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RESEARCH ARTICLE - MEDICAL TECHNIQUES

The Investigation of Covid-19 Infection in β-Thalassemia Minor Patients

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Article Info.	Abstract
A 1 . 1	Coronavirus or COVID-19 is one of the most important SARS group viruses that cause many severe contagious diseases
Article history:	like acute respiratory pneumonia leading to death in many cases worldwide, COVID-19 was first detected in Wuhan,
Received	China at the end of 2019. Thalassemia is a type of anemia with hereditary origin characterized by abnormal hemoglobin structure and level that leads to poor growth and general weakness.
22 May 2023	To prove the role of zinc in the body of β -thalassemia minor nations and the function of abnormal hemoglobin (HBA2)
	in red cells of those patients in resistance to coronavirus infection.
Accepted	100 blood samples were 50 collected from thalassemia patients who were diagnosed as β -thalassemia minor in this study &
25 August 2023	50 were collected from apparently healthy controls. Zinc tests and HB electrophoresis tests were done on all. The results
Publishing	showed males were 61% and females were 39% including 60% males in β -thalassemia minor patients& 40% were females,
01 March 2024	the age group of this study was (20-59) years old and the age group (20-49) were the most age distributed in this study in $50(1000)$ for the last of
	about 90% of patients & controls, $50(100\%)$ of patients having p-malassemia minor disease with 94% showing normal Hb (12.14 g/ll). 74% having normal response QPC count ≥ 5 calls/um2 with the abnormal rising of Hb A2 pretain ≥ 20
	(12-14 g/u), 74% having increase KDC count >5 censimins with 52% with the another insing of rioZ2 potent >5%. These results also showed that 38% of patients had normal zinc levels in serium 50% of patients showed increased levels
	above normal >127 ug/d and 80% of controls had normal zinc levels between (60-127 ug/d).
	The β -thalassemia minor patients' results were they have increasing immunity by the results of serum zinc rising level
	above normal in about 50% of those patients enhancing the immune response against coronavirus infection. The results of
	this study explained that the patients and controls were comparable in age, gender, and smoking status with no significant
	differences ($P > 0.05$). while results reported that there was a greater significant difference ($p < 0.01$) in levels of the mean
	for fasting blood sugar (FBS), glycated hemoglobin (HbA1c), and a significant difference (p <0.05) in urea and creatinine
	levels between the type 2 diabetic patients and the controls there was a significant difference detected in values of institut and domains $2(n, O(S))$ between the patient and control around S to an b according deform our study that these who had
	and dopamine $2(p < 0/05)$ between the patient and control groups. It can be concluded from our study that those who had type 2 diabetes mellitus had higher levels of dopamine and insulin when compared with the control group
	type 2 diabetes memus had mener levels of dopanine and insum when compared with the control group.

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Keywords: Hemoglobin; SARS; COVID-19; β-thalassemia; Zinc.

1. Introduction

Coronaviruses are a group of related viruses that cause diseases in mammals and birds. In humans, coronaviruses cause respiratory tract(RT) infections that range from mild to severe. The novel coronavirus rapidly became widespread in distribution, resulting in an epidemic in China, followed by a pandemic, raising the number of cases in several countries throughout the world. Coronavirus disease 2019 (COVID-19)is widespread in large droplets produced during sneezing and coughing by patients, also asymptomatic persons before starting their symptoms [1]. Coronaviruses are large, single-stranded RNA viruses with envelopes, found in humans and mammals, such as dogs, pigs, cattle, cats, and birds. Coronaviruses cause respiratory pneumonia and neurological and gastrointestinal diseases. The distribution of severe acute respiratory syndrome (SARS) in 2002 and the Middle East respiratory- syndrome (MERS) in 2012 have demonstrated the possibility of human to human & animal to human transmission of novel emerging coronavirus [2,3]. SARS-CoV-2 belongs to the family Coronaviridae & order Nidovirales, The family involves two subfamilies, i.e., Coronaviruse & Torovirinae. The members of the sub-family Coronaviridae are also subdivided into 4 genera: (i) α coronavirus, 229E, and NL63 are the human coronaviruses that are responsible for the common cold and croup, (ii) In comparison, SARS-CoV-2, MERS-CoV, are classified as β - coronaviruses, (iii) Gamma(γ) coronavirus includes viruses birds, and (iv) Delta (δ) coronavirus include viruses isolated from pigs [4]. The conjugation of 3 major structural proteins defines the compound structures of the virus. The 3 proteins accounting for its structure are nucleo-capsid (N) protein that is phosphorylated internally; (S) surface glycoprotein, comprising the spike; and (M) matrix glycoprotein, which is an abnormal trans-membrane protein [5].

Thalassemia is an autosomal recessive gene inherited disorder caused by abnormal synthesis of one or more globin of hemoglobin chains. The impairment changes hemoglobin production. Thalassemia causes several varieties of anemia, which range from mild to life-threatening. Mediterranean, Southeast Asian, and African, Middle Eastern populations are at rising risk of carrying thalassemia genes [6]. Thalassemias types as α and β - Thalassemia, the cause of these changes is mutations in the gene of globulin portion causing malfunctioning or low levels of α and β globin proteins, respectively. α and β globin chains form a globin pocket for haeme (Fe⁺⁺) linking to transfer oxygen [7]. There are 4 types of globin chains alpha (α) beta (β) gamma (γ) and delta (δ). Depending upon which chain synthesis is altered, the thalassemia is named

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Nomenclature & Symbols							
SARS	Severe -Acute Respiratory Syndrome	RNA	Ribonucleic Acid				
covid	Coronavirus	RBC	Red Blood Cell				
HBA ₂	Adult Hemoglobin A2	IgG	Immunoglobulin G				
MERS	Middle East Respiratory- Syndrome	IgM	Immunoglobulin M				
RT	Respiratory Tract	TDT	Transfusion-Dependent B-Thalassemia				
Hb	Hemoglobin	NTDT	Non Transfusion- Dependent B-Thalassemia				
PCR	Polymerase Chain Reaction	Zn	Zinc				
MCV	Mean Cell Volume	Fe	Haeme				

 α -, β -, γ -, δ -, $\delta\beta$ -, thalassaemias. Mainly genetic inherited as a recessive pattern, the most common types are α - and β -thalassemias, the result from the deficiency of α or (β) globin proteins that are essential in the production of normal Hb molecules like (HbA, $\alpha_2\beta_2$) in an adult [8].

Hemoglobin (Hb), is the pigment color in the red blood cells that carry oxygen to & from the tissues. In adults, 2 components were found: a major hemoglobin (Hb A) and a minor hemoglobin (Hb A₂). The bulk of the hemoglobin (Hb) during fetal life is (Hb F). This hemoglobin consists of 2 pairs of unusual globin chains. The adult hemoglobin's and fetal HbF have α chains complexed with β (Hb A, $\alpha_2\beta_2$), δ (Hb A₂, $\alpha_2\delta_2$), and γ chains (Hb F, $\alpha_2\gamma_2$) [9]. β -thalassemia is a heterogeneous group of genetic anemia caused by gene mutations altering the production of α -& β -globin [10]. Three main types of thalassemia are described: nontransfusion- dependent β -thalassemia (NTDT; thalassemia intermedia)transfusion-dependent β -thalassemia (TDT; thalassemia major), and thalassemia minor(trait). Persons with (TDT) are usually found within the 2first years of life with severe chronic anemia, requiring regular blood transfusions. Regular blood transfusion leads to iron overload complications including liver, heart & endocrine glands [10].

The number of patients infected with thalassemia is not known, In addition, in Northern Italy, 10 Italian patients with (TDT) and 1 with (NTDT)suffered from a COVID-19 disease, while no or very rare number & asymptomatic patients with COVID-19 among β -thalassemia minor(trait) group [11]. Groups of individuals at increased risk of coronavirus infections include those who need blood transfusions like chronic hemolytic anemia and thalassemia major persons with chronic diseases like diabetes, splenectomized patients & iron overload, and those with chronic liver disease and heart complications [11]. Zinc (Zn) is a necessary element for cell growth, proliferation, and survival. It is a structural compound of several proteins. Zinc raises growth in children. It is known that enough zinc levels are essential for maintaining suitable values of insulin-like growth factors in the body & growth hormone [12].

As well as Zinc is also essential in nucleic acid synthesis, the metabolism of lipids &cell division also proteins& carbohydrates. It is also necessary in homeostasis & bone growth in the building of connective tissues, decreased Zn level may impair growth & immune functions [13]. Zn is called to be important in the promotion of the immune system, although its mechanism of action is not understood [13].

Several factors are involved in zinc deficiency in thalassemia patients. One of these main probable factors is chelation therapy, Although, some studies revealed no significant correlation between zinc level & serum ferritin level in thalassemia patients, other study results reported that Zinc can be at a normal level in some patients especially those are do not need regular transfusions of blood like thalassemia minor(trait) [13,14].

2. Materials and Methods

2.1. Collection of samples

100 blood samples were included in this study, 50 samples were collected from β -thalassemia minor (trait) patients previously diagnosed with hemoglobin electrophoresis, and 50 samples were collected from apparently healthy individuals as controls, all samples were investigated with Hb electrophoresis, covid-19 (IgG and IgM) antibody titer and serum zinc level. The age range was between (20-59) years during a period from February 2021 to April 2022, thalassemia samples were collected from the thalassemia centre in Baghdad & Baghdad medical city while controls were collected randomly from apparently healthy diagnosed persons.

2.1.1. Thalassemia identification

50 blood samples were identified as patients having β -thalassemia minor via Hb electrophoresis, blood film, and complete blood pictures including Hb level, RBC count, and mean cell volume (MCV) [15].

2.1.2. Covid-19 investigation

100 samples of β -thalassemia minor patients & controls were investigated for infection with coronavirus disease by detecting IgG & IgM titer, conventional PCR, and gel electrophoresis [16].

2.1.3. Zinc tests

A zinc test was done on all samples in the study by estimating the serum zinc level in the blood & the normal level was (60-127 µg/dl) [17].

2.2. Statistical analysis

The data were statistically analyzed in this case-control study by using (SPSS) ver. (22.0), to estimate the correlation between the presence of COVID-19 in patients with previously diagnosed as β -thalassemia minor as well as the role of zinc in the rising immunity of those patients against coronavirus infection accompanied with apparently healthy control samples. *P* values of less than 0.05 were regarded as statistically significant.

2.3. Ethical clearance

The study's goal and procedures were explained to each subject group individually. They gave their approval to take part in the study. The study was consented to by the Research Committee of the College of Health and Medical Techniques – Baghdad.

3. Results

3.1. Gender distribution in all study groups

All 100 blood samples were 50 (50%) collected from β -thalassemia minor patients previously diagnosed and 50 (50%) were from apparently healthy persons as a control group. The number of males in all study groups was 61 (61%) and females there were 39 (39%) in all study groups, in the β -thalassemia minor group the number of males was 30 (60%) while females were 20 (40%), while in control group the males were 31 (62%) while females were 19 (38%), due to the randomly selection of samples there are no significant differences in distribution of study groups depending on gender at (*p*=0.9), as shown in (Table 1).

Table 1. Distribution of all study groups						
sex	β-thalassemia minor group	control group	Total	<i>P</i> -value		
Male (No /%)	30 (60%)	31 (62%)	61 (61%)	** 0 0		
Female (No /%)	20 (40%)	19 (38%)	39 (39%)	** 0.9 NG		
Total (No /%)	50 (100%)	50 (100%)	100 (100%)	INS .		

*Highly significant;**non-significant

3.2. Distribution of study groups regarding age group

The ages of all 100 samples were divided into 4 groups from (20-59) years old including both males and females were distributed according to age/years, 10(20%) of 30(60%) males in β -thalassemia minor patients were more distributed in age groups (40-49) years old whereas 15(30%) males of all control group males 31(62%) were more included in age (20-29) years old but the distribution of females in the β -thalassemia minor study group was 11(22%) more in (40-49) years old like as males distribution in the patients, as well as the age group (20-29) years old, were more females at 9 (18%) in control healthy group, these results show a highly significant correlation between gender and distribution of age groups particularly in (40-49) and (20-29) years old in patients & controls respectively at p=0.002 (Table 2).

Table 2. Distribution of gender regarding age groups								
	Age	Study	group	Contro	l group			
Age group	(20.50)	male	female	male	female	<i>p</i> -value		
	(20-59) No(%)	No(%)	No(%)	No(%)				
	20_29	7(14%)	2(4%)	15(30%)	9(18%)			
A go Croups	30_39	9(18%)	6(12%)	7(14%)	5(10%)	$P_{-0,002}$		
(Per yrs.)	40 _ 49	10(20%)	11(22%)	6(12%)	3(6%)	P=0.002 *US		
	50_59	4(8%)	1(2%)	3(6%)	2(4%)	113		
	Total	30(60%)	20(40%)	31(62%)	19(38%)			

*Highly significant;**non-significant

3.3. β-thalassemia minor diagnosis

The diagnosis of β -thalassemia minor patients depends on many parameters including blood film, complete blood picture, and hemoglobin electrophoresis including (Hb A, Hb A₂& Hb F), the control group was also subjected to these tests or parameters and showed a normal level of all measurements according to normal laboratory values in adult samples of this study: hemoglobin (12-14 g/dl), red blood cell count (RBC $10^{6}/\mu$ L =3.8-5.5 cells/mm³), Hb A (95-98%), Hb A₂ (2-3%) & Hb F (0.4-2%). The result of this study including blood film revealed hypochromic microcytic cells with frequent target cells and adequate platelets, In addition, to increase blood volume, bone marrow produce abnormal shape of cells as well as normal level of Hb or slightly decreased β -thalassemia minor patients with 47(94%) had 10-12 g/dl hemoglobin from the total 50 (50%) patients with mild anemia they don't require blood transfusion while in control group normal blood film with normochromic normocytic cells and about 32(64%) from 50 controls were had normal level of hemoglobin approximately 12-14 g/dl, according to red blood cell count (RBC) in β -thalassemia minor patients about 37(74%) of patients had very high number of red cells more than 5.5 cells/mm³ while in control group there were normal red cell count. According to Hb analysis in β -thalassemia minor patients that revealed decreased amount of HbA about 48(96%) were less than normal level and increased Hb A₂ more than 3% in 46(92%) of patients considered characteristic features that confirm the diagnosis compared with normal Hb electrophoresis types in controls that participated in this study; statistically there is a highly significant differences between study and controls in diagnosis of β -thalassemia minor diseases at p=0.000as shown in (Figs. 1, 2) & (Table 3).

3.4. Estimation of zinc in all study groups (60-127 µg/dl)

Serum zinc level is very important in increasing immune system action, the measured expected level is about ($60-127\mu g/dl$), The results of this study revealed a high level of serum zinc in patients with β -thalassemia minor disease reached 25(50%) of patients having >127 $\mu g/dl$, 19(38%) having normal zinc level and only 6(12%) of patients having zinc level <60 $\mu g/dl$ because of those patients suffering from other chronic diseases like diabetes II, statistically there is a highly significant correlation between β -thalassemia minor patients and increase serum zinc level at p=0.0008as shown in Table 4.

3.5. Investigation of virus COVID-19 in β -thalassemia minor patients and controls

These study results showed very rare coronavirus infection in patients with β -thalassemia minor diseases from all 50 (50%) patients only 1(2%) having positive IgM rapid tests, 2(4%) of patients having positive IgG previous infection with only 2(4%) showed positive gel electrophoresis results of PCR and the remainder (98%), (96%), (96%) having negative results for IgM, IgG & PCR gel electrophoresis respectively, whereas in apparently healthy control the results were 10(20%) IgG positive were had previous infection at highly significant association (p=0.000) as in (Table 5 and Fig. 3).





Fig. 1. Diagnosis of β -thalassemia minor regarding Hb electrophoresis



Fig. 2. Hb electrophoresis of all study groups

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Table 3. Diagnosis of	β-thalassemia	minor in the stud	v compared with	the control grout
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Tests	Croups	Stu	udy	Con	trol	Total	P voluo
Normal range	Groups	No.	%	No.	%	No.(%)	1 -value
LID	<10	1	2	0	0	1(1%)	
ПD	10-12	47	94	18	36	65(65%)	P=0.000
g/ui	>12	2	4	32	64	34(34%)	*HS
(12-14)	Total	50	100	50	100	100(100%)	
DDC	<3	2	4	3	6	5(5%)	
KDC 106/1	3-5	11	22	46	92	57(57%)	B 0.000
3.8-5.5	>5	37	74	1	2	38(38%)	P=0.000 *HS
Cells/mm ³	Total	50	100	50	100	100(100%)	
HB Electrophoresis		No.	%	No.	%		
	<95	48	96	47	94	95(95%)	
ПD А 05 090/	>98	2	4	3	6	5(5%)	
93-98%	Total	50	50	50	50	100(100%	<i>B</i> -0.000
LID A.	2-3	4	8	49	98	53(53%)	F=0.000 *US
нв А ₂ 2-3%	>3	46	92	1	2	47(47%)	· ns
	Total	50	100	50	100	100(100%)	
	0.4-2	49	98	47	94	96(96%)	
HB F%	>2	1	2	3	6	4(4%)	
0.4-2	Total	50	50	50	50	100(100%)	

*Highly significant;**Non significant

Table 4. Zinc level in β -thalassemia minor patients							
Zinc test	Zinc test Family		Study n=50 Contro		ntrol	Total	
Normal level (60-127	History of	(patients)		n=	=50	n=100	n-vəluo
μg/dl	patients	No.	%	No.	%	No. (%)	<i>p</i> -value
Low < 60	Diabetes II , thalassemia &hypertension	6	12	3	6	9(9%)	
Normal(60-127)	Thalassemia only	19	38	40	80	59(59%)	0.0008
High >127	Thalassemia only	25	50	7	14	32(32%)	*HS
-	50	100	50	100	100(100%)		

*Highly significant;**Non significant

Table 5. Rapid antibody tests an	d PCR screen	ing for corona	avirus infection
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Study	Fourthe history		Rapid aı	Conventional PCR Gel electrophoresis			
groups	Family history	IgM (No. %)		IgG (No. %)		(No. %)	(No. %)
		+ve	-ve	+ve	-ve	+ve	-ve
Study	β-thalassemia minor	1(2%)	49(98%)	2 (4%)	48(96%)	2(4%)	48(96%)
Control	Apparently healthy	0(0%)	50(100%)	10 (20%)	40(80%)	0(0%)	50(100%)
	Samples $= 100$	1(1%)	99(99%)	12(12%)	88(88%)	2(2%)	98(98%)
Total	50 study + 50 control	100		100		1	00
<i>p</i> -value	0.000 (*HS)						

*Highly significant;**Non significant



Fig. 3. Gel electrophoresis of thalassemia samples for covid-19 screening (only sample no. 5,8, and 9 was positive)

4. Discussion

This study involved 100 samples categorized into 2 groups: 50 samples considered as the study group collected roughly from thalassemia patients who were diagnosed as β -thalassemia minor and 50 samples as apparently healthy controls, this work was conducted in the thalassemia centre of Baghdad and Baghdad Medical city from February 2021 to April 2022.

In this study, ages of all groups were between (20-59) years old including both males and females, table 1 shows that the distribution of males was 61(61%) from all samples including 30(60%) in β -thalassemia minor group & 31(62%) in the control group while the number of females in all samples was 39(39%) including 20(40%) in patients and 19(38%) in controls with non-significant different at p=0.9, the reason for this random distribution due to the random selection of samples in the study, this study agreed with another study performed in Iraq / Nineveh by *Bassma Adnan Yonus* [18] for screening of β -thalassemia trait in premarital persons that involved 47 subjects were 31(66%) males and 16(34%) were females.

According to the distribution of age groups in this study samples about (20-60) years old, in the thalassemia patients group were 21(42%) distributed in age groups between (40-49) years old while in the control group the age range (20-29) years old involved 24(48%) subjects with statistically highly significant distribution of all ages in this study at p=0.002, the results of our study not agreed with study a performed in Iran 2016 [19] by MEHRNOUSH KOSARYAN, HOSSEIN KARAMI and others who recorded demographic data of 1725 patients with thalassemia and the mean age was 30 ± 9 (21-39) and the females were (51.5%) more than males (48.5%), the reason for this disagreement is in our study mean age was 20-59 and distributed to groups in both study and controls according to the random style of the sample selection but our results agreed with the same study of MEHRNOUSH KOSARYAN in the distribution of gender of our study in the age group (20-39) years old.

Determination of thalassemia in patients depends upon many tests like blood film , complete blood count and electrophoresis of hemoglobin , in this study 50(50%) patients were diagnosed with β -thalassemia minor (trait) according to results in table3 showed that 94% of patients had hemoglobin level between 10-12 g/dl and red blood cell count in complete blood picture in > 5 cells/mm³ were about 74% of patients also hemoglobin electrophoresis involved a high level of HbA₂ (>3%) in about 92% of total patients this is considered a characteristic diagnostic features of β -thalassemia minor (trait) disease , the blood film also showed hypochromic microcytic cells with mild anemia the causes of these mild anemia are due to increase blood volume in thalassemia trait patients by increased formation of abnormal small red cells in the bone marrow to compensate for the decrease of hemoglobin so the hemoglobin is slightly normal but decreases mean cell volume (MCV) and increases microcytic cells , this study in agreement with other studies accomplished in Pakistan/ Islamabad [20] by Raiz Ahmed Qazi depends on taking different laboratory parameters in diagnosis and screening of β -thalassemia minor (trait) like level of Hb , RBC count , MCV and Hb electrophoresis.

Zinc or (Zn) is one of the most important chemical elements atomic number is 30 and located in the first series of the periodic table and it's very essential in the human body life [12], hair growth and the enhancing immune system against pathogens like viruses by inducing and promoting immune response [13], zinc in this study can be organized as a very important tool in β -thalassemia minor patients that increases immune response against any pathogens or microorganisms like bacteria, viruses like corona by rising its level above normal level in thalassemia trait that doesn't needs to blood transfusion like thalassemia major, so in this study increase level of serum zinc >127 in 50% of patients and increase level of HbA₂>3 in 92% of patients considered as specific criteria that those patients resist or prevent covid 19 infection, these conclusions coordinated with the hypothesis of another study in France [21] by Edouard Lansiauxa who proved that several cities in Italy have a high incidence of thalassemia trait that resists covid19 infection, the cause of this resistance may due to that viral surface glycoproteins binds to abnormal hemoglobin (HbA2) that attacks heame portion and porphyrin in Hb that synthesized in the 1-beta of hemoglobin in mitochondria that form a strong rigid complex that catch the virus and prevents its invasion of the host cells as well as genetic mutations in hemoglobin structure leads to the synthesis of abnormal alpha and beta chains of hemoglobin that form another strong complex that provide immunity against viruses, another study in Iran [22] by Somayeh Rahimi in 2021 its results also agreed with our study and concluded that high level of HbF result in a lower prevalence of coronavirus in beta thalassemia patients compared with the general population, also have in agreement with a study published in Thailand by Konstantinos I in 2020 [23] results show that abnormal HbE level resists covid19 infection, but our study results disagreed a with study published in Greece by Sotirios Sotiriou in 2021 [24]whose results revealed a high susceptibility of β-thalassemia heterozygote to covid19 infection and mortality.

In thalassemia patients normal serum zinc level is between (60-127 μ g/dl) in 38% of patients & an increase level in this study >127 μ g/dl in 50% of patients meaning 88% of patients have good zinc levels and immunity against covid infection agreed with the study of AKM AMIRUL MORSHED was published in Bangladesh [25] who reported a normal level of zinc in thalassemia patients and control but in disagreement with the study of Mohamed ElMissiry reported in Iran [17] and approved 70% of thalassemic adolescents have a zinc deficiency. Finally, COVID-19 infection in thalassemia patients & healthy controls can be screened by several laboratory parameters such as rapid antibody (IgG, IgM) screening tests and conventional PCR with gel electrophoresis, and our results showed very rarely coronavirus infection in patients with about 2% IgM positive recent infection & 4% IgG positive previous infection with mild flue like symptoms.

5. Conclusion

In the results of this study, we conclude that more than 90% of β -thalassemia minor (trait) patients were resistant to covid-19 infection and those patients having about 88% normal and increased levels of zinc in the blood considered the most probable reason for enhancing and promoting an immune response against viral infection as well as 92% of those patients revealed an increase in the HbA₂ level above 3% when analyzed by electrophoresis of hemoglobin that causes genetic modulation and mutation in the porphyrin of heme and forms rigid complex that catch the coronavirus and prevent infection in those β -thalassemia minor patients.

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