Evaluation of vitamin-D3 Concentration in Patients with Thalassemia in Baghdad City

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Abstract

Background: Thalassemia is inherited blood disorders characterized by abnormal hemoglobin production.
Objective: To evaluate decreasing in vitamin-D3 concentration in patients with thalassemia among healthy men in Baghdad city.
Patients and Methods: In this study containing 99 thalassemia patients from Baghdad city (68 female and 31 male patients) and (31 healthy males and 10 healthy women) as a control group collected from Al-Kadhymia hospital. Their age ranged (15-40) years old which are compatible to age of study group. Serum samples taken from the patients to determined vitamin-D concentration by using Enzyme Linked Immuno Sorbent Assay (ELISA) technique, during the period 1st April 2016 to 28th January 2017.
Results:The study revealed highly significant (P<0.01) low of vitamin-D3 concentration in patients with thalassemia (89.9% decrease vit-D3 compared with 10.1% control group vit-D3 concentration).
Conclusion:The decreasing in vitamin-D3 concentration associated with thalassemia patients was due to less exposure to sun light that affected the vitamin metabolism in their bodies.**Key words:** Thalassemia,Vitamin-D3 concentration , ELISA.
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Introduction

Thalassemias are various group of hemoglobinopathies which are genetically restricted described by absolute or low production normal hemoglobin and this leads to microcytic anemia of different grade [1]. The symptoms of the disease can be stand on type and can be differ from nothing to severe. Mostly there is moderate to acute anemia (depression in red blood cells). Anemia results from feeling fatigue and yellow skin. Bone problems may also be present, an extended spleen, pale skin, deep urine, and tardy growth between children [2].The disease is genetic disturbances comes from parents of the person [3]. Two main types of thalassemia

founded, alpha thalassemia and beta thalassemia [4], and the causative agents of the affliction with thalassemia is the decreased synthesis of alpha/beta globin chain imponderables [4]. The stringency of these types depends on how many genes are absent which are four genes for alpha globin or two genes for beta globin [5]. The cause of inactive erythropoiesis and hemolytic anemia is the aggregation of too much free alpha or beta globin chains and their output and precipitation on the membrane of red blood cell [6]. Diagnosis is standard happened by tests which done for blood containing a complete blood count, special hemoglobin tests and genetic tests [7]. Before delivery the diagnosis may take place during prenatal testing [8].

Treatment for thalassemia bases on kind and rigidity of the disease and treatment for extra acute cases mostly involves regular blood transfusions iron chelation, and folic acid. Iron chelation may occur by deferoxamine or deferasirox. Sometimes transplantation for bone marrow could be a choice [9]. The complications can predicate an increase in iron intake because of chronic hemolytic anemia which leads to Iron oversize load and precipitation of it in organs those progresses in significant iron-related organ complications such as cardiac and endocrine dysfunctions included diabetes mellitus, hypoparathyroidism, hypogonadism and infertility [10 &11]. So the ineffective erythropoiesis iron oversize load and chronic hemolytic anemia are the major complications of the disease [12&13]. As in 2013 the disease affected in about 208 million people with about 4.7 million having acute thalassemia [14].

Thalassemia is universe disease in inhabitance, especially Mediterranean range, far-eastern and south East Asian countries [15, 16 &17]. The disease had similar rates in both gender [18]. It was conducted in 25,000 deaths in 2013 down from 36,000 deaths in 1990[19]. People with minor degrees of thalassemia were similar to people with sickle-cell trait that has some protection against malaria clarifying that why they were more common in areas of world where malaria coexists [20].

Alpha-thalassemias: The α-thalassemias includes the genes HBA1 and HBA2 [21 & 22], inherited in a Mendelian recessive way. Two gene loci and so four alleles present. In addition to that it is correlated to the deletion of the 16p chromosome. α-Thalassemias will give a lower alpha-globin production, wherefore less alpha-globin chains are resulted, producing an increase of βchains in adults and an increase of γ chains in.

Beta-thalassemia: Beta thalassemia is a genetic autosomal recessive blood illness. In beta. thalassemia and hemoglobionpathies, the occurrence of mutations or deletion products in reduced average of synthesis or no combination of one of the globin chains that produce hemoglobin could be considered as hereditary disorders [23].

Beta thalassemias are caused by mutations of the HBB gene on chromosome 11 [24] and transmitted in an autosomal recessive manner. The nature of the mutation and the occurrence of mutations in one or two alleles determine the severity of thalassemia mutated alleles are named β+ as partial function is preserved (either the protein has an abbreviated role, or it is role be ordinary but is resulted in very little amount) or β⁰when no producing for effective protein. B thalassemia major (Mediterranean anemia or Cooley anemia) is caused by a βo/βo genotype. It is the most stringent fashion of β newly born. The overmuch βchains form unsteady tetramers (called hemoglobin H (HbH) of 4 beta chains), which have atypical oxygen disassembly.curves. thalassemia because no production of functional βchains, and hence hemoglobin A is cannot be aggregated [25].

Vitamin D: Vitamin D regards as a collection of fat-soluble secosteroids answerable for elevating the absorption of calcium, iron, magnesium, phosphate, and zinc in intestine. Vitamin D3 as it is additionally well-known as (cholecalciferol) and vitamin D2 (ergocalciferol) which represent the major important composite of this collection in human [1]. We can accomplished Cholecalciferol and ergocalciferol through ingestion from the meal and supplements [26][27]&[28] . Limited food contain vitamin D nevertheless synthesis of vitamin D (specifically cholecalciferol) is the most significant normal source of the vitamin in skin. Dermal synthesis of vitamin D from cholesterol is dependent on sun exposure (specifically UVB radiation).

The source for vitamin D from the diet or dermal synthesis through sunlight is inefficient biologically, the motivation demands an enzymatic transition (hydroxylation) in liver and kidney. Negative feedback loop that forbids toxicity can organize the synthesis of vitamin D from exposure to sunlight and this was revealed by evidence, but because of suspicion from cancer′s risk affliction by sunlight, no recommendations are issued by the Institute of Medicine (US) for the quantity of showing up to sun needed to reach vitamin D neediness. Wherefore, the diet system reference intake for vitamin D presumes no occurrence for the vitamin D synthesis and the whole vitamin D for person was taken from food. The majority of mammals showing up the sunlight synthesized vitamin D insufficient quantity [citation needed] it is not exactly a vitamin, and may be regarded as a hormone when its synthesized and the vivacity happen in different sites.[misguiding]. This vitamin has a meaningful situation in homeostasis of calcium and metabolism. The detection of vitamin D was belonging to exertion to detect the dietary item lacking in rickets (the juvenile model of osteomalacia) [29].

Vitamin D (Vit-D) Deficiency: Deficiency in vitamin D through diet in association with insufficient appearance to sun light leads to osteomalacia (or rickets at the time when it takes place in children), which is a

looseness in the bones. It is an usual illness in developed world [26&27]. Nevertheless, the deficiency of vitamin D has come to be a worldwide trouble in the aged people but subsist as a universal in juveniles and adults [28&29]. Decrease in blood calcifediol (25-hydroxy-vitamin D) it is an outcome takes place from avoiding the sun [30]. Decline in vitamin D leads to impairment in mineralization of bone and destruction of bone, this will handle to illness in looseness of bone containing rickets and osteomalacia [31, 32].

Patients and Methods

Serum samples taken from patients to determined vitamin-D concentration by using Enzyme Linked Immuno Sorbent Assay (ELISA) technique, during the period 1st April 2016 to 28th January 2017. Their age ranged (15-40) years old.

(Vit-D) test Procedure [30]

1. Choice the demanded number of strips for the run. The unused strips should be plugged up in the bag with a desiccant and conserved at 2-8°C.
2. Obtain the strips into the holding frame.
3. Fifty microliter of calibrator, control and sample were transferred into the suitable wells.
4. 150 µl of incubation buffer were pipetted into all wells.
5. The plate shaker (300 to 700 rpm) was incubated for 2hr. at room temperature then added the working HRP conjugate solution once the incubation is beginning (within 15 minutes).
6. The well were aspirated and then washed 3 times with 0.35ml of the washing solution.
7. 200 µl of the working HRP conjugate solution were pipetted into each well then incubated for 30 minutes at room temperature, on a plate shaker (300 to 700 rpm).
8. The wells were emptied and then washed as described in no. 6.
9. 100 µl of the chromogenic solution were pipetted into each well after washing step during 15 minutes.
10. The microtiter plate was incubated for 15 minute at room temperature, on a plate shaker (300 to 700 rpm), keep out of direct exposure to sun.
11. 100 µl of stop solution were pipetted into each well.
12. The absorbance was read at 450 nm (reference filter 630 nm or 650 nm) during one hour. The calibration curves were determined the concentrations of the samples

Statistical Analysis

Data were collected, tabulated and statistically analyzed by:

(1)Descriptive P statistics.

(2) Chi-square [3].

Results

Table (1) and figure (1) describes the number, gender and percentage of studied group (Patients and control groups) which revealed highly significant ($P<0.01$) between male and female infected with Thalassemia compared with control groups.

Table (1): The numbers and percentage of studied groups (Patients and control groups).

Gender		Studied groups		Chi-Square (P-value)
		Control	Thalassemia	
Male	N	31	31	P=0.00 Highly sign. (P<0.01)
	%	75.6%	31.3%	
Female	N	10	68	
	%	24.4%	68.7%	
Total	N	41	99	
	%	100%	100%	

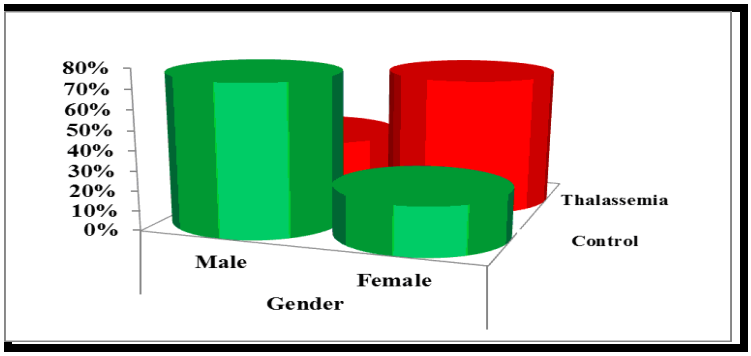

Figure (1): The percentage of studied groups (Patients and control groups).

While table (2) and figure (2) revealed highly significant with p-value (P<0.01) in percentage of Vitamin D3 concentration compared among Thalassemia patients and control group.

Table (2): Percentage of Vitamin D3 concentration compared among Thalassemia patients and control groups.

Vitamin D3		Studied groups		Chi-Square (P-value)
		Control	Thalassemia	
Normal	N	20	10	P=0.00 Highly sign. (P<0.01)
	%	48.8%	10.1%	
Decrease	N	21	89	
	%	51.2%	89.9%	
Total	N	41	99	
	%	100%	100%	

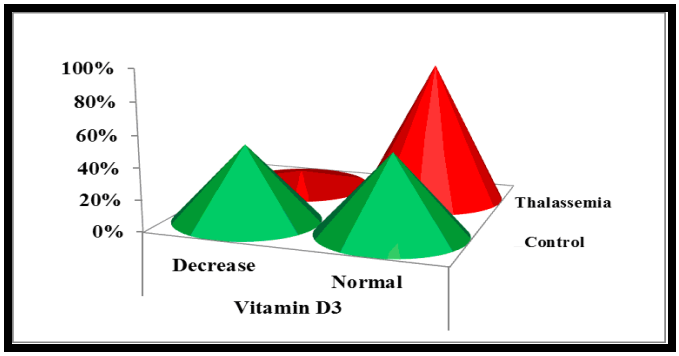

Figure (2) Comparison of percentage of Vitamin D3 concentration among Thalassemia patients and control group.

In table (3) and figure (3) vitamin D3 concentration in Thalassemia patients especially female appeared highly significant (P<0.01) than male.

Table (3): Percentage of Vitamin D3 concentration compared with male and female Thalassemia patients.

Parameters		N	%	Binomial (Z) test (P-value)
Gender	Male	31	31.3%	P=0.00 Highly sign. (P<0.01)
	Female	68	68.7%	
	Total	99	100%	
Vitamin D3	Decrease	89	89.9%	P=0.00 Highly sign. (P<0.01)
	Normal	10	10.1%	
	Total	99	100%	

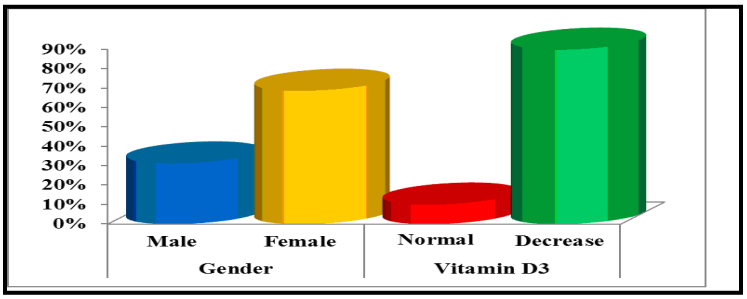

Figure (3) Percentage of Vitamin D3 concentration compared among male and female Thalassemia patients.

Discussion

This study has shown highly significant (P<0.01) between female and male with thalassemia as shown in table (1)and figure (1), that revealed increase percentage of women with thalassemia than men. This result disagree with some researchers like

in Turkey [32] which represented the ratio of male patients was advanced more than the female with thalassemia they believe that the people are more interested with the health of the male progeny and for that are more probable to request medical protection for them. In table (2) and figure (2) results indicated an elevated significances ($P<0.01$), decrease vitamin D3 concentration among thalassemia patients and control group. Some studies clarified that vitamin D deficiency was attributed to defective 25 hydroxylation of vitamin D in the liver because of excessive load of iron [33]. Other mechanisms contain low intake, weak absorption, or production of skin was reduced [34]. As we know that the liver is the main place for hydroxylation of vitamin D, it is likely that excessive amount of iron in liver may direct to shortage of vitamin D proposing that presence of excessive amount of iron in liver may affect the metabolism of vitamin D and probably may do elevation in danger of the clinical end points correlating to vitamin D insufficiency/deficiency [35]. Table (3) and figure (3) revealed increase percentage of vitamin D3 concentration decreasing in female patients more than.male.patients.

Our explanation to demonstrate results in the women are less exposed to sun light than men may be because they covering all their bodies with clothes even their heads that prevent the contact with the sun light directly specially in Islamic places like in Baghdad city (most people are Muslims). Several studies have reported an elevated hazard of vitamin Vit-D deficiency are due to inherited and ethno-cultural factors, deep skin or concealing clothing that may cause a limited exposure to sun light [36, 37& 38].

Conclusions

The decreasing in vitamin-D3 concentration associated with thalassemia patients was due to less exposure to sun light that affected the vitamin metabolism in their bodies.

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