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Effects of Iron Chelating Therapy And/or HBV- Vaccination On β-Thalassemia Major Patients

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Abstract

Introduction: Thalassemia is a genetic inherited blood disorder in which the body makes abnormal hemoglobin with excessive destruction of red blood cells, which leads to anemia. For many years, hepatitis B virus was a major problem for patients with thalassemia substantially contracted from blood transfusions. The development of effective vaccine has further reduced the magnitude of the problem of hepatitis B. Iron chelators are used to remove excess iron that accumulates due to repeated blood transfusion.

Aim of the work: To compare thalassemic patients either have or haven't HCV with healthy persons as regards biochemical indices taking in consideration effect of vaccination against HBV or not and using iron chelating therapy or not by the studied persons.

Methodology: A case control study in which 40 thalassemic, blood transfusion dependent patients were chosen randomly to act as a case group from thalassemic patients attending the VACSERA Company. The cases (40 patients) they were classified to patients having HCV, patients were HBV vaccinated ,other non vaccinated, using iron chelating therapyor don't use it. Another 10 healthy and non thalassemic persons were chosen randomly among persons attending the same company as a control group to be matched with the case group.

Results: 50% of studied thalassemic patients had HCV seropositivity. Biochemical blood indices which were found to be significantly elevated among thalassemic patients than controls were ALT, AST, ALP, GGT and LDH enzymes in addition to serum iron, ferritin and globulin mostly in HBV non-vaccinated and iron chelating therapy non dependent patients while, other biochemical indices which were significantly decreased among thalassemic patients compared to controls included: total cholesterol, total protein, albumin and albumin/globulin ratio mostly in HBV vaccinated and iron chelating non dependent patients.

Conclusion: this study illustrated the effectiveness of iron chelators agentsand the importance of vaccination for reduction of morbidity and mortality.

Introduction

Thalassemia is a genetic blood disorder passed down through families (inherited) in which the body makes an abnormal form of hemoglobin (the protein in red blood cells that carries oxygen). The disorder results in excessive destruction of red blood cells, which leads to anemia.[1]

Hemoglobin is made of two proteins: Alpha globin and beta globin. Thalassemia occurs when there is a defect in a gene that helps control production of one of these proteins. [2]

There are two main types of thalassemia: Alpha and beta that occur when a gene or genes related to the alpha or beta globins are missed or mutated respectively[3,4].

Beta thalassemias are common in persons of Mediterranean origin, and to a lesser extent, Chinese, other Asians, and African Americans. Both alpha and beta thalassemia include two forms: Thalassemia major (homozygous) and thalassemia minor (heterozygous). Beta thalassemia major is also called Cooley's anemia.[5]

Some surveys have also indicated that many of the developing countries have no facilities for the diagnosis, control, and management of the common hemoglobin disorders.[6] In short, the global situation regarding the control and management of these conditions is extremely unsatisfactory and cannot continue to be ignored by the international hematology community and health agencies.[7]

The thalassemic children become wasted and pot bellied due to hepatosplenomegally. Hypersplenism leads to worsening of anemia with a greater tendency to infection and hemorrhage because of granulocytopenia

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and thrombocytopenia respectively.[8] Progressive tissue and organs damage occurs leading to progressive hepatic fibrosis, cardiac arrhythmia, preicarditis and congestive heart failure. Endocrinal dysfunction includes diabetes mellitus, hypothyroidism hypoparathyroidism, adrenal insufficiency and hypogonadism. Red blood cells will appear small and abnormally shaped when looked at under a microscope. A complete blood count (CBC) reveals anemia. Hemoglobin electrophoresis shows the presence of an abnormal form of hemoglobin.[9]

\a treatment called chelation therapy to remove excess iron accumulated in the body.[14,15]

Iron overload could be defined as an increase of total body iron stores which can be normally maintained within the range of 200-1500 ng/ dl. This normal range is maintained by re-utilization of iron from RBCs destruction and iron uptake from diet. In adults, 95% of iron required for RBCs production is recycled from breakdown of RBCs and 5% from diet.[16]

Causes of iron overload may be primary or secondary. In primary cause, there is a primary defect in iron balance regulation e.g. iron is absorbed in excess due to increased iron transfer from the enteral cells to the blood. In secondary types, the best examples are major thalassemia and sideroblastic anemia due to ineffective erythropoisis and repeated blood transfusion.[17]

Organ damage is related to amount of iron deposited in the parenchymal cells in different organs as liver, pituitary glands, pancreas, spleen and heart, and the latter has less antioxidants mechanisms and consequently more susceptible to iron induced or oxidative damage that may lead to congestive heart failure which is the main cause of death in thalassemia major patients.[18]

Iron overload can be diagnosed clinically and by laboratory investigations. Clinical findings which suggest parenchymal iron overload deposition include hepatocellular dysfunction and even cirrhosis, bronze skin pigmentation, diabetes mellitus (50 -60% of iron overload patients are diabetic) cardiomyopathy, arthritis, abdominal pain and chondrocalcinosis, while laboratory diagnosis includes detecting the three biochemical iron marks; serum iron, transferring saturation and serum ferritin.[19]

Iron overload deposition in liver was reported and suggested to be a direct cause of liver damage, affecting the disease outcome and the response to interferon therapy.[20]

It was reported that serum iron reflects the degree of current hepatic inflammation and necrosis where transferrin saturation is the best predictor for the status of hepatic iron deposition.[21]

In general, chelators are small molecules that bind very tightly the metal ions preventing its poisoning effect. Iron chelators are used to remove excess iron that accumulates due to repeated blood transfusion with a large preference to other metal ions such as calcium.[22]

Aim of the work

To compare between the apparently healthy persons and thalassemic patients with or without HCV who were subjected to vaccination against HBV or using iron chelating therapy as regards some biochemical indices.

Patients and methods:

-A case control study, conducted in the holding company for biological products and vaccines (VACSERA).

Forty thalassemic blood transfusion dependent patients (aged 4-30 years, mean ±SD = 14±4.5 years) was chosen (according to selection criteria) among all patients attending the blood bank, therapeutic unit of the above mentioned company. Of them 20 patients having hepatitis C and the remaining 20 patients were hepatitis C free.

Thalassemic cases were chosen as 50 % of them were HBV vaccinated and the other half were not vaccinated.

According to liver affection, HBV vaccination and using iron chelating agent (as a treatment by thalassemic patients) the case group (40 cases) was finally divided into 4 main subgroups:

*Subgroup 1 (n=10): Patient who had thalassemia + hepatic affection + desferal dependent (5 HBV vaccinated & 5 HBV non- vaccinated).

* Subgroup 2 (n=10): Patient who had thalassemia + hepatic affection + desferal non- dependent (5 HBV vaccinated & 5 non-vaccinated).

* Subgroup 3 (n=10): Patient who had thalassemia + non hepatic affection + desferal dependent (5 HBV vaccinated & 5 non-vaccinated).
* Subgroup 4 (n=10): Patient who had thalassemia + non hepatic affection + desferal non- dependent (5 HBV vaccinated & 5 non- vaccinated).
-Another sample of 10 healthy persons were chosen randomly according to selection criteria (to be nearly at same age range as the case group, healthy and free from any chronic disease to act as a control group). The control group was chosen so that half of them (5 persons) were HBV vaccinated and the other (5) persons were not vaccinated.
-Blood samples were collected from thalassemic patients (before blood transfusion) as well as control persons. Blood samples were processed to detect:
*HCV Ab by rapid technique method.[23]
*Serum ferritin using Elisa test.[24]
*Blood chemistry indices included: serum liver enzymes, serum cholesterol, serum proteins, serum LDH & serum total iron.[25]
-Official permissions as well as ethical considerations were followed with the examined persons.
-Statistical analysis used SPSS program version 16.0 and included statistical parameters (means & S.E.) and statistical tests: independent sample t- test & Duncan's multiple range test (a one way ANOVA post hoc test used to determine which means differ).[26]

Results

-Non vaccinated control group showed a significant decrease in both total protein , globulin and total cholesterol levels and showed a significant increase in ferritin level in comparison with vaccinated group (table 6, 8, 12).
-Non vaccinated patients with HCV &iron chelating dependent showed a significant decrease in ALT, AST, alkaline phosphtase, GGT, LDH activities and recorded a significant increase in total cholesterol and serum iron levels when compared with vaccinated and control groups (table 3, 4, 5, 10, 11).
-Concerning non vaccinated thalassemic patients with HCV infection and iron chelating independency, they showed a significant increase in ALT, AST, alkaline phosphtase, GGT activities, total cholesterol, iron and ferritin levels when compared with vaccinated thalassemic HCV positive, iron chelating independent and control group.
- Thalassemic non vaccinated patients without HCV and iron chelating dependent showed a significant increase in ALT, AST, albumin/globulin ratio and a significant decrease in globulin and ferritin levels compared with vaccinated and control groups (tables 1, 2 , 8, 9 , 12).
-While in non vaccinated thalassemic patients without HCV infection and iron chelating independency, they showed a significant increase in albumin, total protein, iron levels and. It also recorded a significant decrease in LDH activity compared with vaccinated thalassemic, non HCV and iron chelating independency and control group (tables 5, 6, 7, 10, 11).

Discussion

Thalassemic cases may still develop liver dysfunction due to infection with blood born agents either known, undiscovered or due to transfusional iron overload.[27]
The elevated liver function tests specially ALT & AST activities reflected hepatic damage secondary to hemosderosis or viral hepatitis C. Also, it was detected that liver disease ranks second as a cause of death among adults with thalassemia.[28]
These parameters specially ALT represent biochemical changes of the inflammatory activity of chronic hepatitis and its elevation reflects the activity of the disease.[29]
Alanine aminotransferase (ALT) abnormalities were observed also in subset of patients with lower ferritin concentration which suggests that even relatively small increase of iron burden may cause hepatocellular damage. However, ferritin measurement may be underestimated in body iron stores of those patients.[30]
Some studies stated that HCV seropositivity had a significant correlation with higher serum AST and lower albumin level, but not with ALT & ALP levels. High AST level in HCV infected cases is most probably by liver disease rather than any other cause.[31]
The present study showed that there was a highly significant decrease in total cholesterol level in most case subgroups specially HCV+ve, iron chelating therapy

non dependent cases compared with control persons.

Total cholesterol is one of the important parameters of lipids profile in B- thalassemia and appeared below normal compared with relevant controls and is considered peculiar of the disease.[32]

The decreased cholesterol level may be due to effect of iron chelating therapy. Kim *et al*.[33] mentioned that, an impaired liver function might be likely explanation of decreased lipoprotein level, while a reduced extra-hepatic lipolytic power may result in elevation of cholesterol level in circulation.

Interestingly, Behera*et al*.[34] stated that lipids pattern in B-thalasseia usually refers to hypocholesterolemia and this can lead to reduction of vitamins A &E, but fortunately this may be associated with lower risk of cardiovascular diseases.

In other studies, these results give evidence that oxidative alterations to cell components can be shown in serum as a marked increase of conjugated dine lipid hydroperoxides.[35]

So, it is worthy note that iron induced liver damage in thalassemia may play a role in depletion of lipid soluble antioxidants irrespectively age of patients.[36]

The present study showed that total protein and albumin levels in blood were significantly decreased among HCV-ve, iron chelating therapy nondependent, either vaccinated or non vaccinated cases in comparison with control persons.

Other studies such as Leecharoenkiat*et al*.[37] revealed that anti-HCV positive patients have a significant higher serum globulin and lower serum albumin. This pattern is mostly due to thalassemia and liver dysfunction and not due to repeated blood transfusion because time interval between transfusions was not different for positive and negative HCV patients.

Shams *et al*.[38], reported that albumin is the most abundant protein in serum. It serves as a store of nutritive and structural protein in time of need. The main function of albumin is to stabilize blood volume and to regulate vascular fluid exchange and this mechanism accounts for 75% of osmotic pressure. Almost all diseases show some degree of low albumin level but marked low levels usually refer to hepatic, renal or systemic disease.

In our study, there was a highly significant activity of lactate dehydrogenase enzyme (LDH) in blood in thalassemic patients either vaccinated or non-vaccinated compared with healthy persons.

Tselepis*et al*.[39] mentioned that (LDH) enzyme is usually elevated in thalassemic patients (due to liberation of erythrocyte enzyme during hemolysis) but it is not a specific finding as it is also elevated in other medical conditions associated with tissue injury.

Pennell *et al*.[40] stated that physical examination of thalassemic patients revealed that there was a some sort of cardiac dysfunction and then cardiac function deterioration evidenced by elevated cardiac function tests and this occurs due to iron accumulation. Cardiac damage is best prevented in thalassemic patients by maintaining a constant level of chelator in the circulation.

The effect of using deferazirox (as an iron chelating therapy) in patients with cardiac diseases associated with thalassemia major as regards survival rate was reported since early 1980s. At the same time, subsequent follow up studies which were conducted to observe the reduction of morbidity and mortality with the use of deferoxamine (as an iron chelator) were too short to give definite conclusion. However, in the present decades patients who started deferoxamine at early childhood reach a long term survival rate with great certainty.[41]

Concerning iron level in our study, it was detected in an increased significant values ranging between 124-190 ug/dl especially in non vaccinated thalassemic HCV-ve and iron chelating nondependent patients. The results of this study was supported by Win *et al*.[42] who stated that iron induced hepatic damage is influenced by HCV infection which is the most frequent cause of hepatitis in thalassemic patients.

Iron is an essential element for all cells functioning and survival, but excess tissue iron is potentially toxic, mutagenic, mitogenic and liver is primarily vulnerable .Iron overload and the beneficial role of iron chelation therapy had been demonstrated in chronic HCV patients.[43]

Normally, serum ferritin concentration should be ranging from 200-300 ng/ml, and concentration exceeding that level had

clinically relevant to liver damage. Increased ferritin concentration above normal would put thalassemic patient at risk of developing cardiac disease.[44]

The present data were in agreement with Vichinsky*et al.*[45] who mentioned that serum iron, ferritin and calculated transferrin saturation were significantly elevated in chronic hepatic patients versus controls. Among these studies about (46%) of patients had elevated serum ferritin above the cut off value (156 ug/dl) for iron chelating either dependent or nondependent patients.

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Tables

Table (1): Comparing thalassemic subgroups with control group as regards the liver enzyme ALT (alanine aminotransferase U/L) and HB vaccination status.

Case subgroups and controls	HB vaccination status		P
	Vaccinated (n=25) ALT (mean ±SE)	Non vaccinated (n=25) ALT(mean ±SE)	
Subgroup 1(n=10) Thalassemic , HCV +ve ,iron chelating dependent	38.2 ± 2.2 [a*]	19.0 ±1.6 [bc*]	**
Subgroup2(n=10) Thalassemic , HCV +ve ,iron Chelating independent	13.8 ± 0.6 [c*]	32.4 ±2.3 [a*]	**
Subgroup 3 (n=10) Thalassemic , HCV +ve ,iron Chelating dependent	7.2 ± 0.5 [d*]	23.8 ±3.2 [b*]	**
Subgroup 4 (n=10) Thalassemic ,HCV+ve, iron chelating independent	20.6 ±1.4 [b*]	18.0 ±1.8 [bc*]	NS
Control group (n=10) Healthy non thalassemic	15.0 ±1.1 [c*]	15.4 ±1.1 [c*]	NS

P= probability between means of vaccinated & non vaccinated groups. NS: Non significant
*Duncan test : where similar letters in each column denote that means aren't significantly different.
** significant at P< 0.05 *** significant at P< 0.01

Table (2): Comparing thalassemic cases with controls as regards the liver enzyme AST (aspartate aminotransferase U/L) and HB vaccination status.

Case subgroups and controls	HB vaccination status		P
	Vaccinated (n=25) AST(mean±SE)	Non vaccinated (n=25) AST(mean± SE)	
Subgroup 1(n=10) Thaalassic , HCV+ve ,iron chelating dependent	39.8±3.0 [a*]	22.2±2.2 [b*]	**
Subgroup 2 (n=10) Thaalassic , HCV+ve ,iron Chelating independent	15.2±1.3 [c*]	40.6±2.1 [a*]	***
Subgroup3 (n=10) Thaalassic , HCV-ve,iron Chelating dependent	9.6±0.7 [d*]	28.4±3.6 [b*]	***
Subgroup4 (n=10) Thalassemic , HCV-ve, iron chelating independent	21.5±1.1 [b*]	22.0±2.0 [b*]	NS
Control group (n=10) Healthy non thalassemic	11.0±1.1 [cd*]	11.8±0.9 [c*]	NS

Table (3): Comparing thalassemic cases with controls as regards the enzyme ALP (alkaline phosphotase U/L) and HB vaccination status.

Case subgroups & controls	HB vaccination status		P
	Vaccinated (n=25) ALP(mean±SE)	Non vaccinated (n=25) ALP(mean±SE)	
Subgroup 1(n=10) Thalassemic, HCV+ve ,iron chelating dependent	246±5.6[a*]	136±7.8[c*]	***
Subgroup 2 (n=10) Thalassemic, HCV+ve ,iron Chelating independent	168.8±19.7[b*]	246.6±17.4[a*]	**
Subgroup3 (n=10) Thalassemic ,HCV-ve ,iron Chelating dependent	123.8±5.6[c*]	118.6±7.5[c*]	NS
Subgroup4(n=10) Thalassemic , HCV-ve, iron chelating independent	169.2±8.2[b*]	183.0±7.7[b*]	NS
Control group (n=10) Healthy non thalassemic	82.2±3.4[d*]	75.2±14.2[d*]	NS

Table (4): Comparing case subgroups with controls regarding the enzyme GGT (gamma- glutamayi transfers U /L) and HB vaccination status.

Case subgroups & controls	HB vaccination status		P
	Vaccinated (n=25) GGT(mean±SE)	Non vaccinated (n=25) GGT(mean±SE)	
Subgroup 1(n=10) Thalassemic , HCV+ve ,iron chelating dependent	28.2±3.2[a*]	9.6±0.8[b*]	***
Subgroup 2 (n=10) Thalassemic , HCV+ve ,iron Chelating independent	5.0±0.4[b*]	29.2±4.3[a*]	***
Subgroup3 (n=10) Thalassemic , HCV-ve ,iron Chelating dependent	5.2±0.5[b*]	7.0±0.8[b*]	NS
Subgroup4(n=10) Thalassemic , HCV-ve, iron chelating independent	5.1±0.6[b*]	6.5±1.04[b*]	NS
Control group (n=10) Healthy non thalassemic	7.0±0.6[b*]	9.0±0.7[b*]	NS

Table (5): Comparing thalassemic groups with controls as regards lactate dehydrogease enzyme LDH (U/L) and HB vaccination status.

Case subgroups & controls	HB vaccination status		P
	Vaccinated (n=25)	Non vaccinated (n=25)	
	LDH (Mean±SE)	LDH (Mean±SE)	
Subgroup 1(n=10) Thalassemic , HCV+ve ,iron chelating dependent	820.8±28.5 [a*]	697.4±45 [b*]	**
Subgroup 2 (n=10) Thalassemic , HCV+ve ,iron Chelating independent	594±27 [b*]	577.8±19.3 [c*]	NS
Subgroup3(n=10) Thalassemic , HCV-ve ,iron Chelating dependent	521±34.4 [bc*]	597.8±20.8 [c*]	NS
Subgroup4(n=10) Thalassemic , HCV-ve, iron chelating independent	485.6±21 [c*]	836.5±37.5 [a*]	***
Control group (n=10) Healthy non thalassemic	224.4±14.5 [d*]	269.2±19.6 [d*]	NS

Table (6): Comparing case subgroups with controls as regards serum iron level (ug /dl) and HB vaccination status.

Case subgroups & controls	HB vaccination status		P
	Vaccinated (n=25)	Non vaccinated (n=25)	
	serum iron (Mean±SE)	serum iron (Mean±SE)	
Subgroup 1(n=10) Thalassemic , HCV+ve ,iron chelating dependent	124.2±3.2 [bc*]	178.4±4.01 [b*]	***
Subgroup 2 (n=10) Thalassemic , HCV+ve,iron Chelating independent	134.4±2.5 [b*]	153.4±3.5 [c*]	**
Subgroup3(n=10) Thalassemic , HCV-ve ,iron Chelating dependent	127.4±1.2 [b*]	130.6±2.4 [d*]	NS
Subgroup4(n=10) Thalassemic , HCV-ve, iron chelating independent	156.6±3.8 [a*]	190.2±4.1 [a*]	***
Control group (n=10) Healthy non thalassemic	112.8±6.6 [c*]	100.2±2.8 [e*]	NS

Table (7): Comparing case subgroups with controls as regards serum ferritin level (ng /ml) and HB vaccination status.

Case subgroups & controls	HB vaccination status		P
	Vaccinated (n=25)	Non vaccinated (n=25)	
	Serum ferritin (Mean±SE)	Serum ferritin (Mean±SE)	
Subgroup 1(n=10) Thalassemic , HCV+ve ,iron chelating dependent	2454.6±117.2 [b*]	2737±140.9 [a*]	NS
Subgroup 2 (n=10) Thalassemic , HCV+ve ,iron Chelating independent	1624.6±115 [c*]	2274.2±128.7 [b*]	**
Subgroup3(n=10) Thalassemic , HCV-ve ,iron Chelating dependent	3223±104.8 [a*]	1501.2±162.9 [c*]	***
Subgroup4(n=10) Thalassemic , HCV-ve, iron chelating independent	2208.8±100.7 [b*]	2411±184.8 [ab*]	NS
Control group (n=10) Healthy non thalassemic	112±3.6 [d*]	148.8±5.8 [d*]	***

Table (8): Comparing thalassemic patients with controls regarding globulin level in blood (g/dl)and HB vaccination status.

Case subgroups & controls	HB vaccination status		P
	Vaccinated (n=25)	Non vaccinated (n=25)	
	Globulin level (Mean±SE)	Globulin level (Mean±SE)	
Subgroup1(n=10) Thalassemic , HCV+ve ,iron chelating dependent	2.9±0.03 [c*]	3.0±0.07 [a*]	NS
Subgroup 2 (n=10) Thalassemic , HCV+ve ,iron Chelating independent	3.1±0.09 [b*]	3.1±0.08 [a*]	NS
Subgroup3 (n=10) Thalassemic , HCV-ve ,iron Chelating dependent	3.4±0.16 [a*]	3.0±0.12 [a*]	**
Subgroup4(n=10) Thalassemic,HCV-ve, ironchelating independent	2.2±0.06 [d*]	2.3±0.07 [b*]	NS
Controlgroup(n=10) Healthy non thalassemic	2.8±0.05 [c*]	2.4±0.11 [b*]	*

Table (9): Comparing case subgroups with controls regarding albumin level in blood (g /dl) and HB vaccination status.

Case subgroups & controls	HB vaccination status		P
	Vaccinated (n=25)	Non vaccinated (n=25)	
	Albumin level (Mean ± SE)	Albumin level (Mean ± SE)	
Subgroup 1(n=10) Thalassemic ,HCV+ve ,iron chelating dependent	4.8±0.11 a*	5.0±0.08 ab*	NS
Subgroup 2 (n=10) Thalassemic ,HCV+ve ,iron Chelating independent	4.9±0.10 a*	5.1±0.02 a*	NS
Subgroup3(n=10) Thalassemic , HCV-ve ,iron Chelating dependent	4.4±0.17 b*	4.8±0.16 abc*	NS
Subgroup4(n=10) Thalassemic , HCV-ve, iron chela independent	4.0±0.11 b*	4.4±0.11 c*	**
Control group (n=10) Healthy non thalassemic	5.0±0.07 a*	4.7±0.17 bc*	NS

Table (10): Comparing case subgroups with controls regarding total proteins(g/dl) and HB vaccination status.

Case subgroups & controls	HB vaccination status		P
	Vaccinated (n=25)	Non vaccinated (n=25)	
	Total protein (Mean±SE)	Total protein (Mean±SE)	
Subgroup 1(n=10) Thalassemic , HCV+ve ,iron chelating dependent	7.7±0.13 a*	8.04±0.14 a*	NS
Subgroup2(n=10) Thalassemic , HCV+ve ,iron Chelating independent	8.08±0.08 a*	8.2±0.08 a*	NS
Subgroup3 (n=10) Thalassemic, HCV-ve ,iron Chelating dependent	7.8=0.3 a*	7.7±0.2 a*	NS
Subgroup4(n=10) Thalassemic , HCV-ve, iron chelating independent	6.2±0.09 b*	6.7=0.08 b*	***
Control group (n=10) Healthy non thalassemic	7.8±0.12 a*	7.1±0.22 b*	**

Table (11): Comparing case subgroups with controls regarding albumin /globulin ratio (%)and HB vaccination status.

Case subgroups & controls	HB vaccination status		P
	Vaccinated (n=25)	Non vaccinated (n=25)	
	albumin /globulin ratio (Mean±SE)	albumin /globulin ratio (Mean±SE)	
Subgroup 1(n=10) Thalassemic , HCV+ve ,iron chelating dependent	1.6±0.04^{ab*}	1.6±0.03^{b*}	NS
Subgroup2(n=10) Thalassemic, HCV+ve ,iron Chelating independent	1.5±0.07^{b*}	1.6±0.04^{b*}	NS
Subgroup3(n=10) Thalassemic , HCV-ve ,iron Chelating dependent	1.2±0.05^{c*}	1.6±0.08^{b*}	**
Subgroup4(n=10) Thalassemic , HCV-ve, iron chelating independent	1.8±0.09^{a*}	1.9±0.09^{a*}	NS
Control group (n=10) Healthy non thalassemic	1.7±0.02^{ab*}	2.0±0.12^{a*}	NS

Table (12): comparing thalassemic subgroups with controls as regards total cholesterol level (mg/dl) in blood and HB vaccination status.

Case subgroups & controls	HB vaccination status		P
	Vaccinated (n=25)	Non vaccinated (n=25)	
	Total cholesterol (Mean ± SE)	Total cholesterol (Mean ± SE)	
Subgroup 1(n=10) Thalassemic , HCV+ve ,iron chelating dependent	89.2±3.2^{b*}	106±5.8^{b*}	**
Subgroup 2 (n=10) Thalassemic , HCV+ve ,iron Chelating independent	85.4±4.2^{b*}	118.6±3.04^{b*}	***
Subgroup3(n=10) Thalassemic, HCV-ve ,iron Chelating dependent	94.8±2.08^{b*}	103.6±5.9^{b*}	NS
Subgroup4(n=10) Thalassemic , HCV-ve, iron chelating independent	89.8±4.1^{b*}	74.5±3.09^{c*}	**
Control group (n=10) Healthy non thalassemic	172.4±3.2^{a*}	156.2±6.1^{a*}	**