Evaluation of Vitamins (B6, B12, B9) and Some Biochemical Parameters in Thalassemic Children

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Abstract :

Due to the various and multiple vitamin roles that is used to improving drug action and decreasing the negative role cause by vitamins deficiency concentration which lead to many types of anemia, included this study measuring their level .

The present study was conducted in- Ibn AlBalady hospital. It includes 50 patients suffering from thalassemia major aged (13-15) years. The patients were diagnosed by the type of hemoglobin (Hb), Mean corpuscular hemoglobin concentration (MCHC) and Red blood cell count (RBC), which showed alteration in those patients . All patients were males matched in age and sex . some biochemicals parameters were studied including vitamins B6, B12 and Folic acid (B9) .

Some important enzymes were tried including GOT and GPT, The study also included the measurement of Bilirubin in sera of patients and healthy controls. The results showed lower significantly in Hb levels in sera of major thalassemia patients compared to control as in RBC and MCHC. The activity of both enzymes GOT and GPT were higher in sera of patients compared to control. A significant increase of Bilirubin was found in sera of a patients.

Key words: thalassemia , Bilirubin , B6 , B12 , B9 , GOT , GPT

تقييم الفيتامينات (B6,B12,B9) وبعض العوامل الحيوية في الاطفال المصابين بالثلاسيميا

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المستخلص:

نظرا لدور الفيتامينات المتعدد والكبير واستخدامها في تحسين الكثير من العلاجات لكثير من الامراض ولتقليل الدور السلبي الذي يؤدية نقص بعض الفيتامينات في اظهار انواع مختلفة من فقر الدم تضمنت هذة الدراسة قياس مستواها وتم اجراء هذ ِه الدراسة في مستشفى ابن البلدي وشملت ٥٠ حالة مرضية من مرض فقر دم البحر الابيض المتوسط (الثلاسيما العظمى) من الذكور فقط والذين يتروح اعمارهم بين (١٣ - ١٥) سنة و تم التشخيص من خلال دراسة مستوى الهيموكلوبين (Hb) ومعدل تركيز الهيموكلوبين في كريات الدم الحمراء(MCHC) وعدد كريات الدم الحمراء(RBC) . وكذلك اخذت نماذج دم لخمسين متطوع اً من الاصحاء المقاربين في العمر ومن جنس المرضى نفسه.

درست مستويات بعض الفيتامينات حامض الفوليك (B9)(غير مدرج كعلاج لمرضى الثلاسيميا على الرغم من تاكيد الدراسات بخصوصه) و فيتامين B6 و فيتامين B12 ودرست فعالية بعض الانزيمات المهمة مثل انزيمات نقل مجموعة الامين GOT و GPT ، وتضمنت الدراسات قياس المادة الصفراء (Bilirubin) في مصل مرضى الثلاسيميا ومجموعة الاصحاء .

اظهرت النتائج انخفاض اً معنوياً حاداً في مستوى الفيتامينات B6،B12،B9 في مصل المجموعة المرضية مقارنة مع الاصحاء . ان النتائج الخاصة بالبيلوربين (TSB) اظهرت زيادة معنوية في امصال المرضى مقارنة مع الاصحاء . إنّ هدف الدراسة هو التحري عن اي سبب لحدوث الانيميا المكتسبة لمرض ى يعانون اصلا من انيميا وراثية للتاكيد على توسيع العلاجات لتتضمن الفيتامينات كاحد العلاجات المرافقة لدى مرضى الثلاسيميا لتقليل الاثار الخطيرة للانيميا الذي يعانون منها.

الكلمات المفتاحية: (thalassemia , Bilirubin , B6 , B12 , B9 , GOT , GPT)

Aim of Study

The aim of the present study was to investigate any reason that causes anemia with thalassmic patients that sources anemia for expansion of taking drug to include vitamins with thalassmic patients to decrease the influence of dangerous anemia.

Introduction:

Anemia is a decrease in number of the Red blood cells (RBCs) or the presence of less them normal quantity of hemoglobin in the blood hemoglobin. However it can include decreased Oxygen-binding ability of each hemoglobin molecule due to deformity or lack in numerical development as in some other types of hemoglobin deficiency [(1)]. This may occur in acute or chronic hemorrhages, or may be produced by toxic factors which cause hemolysis and increased erythrocyte destruction [(2)].Iron deficiency anemia is the most common anemia; it occurs when iron intake or absorption is insufficient, and since iron is essential for the formation of hemoglobin, then the latter cannot be formed properly[(3).] Nutritional anemia results from a deficiency in folic acid or vitamin B_{12}[(4)]. Thalassemia genetically determine defect in hemoglobin synthesis. There is an inability to manufacture sufficient globin chains [(5)] .The defect may affect α, β and δ chain or may affect some combination of the α , β and δ chain in the same patient, but never α and β chain together, unmatched globins crepitate and damage RBC membranes causing their destruction while still in the marrow [(6)].

Beta Thalassemia Major (Homozygous) (B^o)

These patients are well at birth but develop a life threatening anemia by one or two months. In beta thalassemia major there is a complete failure of beta chain production. Hence there is very little, if any, Hb A present. Raised levels of Hb A2 and Hb F. Hemoglobin F has a very high affinity for oxygen and it is a poor oxygen deliverer. As a result, the only functional hemoglobin present is Hb A2; hence the patient is hypoxic which causes increased erythropoietin secretion. The excess erythropoietin stimulates the marrow to the maximum, and ultimately to the point that extramedullary hemopoiesis occurs with splenomegaly. Even with an increased production of hemoglobin's A2 and F there is still an excess of alpha chains and they precipitate in developing norm blasts. This results in

intramedullary hemolysis and their premature removal from the marrow by Reticuloendothelial cells (RE) [7].

Genetic Pattern of Inheritance of β-Thalassemia:

The pattern which recognized that the parents of children with thalassemia major had thalassemia minor with one β-thalassemia gene is medically referred to as an autosomal recessive pattern. Where these parents, have children, they have a 25% chance of having a thalassemia major child (with both genes for β-thalassemia), a 50% chance of having children with thalassemia minor (with only one gene for β-thalassemia) and a 25 % chance of having a child without the thalassemia major or minor (with both genes for normal β-chains [8].

Treatment of Thalassemia:

Treatment for thalassemia depends on the type and severity of the disease. People who are carriers (thalassemia trait) usually have no symptoms and need no treatment. Those with moderate forms of thalassemia may need transfusion occasionally, those with severe thalassemia have a serious and life- threatening illness. They are treated with regular blood transfusion, iron chelating therapy, and bone marrow transplants [9]. Folic acid is used in the body to synthesize blocks of DNA, the body's genetic information, and blocks of RNA, needed for protein synthesis in all cells fast growing tissues, such as those of a fetus, and rapidly regeneration cells like red blood cells and immune cells, have a high need for folate [10] .

Bilirubin:

Degradation of heme after 120 days in the circulation is to produce green pigment biliverden after two steps of oxidation reactions. Biliverden is reduced forming bilirubin, the iron is either used to make new hemoglobin molecule in the red bone marrow or stored in the liver as an iron – protein complex by protein ferritin. Bilirubin binding to albumin and enters a hepatocyte, in hepatocyte the solubility of Bilirubin is increased by the addition of two molecules of glucorinc acid and excretion of bilirubin into bile .Bilirubin can be measured as direct (conjugated). Sometimes the total amount of bilirubin in the blood is measured [11]

Aspartate Transaminase (AST):

This enzyme is also called glutamate oxaloacetate transaminase (SGOT). It is found in high concentration in cells of cardiac and skeletal muscle,liver,kidney and erythrocytes. Damage to any of these tissues may increase plasma AST levels [12]. There are two forms of AST .The Mitochondrial and the soluble forms. Pathologically, AST Activity is used as a pathological marker for myocardial infarction disease and muscle diseases Thus, increased concentration of aminotransferases signify pathology Markers of liver damage" transaminases and alkaline phosphates" predominate rise in patients with different demagogical disorders cancers [13].

Alanine Transaminases (ALT):

Also called serum glutamate-pyruvate transaminase (SGPT). Having molecular weight approximately 101.000.D ALT is present in high concentration in liver in a lesser extent in skeletal muscle, kidney, and heart. Measurement of ALT activity in serum is used as an indicator of hepatocellular damage [14].

Materials and Methods:

Subjects have been classified into two groups: control group included (50) healthy individual (male) (13-15 age) with no previous disease which may interfere with the parameters analyzed in this study and β. Thalassemic major group: included (50) patients (male)(13-15 age) suffering from β–thalassemia. Vitamins were determined by high performance liquid chromatography (HPLC) [15] at Research Center of Medical College /Al-Nahrain University . Soluble vitamins (Folic acid, Vitamin B_6 and vitamin B_{12}) were separated and quantitatively determined according to the method an Augustin et at [16].

Result:

The HPLC analytical method gives a linear response curve for the standard solution for vitamins (Fig.1)(17)

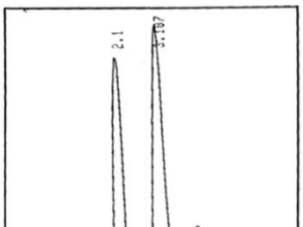


Fig (1) Separation of Soluble vitamins (folic acid, B_6 and B_{12})
Reversed phase (250 ×4.6mm inside diameter), 5 µm particle size

Mobile phase 5mM octyl sulfonate: acetonitrile
Detection: UV at 210 nm
Peaks Indentity: 1. Folic acid (Retention time) =2.1 min
2. Vit B6 (Retention time) = 3.1 min
3. Vit B12 (Retention time) = 4.09 min

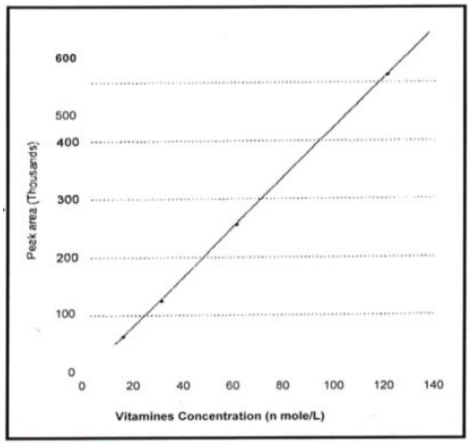


Fig (2) Standard curve of one vitamins (folic acid, B_6 and B_{12})

Table (1) shows the B6 level measured by HPLC in sera of the two studied groups. A significant decrease in the thalassemic group compared to control group (40.93±06.19), (62.55± 15. 58)

Table (2) shows the vitamin B12 level measured by HPLC in serum of two studied groups

A significant decrease of thalassemic group compared to control group, in thalassemic patients in this study is compatible with many other studies Kondo.H, Wrf SS. [18], [19].

Table (3) shows the level of folic acid in two groups, thalassemic and control and there was significant decrease in thalassemic patients compared to control group (27.12 ± 4.19) (57.16 ±11.66).

Determination of Bilirubin:

Total bilirubin is determined in the presence of caffeine by the reaction with diazotized sulphanilic acid. Direct (conjugated) bilirubin is determined in the absence of caffeine.

Determination of AST Activity (SGOT):

Colorimetric method for determination of serum aspartate aminotransferase. By monitoring the concentration of oxaloacetate hydrazine formed with 2,4 dinitrophenyl- hydrazone (DNPH) [20].

Determination of ALT Activity:

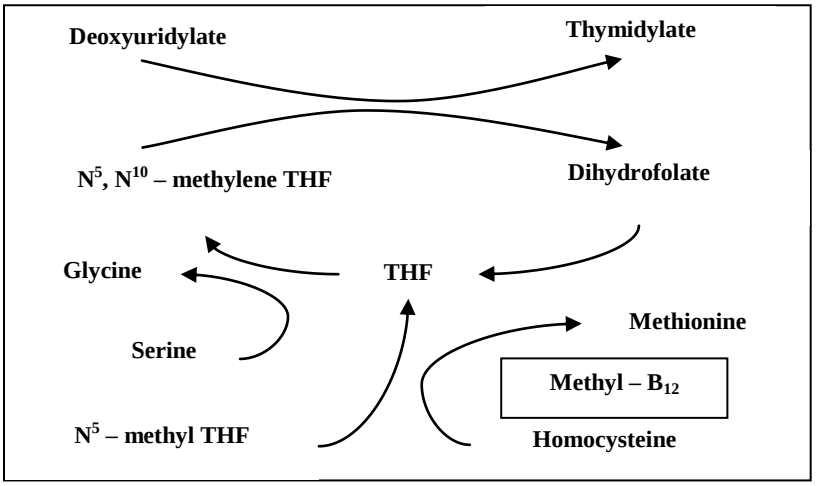
Colorimetric method is used for the determination of serum alanine aminotransferase [21].

Discussion :

Vitamin B6 is one of the vital vitamins for healthy individuals. Results showed that thalassemic patients are suffering from lower levels of vitamin B6 in comparison with control group. This vitamin is required for both mental and physical health and decreased level can cause what is called sideroblastic anemia which is category of anemia featuring a buildup of iron containing immature red blood cells one type of sideroblast anemia is due to genetic defect in an enzyme that uses vitamin B6 as a cofactor [22]. Vitamin B6 deficiency can be restored either by food or by drug supplementation using drug supplementation is preferred to be with other vitamins and minerals like vitamin C, potassium, calcium and fatty acid[23]. Cortisone administration should be avoided during the course of vitamin B6

administration because cortisone will impair the addition absorption of this vitamin [24]

Vitamin B12 is required in humans for two essential enzymatic reactions, the synthesis of methionine and the isomerization of methylmalonly CoA that is produced during the degradation of some amino acids and fatty acids with odd .The megaloblastic state results from an imbalance between supply of co-enzyme necessary for DNA production. The two cofactors which are folate and Vit B12.When these are deficient, megaloblastic change results. On the other hand increased damage for DNA in physiologically hyper proliferative states, such as cancer & hemolytic anemia, can cause megaloblastic change even in the face of free available folate & B12. All vitamin B12 comes from the diet and vitamin B12 present in all foods of animal origin [25]. Vitamin B12 should supplement with at least 2 to 3 mg per day[26]. Deficiency of vitamin B12 produces megaloblastic anemia due to its role in folate metabolism [27]. During the many transformations of folate from one form to another, a proportion gets accidentally converted to N^5 – methyl – THF, an inactive metabolite. This is called the "folate trap" since there is no way for active N5, N^{10} – THF to be regenerated except through a reaction for which a form of vitamin B12, methyl – B12 is a coenzyme Fig(4)[28].



Fig(4)Tetra Hydrofolate Pathway

Deficiency of B12 then produces a situation where more and more folate is trapped in an inactive form with no biochemical means of escape. The end result is failure to synthesize adequate DNA [29]. Some studies have found people to be frequently deficient in folic acid, and vitamin B12 [30]. Folate is necessary for production and maintenance of DNA new cells. Folate is needed to replicate DNA. It also helps prevent changes and that may lead to cancer [31].

Folate deficiency is usually found in individuals with poor dietary habits and is frequently associated with chronic alcohol abuse. Thus many patients show signs of chronic liver disease [32]. Causes of folic acid deficiency included inadequate diet, increased vitamin requirements, impaired absorption or defective folate metabolism. The folate deficiency hinders DNA synthesis and cell division, affecting most clinically the bone marrow, a site of rapid cell turnover. Because RNA and protein synthesis are not hindered, large red blood cells called megaloblasts are produced, resulting in anemia [33].

Defective folate metabolism results in deficient interconversion; the activity of folic acid depends on its conversion to tetrahydrofolic acid and to certain one carbon methylated derivatives. This conversion is inhibited by liver disease, vitamin B12 deficiency, and drugs such as methotrexate, and ethanol [34].

Folic acid deficiency might be caused by impaired absorption from the intestine or might be caused by a disease which are associated with red blood aggregation such as thalassemia and leukemia. Folic acid deficiency symptoms might occur several weeks after not taking this vitamin in food. Increased requirements for folate are also a common cause. Requirements for folate may be increased three to five folds in patients with chronic hemolytic anemia (thalassemia), pregnancy, chronic exfoliative dermatitis and chronic infections [35]. The results of bilirubin measure of the two studied groups are shown in table (4) that there was a significant increase of bilirubin in thalassemia major patients compared to control individual .

Thalassemic patients will have an increased amount of bilirubin in the blood. This is due to the increased destruction of red blood cells

(hemolysis) by the spleen. This is the main cause of hyperbilirubinemia. On the other hand, liver cells are damaged as a side effect of iron overload, bilirubin can escape into blood stream [36]. The liver has the capacity to conjugate and excrete over 3000 mg of bilirubin per day; whereas the normal production with covers ponding increase in conjugation and secretion of bilirubin diglucuonide. However, massive lysis of red blood cells (for example in patients with thalassemia,malaria) may produce bilirubin is excreted into the bile, the amount of urobilinogen entering the enterohepatic circulation is increased, and urinary urobilinogen is increased. Unconjugated bilirubin levels become elevated in the blood causing jaundice [37]. These results are in agreement with other studies Olivieri N.et al. [38,39]

Table (5) shows the result of GOT activity in serum of the two studied group. Elevated activity of GOT in serum of TM patients compared to control was found. Transaminases are expressed as multiplied by the upper level of the normal range to identify the role of iron overload in the natural history of liver fibrosis .These results are in agreement with other studies Ansor M., Cunningham M. [40,41]. Table (6) shows the results of ALT activity in sera of thalassemia major patients group and control group A significant increase in ALT activity in the serum of TM patients compared to control was found. The mean ALT activity in thalassemic patients was reported to increase and this increase in ALT was generally transient and occurred more commonly in patients with hepatitis C [42]. ALT activity was elevated in all thalassemic patients, which is due to the symptoms of liver damage [43].

Transfusional iron overload occurs with severe conditions that fulfill this criteria include thalassemia major. The iron is stored in the liver as ferritin . Ferritin is normally found mainly inside the cells, will only a small amount in the blood when there is damage to organs that contain ferritin (especially the liver, spleen and bone marrow)[44]. B-thalassemia major is associated with varying degrees of liver damage which causes the elevated plasma transaminase activities in those patients .

Tables:

Table (1) B6 level in sera of control group and thalassemic group.

Groups	No.	B6 n mol/l Mean ± SD	P value
Control	35	62.55 ± 15.58	
Thalassemia	35	40.93 ± 6.19	< 0.001*

***Represent P value between control group and TM group.**

Table (2) Vitamin B12 level in sera of control group and major group.

Groups	No.	Vit B12 n mol/l Mean ± SD	P value
Control	35	65.75+23.90	
Major	35	19.8 5+ 9.16	< 0. 001*

***Represent P value between control group and patient group.**

Table (3) Folic acid in sera of control group and thalassemic group.

Groups	No.	B9 n mol/l Mean ± SD	P value
Control	35	57.16+11.66	
Thalassemia	35	27.12+4.19	*<0.001

***Represent P value between control group and thalassemic group.**

Table (4) bilirubin levels of control and thalassemic group.

Groups	No.	Bilirubin n mol/l Mean ± SD	P value
Control	35	8.74 ± 1.55	
Major	35	18.87 ± 8.92	< 0.001*

Table (5) GOT activity in sera of control group and major group.

Groups	No.	AST (GOT) U/L Mean ± SD	P value
Control	35	33.20 ± 8.42	
major	35	63.68 ± 19.81	< 0.001*

Table (6) GPT activity in sera of control group and major group.

Groups	No.	ALT (GPT) U/L Mean ± SD	P value
Control	35	10.54 ± 1.48	
Major	35	25.48 ± 12.95	< 0.001*

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