

Hematological and Immunological Studies on the Effect of Hepatitis B Virus Vaccination in Hepatitis and Non-Hepatitis, Iron Chelating Dependent or Independent Egyptian Thalassemia Patients

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Abstract

Background: Regular transfusion in thalassemia major patients increases life expectancy and improves quality of life. Blood transfusion is the main sources for viral transmission to Thalassemia patients. So, detection of viral antigens using more than one technique must be adopted. Iron and its binding proteins have immune regulatory properties and shifting of immune regulatory balance by iron excess or deficiency may produce severe deleterious physiological effects. Thus, the aim of this study was to assess the efficacy of immunization and determine the immune response of beta-thalassemia patients. Also, to evaluate the effects of iron overload chelating therapy and hepatitis B virus (HBV) vaccination on some immunological and hematological parameters in hepatitis and non hepatitis Egyptian thalassemia patients.

Methods: Forty homozygote Thalassemia patients attending blood bank, therapeutic unite, Holding company for Biological production and Vaccine, VACSERA were chosen for this study (age range 4-30years, mean 14 years, 18 females 46% and 22 males 54%).

Results: There was no significant correlation between HBs Ab level in control and non vaccinated groups that include Thalassemia, hepatitis, non hepatitis, either iron chelating therapy dependent or independent patients. In the same time, there was no significant correlation between Ab level in vaccinated control and Thalassemia groups. HbF and HbA₂ % showed significant and highly significant increases respectively, in most of groups especially, Thalassemia, hepatitis and iron chelating independent, vaccinated or non vaccinated groups. While HbA may be present in small amount or completely absent. RBCs count, Hb%, Hematocrit and MCV values were decreased significantly in all patient's groups vaccinated or non vaccinated compared to control group, while MCH and MCHC were not changed in patient' groups compared to control group. Platelets count was increased significantly in most patient' groups (except non vaccinated, Thalassemia, hepatitis, iron chelating dependent) compared to control group. Also, WBCs count was increased significantly in most groups (except non vaccinated, Thalassemia, hepatitis or non hepatitis, iron chelating dependent or independent) in comparison with the control group. All patients (hepatitis, vaccinated or non vaccinated) had high significant increase in gamma globulins compared to control group.

Conclusion: Hematological and immunological measurements for hepatitis Thalassemia patients are important to monitor and treat the disease.

Key words: Blood transfusion, Thalassemia patients, iron excess, HBs, gamma globulins.

Introduction:

Thalassemia (is also known as Cooley's anemia) is a group of inherited hemoglobin disorders characterized by reduced synthesis of one or more of the globin chains leading to imbalanced globin synthesis which is the major factor in determining the severity of the disease in the Thalassemia syndromes. In Egypt, β -thalassemia is the commonest cause of chronic hemolytic anemia and it represents a major genetic disease and a public health problem which engulfs a large portion of the country's health financial

plan^(1,2) and the rate of new birth with Thalassemia per year is 1000/1.5million⁽³⁾.

The thalassemias are classified according to which chain of the hemoglobin molecule is affected. In α - thalassemias, production of the α globin chain is affected, while in β - thalassemia production of the β globin chain is affected. Thalassemia is the most common type of hemoglobinopathy transmitted by heredity. The decrease or loss of α or β chain has unfavorable effects on the production and the survival of red blood cells (RBCs) and may cause a decrease in the concentration of the globin chain and of hemoglobin, resulting in microcytosis and hypochromia⁽⁴⁾.

The management of thalassemia major essentially comprises of regular “safe blood transfusion” and a life long iron-chelation

therapy⁽⁵⁾. HCV infection has gained importance particularly as one of the major complications in multiply transfused patients during the last decade. This is especially true for countries where HCV is more prevalent in general population and therefore also amongst blood donors. The prevalence rate of seropositivity increases with the number of transfusions^(6,7,8). This post-transfusion hepatitis has significantly contributed to morbidity in thalassemia⁽⁹⁾. It should be remembered that HCV hepatitis is more threatening than HBV hepatitis due to a greater risk of chronic liver disease such as cirrhosis and hepatocellular carcinoma⁽¹⁰⁾.

In case of hepatitis B, since an effective vaccine is available, immunization against this virus before transfusion management is started which could be effectively protected against transfusion hepatitis B. However, since no such vaccine is so far available against hepatitis C, the only effective protective measure against this virus is provision of HCV negative blood for transfusion. Therefore, screening of transfused blood for HCV is not only mandatory, but also it is essential to use the most sensitive screening methods with least possible false-negative results⁽⁷⁾.

Transfused antibodies may inhibit the recipient's sensitization and primary immune response to the homologous antigen, especially when the antibody level in the transferred blood is high whereas the secondary immune response is not affected⁽¹¹⁾.

Fetal hemoglobin (HbF) is the main hemoglobin component throughout fetal life and at birth, accounting for approximately 80% of total hemoglobin in newborns. HbF is produced from the sixth week of gestation and during the rest of fetal life, replacing the embryonic hemoglobins⁽¹²⁾. After birth, HbF synthesis rapidly declines and HbF is gradually substituted by adult hemoglobin (HbA) in the peripheral blood, so that within the first two years of life, the characteristic hemoglobin phenotype of the adult (96-98%) with very low levels of HbF (less than 1%) is found⁽¹³⁾ and most of the remainder is structurally different hemoglobin called hemoglobin A₂ (HbA₂)⁽¹⁴⁾. HbF measurement is clinically useful in the study and diagnosis of some important globin gene disorders where HbF levels may vary considerably (mainly, β - and $\delta\beta$ -thalassemia)⁽¹²⁾.

Iron and its binding proteins have immunoregulatory properties, and shifting of immunoregulatory balances by iron excess or deficiency may produce severe, deleterious

physiological effects. Effects of iron overload include decreased antibody-mediated and mitogen-stimulated phagocytosis by monocytes and macrophages, alterations in T-lymphocyte subsets and modification of lymphocyte distribution in different compartments of the immune system which is associated with HCV positively⁽¹⁵⁾. The poor ability of lymphocytes to sequester excess iron in ferritin may help to explain the immune system abnormalities in iron overload patients⁽¹⁶⁾. In patients with chronic hepatitis (CHC) with no other cause for iron overload, iron may be a cofactor in the development of liver injury and correlate with disease severity⁽¹⁷⁾.

Since iron overload and hepatitis C virus (HCV) are the main causes of chronic liver diseases in β -Thalassemia patients. The aim of this study was to evaluate the effects of iron overload chelating therapy and hepatitis B virus (HBV) vaccination on some immunological and hematological parameters in hepatitis and non hepatitis Egyptian Thalassemia patients.

Material and Methods:

Study subjects included 40 patients with age range 4-30 years (mean 14 years), 18 females 46% and 22 males 54%. All patients were Thalassemia Major. Every patient received approximately blood transfusion (3-4 week intervals) the range of hemoglobin was 6.5-7.5g/dl. The samples were collected before the blood transfusion.

Twenty patients of all were positive hepatitis C virus (HCV). These 20 patients were divided into 2 subgroups, 50% of them used iron chelating therapy (iron chelating dependent) and the other 50% were iron chelating independent. 50% of every subgroup were vaccinated with HBV. Other 20 (non HCV infection) also divided to 2 subgroups, 50% of them used iron chelating therapy (iron chelating dependent) and the other 50% were iron chelating independent. 50% of every subgroup were vaccinated with HBV. In addition 10 healthy individuals 4 females (40%) and 6 males (60%) were enrolled in the study to act as a reference group. The control group was divided into 2 subgroups (5 of them were HBV vaccinated and 5 were non vaccinated).

A-Immunological parameters;

1- Rapid detection for HCV Ab:

Qualitative detection of HCV antibodies (HCV Ab) were carried out by the HCV rapid screen test (RST) according to Acon comp, USA. Briefly, RST is a chromatographic immunoassay for detection of antibodies of

hepatitis in human serum or plasma. HCV recombinant antigens are precoated on to membrane as a capture reagent to specific antibodies (Ab) on the test region.

2- Rapid detection for HBs Ag:

RST for direct qualitative detection of antibodies to hepatitis B surface antigen (HBs Ag) is precoated on to membrane as a capture reagent content on the test region. Specimen is allowed to react with colloidal gold particles, which have been labeled with other specific antibodies.

3- Determination of HBs Ab by Axyme radioimmunoassay :

HBs Ab was determined by using an automated instrument model (ER-HYD-1525 - 199) closed system, according to (Abbott laboratories diagnostic division, operations manual, volume 1997, USA).

4- Detection of hemoglobin A₂ and F (HbA₂/F) variants by HPLC:

HbA₂ /F were achieved by using technical hemoglobin test High Performance Liquid Chromatography (HPLC) system HbA₂/F according to ⁽¹⁸⁾.

B- Hematological parameters:

Blood counts were done by coulter counter AC-T8 which include red blood cells count (RBCs) count ($\times 10^6/\text{cm}^3$); hemoglobin concentration (Hb g/100ml), hematocrit (%); RBCs indices (MCV, MCH and MCHC); total leukocyte count ($\times 10^3/\text{cm}^3$) and platelet count ($\times 10^3/\text{cmm}$). Also; smears of blood stained with Leishman's stain were prepared for differential leukocyte count according to Dacie and Lewis ⁽¹⁹⁾. Blood slides were also examined to detect variations in structure, size, shape and content of RBCs ⁽²⁰⁾.

Statistical analysis was carried out using SAS program (SAS, 1988). Student's *t*-test was run to test the effect of vaccination within each treatment. One way analysis of variance

(ANOVA) followed by Duncan's multiple range test were used to test the effect of treatments on different measurements in non vaccinated and vaccinated groups. Cross tabulation and Chi square analysis were used to compare the prevalence of HBs between different groups.

Results:

Immunological parameters changes:

In the present study, the HBv antibody level was detected in Thalassemia patients and it was noted that the Ab level in sera of patients (100%) ranged from (0-100) mIU/ml. Immune response of patients was classified according to the Ab level as follow: (0-10 mIU/ml) is -ve immune response or non-vaccinated and it was detected in 40% of non vaccinated groups (thalassemic, non hepatitis, iron chelating independent); 50% of non vaccinated group were positively reacting to the vaccine but recommended to be revaccinated; the Ab level was (10-100 mIU/ ml) and the rest 50% were + ve reacting to the vaccine. There was a significant correlation between ABv Ab level and non vaccinated patient groups (table 1).

The recorded data revealed that there was no significant correlation between Ab level in control and non vaccinated groups that include Thalassemia, hepatitis, non hepatitis, either iron chelating therapy dependent or independent patients. In the same time, there was no significant correlation between Ab level in vaccinated control and Thalassemia groups.(table,1).

It is noticed that, serum ferritin level in all patient groups (non vaccinated and vaccinated) was elevated significantly ($P \leq 0.001$) compared to control groups. On the other hand, vaccinated control group had decreased serum ferritin compared to non vaccinated control group. The highest serum ferritin level was detected in vaccinated, Thalassemia, non hepatitis, iron chelating compared to other patient groups.

Table (1): Assessment of hepatitis B surface antibody level(%) and ferritin (ng/mL) i n the sera of Thalassemia patients in relation to control groups

Group	HBs Ab titer	Hepatitis B surface antibody level		.Ferritin		
		Non vaccinated	vaccinated	Non vaccinated	vaccinated	
Control group.	Negative (≤ 10)	40	20	148.8 \pm 5.817	112.0 \pm 3.661***	
	Revaccinated (10- > 100)	60	40			
	Immune response (≥ 100)	0	40			
Thalassemic, hepatitis, iron chelating dependent group	Negative (≤ 10)	60	*	2737.0 \pm 140.941***	2454.6 \pm 117.269***	
	Revaccinated (10- ≥ 100)	20				0
	Immune response (≥ 100)	20				60
Thalassemic, hepatitis, iron chelating independent group	Negative (≤ 10)	60	*	2274.2 \pm 128.716***	1624.6 \pm 115.017***	
	Revaccinated (10- ≥ 100)	20				20
	Immune response (≥ 100)	20				60
Thalassemic, non hepatitis, iron chelating dependent group.	Negative (≤ 10)	40	*	1501 \pm 162.984***	3223.0 \pm 104.845***	
	Revaccinated (10- ≥ 100)	20				40
	Immune response (≥ 100)	40				20
Thalassemia, non hepatitis, iron chelating independent group	Negative (≤ 10)	50	*	2411.0 \pm 184.806	2208.8 \pm 100.751	
	Revaccinated (10- ≥ 100)	25				0
	Immune response (≥ 100)	25				100

Ferritin results are M \pm S.E. of 5 patients.

* Significant at P \leq 0.05 compared to control groups.

*** Significant at P \leq 0.001 compared to control groups.

In the present study, the HbF was 100% in all Thalassemia patients. HbF were 0.1% and 0.08% in control non vaccinated and vaccinated control group respectively, on the other hand, these percentages were increased up to 12.6% in vaccinated Thalassemia, non hepatitis, iron chelating independent and to 41.5% in vaccinated Thalassemia, hepatitis, iron chelating independent patients. There was a significant correlation between HbF values in non vaccinated (Thalassemia, hepatitis, iron chelating independent group and Thalassemia, nonhepatitis, iron chelating therapy dependent group). Also, a non significant correlation between Thalassemia, hepatitis, iron chelating therapy dependent group was detected (table 2). On the other hand, in vaccinated groups a highly significant correlation between Thalassemia, nonhepatitis, iron chelating therapy dependent and independent and control groups. Also, a non significant correlation between Thalassemia, hepatitis, iron chelating therapy dependent and Thalassemia, nonhepatitis, iron chelating therapy dependent groups were detected (table 2).

Regarding the vaccination effect on HbF values, the non vaccinated patient group tend to have lower HbF% than vaccinated one, the difference was significantly increased (P \leq 0.05) in Thalassemia, non hepatitis, iron chelating therapy dependent or independent, while other groups showed non significant changes compared to vaccinated groups (table 2 and plate 1 pictures 1-5).

In this study, the HbA₂ % in non vaccinated group showed significant increase value in comparison with the control group, while HbA₂% value in Thalassemia, hepatitis and iron chelating therapy independent patients was significantly higher than its values in Thalassemia, non hepatitis patients either iron chelating therapy dependent or independent.

Similarly, HbA₂ % value in the last two groups was no significant related to its values in Thalassimia, hepatitis and iron chelating therapy dependent patients. While in vaccinated groups significant correlative values of HbA₂ % were noted compare to control groups (table2 and plate1 pictures 1-5).

Table (2): Comparative evaluation of HbA₂ % and HbF % variants in Thalassemia patients in relation to control groups.

Groups	HbA ₂ %		HbF %		HbA%	
	Non vaccinated	Vaccinated	Non vaccinated	Vaccinated	Non vaccinated	Vaccinated
Control group	2.180± 0.097	2.380± 0.080	0.100± 0.063	0.080± 0.049	83.980± 1.474	83.420± 0.881
Thalassemic, hepatitis, iron chelating dependent	7.200± 0.679*	5.140± 0.623*	19.740± 3.718*	20.56± 02.096*	71.000± 2.816*	69.86± 2.484*
Thalassemic, hepatitis, iron chelating independent	9.660± 0.344*	7.180± 0.689*a	35.860± 1.904**	41.580± 5.209**	69.660± 2.267*	60.440± 0.703**
Thalassemic, non hepatitis, iron chelating dependent	7.940± 0.469*	7.780±0. 629*	18.740± 0.841*	15.20± 01.159**	69.560± 2.494*	74.260± 3.799*
Thalassemic, non hepatitis, iron chelating independent	9.225± 0.549**	7.467± 0.249*a	22.475± 2.089	12.617±0 .695**	65.050± 3.401*	70.983± 3.205*

Mean of 5 patients/ group ± S.E.

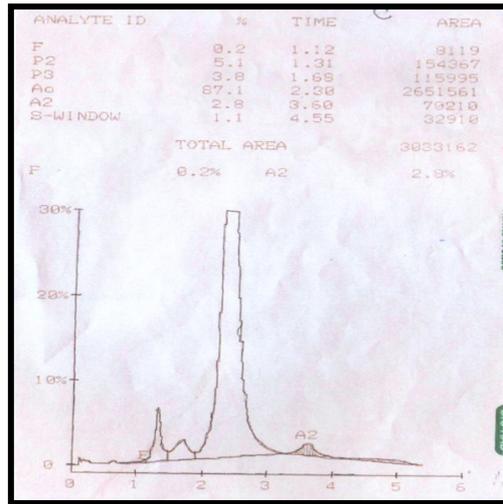
* = significant at $p \leq 0.05$ compared to control group.

** = significant at $p \leq 0.01$ compared to control group.

a significant at $P \leq 0.05$ compared to non vaccinated similar groups.

Regarding the vaccination effect on HbA₂%, the vaccinated patient group tended to have lower HbA₂ % than non vaccinated one, the difference was significantly decreased ($P \leq 0.05$) in Thalassimia, iron chelating therapy independent either with or without hepatitis. While, other groups showed non significant changes compared to vaccinated groups (table 2).

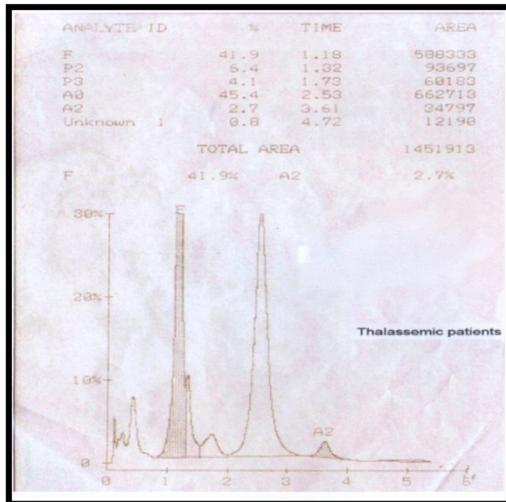
Table 2 represents the changes in HbA % in different patients groups. HbA % was decreased in all patient groups ($P \leq 0.05$) compared to control group. In both non vaccinated and vaccinated groups, HbA% showed non significant differences between Thalassimia, hepatitis or non hepatitis either chelating therapy dependent or independent. The lowest HbA% was recorded in vaccinated, Thalassimia, hepatitis, iron chelating independent patients (table 2).



Picture (1): Control group

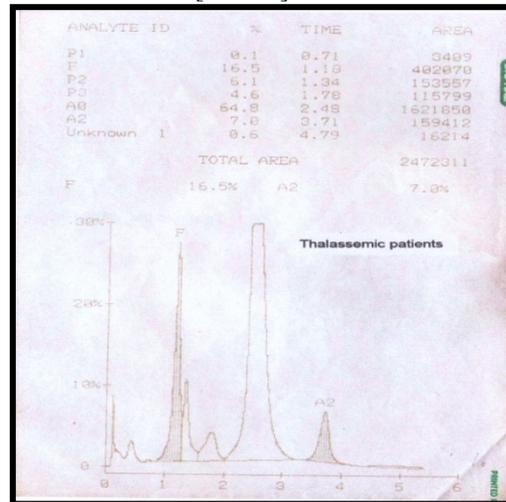
HbF = 0.2%
HbA₂ = 2.8 %

Normal value [0.0-0.5]
Normal value [2.5-3.5]



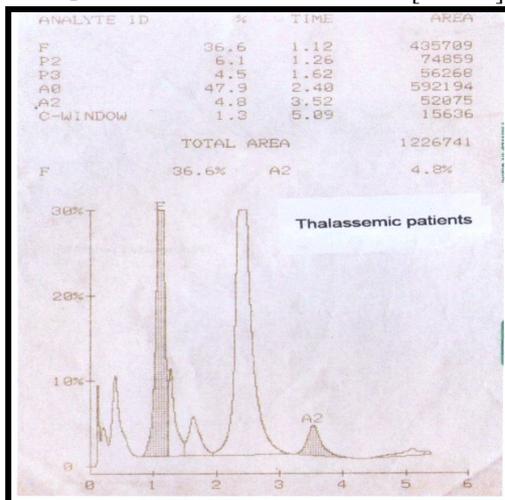
Picture (2): Thalassemia, non hepatic, iron chelating independent group

HbF= 41.9% Normal value [0.0-0.5]
HbA₂=2.7% Normal value [2.5-3.5]



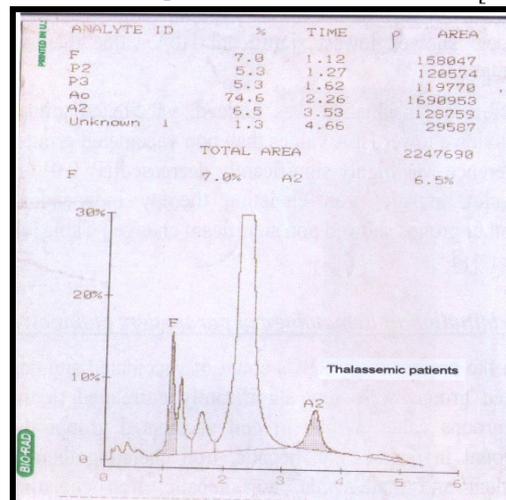
Picture (3): Thalassemia, non hepatic, iron chelating dependent group

HbF= 16.5% Normal value [0.0-0.5]
HbA₂=7.0% Normal value [2.5-3.5]



Picture (4): Thalassemia, hepatic, iron chelating dependent group

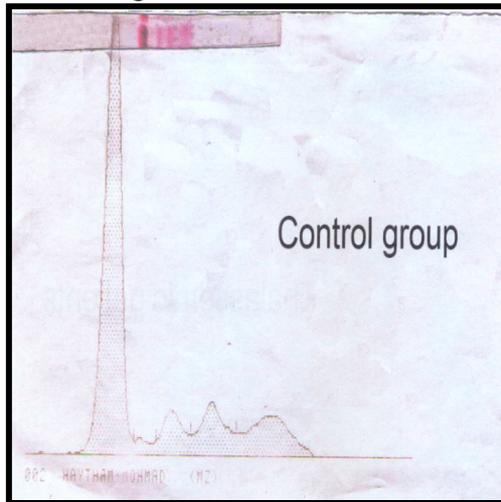
HbF= 36.6% Normal value [0.0-0.5]
HbA₂=4.8% Normal value [2.5-3.5]



Picture (5): Thalassemia, non hepatic, iron chelating dependent group

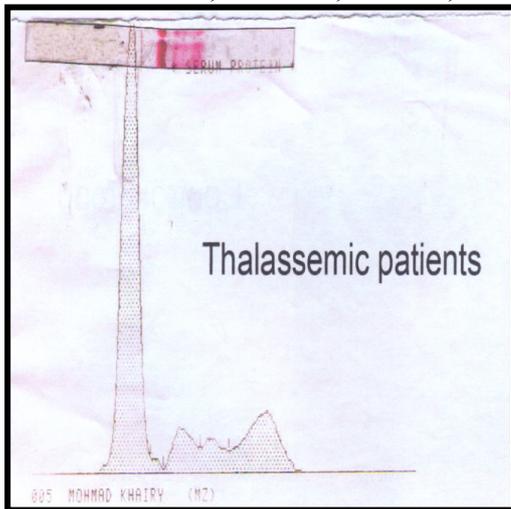
HbF= 7.0% Normal value [0.0-0.5]
HbA₂=6.5% Normal value [2.5-3.5]

Plate (1) HPLC hemoglobin variants to evaluate HbF & HbA₂



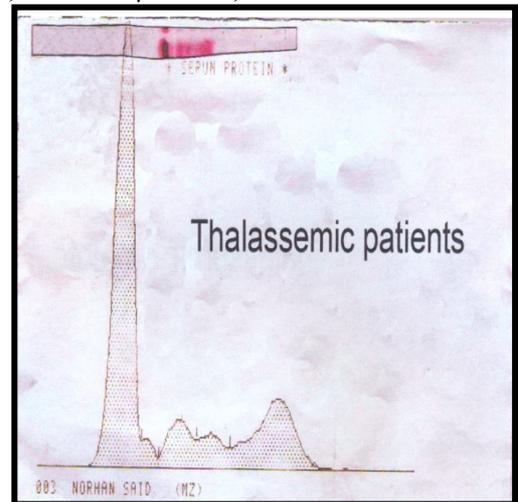
Picture (1): Control group

$\alpha_1 = 2.2, 1.5-4.5\%$; $\alpha_2 = 7.8, 6-12\%$; $\beta = 11.2, 11-17\%$ $\gamma = 14.7, 11-19\%$



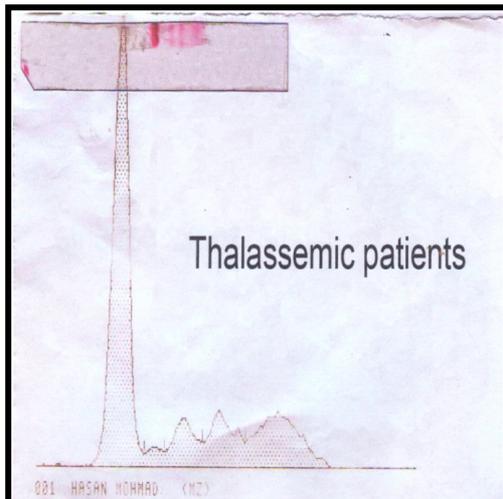
Picture (2):Thalassemia, non hepatic , iron chelating dependent

$\alpha_1 = 1.1, 1.5-4.5\%$ $\alpha_2 = 8.3, 6-12\%$
 $\beta = 6.0, 11-19\%$ $\gamma = 18.1, 11-19\%$



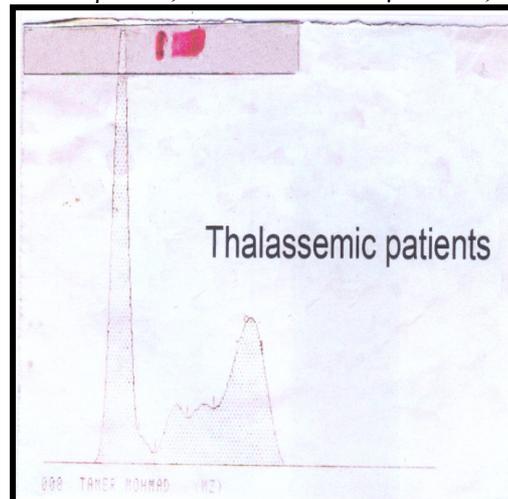
Picture (3):Thalassemia, non hepatic , iron chelating independent

$\alpha_1 = 2.6, 1.5-4.5\%$ $\alpha_2 = 8.4, 6-12\%$
 $\beta = 6.2, 11-19\%$ $\gamma = 21.4, 11-19\%$



Picture (4):Thalassemia, hepatic , iron chelating independent

$\alpha_1 = 2.4, 1.5-4.5\%$ $\alpha_2 = 8.5, 6-12\%$
 $\beta = 11.4, 11-19\%$ $\gamma = 19.5, 11-19\%$



Picture (5):Thalassemia, hepatic , iron chelating dependent

$\alpha_1 = 1.8, 1.5-4.5\%$ $\alpha_2 = 7.5, 6-12\%$
 $\beta = 8.1, 11-19\%$ $\gamma = 75.4, 11-19\%$

Plate (1) HPLC hemoglobin variants to evaluate HbF & HbA₂

Hematological parameters changes:

The RBCs count and indices and other hematological parameters for control and β -thalassaemic patients are given in tables 3 and 4. Red blood cells count in vaccinated and non vaccinated groups were lower than that in control groups ($P \leq 0.05$). Although vaccinated Thallasimia, hepatitis or non hepatitis, iron chelating dependent or independent had lower RBCs count in relation to non vaccinated groups, the differences were not significant (table 3 and fig 2). RBCs count was significantly decreased in vaccinated control group compared to non vaccinated control group,

but, it was still in human normal value ($P \leq 0.05$).

All Thallasimia patient, vaccinated or non vaccinated, hepatitis or non hepatitis, iron chelating dependent or independent had lower Hb contents than control groups (vaccinated and non vaccinated, table3).

The Hematocrit (Ht) value in this study revealed that, all Thallasimia patient, vaccinated or non vaccinated, hepatitis or non hepatitis, iron chelating dependent or independent were significantly lower than the control groups (vaccinated and non vaccinated, table 3).

Table (3) RBCs ($\times 10^6/\text{cm}^3$), Hb (g/100ml) and Ht (%) in Thalassemica patients in relation to control groups.

Groups	RBCs		Hb		Ht	
	Non vaccinated	Vaccinated	Non vaccinated	Vaccinated	Non vaccinated	Vaccinated
Control group	5.486 \pm 0.197	4.832 \pm 0.141*	14.340 \pm 0.220	12.700 \pm 0.529*	42.780 \pm 0.466	37.220 \pm 1.584**
Thalassemic, hepatitis, iron chelating dependent	2.798 \pm 0.077**	2.686 \pm 0.109**	7.220 \pm 0.146**	7.060 \pm 0.051**	21.380 \pm 0.174**	21.620 \pm 0.937**
Thalassemic, hepatitis, iron chelating independent	3.244 \pm 0.134**	2.904 \pm 0.087**	8.040 \pm 0.136**	7.440 \pm 0.361**	24.080 \pm 0.962**	22.660 \pm 1.289**
Thalassemic, non hepatitis, iron chelating dependent	3.318 \pm 0.116**	3.124 \pm 0.218**	7.54 \pm 0.229**	7.960 \pm 0.299**	22.260 \pm 0.754**	22.900 \pm 0.965**
Thalassemic, non hepatitis, iron chelating independent	3.370 \pm 0.237**	3.230 \pm 0.164**	7.225 \pm 0.272**	6.950 \pm 0.159**	21.375 \pm 1.045**	22.567 \pm 1.484**

Results = M of 5 patients \pm SE

* = $p \leq 0.05$ when compared with control group (non vaccinated and vaccinated).

** = $p \leq 0.01$ when compared with control group (non vaccinated and vaccinated).

Blood indices revealed that significant decreases of MCV values in non vaccinated and vaccinated groups were shown compared to the control group, (except non vaccinated, Thallasimia, hepatitis, either iron chelating dependent or independent) which showed insignificant differences (table 4). Also, non vaccinated, non hepatitis either iron chelating therapy dependent or independent had very high significant decreased MCV ($p \leq 0.001$) in relation to vaccinated groups (table 4).

MCH values in non vaccinated groups were significantly decreased compared to control group, except, Thalassemia, hepatitis, iron chelating therapy dependent which had insignificant difference with control group. Also, non vaccinated Thalassemia, iron chelating independent either hepatitis or non hepatitis had the lowest MCH value than other groups. No significant changes were recorded between vaccinated patients and control group except Thalassemia, non hepatitis, iron chelating independent which had decreased MCH (table 4).

Table (4): Blood indices MCV (fl), MCH (pg) and MCHC (g/dl) in Thalassemia patients in relation to control groups.

Groups	MCV		MCH		MCHC	
	Non vaccinated	Vaccinated	Non vaccinated	Vaccinated	Non vaccinated	Vaccinated
Control group	79.400± 0.748	79.400± 1.122	27.780± 0.483	27.440± 0.594	34.760± 0.632	34.520± 0.632
Thalassemic, hepatitis, iron chelating dependent	76.620± 1.593	74.800± 1.006*	26.600± 0.416	26.260± 0.510	33.920± 0.611	33.840± 1.373
Thalassemic, hepatitis, iron chelating independent	76.640± 1.189	75.620± 1.057*	23.520± 0.480*	27.400± 0.869	32.340± 1.094	33.860± 0.777
Thalassemic, non hepatitis, iron chelating dependent	67.780± 1.061*	75.600± 0.639*	25.120± 1.011	27.100± 0.609	33.160± 0.700	34.200± 0.713
Thalassemic, non hepatitis, iron chelating independent	64.275± 0.652*	74.717± 1.009*	24.475± 0.636*	25.267± 0.442*	33.600± 0.824	35.350± 0.448

Results = M± SE

* = $p \leq 0.05$ when compared with control group (non vaccinated and vaccinated).

** = $p \leq 0.01$ when compared with control group (non vaccinated and vaccinated).

No significant changes were recorded in MCHC in all groups (non vaccinated and vaccinated) compared to control groups (table 4).

White blood cells count (WBCs) elevated in both non vaccinated and vaccinated patients compared to control groups (table 5).

At the same time, a significant decrease ($p \leq 0.05$) in Vaccinated Thalassemia, hepatitis, iron chelating dependent compared to non vaccinated Thalassemia, hepatitis, iron chelating dependent (table 5).

Platelet counts in non vaccinated and vaccinated groups were significantly higher than the control groups. Moreover, Thalassemia, hepatitis, iron chelating independent (non vaccinated and vaccinated) recorded the highest count than other groups (table 5).

Table (5) WBCs ($\times 10^3/\text{cm}^3$) and platelets count ($\times 10^3/\text{cm}^3$) in Thalassemia patients in relation to control groups.

Groups	WBCs		PLts	
	Non vaccinated	Vaccinated	Non vaccinated	Vaccinated
Control group	5.922± 0.049	6.058± 0.402	260.600± 14.102	277.800± 8.907
Thalassemic, hepatitis, iron chelating dependent	11.138± 0.766**	8.204± 0.498*	357.800± 24.656**	292.400± 29.578*
Thalassemic, hepatitis, iron chelating independent	12.298± 0.553**	13.250± 0.315**	850.200± 33.942**	840.600± 29.503**
Thalassemic, non hepatitis, iron chelating dependent	10.248± 0.586**	12.068± 0.134**	357.600± 17.119**	414.200± 16.939**
Thalassemic, non hepatitis, iron chelating independent	9.727± 0.276**	8.727± 0.309*	666.750± 23.499**	537.333± 31.972**

Results = M of 5 patients ± SE

* = $p \leq 0.05$ when compared with control group (non vaccinated and vaccinated).

** = $p \leq 0.01$ when compared with control group (non vaccinated and vaccinated).

Neutrophils in non vaccinated and vaccinated groups were significantly lower than the control groups. Vaccinated, Thalassemia, non hepatitis, iron chelating independent group recorded the least percent of neutrophils among the vaccinated groups (table 6).

Eosinophil cells count in non vaccinated groups showed no significant correlation among Thalassemia, hepatitis, iron chelating independent; Thalassemia, non hepatitis, iron chelating dependent and control groups. Moreover, Thalassemia, hepatitis, iron chelating dependent group recorded the lowest value and Thalassemia, non hepatitis, iron chelating independent had the highest value of eosinophils than other groups (table 6).

Vaccinated groups showed significant increases of basophile cells count in Thalassemia, hepatitis, iron chelating dependent and independent groups than the control group and all other groups (table 6).

Table (6): Neutrophils, eosinophils and basophils (%) in Thalassemia patients in relation to control groups.

Groups	Neutrophils		Eosinophils		Basophils	
	Non vaccinated	Vaccinated	Non vaccinated	Vaccinated	Non vaccinated	Vaccinated
Control group	47.200± 0.860	50.200± 2.177	3.200± 0.583	2.800± 0.374	0.000± 0.000	0.000± 0.000
Thalassemic, hepatitis, iron chelating dependent	43.600± 2.315*	37.400± 1.913**	2.600± 0.400	6.600± 1.029**	0.4± 0.400	2.600± 0.748**
Thalassemic, hepatitis, iron chelating independent	34.200± 2.478**	39.200± 0.374**	3.400± 0.872	3.600± 0.244*	1.200± 0.734	2.600± 0.400**
Thalassemic, non hepatitis, iron chelating dependent	34.400± 0.509**	39.600± 0.678**	3.000± 0.547	2.400± 0.509**	1.000± 0.632	0.400± 0.244
Thalassemic, non hepatitis, iron chelating independent	37.250± 0.750**	29.833± 1.249**	4.750± 0.629*	5.500± 0.428**	1.500± 0.288	1.000± 0.258

Results = M of 5 patients ± SE

* = $p \leq 0.05$ when compared with control group (non vaccinated and vaccinated).

** = $p \leq 0.01$ when compared with control group (non vaccinated and vaccinated).

Concerning staff cells count, there was significant increase in non vaccinated groups compared to control group except, Thalassemia, hepatitis, iron chelating dependent which recorded insignificant change. While, there were high significant increases in the staff cells of vaccinated groups except its value in Thalassemia, non hepatitis, iron chelating independent group (table 7).

Lymphocytes increased in all non vaccinated groups and the increase was significant in both Thalassemia, non hepatitis, either iron chelating dependent or independent (table 7). Vaccinated Thalassemia, hepatitis, iron chelating dependent group had high significant decreased lymphocyte count ($p \leq 0.01$). While, vaccinated Thalassemia, non hepatitis, iron chelating independent had high significant increased lymphocyte count ($p \leq 0.01$) compared to control groups (table 7).

Elevated monocyte counts were recorded in all tested groups (non vaccinated and vaccinated) compared to control groups. Non vaccinated Thalassemia, hepatitis, iron chelating dependent group had highly significant decrease in monocyte counts compared to vaccinated Thalassemia, hepatitis, iron chelating dependent group ($p \leq 0.01$), while, monocyte counts increased significantly ($p \leq 0.05$) in non vaccinated Thalassemia, non hepatitis, iron chelating independent compared to vaccinated Thalassemia, non hepatitis, iron chelating independent (table 7).

Table (7) : Lymphocytes, monocytes and staff cells (%) in Thalassemia patients in relation to control groups.

Groups	Lymphocytes		Monocytes		Staff cells	
	Non vaccinated	Vaccinated	Non vaccinated	Vaccinated	Non vaccinated	Vaccinated
Control group	43.600± 1.364	41.60± 2.768	3.800± 0.734	3.400± 0.400	2.200± 0.200	2.00± 0.200
Thalassemic, hepatitis, iron chelating dependent	46.000± 2.607	34.400± 1.860**	4.800± 1.019	10.200± 1.113**	2.600± 0.678	8.800± 0.583**
Thalassemic, hepatitis, iron chelating independent	46.800± 4.104	43.200± 0.969	9.400± 0.927**	7.600± 0.678**	5.000± 0.707**	3.800± 0.200**
Thalassemic, non hepatitis, iron chelating dependent	51.400± 1.503*	47.000± 0.837*	5.800± 0.734*	5.600± 0.244**	4.400± 0.244**	5.000± 0.316**
Thalassemic, non hepatitis, iron chelating independent	46.250± 0.750*	56.167± 1.558**	6.000± 0.408*	4.833± 0.307*	4.250± 0.853**	2.667± 0.333

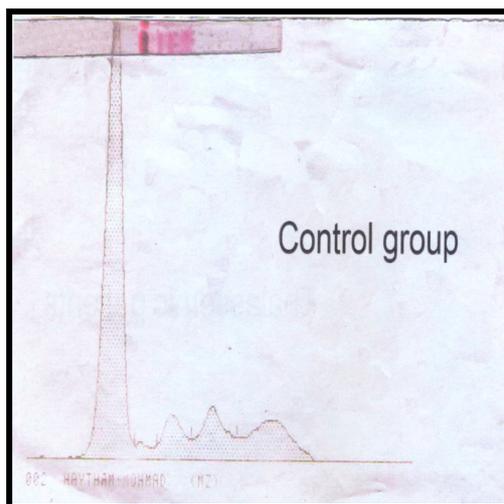
Results = M of 5 patients ± SE , * = $p \leq 0.05$ when compared with control group (non vaccinated and vaccinated). ** = $p \leq 0.01$ when compared with control group (non vaccinated and vaccinated).

In this study, plasma proteins (Alpha1, Alpha 2, Beta chains and Gamma globulin chain) percentages were determined by electrophoresis and represented in table 8 and plate 2 (pictures 1-5). Alpha1 chain % increased in both non vaccinated and vaccinated, hepatitis or non hepatitis, iron chelating therapy dependent or independent patients compared to control groups. Also non vaccinated and vaccinated Thalassemia, hepatitis, iron chelating independent had the highest % of Alpha1 chain. Regarding to Alpha 2 chain%, the results showed significant increases in different patients groups compared to control groups. On the other hand, Beta chain% decreased in different patients groups compared to control groups. Evaluation of Gamma chain % elevated significantly in different patients groups compared to control groups. Moreover, Thalassemia, hepatitis, iron chelating dependent patients detected the highest percentage than all other groups.

Table (8): plasma proteins (Alpha1, Alpha 2, Beta chains and Gamma globulin chain %) in Thalassemia patients in relation to control groups.

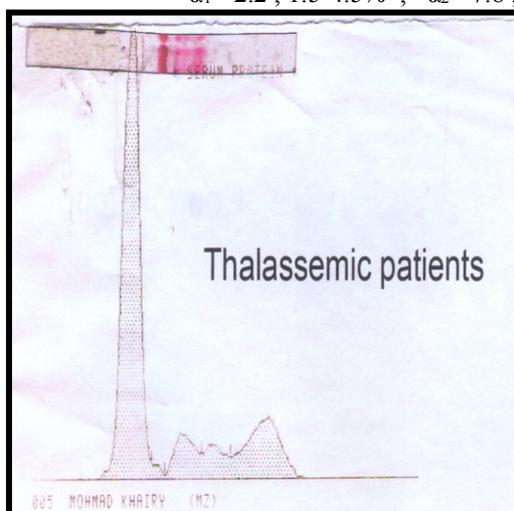
Groups	Alpha1		Alpha 2 chain		Beta chain		Gamma chain	
	Non vaccinated	Vaccinated	Non vaccinated	Vaccinated	Non vaccinated	Vaccinated	Non vaccinated	Vaccinated
Control group	2.420± 0.066	2.660± 0.081	8.200± 0.114	8.540± 0.108	11.520± 0.139	11.720± 0.124	15.220± 0.259	17.700± 0.239*
Thalassemic, hepatitis, iron chelating dependent	2.820± 0.086*	2.900± 0.084	10.340± 0.414**	10.940± 0.220**	7.700± 0.217*	7.640± 0.117*	35.320± 0.646***	30.480± 0.606***
Thalassemic, hepatitis, iron chelating independent	3.600± 0.141**	3.580± 0.111**	9.740± 0.166**	8.980± 0.289**	7.200± 0.176*	8.220± 0.287*	30.120± 0.290***	29.220± 0.404***
Thalassemic, non hepatitis, iron chelating dependent	3.000± 0.130*	2.800± 0.071	9.840± 0.129**	10.520± 0.345**	8.260± 0.175*	9.700± 0.179*	16.960± 0.441*	16.660± 0.213*
Thalassemic, non hepatitis, iron chelating independent	3.375± 0.085**	2.733± 0.092	9.550± 0.253**	10.000± 0.227**	8.275± 0.193*	8.033± 0.161*	17.300± 0.367*	18.717± 0.309*

Results = M of 5 patients ± SE , * = $p \leq 0.05$ when compared with control group (non vaccinated and vaccinated). ** = $p \leq 0.01$ when compared with control group (non vaccinated and vaccinated). *** = $p \leq 0.001$ when compared with control group (non vaccinated and vaccinated).



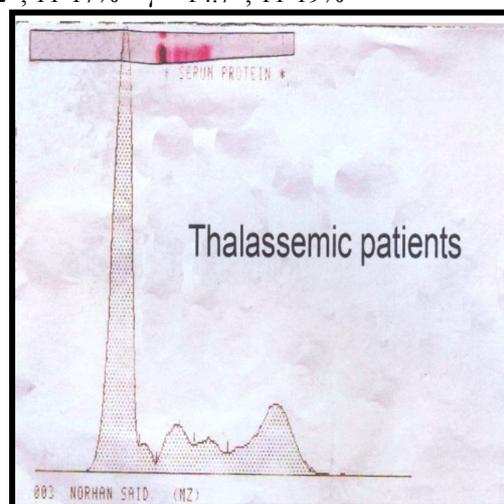
Picture (1): Control group

$\alpha_1 = 2.2$, 1.5-4.5% ; $\alpha_2 = 7.8$, 6-12% ; $\beta = 11.2$, 11-17% $\gamma = 14.7$, 11-19%



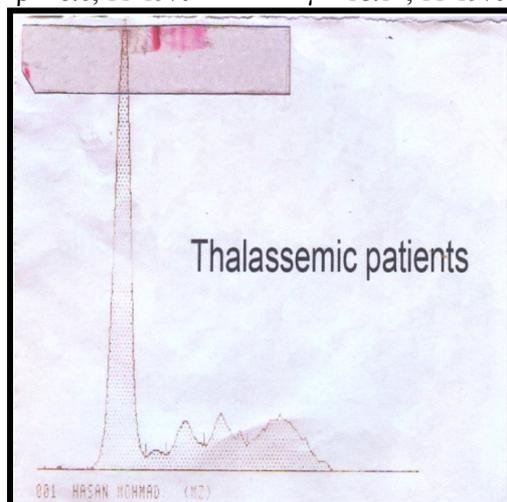
Picture (2):Thalassemia, non hepatic , iron chelating dependent

$\alpha_1 = 1.1$, 1.5-4.5% $\alpha_2 = 8.3$, 6-12%
 $\beta = 6.0$, 11-19% $\gamma = 18.1$, 11-19%



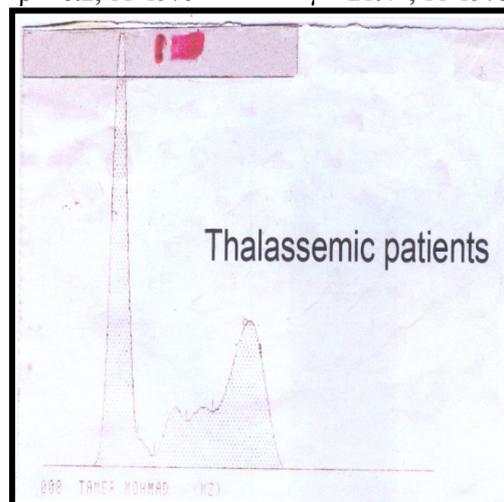
Picture (3):Thalassemia, non hepatic , iron chelating independent

$\alpha_1 = 2.6$, 1.5-4.5% $\alpha_2 = 8.4$, 6-12%
 $\beta = 6.2$, 11-19% $\gamma = 21.4$, 11-19%



Picture (4):Thalassemia, hepatic , iron chelating independent

$\alpha_1 = 2.4$, 1.5-4.5% $\alpha_2 = 8.5$, 6-12%
 $\beta = 11.4$, 11-19% $\gamma = 19.5$, 11-19%



Picture (5):Thalassemia, hepatic , iron chelating dependent

$\alpha_1 = 1.8$, 1.5-4.5% $\alpha_2 = 7.5$, 6-12%
 $\beta = 8.1$, 11-19% $\gamma = 75.4$, 11-19%

Plate (2) Protein electrophoresis to evaluate α , β and γ chains

Discussion

Thalassemia major is a worldwide disease, but it is more common in the Mediterranean region, the Middle East, the Asian subcontinent, and southeastern Asia, as well as southwestern Europe and central Africa. It is one of the most common genetic diseases in the world. It is a major health problem, brings much morbidity and early mortality⁽¹⁴⁾.

Thalassemia patients are considered to be one of the high risk groups suffering from post transfusion viral infection such as HCV. In this study, 50% of patients were +ve for HCV that was much higher than in the healthy blood control. Also, this percentage is higher than that in Thalassemia patients (40.5%) reported in Jordan⁽⁶⁾ and (35%) in Pakistan,⁽²²⁾ but less than the recorded percentage (63.8%) in Iran⁽²³⁾.

Several studies with controversial results regarding immunity level and duration of acquired immunity from hepatitis B vaccination have been performed in different countries⁽²¹⁾. In a study on children in China, serum anti-HBs was 75% within 2 years of vaccination and decreased to 48.2%, 7 years post vaccination. In Taiwan, 15 years after the vaccination of neonates, 75% were anti-HBs positive, but the level was not determined⁽²⁴⁾. The rate of seropositivity of anti-HBc was 2.9%⁽²⁵⁾. This study showed that, 100% vaccinated, Thalassemia, non hepatitis, iron chelating therapy independent recommended to revaccinate because they had less antibodies against HBv vaccine compared to the non vaccinated group and that could not be explained.

Ferritin was prognostic at a cut-off of 2,500 ng/mL⁽²⁶⁾. The present study showed that, high levels of serum ferritin have been reported in HCV- infected patients (1624- 2737ng/mL) vaccinated or non vaccinated, iron chelating therapy dependent or independent. This result is in agreement with Katsanos *et al.*⁽¹⁰⁾ who recorded increased serum ferritin in all β -thalassemia patients (range of 213 to 7105 ng/mL). Also, Tabatabaei *et al.* (2012) found increased serum ferritin in their patients below 2006 ng/mL responding to ribavirin therapy. Olivieri *et al.*⁽²⁷⁾ demonstrated a better prognosis for survival without cardiac disease in transfused patients whose ferritin concentrations remained below 2,500 ng/mL.

Fetal hemoglobin (HbF) is being useful for the diagnosis of β -thalassemia syndromes together with the hematological data (mostly MCV, MCH) and iron status markers⁽¹²⁾. In this study, HbF showed a significant increased values in most groups (12.6- 41.5%) when compared to

control group ($P < 0.05$). This result was in agreement with Attia *et al.*⁽²⁸⁾ who recorded fetal hemoglobin values in the range (12.5–45.38%) for β -thalassemic children. Increased level of HbF, ranging from 10% to over 80% is characteristic of homozygous β -Thallemia⁽²⁹⁾.

Quantitative HbA2% is an important helping factor for the diagnosis of thalassemia. Patients with β -thalassemia will generally have an increased amount of HbA2 usually (4-6%)⁽³⁰⁾. The represented data (table 2) showed significantly elevated HbA2 values in the non vaccinated and vaccinated specially iron chelating independent either hepatitis or non hepatitis. The noticed decreased HbA2 in iron chelating dependent either hepatitis or non hepatitis may be due to the effect of the iron chelating therapy program compared to iron chelating independent (non vaccinated or vaccinated).

Adult hemoglobin (HbA) concentration in the present study was significantly decreased in all patients. This can be explained due to elevation of both HbF% and HbA2 % in all patients. This result was in agreement with Mosca *et al.*⁽³⁰⁾.

Hematological parameters provide information regarding the status of bone marrow activity and hemolysis⁽³¹⁾. Decreased RBCs count, Hb and Ht (table 3), also MCV and MCH (table 4) were recorded for all patient groups. This may due to reduced production of RBCs from the bone marrow⁽²⁸⁾. It was documented that most β -thalassemic patients suffer from chronic hemolytic anemia because of untimely RBCs destruction in the bone marrow and spleen⁽³²⁾. They also added that, malformed RBCs number with anisocytosis and poikilocytosis accompanied by hypochromia are well documented features of β -thalassemia.

The present study showed highly significant increase in the platelet count in all β -thalassemic patients. The data recorded (table 5) were in agreement with Uggun *et al.*⁽³³⁾ who reported that thrombocytosis was common in most β -thalassemic patients.

In this study, white blood cells (WBCs) were slightly elevated or significantly increased in β -thalassemic patients. Sometimes, the elevated WBCs count may be explained by reveals stippling or ragged inclusion bodies in the red blood cells⁽³⁴⁾. The differential WBCs in the blood film shifted to the left of neutrophils and neutropenia with relative lymphocytosis. Neutropenia is more common in patients who had not been splenectomized and using iron

chelating therapy⁽³⁵⁾. Slightly elevated lymphocytes and agranulocytosis were found in some patients who used iron chelating therapy specially Defriprone therapy⁽³⁶⁾.

Alpha1 chain protein was highly increased in patients group that referred to the chronic inflammation which occurred by hepatitis.

The present study showed high significant increase in Alpha₂ chain. Alpha₂ chain is the major constituent of haptoglobin; it is responsible for the binding of hemoglobin released into the circulation when red cells die. Haptoglobin may be elevated, especially during inflammation as part of the acute-phase response⁽³⁾.

Transferrin comprises the most of Beta -band. An increase of Beta -proteins is typical for iron-deficiency anemia due to elevated levels of free transferrin⁽³⁷⁾. In this study, Beta chain was decreased in all patients group. The reasons of this reduction may be due to the presence of transferrin (the iron bound protein) in β -band⁽³⁸⁾, which is considered as to be the major component of the β -globin fraction. Moreover, it agrees with previous study of Al-Mustansiriya⁽³⁹⁾.

hemoglobinopathies

The various immunoglobulin classes (IgG, IgA, IgM, IgD and IgE) are usually of Gamma –band. Gamma-globulins increase in inflammatory disorders like chronic infections (e.g. viral hepatitis)⁽³⁴⁾. This rises in γ -globulin levels were also demonstrated in patients with sickle cell anemia and genetic hemochromatosis by Rivero et al⁽⁴⁰⁾ and Fargion⁽⁴¹⁾ respectively. On the other hand, the γ -globulin band consist of C- reactive protein, which is elevated as much as (1000) fold in response to inflammation, also the acute nature of the inflammatory syndrome as assessed by CRP concentration was confirmed by high level of production of IgM and IgG antibodies^(42,43).

The recommendations from this study are:

1- All thalassemia patients or their parents should receive information regarding the risk of viral infections associated with blood transfusion and other routes.

2- All thalassemia patients should receive hepatitis B immune globulin (HBIG) and hepatitis B vaccine immediately before first blood transfusion.

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