Folic acid deficiency associated with mitochondria DNA damage in beta thalassemia major

Qassim Ibrahim obeas[1], Anwar Jasib Almzaiel[2]

[1, 2]Branch of Medical Chemistry, College of Medicine, University of AL-Qadisiyah, Al-Diywaniyah, Iraq
[3]Branch of Internal Medicine, College of Medicine, University of AL-Qadisiyah, Al-Diywaniyah, Iraq

* Corresponding Author

Name: Qassim Ibrahim Obeas

Email: qassimibrahim1994@gmail.com
Contact: +964-7826574593

Abstract

Background: Folate deficiency has been found in Beta-thalassemia major (β-TM). However, its impact with mitochondria DNA damage status has not been addressed. The study aimed to investigate the serum folic acid in β-TM patients and to determine an association between serum FA deficiency and mitochondria DNA damage.

Methods: A total of 80 patients who were diagnosed with β-TM patients were enrolled in the study and divided into two groups:45 β-TM who were not receiving folic acid (FA)and 35 β-TM who were receiving folic acid 5 mg/day (β-TM +FA), 40 healthy subjects were consider as control. A blood sample (5 ml) was taken from all study groups. 1 ml was used for the complete blood count (CBC) Neutrophils/lymphocytes ratio analysis and mtDNA damage by conventional PCR. Serum was separated from other 4 ml of blood by centrifugation for folic acid analysis by immunochromatographic assay**.** serum Ferritin was measured by an enzyme-linked fluorescence immunoassay.

Results: the results were demonstrated a significant decrease in serum folic acid ($P \leq 0.05$) in both patient's groups as compared with control ($P \leq 0.05$). Serum ferritin levels increased significantly in both patient groups as compared with control. The results of conventional PCR for mtDNA damage showed mtDNA damage in β-TM compared to control.

Conclusion: The folic acid deficiency and iron overload due to repeated blood transfusion in patients with β-TM may cause more mtDNA damage.

Keywords: Folic acid, mitochondrial DNA, Beta thalassemia major

Introduction

Thalassemia are a set of congenital autosomal recessive (inborn) illnesses a condition in which single or more globin subunits of hemoglobin are not synthesized properly. Four genes code for alpha globin chains, whereas two genes code for beta globin chains.

It is a public health problem worldwide. β-thalassemia is a common type thalassemia disease, that occurs when the patient has two abnormal (homozygous) genes, and no β chain is synthesized, so there is no HbA, only HbF and HbA2 form all the Hb. On the basis of ability to synthesize β-genes thalassemia are referred to as(1). β Gene – capable of producing a normal quantity of β-chain , β+ gene – can produce a smaller quantity of β-chain and β0 gene – inability to manufacture β-chain. Alpha thalassemia is caused by reduced or absent alpha globin chain synthesis, whereas beta thalassemia is caused by reduced or

absent beta globin chain synthesis. Hemolysis and erythropoiesis are affected by globin chain imbalances. People with the alpha or beta thalassemia trait and asymptomatic alpha thalassemia carriers do not require treatment. Hemolytic anemia is caused by alpha thalassemia intermedia, often known as hemoglobin H sickness.

β-thalassemia causes hemolytic anemia, poor development, and skeletal abnormalities in children. Affected children will require regular blood transfusions for the remainder of their lives. β-thalassemia intermedia is a lesser form of beta thalassemia than beta thalassemia major(β-TM), although it still requires blood transfusions on a regular basis. Iron overload will develop in transfusion-dependent individuals, necessitating chelation therapy to eliminate the excess iron. Some children because the beta chain is not produced within hemoglobin of red blood cells, it occurs in large numbers of alpha chain, causing the red cells to be destroyed, therefore, a severe anemia will occur, which is blood transfusion dependent since childhood. Bilirubin stones may form in the gall bladder as a result of the patient's chronic hemolysis. In thalassemia major, fetal hemoglobin is produced in place of adult hemoglobin(2).

Folates are a group of structurally related chemicals that are engaged associated with the transfer with one-carbon units to the creation of nucleotides utilized in DNA synthesis, as well as to methyl the number of biological targets &cell proliferation. Folates are especially crucial throughout the anabolic periods of fetal and juvenile development because of these functions. 5-Methyltetrahydrofolate (5-MTHF) is the preferred form of folate supplementation due to its the active form of folate that used by the body's tissues. 5,10Methylenetetrahydrofolate reductase(catalyzes the conversion of 5,10Methylenetetrahydrofolate to 5-Methyltetrahydrofolate (5-MTHF), a co-substrate for homocysteine remethylation to methionine(3). Folate deficiency is commonly indicated in β-thalassemia. Hyperhomocysteinemia (Hhcy) has been defined as a risk factor for these complications. (4). Number polymorphisms of factors encoding for enzymes acting in the remethylation pathway of homocysteine metabolism MTHFR have been shown to cause hyperhomocysteinemia (Hhcy) particularly in patients with deficiency of folate(5). This can result in an increase in hcy, which works as a prooxidant, creates oxidative stress by auto-oxidation, increases lipid peroxidation, lowers endothelial NO, and damages endothelial cells. Hhcy is thought to play a role in the aetiopathogensis of a number of diseases, the most serious of which being cardiovascular and peripheral vascular diseases(6).

Unpaired globin chains and excessive cellular iron concentrations in β-thalassemia patients may cause oxidative damage to red blood cells, resulting in lower blood cell survival. While iron is required for metabolic functions, too much of it can result in the creation of free radicals.

In β-TM, repeated blood transfers create a buildup with heavy iron within human tissues. Peroxidative damage is caused by an increase in the generation of reactive oxygen species inside to red cells, result in oxidative stress(OS).Consequently, growth retardation as well as liver, cardiovascular, endocrine, and neurological problems(10).

Materials and methods

Subject

Eighty patients with β-TM (n=80) with mean age (8.5± 0.39; 48 males, 32 female), were involved in this study whose and divided into two main groups: β-TM patients group without folic acid administration (n=45) and the other one

include β-TM patients who have been administrated folic acid (n=35). 40 healthy subjects as control with mean age (6.93±0.37; 15 males, 20 female). who visiting hospital for routine check-up without any history of hematological diseases, with no chronic diseases, acute illness or infection.

All subjects were enrolled in this study between October 2021 to January 2022, they signed a written informed consent form. All study methods were approved by the Ethical Committee of Babil Teaching Hospital of Maternity and Children and the University of Al-Qadisiyah Al-Diwaniyah, Iraq). Diagnosis of participants are executed by medical senior on the basis clinical characteristic, history of patients, and biochemical tests. Ages, genders, family history and BMI were recorded.

Methods

About five ml blood were collection from each participant in this study through venipuncture. 1 ml of blood was then immediately put in dipotassium-EDTA Vacutainer tubes for CBC and mtDNA damage assessment. Complete blood counts (CBC) ratio analysis was performed directly in Hematology Analyser (spinreat ,Spain) technique, 4 ml after collection the blood was left for 15 minutes stable at room temperature. The blood samples were discrete by centrifuge at 11000 rpm for 5 minutes. Then, the serum transferred into Eppendorf tube (1.5 ml). labeled and stored at (-80) ºC until analyzed. Conventional PCR was applied for measurement the concentration mtDNA damage. Serum levels folic acid was analyzed by immunochromatographic assay while an enzyme-linked fluorescence immunoassay was used for ferritin measurements.

Statistical analysis

Data are expressed as means ± standard error of the mean (SEM). Statistical analysis was carried out using SPSS Statistics 23. The Andersen-Darling test was used to check for normality ($P \leq 0.05$). In order to explore significant differences between control and patient groups, one-way ANOVA was carried out as appropriate following by post hoc analysis using Tukey's test, in order to understand the main effects. A P value ≤ 0.05 was considered significant throughout.

Results

The clinical and biochemical assessment.

The clinical and biochemical variables of patients were compared with control as shown in (Table 3.2). There were no significant changes were observed in BMI ($P \geq 0.05$) in patient groups compared to control.

Serum Ferritin was increased in both patient groups($P \leq 0.05$) compared to control (Table 3.1).

The number of red blood cells(RBCs), Hb, PCV, and mean cell volume (MCV) were significantly decreased in the blood of patient groups compared to control ($P \leq 0.05$). (Table 3.1).

Table 3.1: Comparison of the biochemical parameters in study groups.

Parameters Mean ± SEM	Groups			
	β-TM			
	β-TM	β-TM +FA	Control	*P*-value
BMI(Kg)	**13.0± 0.6**	**12.5 ± 0.5**	**15.1±0.48**	***P*>0.05**
Ferritin ng/ml	**2543.4±306.5***	**1988.3±208.2***	**81.6±7.5**	***P*≤0.05**

Red blood cells (10^{12}/L)	**2.9±0.09***	**3.0±0.12***	**4.3±0.05**	***P*≤0.05**
Hb (g/dl)	**7.7±0.2***	**8.0±0.2***	**12.3±0.1**	***P*≤0.05**
PCV%	**22.8±0.6***	**24.1±0.8***	**38.0±0.39**	***P*≤0.05**
MCV (fl)	**72.2±1.1***	**75.7±1.4***	**83.5±0.36**	***P*≤0.05**

* indicates significant difference compared to the control.

Serum Folic acid levels in patients with β-TM.

A significantly decrease was observed in the serum level of FA in β-TM compared to control group ((*P*≤0.01). The lower serum level FA was indicated in patients without folic acid(*P*≤0.01) (Figure 1).

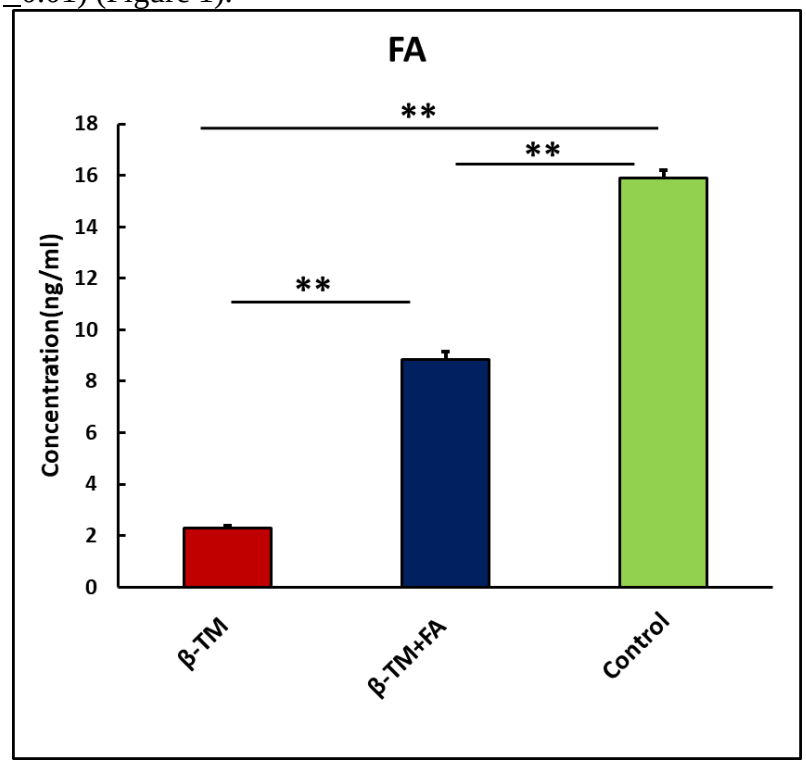

Figure 1: Serum FA levels in patients with β-TM, β-TM +FA, and control groups. The data are expressed as means ± SEM, ** indicates significant differences (*P*≤ 0.01)between study groups.

Mitochondrial DNA damage in patients with β-TM.

The present study has suggested that mtDNA damage might associate with of β-TM pathology . PCR was used to determine the level of mtDNA damage. A mtDNA genes that targeted in this study were D-long, mtDNA-long, region. For long amplification product, respectively, the PCR products were separated on 1%Agarose gels. The relative amplification ratio was determined by normalizing the intensity of the long PCR product a decrease in the amplification indicated increased DNA damage**.** The mtDNA concentration was decreased in both patient groups (*P*≤0.01)

Table 3.5 DNA concentration in patient and control subjects

Groups	DNA(ng)	P-value
β-TM	28.51 ± 2.9	P≤0.01
FA+β-TM	41.86 ±5.0	P≤0.01
Control	68.98±7.9	

In this study, mtDNA content was differ significantly in patients with β-TM compared to control (figure 2.and 3).However, no fragments were observed in DNA bands.

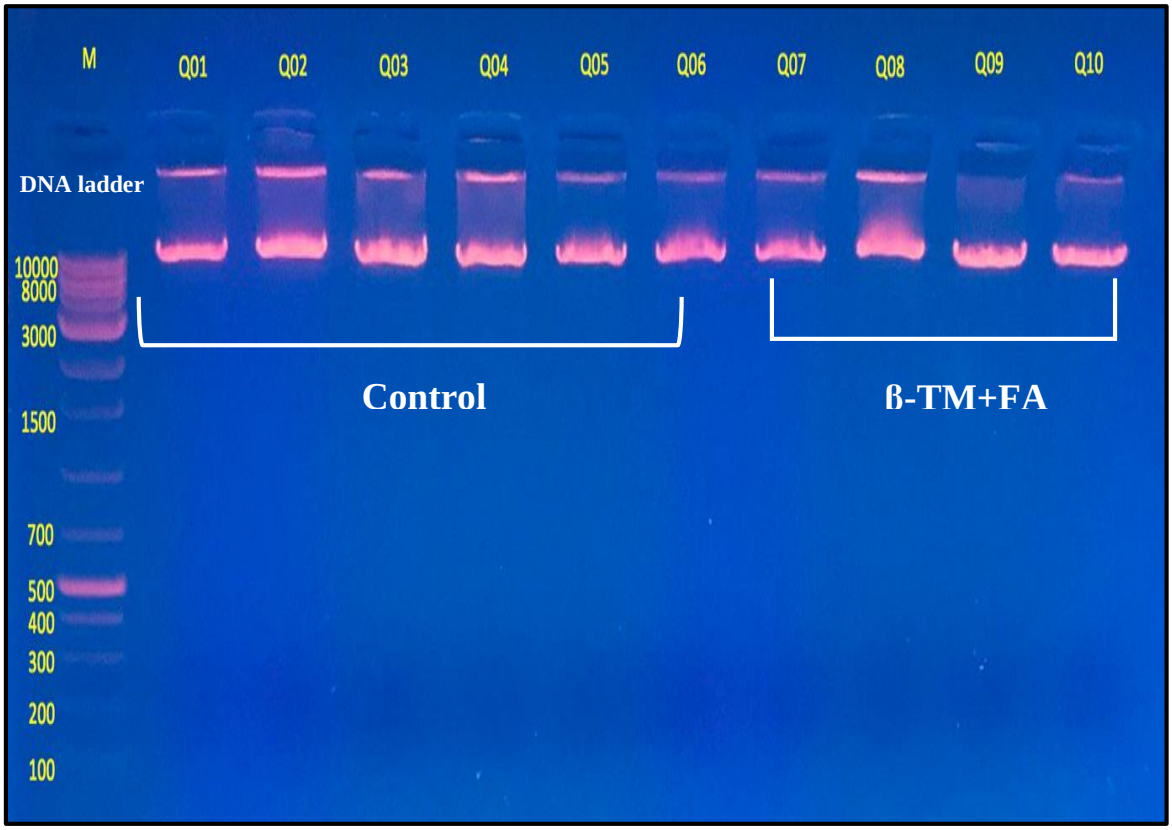

Figure 2. PCR band of mtDNA- long region. M=DNA ladder. PCR bands of mtDNA-long region in β-TM patients and control in 1% agarose gel

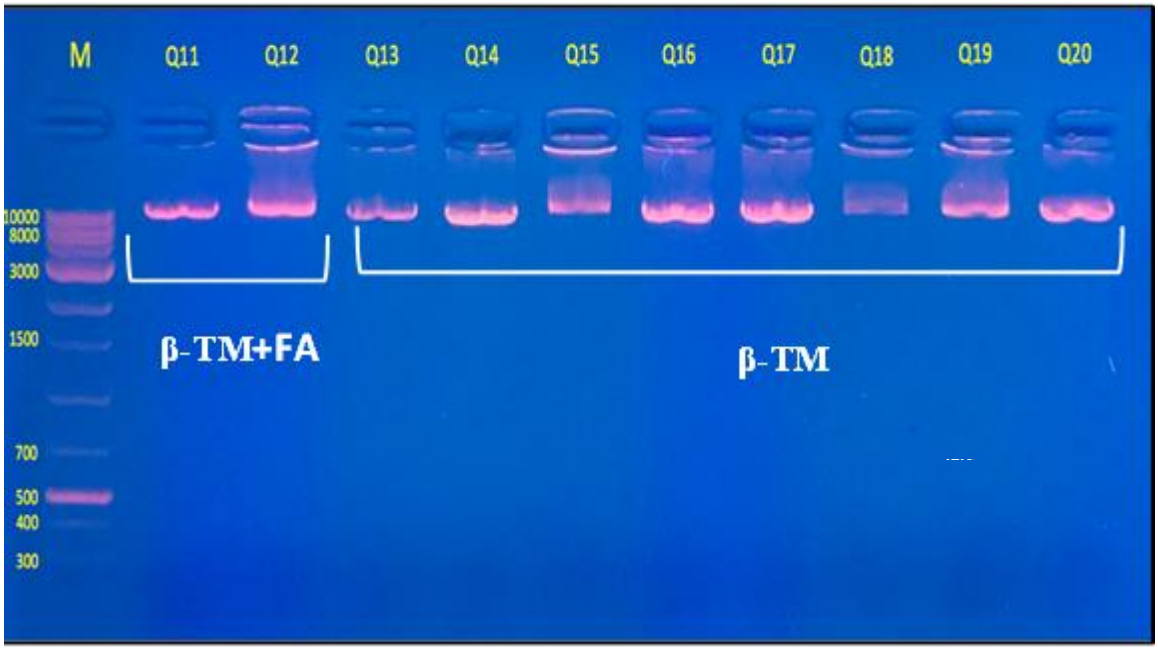

Figure 3. PCR band of mtDNA- long region. M=DNA ladder. PCR bands of mtDNA-long region in β-TM patients with and without FA in 1% agarose gel

Discussion

The recently studies was reported that the number of red blood cells is dimension in β-TM due to hemolysis (141). The results showed a significant decrease in erythrocyte counts in β-TM and β-TM+FA compared to control ($P \leq 0.05$). The hemolytic of red blood cells in β-TM it is cause by absence of β globin chain in the hemoglobin molecules and leads to high accumulation of α globin chain which considerable part of hemoglobin molecules which precipitation on wall of red cells and cause Lysis(142), (143). The presence of excessive destruction in red cells which driving to out of hemoglobin from inside cells to blood circulation and that result defect in normal function of red blood cells for transmission of oxygen to any parts in body so as that leads to hypoxia lower reach oxygen to vital organs(144). Hemoglobin&hematocrit and mean cell volume was significantly decrease in β-TM and β-TM+FA compared to control ($P \leq 0.05$). The hemolytic of red blood cells in β-TM compared to control ($P \leq 0.05$). HB, HCT and MCV is well correlated with diseases that causes hemolytic anemia such as hemoglobinopathies β-TM which considerable genetic disorder that transmission from parents to children(151). The reason for the lower concentration of hemoglobin and percentage of hematocrit and mean corpuscular volume returns to the hereditary mutations that happen in the genes responsible for the synthesis of protein chains in Hb which drive to disorder in biosynthesis of globins chain (145). and the loss imbalance in the manufacture of hemoglobin and this effected the count and shape and size of erythrocytes during the formation stages in the bone marrow, which make the red cells microcytic hypochromic and collect the globin chains in put red cells to cause formation inclusion bodies that are prone phagocytosis by macrophages which appearance numerously in bone marrow and destruction the red cells during the mature stages in the blood circulation or through to pass across spleen by macrophages so the patients of severity beta thalassemia lead to splenectomy for the dimension the number of broken red blood cells(152).

Serum ferritin in β-TM patients.

The serum ferritin is the major iron storage molecule of humans, which can be detected in serum under number of cases, comprising inflammatory, neurodegenerative, hemolytic anemia, malignant diseases, and blood transfusion diseases(140). Several studies showed a relationship between the elevated serum levels of ferritin and β-TM like (142) (143) (144). Mechanism elevation serum levels ferritin and iron overload in our study in beta thalassemia major patients it is caused by multiple blood transfusions, ineffective erythropoiesis and increase gastrointestinal iron absorption according to these causes that will be leads to iron overload in the body. Iron overload impairs the immune system, make the patients at greater risk of infection and illness. So the regulation the concentration of ferritin in the body through hormone is name Hepcidin it is product in the liver, regulates iron hemostasis in the body depended on hypoxia, anemia, iron storage and controls the serum amount distribution tissue of iron. Ineffective erythropoiesis in β-TM alters the secretion of Hepcidin to causes excess of this hormone produce from the hepatic cells(145)(146). The result of iron accumulation in vital organs same as liver, heart, and endocrine glands that cause function deteriorates progressively. So that the patients of β-TM which depended on blood transfusion it is required for treatment continuous for removal this iron from body, the best therapy used for this condition is desferrioxamine as a chelator in the management of thalassemia patients with acute or chronic iron toxicity this DFO is poorly absorption from intestinal in case take orally, for this reason it must be given intramuscular, subcutaneously, or intravenous(147)(148).

Folic acid in β-TM patients.

low levels of FA in β-TM patients is indicated a MTHFR deficiency which leads to a decrease of 5-MTHF and increased serum level of homocysteine, This can result in elevated homocysteine which acts as prooxidant, generates free radicals by auto-oxidation, induces lipid peroxidation(12), decreases endothelial NO and causes endothelial cell damage(13). homocysteine has been claimed to have a part in the aetiopathogensis of several disorders including most importantly cardiovascular and peripheral vascular diseases(14). There is some evidence to support the assumption that homocysteine can be toxic for endothelium, and that this is mediated by free radicals generated during the oxidation of homocysteine. Moreover, high levels of homocysteine may also promote thrombosis due to an increase generation of thrombin(15). Prevent of folic acid supplementations in β-TM patients can lead to a significant decrease in serum folic acid and increase in homocysteine levels. According to our findings effects folic acid in patients with β-TM, it is recommended to use the folic acid supplementation thalassemia (16).

mtDNA damage in patients with β-TM.

In individuals with β-TM, not paired globin chains & high concentration cellular iron which promote oxidative damage to erythrocytes with corresponding little survival in circulation of blood. Although iron it is important role for metabolic process but excessive iron can lead to the production the free radicals(26). Recurrent the transfusion of blood in β-TM driving to accumulation of overflowing iron in the tissues body. This second iron overload is responsible to peroxidative damage by increase produce the ROS (reactive oxygen species) within the red cells, command to oxidative stress(27). This

oxidative stress will cause failure to vital organ growth such as endocrine, cardiovascular, liver, and neurological complications in β-TM patients. It is clear from previous studies that iron overload is the main causative agent accountable for raise production the free radical and ROS and subsequent oxidative stress, which compensated by number antioxidants present in the body(28). Oxidative stress is defined as the interruption of balance among them oxidants & reductants within the body due to the excessive make of free radicals and peroxides. This the defect in balance will cause damage in mtDNA, other cellular components and tissue in the body, progression to oxidative stress(29).

Conclusion

The present results, suggest a link between folic acid deficiency and mtDNA damage. Also ferritin abnormality was indicated, therefore administration of FA may reduce the pathological events in β-TM and mtDNA damage.

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