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Evaluation of Hepcidin Hormone and Some Biochemical Parameters in Iraqi Children Patients with β-Thalassaemia Intermedia Before and After Blood Transfusion

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Abstract
Background:"thalassaemia" refers to a group of blood diseases characterized by low or absent synthesis of normal globin chains. Depending on the chain whose synthesis is impaired, the thalassaemias are called α–, β–,ɣ– ,σ– or εɣσβ-thalassaemias . Most thalassaemias are inherited as recessive traits.Objective: the aim of this study is evaluated hepcidin levels, transferrin, ferritin, serum iron and total iron bending capacity [TIBC] before and after blood transfusion of thalassaemia patients.Methods: In this study, we take 80 persons the age of them were mean±SD (9.68±2.08), 40 patients with β-thalassaemia intermedia, and 40 healthy persons as control. We take blood 5ml from thalassaemia's patients before blood transfusion and after transfusion of blood by 3 days. The total number of the samples we taken are 120, 40 samples before blood transfusion, 40 samples after blood transfusion and 40 control samples. Then we separated each sample into two tubes.Result: increase iron absorption of thalassaemia patient after blood transfusion due to decrease level of hepcidin.Conclusion: from this study was appear iron absorption meanly affect by hepcidin level, so that hepcidin was highly significant change in thalassaemia patient after blood transfusion and that lead to iron overload. So conclude high levels of ferritin after blood transfusion due to blood uptake lead to increase iron storage (ferritin).
Keywords: β-thalassaemia intermidia, hepcidin, serum iron and ferritin (iron storage).

Introduction:
"thalassaemia" refers to a group of blood diseases characterized by low or absent synthesis of normal globin [1]. Depending on the chain whose synthesis is impaired, the thalassaemias are called α–, β– ,ɣ– ,σ– or εɣσβ-thalassaemias . Most thalassaemias are inherited as recessive traits. These primary quantitative defects are no longer rigidly differentiated by the structural variants produced at reduced rate [such as HbE and Hb lepore]. From a clinical point of view, the most types are α- and β-thalassaemias, decrease one of the two types of polypeptide chains [α or β] that form normal haemoglobin molecule [HbA, α2β2] [2].

The recent study of thalassaemias, which constitute a major problem in the countries around the Mediterranean sea, the middle east and the trans-caucasus, India and far East. The most carrier frequency of β-thalassaemia was reported in Maldives [18%] , Cyprus [14%], Sardinia [10.3%] and southeast Asia [3-5%][3]. Because the frequency in these regions is most likely related to the selective pressure from plasmodium falciparum malaria . So that, population migration and intermarriage between different ethnic groups has introduced thalassaemia in almost every country of α-talassaemia[4].

Often, heterozygotes of ether α- or β-thalassaemia asymptomatic and do not need treatment. Interactions of thalassaemia and corresponding haemoglobinopathies e.g. Hb E, Hb C or Hb S with β-thalassaemia or Hb constant spring [Hb CS] with α-thalassaemia also give increase to varies thalassaema syndromes[5]. Depend severity and transfusion requirement , these thalassaemia syndromes can be classified phenotypically into two main group :

A- Transfusion dependant thalassaemia [TDTs]
B- Non-transfusion dependant thalassaemia [NTDTs]. As shown in figure 1-1.

The TDTs need regular blood transfusion to survive so without adequate causes suffer several complications and a short life span [6]. This group includes 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[7].

Therefore Hepcidin is a protein in humans is encoded by the hepcidin antimicrobial peptide [HAMP] gene, is a key regulator of the entry of iron into the circulation in mammals. When hepcidin level is abnormally increase inflammation, serum iron falls due to iron trapping within macrophages and liver cells and low gut iron absorption, this lead to anemia due to not enough amount of serum iron being available for developing red cells [8]. But when the hepcidin level is abnormally low like in hemochromatosis, iron overload happen due to increased ferroportin mediated iron efflux from storage and increased gut iron absorption[9].

So hepcidin, a peptide hormone is mainly synthesis in the liver, was discovered in 2000. It can be reduces extracellular iron in the body by several mechanisms:

1) Hepcidin lower dietary iron absorption via reducing iron transport across gut mucosal cells (enterocytes); It reduces iron exit from macrophages, the major site of iron storage;
2) It reduces iron exit from the liver. So in all three instances this is associated by reducing the transmembrane iron transporter ferroportin

Hepcidin found as a preprohormone (84 amino acids), prohormone (60 amino acids), and hormone (25 amino acids). Twenty- and 22-amino acid metabolites of hepcidin also found in the urine. Removal of 5 N-terminal amino acids results deactivation of function[10]. The conversion of prohepcidin to hepcidin is mediated by the prohormone convertase furin. This conversion may be regulated via alpha-1 antitrypsin [11].

Also Iron overload happened when iron intake increased over period of time, result from red blood cell transfusions or increased iron absorption through gastrointestinal [GI] tract. Both of them occur in thalassaemia, whith blood transfusion therapy start the major cause of iron overload in thalssaemia major and increased GI absorption being more important in non-transfusion dependent thalassaemia [NTDT] [12]. But thalassaemia major patients receive regular blood transfusion, iron overload is occurred because the human body lacks a mechanism to remove excess iron[13]. Iron accumulation is toxic to many tissues, lead to heart failure, cirrhosis, liver cancer, growth retardation and multiple endocrine abnormalities[14].

In addition, Ferritin is a global intracellular protein that stores iron and releases it in a controlled fashion. Produced by almost all living organisms, like algae, bacteria, higher plants, and animals. In humans, the important role as buffer against iron deficiency and iron overload. Ferritin is exist in most tissues as a cytosolic protein, but small amounts are secreted into the serum where it functions as an iron carrier. Plasma ferritin is biomarker of the total amount of iron stored in the body, hence serum ferritin is utilized as a diagnostic test for iron-deficiency anemia[15].

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

Material and method

In this study, we take 80 persons the age of them were mean±SD (9.68±2.08), 40 patients with β-thalassaemia intermedia, and 40 healthy persons as control. We take blood 5ml from thalassaemia's patients before blood transfusion and after transfusion of blood by 3 days. The total number of the samples we taken are 120, 40 samples before blood transfusion, 40 samples after blood transfusion and 40 control samples. Then we separated each sample into two tubes.

The 1st tube for determine the ferritin and hepcidin value and the 2nd tube to determine iron.

Separate serum of the samples by used centrifuge and assay was employ enzyme-linked immune sorbent assay (ELISA) to determine ferritin and hepcidin level.

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

Statistical analysis

results of this study was expressed as [mean ± SD], also T.test was utilized for compared between three studied groups, so T.test less than 0.05 and 0.001 was considered significant and highly significant respectively.

Result

In this study patients were differentiated to three clans, the fist clan [C1] includes before blood transfusion patients samples, the second clan [C2] includes after blood transfusion patients samples and the third clan [C3] was control samples, as show in table 1. Also calculated mean ± standard division for all parameter result of all patient {male and female}, then evaluate t test value to all parameter that utilized to show the differences between clans; variation was considered significantly when T. test values was ≤0.05. That was shown in table 1.

Table 1, parameter result of all patients. BMI [body mass index], HS [high significant], NS [non significant].

Biometers	[C1] Mean±SD	[C2] Mean±SD	[C3] Mean±SD	Ttest C1VsC2	Ttest C1VsC3	Ttest C2VsC3
BMI Kg/m^2	11.35±1.47	11.35±1.47	16.9±2.15		HS	HS
S. Hep pg/ml	13.37±3.25	11.32±1.62	25.15±3.51	HS	HS	HS
S. Trans. %	48.67±8.7	50.85±7.25	30.39±3.31	NS	HS	HS
S. Fer ng/ml	1942.78±704.11	1734.08±298.24	169.62±16.46	NS	HS	HS
S. Iron mcg/dl	84.2±7.1	91.21±7.57	52.6±4.69	HS	HS	HS
S. TIBC mcg/dl	180.27±18.96	182.29±26.31	173.87±10.16	NS	NS	NS

Discussion

The present study show mean ± SD of the body mass index of female (10.93±1.59) and male (11.77±1.23) patients that lower than control (Female= 15.75±1.45) and (Male=18.05±2.14) clan as shown in table 1.

The results of study appeared decrease highly significant with control of T.test value ($p< 0.05$) that agreement with Baldini M study, Baldini M was suggest thalassaemia patients have abnormal secretion of pituitary gland that lead to decrease weight of patients[16], that shown in figure 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.

The nearby study was expose mean ± SD of hepcidin for female (13.14±3.47) and male (13.6±3.09) patients before and after blood transfusion (Female=11.14±1.82 and Male=11.49±1.45) was lesser than control (Female=25.04±3.55 and Male=25.26±3.56) as appears in table 1.

The result of female and male patients in the recent study was decrease highly significant ($p< 0.05$) 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. study[17]. The control had normal level of hepcidin so absorption of iron in stomach was normal so that not found excess iron inside body compare with thalassaemia patients, that shown in figure 2.

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

The result of this study for female and male patients was non-significant ($p > 0.05$) of T.test value between C1 and C2, that agreement with Porter JB, 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[18], that also illustrated in figure 3.

In addition, transferrin test materialize increase highly significant ($p< 0.05$) value with clan 3 that approved by Khatami S, also he explains the increase of transferrin level in thalassaemia patients due to iron overload that stimulate to production transferrin in order to carry it to the target organ[19], that also appear in figure 3.

The nearby study was display mean ± SD of ferritin value for female (1888.85±756.102) and male (1996.7±663.192) thalassaemia patients before and after blood transfusion (Female=1714.5±313.1and Male=1753.65±289.38) was more than control (Female=169.29±17.47 and Male=170.56±15.81) as illustrated in table 1.

The result of this study for female and male patients was non-significant ($p > 0.05$) of T.test value between C1 and C2, that agreement Paolo Ricchi study, also he concluded the drop off ferritin level of female and male patients after blood transfusion due to increase iron storage because blood up take lead to increase ferritin level in patients after blood transfusion[20].

Moreover ferritin was appear increase highly significant ($p< 0.05$) with control that approved by Yathiraj PH. Study, so that he suggest the iron overload lead to increase ferritin production, that shown in figure 4 [21].

The current study was display mean ± SD of iron value for female (83.18±7.56) and male (85.19±6.65) thalassaemia patients before and after blood transfusion (Female=90.86±7.55 and Male=91.55±7.76) was more than control (Female=52.84±4.53 and Male=52.4±4.95) as show in table 1.

The result of this study for female and male patients was decrease significant ($p \leq 0.05$) of T.test value between C1 and C2, that agreement with Nyoman S. study, also he explain the blood transfusion of thalassaemia patients lead to increase of serum iron[22].

Additionally, iron test was result decrease highly significant ($p< 0.05$) with control, that agreement with Inthawong K, also he discus the aggregation of iron in thalassaemia patients that lead to increase iron concentration[23], as appear in figure 5.

Serum iron increased in the thalassaemia patients because of iron overload that due to decrease hepcidin leaded to increase absorption of iron

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

The result of this study for female and male patients were appear non significant ($p > 0.05$) of all T.test value, that agreement with Aparna A study when research about parameters that used to diagnosis thalassaemia[24]. That was show in figure 6.

Total iron binding capacity test of thalassaemia patients before and after blood transfusion appeared higher than control as shown in figure 6.

Conclusion

From this study was appear Iron absorption mainly effected by hepcidin levels so that hepcidin was high significant change in thalassamia intermdia for Iraqi children patients after blood transfusion this versus high significant increase of serum iron and lead to iron overload and increase iron storage (ferritin) because blood uptake, but non-significant in transferrin or TIBC respectively.

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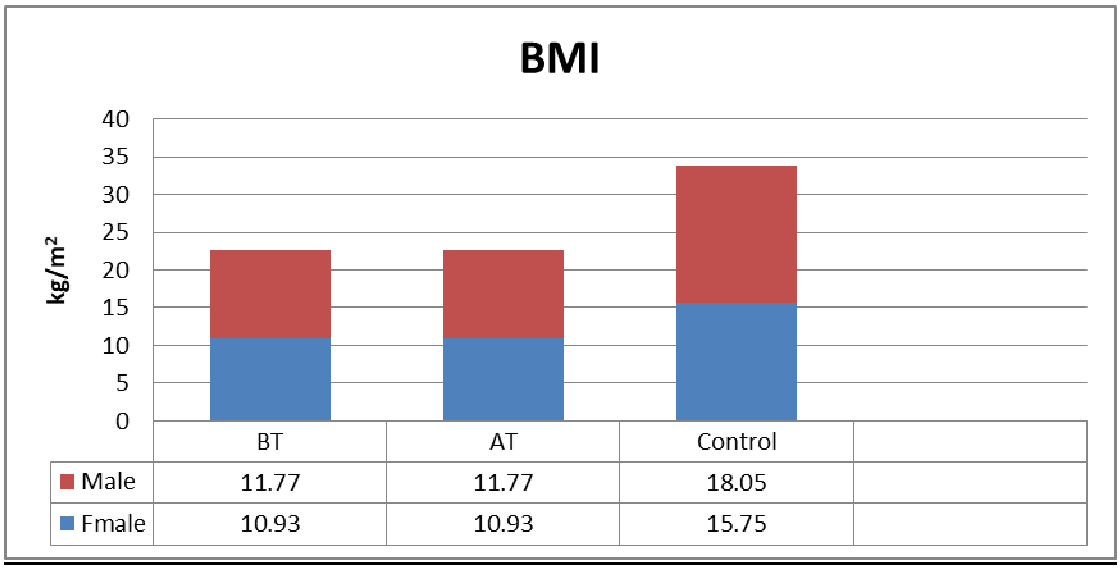

Figure 1, show alteration of BMI in male patient, female patient and control.

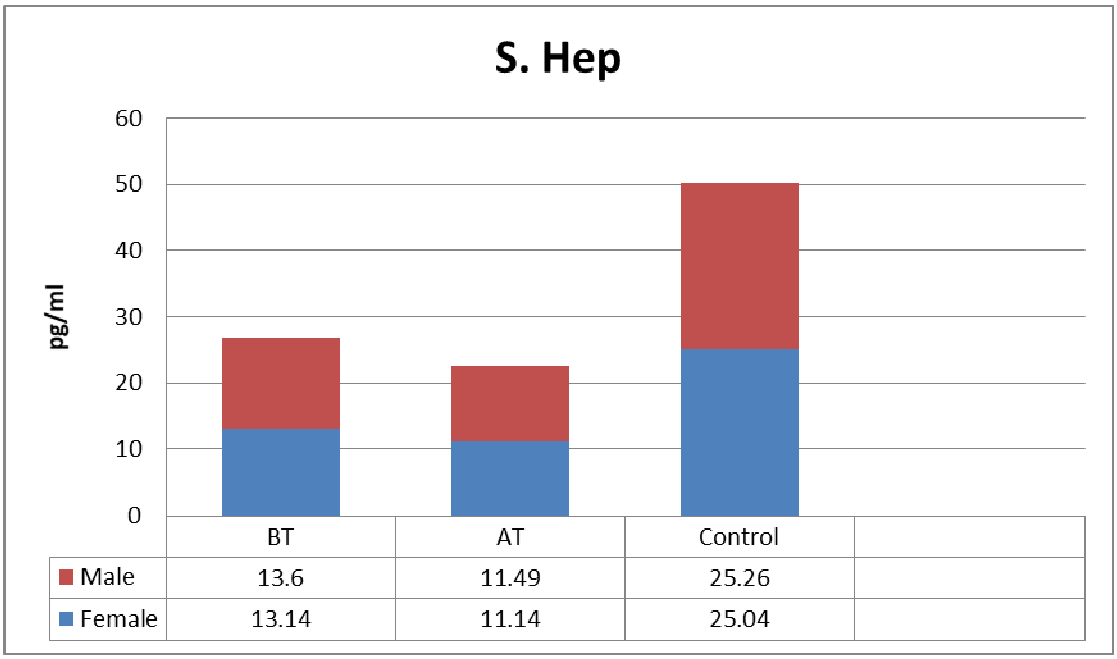

Figure 2, show alteration of serum hepcidin in male patient, and female patient and control.

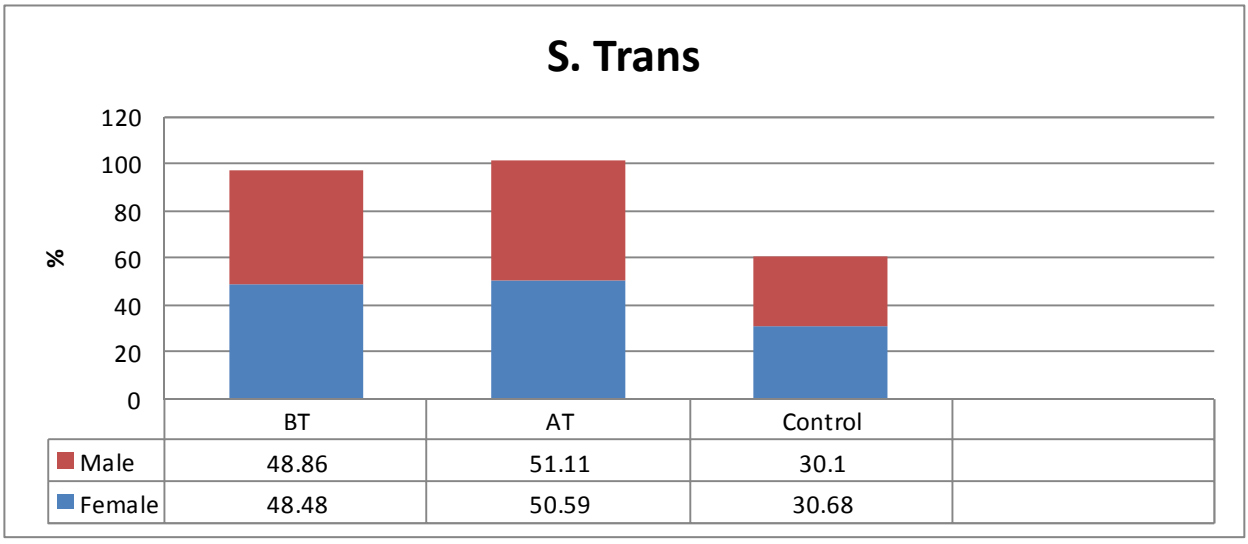

Figure 3, show alteration of serum transferrin in male patient, and female patient and control.

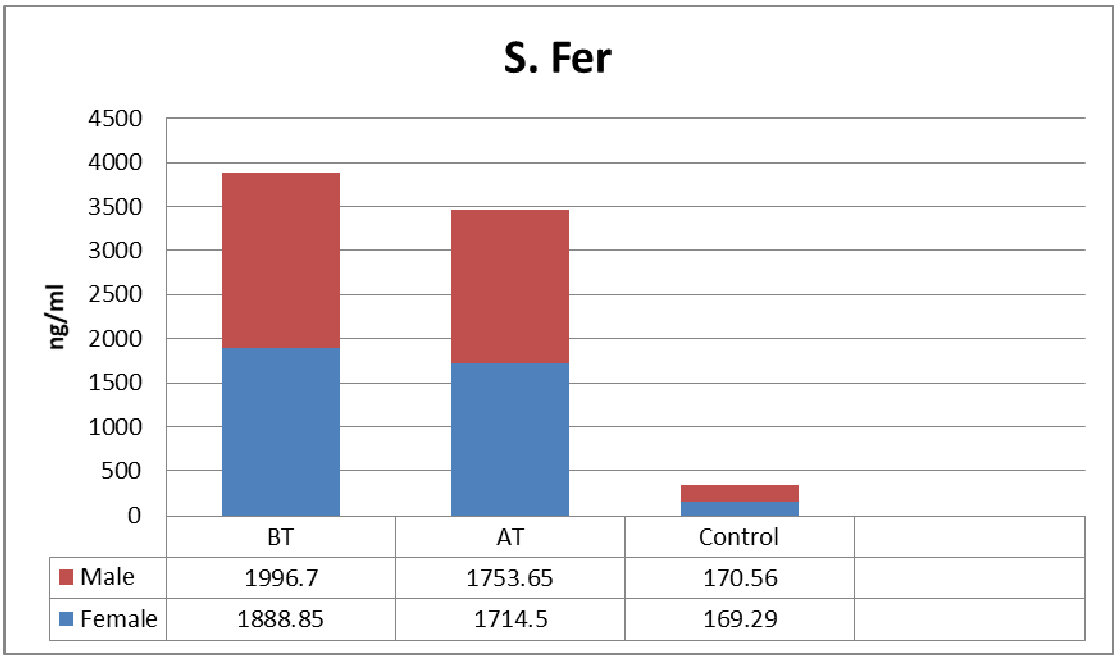

Figure 4, show alteration of serum ferritin in male patient, and female patient and control.

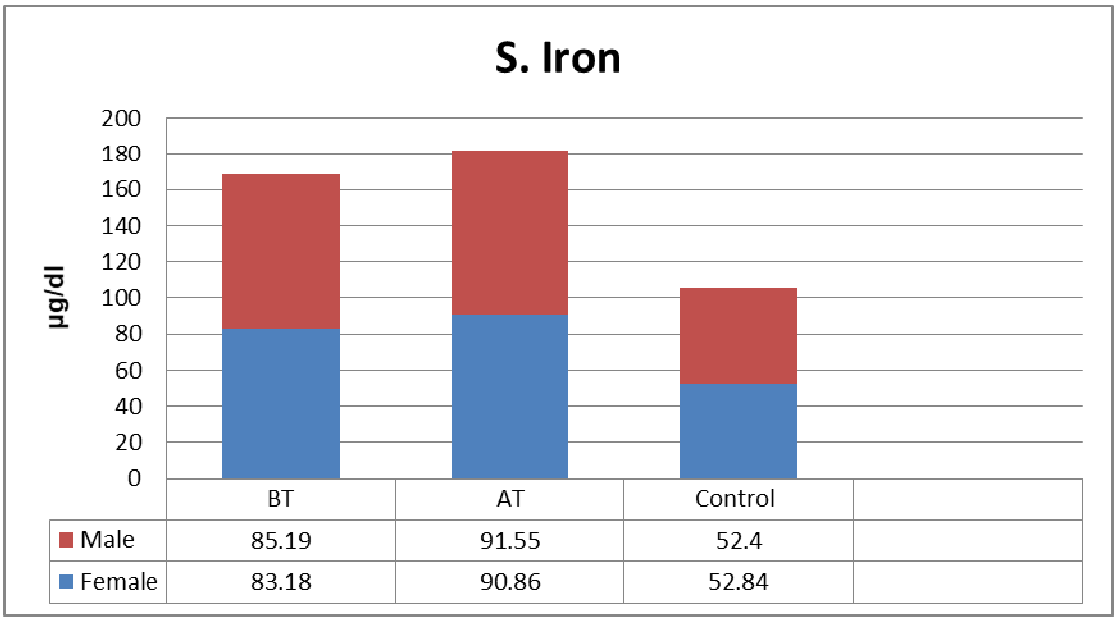

Figure 5, show alteration of serum iron in male patient, and female patient and control.

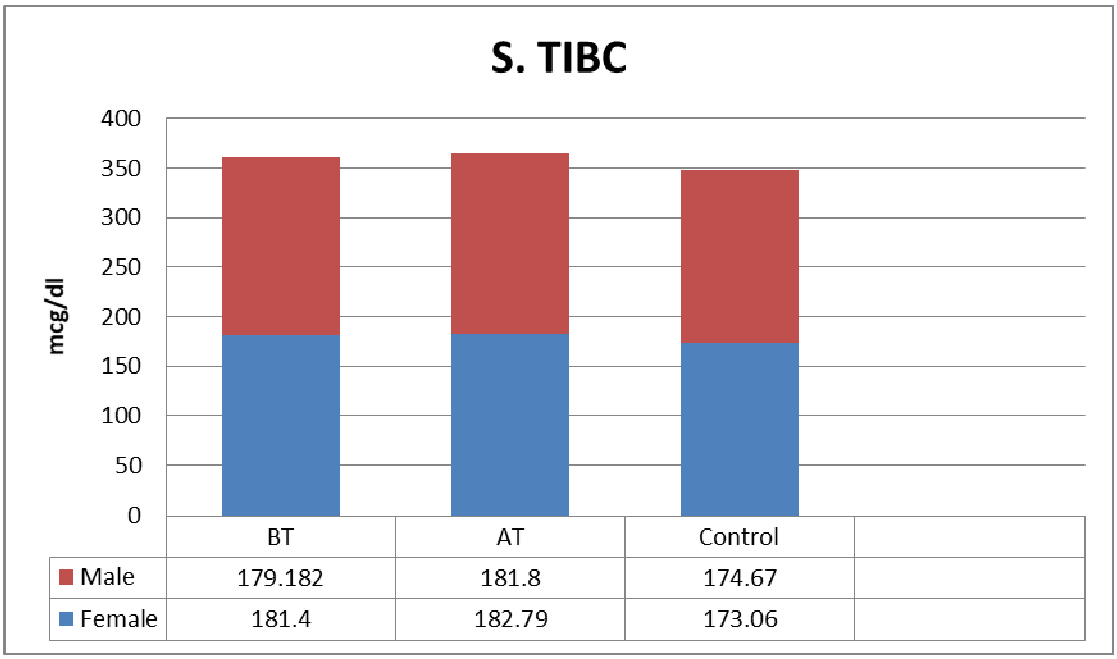

Figure 6, show alteration of serum total iron bending capacity in male patient, and female patient and control.