستوى الهبسدين، حديد مصل الدم، TIBC الترانسفرين وايضا الفرتين لمجموعة من اطفال عراقيين حاملين للبيتا ثلاسيميا المتوسطة وكذلك ايجاد العلاقة بين هرمون الهبسدين وعدد مرات نقل الدم الدوري للمرضى وذلك للتنبأ بتاثير نقل الدم الدوري للمرضى على مستوى الهبسدين لكل المجاميع
- تضمنت الدراسة ٨٠ طفل عراقي مصاب بالثلاسيميا راقدين في مستشفى ابن البلدي خلال المدة (١-تشرين الاول للعام ٢٠١٧) الى (١-نيسان لعام ٢٠١٨) تراوحت اعمارهم (٦-١٢) سنة. تم تقسيم العينات الى ثلاث مجاميع المجموعة الاولى تضمنت اربعون عينة (٢٠ اناث ،٢٠ ذكور) قبل اضافة الدم للمرضى كمجموعة اولى واربعون عينة لنفس المرضى لكن بعد اضافة الدم كمجموعة ثانية اما المجموعة الثالثة فتضمنت اربعون شخص سليم (غير مصاب) ٢٠ انثى ، ٢٠ ذكر. في هذه الدراسة Hb وPCV تم قياسهم بجهاز بصورة تلقائية، بينما الهبسدين والفرتين تم قياسهم بواسطة تقنية الاليزا ، اما نسبة الحديد في المصل وTIBC كان قياسهم بواسطة الطرق الضوئية اما الترانسفرين فتم حسابه من خلال معادلة تقسيم الحديد على TIBC.
- اظهرت نتائج هذه الدراسة تاثير سلبي للهيموغلوبين وPCV لمجاميع المرضى قبل وبعد اضافة الدم مقارنة بالأشخاص الطبيعيون الاناث والذكور كذلك اظهرت تأثير ايجابي للهبسدين لمجاميع المرضى الاناث والذكور مقارنة بالمجاميع الطبيعية. في حين الترانسفرين كان له تأثير ايجابي لمجاميع المرضى الاناث والذكور مقارنة بمجموعة الاصحاء وكذلك الفرتين اظهرت نتائجه تأثير سلبي لمجاميع المرضى بالمقارنة مع مجموعة الاصحاء، وكذلك نسبة الحديد اظهرت نفس النتيجة بالمقارنة مع مجموعة الاشخاص الطبيعيين لكن قيمة TIBCكانت ليس لها تاثير معنوي بالمقارنة مع المجموعة الطبيعية.
- يزداد تاثير Hb وPCV بصورة ملحوظة من خلال إضافة الدم للمرضى المصابين فضلا عن زيادة امتصاص الحديد تتأثر بمستوى الهبسدين لذلك هرمون الهبسدين له تأثير ملحوظ على مرضى الثلاسميا المتوسطة لأطفال العراق وبالتالي سيؤدي الى ارتفاع ملحوظ في مستوى الحديد داخل الجسم مما يؤدي الى تراكم الحديد مع زيادة مخزون الحديد داخل الجسم (الفرتين) وذلك بسبب الإضافة المستمرة للدم، لكن لم نلاحظ من خلال النتائج ان هناك تأثير ملحوظ للترانسفرين وTIBC.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بغداد

كلية التربية ابن الهيثم للعلوم الصرفة

قسم الكيمياء

دراسة القياسات الاحيائية الحديثة هيبسيدين -٢٥ في البيتا ثلاسيميا المتوسطة قبل وبعد عملية نقل الدم لاطفال العراق المصابين

الرسالة

تقدم إلى كلية التربية ابن الهيثم للعلوم الصرفة في جامعة بغداد لانجاز جزئي لمتطلبات درجة الماجستير في علوم الكيمياء

أعداد

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بإشراف

أ.م.د. بشرى حميد علي

ايار ٢٠١٨ م   شعبان ١٤٤٠ هـ