

Article

Seroprevalence of toxoplasmosis related with uric acid in B-thalassemia major patients

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ABSTRACT

Laboratory experiments were performed to study 66 samples were collected for beta-thalassemia patients, with 30 samples for control of healthy people, male and female, and 96 samples were examined in the Cobas e411 Advice for both IgG and IgM, 20 samples were obtained positive for IgG in patients with beta-thalassemia, and one sample was positive for IgM, then the Uric Acid examination was conducted for all. After that, 12 male and female samples were selected with a high percentage of Uric Acid, 6 of which were positive for Toxoplasmosis and 6 negatives for the disease. To perform the Performance Liquid Chromatography (HPLC) technique and use the standard substance allantoin to observe the relationship between Uric Acid and Allantoin, where the relationship is inverse between them; in the case of infection and the presence of the parasite in the blood, the Uric Acid oxidizes and combines with the free radicals (reactive oxygen species) in the blood such as oxygen and hydrogen peroxide that are harmful to the cell or tissue. Free radicals in abundance and as a guide to the extent of damage performed by the parasite.

Keywords: beta-thalassemia, Uric Acid, Allantoin, Toxoplasmosis, IgG and IgM.

INTRODUCTION

Only the asexual stages of *Toxoplasma gondii* (tachyzoites or trophozoites, bradyzoites or cystozoites) were known until 1970, when the sexual cycle and the environmentally resistant stage, the oocyst, were identified ¹. Even though disease rates differ significantly among countries, serological studies estimate that up to 33% of the global population has been exposed to *T. gondii* and may be permanently infected ².

Toxoplasma gondii is transmitted mostly through the eating of degraded raw/half-cooked meat with tissue blisters, as well as through the ingestion of tainted vegetables/water with oocysts ³. Patients with -thalassemia major who receive blood transfusions regularly are at risk of contracting a variety of transplant-transmitted diseases, including Toxoplasmosis, a common and serious parasitic disease with a high prevalence that can be transmitted through blood transfusions from healthy asymptomatic donors ⁴.

Thalassemia is an autosomal recessive condition that causes severe anemia due to a lack of normal hemoglobin production⁵. The problem of transfusion-transmitted infection, which is proportional to the prevalence of infection in the blood donor, may have a role increase in mortality and morbidity rate among thalassemia patients such as Toxoplasmosis caused by *T. gondii* is an obligate intracellular protozoan parasite⁶.

Macrophages are capable of producing vast quantities of highly hazardous chemicals like reactive oxygen species (ROS) and include peroxide (O₂-) radicals, hydroxyl (OH) and hydrogen peroxide (H₂O₂) RNS (reactive nitrogen species), radicals and universal oxide nitrate (NO)⁷.

Macrophages are stimulated by parasites that synthesize a significant amount of nitric oxide (NO). These steroids have cytotoxic effects, as Reactive oxygen species (ROS) and RNS (Reactive nitrogen species) can deconstruct many biological molecules such as DNA, carbohydrates, and proteins are all included. Moreover, the types of oxygen and active nitrogen ROS and RNS can damage membrane lipids' polyunsaturated fatty acids that cause peroxidation of lipids and disrupt cell building and functions⁸.

Thalassemia is a term that alludes to a collection of hereditary blood maladies portrayed by anemia because of enriched red platelet obliteration, hemoglobin, the oxygen-conveying segment of the red platelets comprised of tetrad discrete protein chains, 2 α globin and 2 β globin, tetrad genes are entailed to produce ample alpha globin protein chains while duplet genes (one from both progenitors) are required to form adequate β globin protein chains, the two prominent categories of thalassemia α and β , are referred to in the wake of flaw in these protein chains^{9,10}.

MATERIALS AND METHODS

The Study's Experimental Design

The study's overall processes are summarized in Figure 1

Collection of samples :

Ninety-six blood samples were taken from Babel Hospital for Women and Children in Al-Hilla City, Babylon province. Their age groups ranged from 10 to 25 years. Five (5) milliliters of blood were drawn from each patient before receiving a transfusion quantity of healthy people (control) to compare them, and the samples were divided into two parts as follows:-

1. Fill an EDTA tube with 2 mL of blood for a blood test for hematological assay.
2. Place 3 mL of blood in a gel tube for serological and biochemical tests¹¹ to get the serum.

Collection of blood samples

Before obtaining a blood sample, all men and women must provide some information

questionnaire sheet :

Took five milliliters of blood for Beta-thalassemia patients, including men and women.

For serological and biochemical testing, use a syringe with five milliliters as three milliliters.

Three milliliters of blood were placed in a gel tube and allowed to clot for 20 minutes at room temperature before being centrifuged at 3000 rpm for five minutes to extract serum.

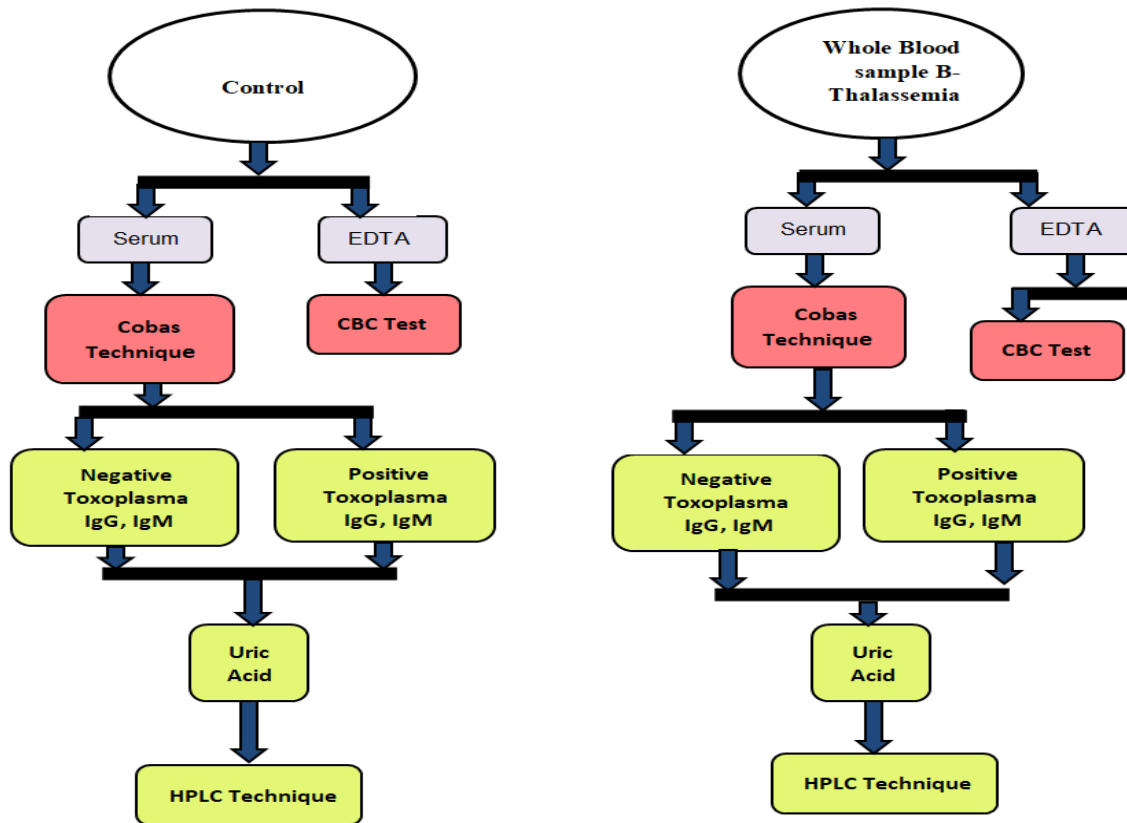


Figure 1. Experiment design of the study

To avoid melting the samples and repeating the freeze, the serum obtained was separated into multiple pieces for different serological tests until it did not impair the quality of the result.

All sera were kept in the deep freezing -20°C until being analyzed Toxoplasma antibodies.

The antecubital vein was selected to draw the blood sample by sterile syringe ¹².

Hematological Assay

The blood test includes the following tests (red blood cell count, hemoglobin, PCV agglutinated corpuscle volume, MCV corpuscular volume average, MCH corpuscular hemoglobin average, MCHC concentration of corpuscular hemoglobin, percentage of erythrocyte width of distribution RDW, number Cells of the white blood (WBCs) and platelets (PLT) were examined using. Humacount Auto Analyzer, after adding (1) ml of blood into an EDTA tube and placing the tube in the specified place on the device, then gave the start instruction, as the device reads the results automatically and when it appears. The results have been given a directive (print) for the device to print it, according to what the German company Human brought Origin ¹³.

Complete Blood Counts(CBC).

The hematological diagnosis shows anemia due to microcytic anemia. Mediterranean anemia is characterized by a decrease in both the mean MCV and MCV levels of corpuscular hemoglobin (MCH), as hemoglobin level (5-10) gm/dl, MCV level (60-90) fl, and hemoglobin level MCH (19-29) pg. ¹⁴.

Cobas e 411 analyzer

The assay was carried out with the help of two kits, one for detecting IgG antibodies and the other for detecting IgM-specific antibodies against Toxoplasma antigen in the patient's serum.

Cobas e 411 Technique for *T. gondii* Antibody (IgG) Detection

1. The detector Toxoplasma IgG, Toxoplasma IgM and Diluted is selected inside the device.
2. Put 1 ml of the serum in the sample cup and then put it in a designated place in the device.
3. The device takes 20 microliters of sample volume.
4. The device is programmed and left for 20 minutes for each sample.
5. The readings are recorded on the device screen and read with a unit of Toxoplasma IgG (Iu/ml) and Toxoplasma IgM (CoI) ¹⁵.

Preparation of Allantoin from Uric acid by oxidation as (standard material) Procedure depends on ^{16, 17, 18} :

A 12-liter round-bottomed flask with a mechanical stirrer is filled with 100 grams of uric acid (0.595 moles) and 4.5 liters of hot water (70–85°). A solution of 80 gm (2 moles) commercial sodium hydroxide in 120 ml of water is added to the stirrer. Continue stirring until the uric acid is dissolved.

After that, a stream of water is directed against the flask to chill the solution. When the temperature drops to 25–30 C°

to the vigorously agitated solution, 50 gm (0.32 mole) potassium permanganate is added simultaneously. Continue stirring for another 15 to 20 minutes.

A 19-cm Buchner funnel is used to filter the mixture all at once. A minor amount of manganese dioxide is present in the initial part of the filtrate. This fraction must be collected and returned to the funnel individually.

6. The filtrate is collected in a 12-l round-bottomed flask containing 130 cc. (137 gm, 2.2 moles) of glacial acetic acid as soon as it becomes clear. To ensure that the filtrate is acid, it is litmus tested and evaporated to a volume of 1.5–2 liters on a steam bath under reduced pressure (20–30 mm.).

7. The resulting solution is kept cool overnight, and the allantoin that crystallizes is filtered through a 9-cm filter funnel Buchner.

8. Allantoin is dissolved in 800–900 mL boiling water, treated with 5-gram Norite, and filtered quickly through fluted filter paper in a steam funnel. Allow the filtrate to sit in a cool area overnight; Chapter Two Materials and Methods 63 Suction filtration separates the white crystals of allantoin.

9. At 230–231°C, the yield of the product melts to 60–71 grams (64–75%) of the theoretical amount. If the purification fluid filtrate is concentrated to 100 mL, an extra 3–5 grams of allantoin is produced. (and then tested some grams of allantoin in a Melting point apparatus(SMP30) to ensure that this material's allantoin melted at 230–231 C°.

10. By FTIR-8400S (Fourier Transform Infrared Spectrophotometer), the sample was and then by this device and from peaks revealed that this sample returned to allantoin, UV visible of uric acid it is equal 294.46 nm and λ_{\max} = 380 nm). At the same time, the UV is visible at 175-800 nm and λ_{\max} = 299.01 nm ^{16, 17, 18}.

The concentration of allantoin in serum patients and control groups was determined using a High-Performance Liquid Chromatography method (HPLC).

Preparation of samples

A 100 mL serum sample was combined with 400 mL solvent C (KH₂PO₄/H₃PO₄-buffer with 50 mmol/l phosphate, pH 4.60) and filtered

through a 0.22 m membrane filter (Germany). An aliquot (50 mL) of the filtrate was directly injected into the HPLC injector, and the quantification was done using the peak areas determined for wavelengths ^{19, 20, and 21}.

Preparation of standard solutions

Using the dilution law, 2 mg of each standard was placed in a volumetric flask (25 ml), and the volume was supplemented with methanol (HPLC 99.9%) until the stock solution concentration (80 ppm) was reached. The quantities introduced into the HPLC were prepared as $C_1 V_1 = C_2 V_2$ ^{22, 23}.

HPLC Condition

HPLC model SYKAMN (High-Performance Liquid Chromatography) (Germany). The mobile phase was isocratic acetonitrile–0.1M phosphate buffer containing 0.5 percent glacial acetic acid (30: 70) at a flow rate of 1.2 mL/min, the column was C18 – ODS (25 cm * 4.6 mm), and the detector was UV–360 nm ^{24, 25}.

Statistical Analysis

Data was analyzed using SPSS(version 23, SPSS Inc. Chicago, Illinois, USA). Descriptive statistics One-way ANOVA was used to compare differences (mean, standard error). It was also carried out using the student test for comparing the two groups, followed by chi-square. The value of $p \leq 0.05$ was thought to have statistical significance. The relationship between studied parameters was determined by Pearson's correlation coefficient (r).

RESULTS

According to the genetic factors(father, mother, father and mother), the highest rate in males (87.5%) and 12.5%) in patients with Toxoplasma, and the highest rate in father and mother (9.09%) in patients without Toxoplasma, whereas the highest rate in females, in father (75%), and in mother (16.67%) in a patient with Toxoplasma, and the highest rate in father and mother (29.17%) in patients without Toxoplasma, from the above there is a significant difference between the genetic factors in the patients without Toxoplasma (Yousefi et al., 2017).

According to the blood, transport shows the highest rate in males, in one every month (45.45%) in patients without Toxoplasma, in two every month (62.5%) in patients with Toxoplasma, and in four every month (4.55%) in patients without Toxoplasma. In contrast, in females the percentage rate of one every month (58.33%) equal percentage in patients with and without Toxoplasma, and the percentage rate of two every month (41.67%) equal percentage in patients with and without Toxoplasma, and no rate of four every month (0%) in patients with and without Toxoplasma, from the above there is a significant difference between the blood transport in the patients with Toxoplasma (Saleh and Al-Numan, 2019).

Beta-thalassemia patients with Toxoplasma in males, there are decrease level in RBCs($\times 10^6/\text{mm}^3$), Hb(g/dl), PCV(%), MCV(fl), MCH(pg), MCHC(g/dl), show (3.25 \pm 0.4), (7.8 \pm 1.1), (0.45 \pm 0.01), (77.7 \pm 6.5), (25.2 \pm 2.4), (32.4 \pm 1.1) respectively, whereas increase level of the following WBCs($\times 10^3/\text{mm}^3$), PLT($\times 10^3/\text{mm}^3$), RDW(%), show (7.79 \pm 0.6), (366.0 \pm 114.5), (15.7 \pm 5.4) respectively compared to the control (Hamza et al., 2020).

| Groups | B-thalassemia patients with <i>Toxoplasma</i> | | B-thalassemia patients without <i>Toxoplasma</i> | |
|--------------------------|---|---------------|--|---------------|
| | Male (male=8) | Female (n=12) | Male (n=22) | Female (n=24) |
| Characteristics | | | | |
| Resident area | | | | |
| Urban | 2(25%) | 8(66.67%) | 8(36.36%) | 11(45.83%) |
| Rural | 6(75%) | 4(33.33%) | 14(63.64%) | 13(54.17%) |
| Total | 8 | 12 | 22 | 24 |
| p-value | 0.068 | | 0.515 | |
| Age (years) | | | | |
| 10-15 | 4(50%) | 6(50%) | 9(40.91%) | 14(58.33%) |
| 16-20 | 2(25%) | 4(33.33%) | 11(50%) | 7(29.17%) |
| 21-25 | 2(25%) | 2(16.67%) | 2(9.09%) | 3(12.5%) |
| Total | 8 | 12 | 22 | 24 |
| p-value | 0.479 | | 0.033* | |
| Cats presence | | | | |
| Yes | 1(12.5%) | 2(16.67%) | 2(9.09%) | 1(4.17%) |
| No | 7(87.5%) | 10(83.33%) | 20(90.91%) | 23(95.83%) |
| Total | 8 | 12 | 22 | 24 |
| p-value | 0.021* | | 0.023* | |
| Genetics | | | | |
| Father | 7(87.5%) | 9(75%) | 18(81.82%) | 14(58.33%) |
| Mother | 1(12.5%) | 2(16.67%) | 2(9.09%) | 3(12.5%) |
| Father and mother | 0(0%) | 1(8.33%) | 2(9.09%) | 7(29.17%) |
| Total | 8 | 12 | 22 | 24 |
| p-value | 0.816 | | 0.031* | |
| Blood transport | | | | |
| One every month | 3(37.5%) | 7(58.33%) | 10(45.45%) | 14(58.33%) |
| Two every month | 5(62.5%) | 5(41.67%) | 11(50%) | 10(41.67%) |
| Four every month | 0 | 0(0%) | 1(4.55%) | 0(0%) |
| Total area | 8 | 12 | 22 | 24 |
| p-value | 0.041* | | 0.311 | |

Table 1. Epidemiological parameter Percentage rate Infection of Prevalence of Toxoplasmosis with two groups (Beta Thalassemia Major patients with *Toxoplasma* and without *Toxoplasma*).

| Groups | B-thalassemia patients with <i>Toxoplasma</i> | | B-thalassemia patients without <i>Toxoplasma</i> | | Control | | LSD (0.05) (group*Gender) |
|-----------------------------------|---|-------------|--|------------|-----------|------------|------------------------------|
| | male | Female | male | Female | male | Female | |
| | Mean±S.E | | | | | | |
| WBCs($\times 10^3/\text{mm}^3$) | 7.79±0.6 | 10.33±1.0 | 21.73±2.6 | 15.36±1.3 | 7.25±1.8 | 8.01±3.1 | 5.351 |
| RBCs($\times 10^6/\text{m}^3$) | 3.25±0.4 | 3.22±0.5 | 3.16±0.3 | 3.24±0.2 | 5.69±0.2 | 4.92±0.1 | 0.157 |
| PLT($\times 10^3/\text{m}^3$) | 366.0±114.5 | 371.68±21.2 | 459.8±31.1 | 495.5±26.7 | 318±22.4 | 331.2±12.0 | 79.121 |
| Hb (g/dl) | 7.8±1.1 | 8.2±1.1 | 7.9±0.7 | 8.1±0.7 | 15.03±0.5 | 13.04±0.2 | 0.367 |
| PCV (%) | 0.45±0.01 | 0.39±0.01 | 24.8±2.3 | 25.2±3.1 | 25.1±2.9 | 25.6±2.3 | 0.822 |
| MCV(fl) | 77.7±6.5 | 78.6±2.9 | 79.46±3.2 | 80.0±3.3 | 79.7±4.1 | 81.08±2.1 | 2.342 |
| MCH(pg) | 25.2±2.4 | 25.6±1.4 | 24.9±1.3 | 25.0±1.5 | 26.76±1.2 | 26.92±0.7 | 0.888 |
| MCHC(g/dl) | 32.4±1.1 | 32.7±1.8 | 31.4±1.6 | 31.3±1.6 | 33.60±0.3 | 33.02±0.3 | 0.562 |
| RDW(%) | 15.7±5.4 | 13.13±0.5 | 14.1±3.3 | 13.5±2.6 | 12.93±0.7 | 12.4±1.2 | 1.076 |

Table 2. Hematological parameter (Mean, standard error) Infection of Prevalence of Toxoplasmosis with two groups (Beta Thalassemia Major patients with *Toxoplasma* and without *Toxoplasma*).

Beta-thalassemia patients with *Toxoplasma* in females, there are decrease level in RBCs($\times 10^6/\text{mm}^3$), Hb(g/dl), PCV(%), MCV(fl), MCH(pg), MCHC(g/dl), show (3.22±0.5), (8.2±1.1), (0.39±0.01), (78.6±2.9), (25.6±1.4), (32.7±1.8) respectively, whereas increase level of the following WBCs($\times 10^3/\text{mm}^3$), PLT($\times 10^3/\text{mm}^3$), RDW(%), show (10.33±1.0), (371.68±21.2), (13.13±0.5) respectively compared to the control (Getso et al., 2021).

Beta-thalassemia patients without *Toxoplasma* in males, there is a decreased level in RBCs($\times 10^6/\text{mm}^3$), Hb(g/dl), PCV(%), MCV(fl), MCH(pg), MCHC(g/dl), show (3.16±0.3), (7.9±0.7), (24.8±2.3), (79.46±3.2), (24.9±1.3), (31.4±1.6) respectively, whereas increase level of the following WBCs($\times 10^3/\text{mm}^3$), PLT($\times 10^3/\text{mm}^3$), RDW(%), show (21.73±2.6), (459.8±31.1), (14.1±3.3) respectively compared to the control (Ehsan et al., 2020).

Beta-thalassemia patients without *Toxoplasma* in females, there is a decreased level in RBCs($\times 10^6/\text{mm}^3$), Hb(g/dl), PCV(%), MCV(fl), MCH(pg), MCHC(g/dl), show (3.24 ± 0.2) , (8.1 ± 0.7) , (25.2 ± 3.1) , (80.0 ± 3.3) , (25.0 ± 1.5) , (31.3 ± 1.6) respectively, whereas increase level of the following WBCs($\times 10^3/\text{mm}^3$), PLT($\times 10^3/\text{mm}^3$), RDW(%), show (15.36 ± 1.3) , (495.5 ± 26.7) , (13.5 ± 2.6) respectively compared to the control table 2 (Roussou et al., 2013).

| Groups | B-thalassemia patients with <i>Toxoplasma</i> | | B-thalassemia patients without <i>Toxoplasma</i> | |
|-----------------|---|-----------|--|-----------|
| | male | Female | male | Female |
| Characteristics | | | | |
| Blood groups | | | | |
| A ⁺ | 1(12.5%) | 2(16.67%) | 2(9.09%) | 4(16.67%) |
| B ⁺ | 2(25%) | 3(25%) | 4(18.18%) | 8(33.33%) |
| A ⁻ | 0(0%) | 2(16.67%) | 1(4.55%) | 0(0%) |
| AB ⁺ | 1(12.5%) | 0(0%) | 8(36.36%) | 4(16.67%) |
| O ⁻ | 4(50%) | 5(41.67%) | 6(27.27%) | 7(29.17%) |
| O ⁺ | 0(0%) | 0(0%) | 1(4.55%) | 1(4.17%) |
| Total | 8 | 12 | 22 | 24 |
| p-value | 0.564 | | | 0.641 |

Table 3. Hematological parameter of Blood groups percentage rate Infection of Prevalence of Toxoplasmosis with two groups (Beta Thalassemia Major patients with *Toxoplasma* and without *Toxoplasma*).

According to blood groups (A⁺, A⁻, B⁺, AB⁺, O⁺, O⁻) show the highest rate in males, in A⁺ (12.5%), in B⁺ (25%), in O⁻ (50%) in thalassemia patients with *Toxoplasma*, whereas the highest rate in A⁻ (4.55%), in AB⁺ (36.36%), in O⁺ (4.55%) in thalassemia patients without *Toxoplasma*, and in females the highest rate in B⁺ (33.33%), in AB⁺ (16.67%), in O⁺ (4.17%) in thalassemia patients without *Toxoplasma*, whereas in A⁻ (16.67%), in O⁻ (41.67%) in thalassemia patients with *Toxoplasma*, and equal percentage in both A⁺ (16.67%) in thalassemia patients with and without *Toxoplasma*, from the above there is no significant difference between blood groups (Laghari et al., 2018).

| Groups | B-thalassemia patients with Toxoplasma | | B-thalassemia patients without Toxoplasma | | Control | | LSD (0.05) (group*Gender) |
|-------------------|--|-----------|---|-----------|----------|----------|------------------------------|
| | male | Female | male | Female | male | Female | |
| | Mean±S.E | | | | | | |
| Uric acid (mg/dl) | 5.10±0.3 | 4.66±0.2 | 5.36±0.6 | 3.76±0.3 | 4.33±0.3 | 3.47±0.2 | 0.571 |
| Allantoin (ppm) | 56.39±1.9 | 59.52±1.6 | 39.78±1.3 | 37.53±1.2 | 3.08±0.4 | 1.97±0.3 | 0.685 |

Table 4. Biochemical parameter(Mean, standard error) Infection of Prevalence of Toxoplasmosis with two groups (Beta Thalassemia Major patients with *Toxoplasma* and without *Toxoplasma*) that related with Uric acid and Allantoin concentration.

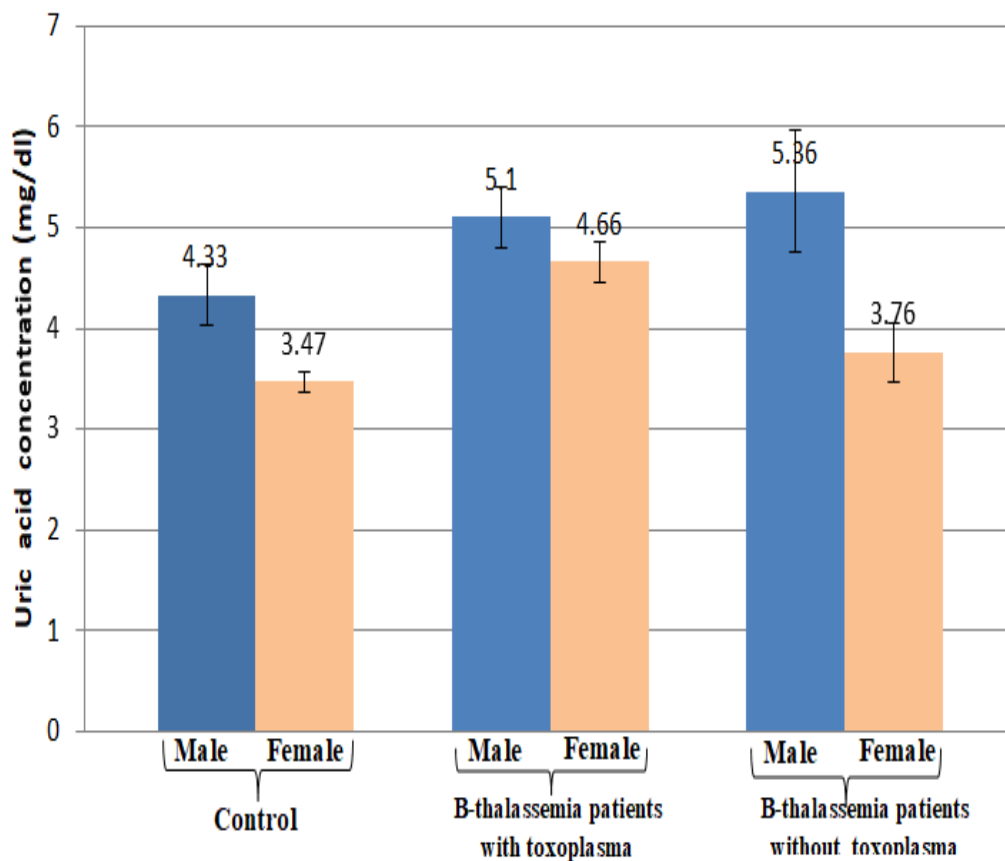


Figure 2. The uric acid concentration in two groups of thalassemia patients

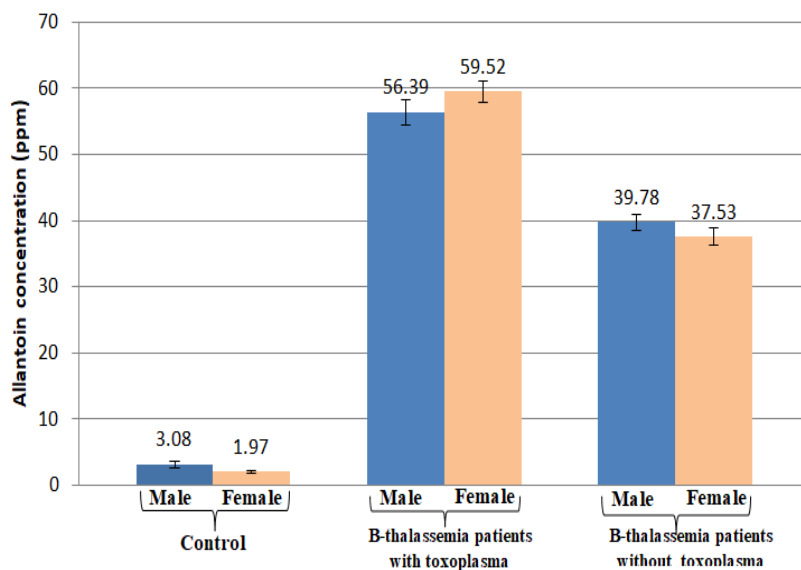


Figure 3. Allantoin concentration in two groups of thalassemia patients

Table 4 shows the concentration of Uric acid and allantoin as oxidative stress related with Beta-thalassemia patients infected with Toxoplasma and Beta-thalassemia patients noninfected with Toxoplasma, revealing the highest concentration (5.10 ± 0.3) mg/dl of Uric acid in males compared with females (4.66 ± 0.2) mg/dl, in the groups of Beta-thalassemia patients infected with Toxoplasma. In contrast, the highest concentration shown in males (5.36 ± 0.6) mg/dl compared with females (3.76 ± 0.3) mg/dl in Beta-thalassemia patients without Toxoplasma Figure 2 (Kontomanolis et al., 2014).

The Allantoin concentration reveals the highest concentration compared with Uric acid, which shows the highest concentration in females (59.52 ± 1.6) ppm compared with males (56.39 ± 1.9) ppm in Beta-thalassemia patients with Toxoplasma. In contrast, the concentration of Allantoin in Beta-thalassemia patients without Toxoplasma reveals the highest concentration in males (39.78 ± 1.3) ppm compared with females (37.53 ± 1.2) ppm Figure 2 (Elad et al., 2008).

| Patients gender | IgG | NO.(%) | IgM | NO.(%) | (IgG and IgM) | NO.(%) |
|-----------------|--------|--------|-----|--------|---------------|--------|
| Male | 8 | 40 | 0 | 0 | 1 | 5 |
| Female | 12 | 60 | 0 | 0 | 0 | 0 |
| Total | 20 | 100 | 0 | | 1 | 5 |
| P- value | 0.2059 | | 1.0 | | 0.3167 | |

Table 5. Percentage rate of IgG, IgM and mixed (IgG and IgM) in Beta Thalassemia patients positive to Toxoplasma using Cobas e411 technique.

In Table 5, the (IgG) P- value (0.2059) the statistical analysis there is no significant difference, the (IgM) P- value(1.0) the statistical analysis there is no significant difference, the (IgG and IgM) P- value (0.3167) the statistical analysis there is no significant difference.

| Groups | B-thalassemia patients with Toxoplasma | | B-thalassemia patients without Toxoplasma | | Control | | LSD (0.05) (group*Gender) |
|-----------------|--|-----------------|---|----------------|--------------------|----------------|---------------------------|
| | male | Female | male | Female | male | Female | |
| Characteristics | Mean±S.E | | | | | | |
| IgG (Iu/ml) | 164.63±2 5.0 | 98.276± 19.4 | 0.79±0.17 | 0.62±0. 15 | 0.128 ±0.0 2 | ±0.02 0.128 | 5.612 |
| IgM (COI) | 0.458±0.0 3 | 0.300±0. 04 | 0.296±0.01 | 0.291± 0.02 | 0.31 ±0.0 1 | 0.30±0. 01 | 0.040 |

Table 6. Immunological parameters related to the concentration of IgG and IgM in two groups (Beta-thalassemia patients with *Toxoplasma* and without *Toxoplasma*) and control groups.

Results obtained with the Elecsys Toxoplasma IgG assay should be interpreted as follows, considering the respective algorithm used for screening Toxoplasma in Beta-thalassemia according to national or regional guidelines or recommendations.

Toxoplasma IgG testing is used as a first-line screening assay

A-Non-reactive: < 1 IU/ml

B-Indeterminate: ≥ 1-< 3 IU/ml

C-Reactive: ≥ 3 IU/mL

Samples with concentrations < 1 IU/ml are considered non-reactive in the Elecsys.

Toxoplasma IgG assay.

Samples with concentrations of IgG antibodies to *T. gondii* in the range of ≥ 3 IU/mL are deemed positive and suggest either acute or latent infection. To rule out early Toxoplasma infection, a Toxoplasma IgM test should be conducted on all samples with concentrations ≥ 3 IU/mL.

Samples with ≥ 3-< 30 IU/ml concentrations and a negative IgM test result: A second sample should be taken within 3 weeks to rule out early Toxoplasma infection, indicated by a considerable increase in Toxoplasma IgG antibody titer.

Samples between 1 IU/mL and < 3 IU/ml are regarded as indefinite. Retesting of the sample is required. If the result is still inconclusive, a second sample should be taken, ideally within 3 weeks (Robert and Guegan, 2021).

The following interpretation of the Elecsys Toxoplasma IgM assay results:

A. < 0.8 COI is non-reactive.

B. ≥ 0.8 -< 1.0 COI is Indeterminate

C. ≥ 1.0 COI is Reactive

In the Elecsys Toxoplasma IgM assay, samples with a cutoff index of less than 0.8 are non-reactive. Indeterminate samples have a cutoff index between ≥ 0.8 and < 1.0. Retesting of the sample is required. If the outcome is still inconclusive,

a second sample should be analyzed, ideally within two to three weeks. In the Elecsys Toxoplasma IgM assay, samples having a cutoff index ≥ 1.0 are reactive.

The Table 6 results mentioned below reveal there are three groups in both IgG and IgM; for IgG the highest concentration of IgG(Iu/ml) in males (164.63 ± 25.0) falls in reactive groups in Beta-thalassemia patients with Toxoplasma, whereas the lowest concentration of IgG(Iu/ml) in females (98.276 ± 19.4) that fall in reactive groups in Beta-thalassemia patients with Toxoplasma, and show the highest concentration of IgG(Iu/ml) in males (0.79 ± 0.17), whereas the lowest concentration of IgG(Iu/ml) in females (0.62 ± 0.15) in Beta-thalassemia patients without Toxoplasma.

The highest concentration of IgM(COI) in males (0.458 ± 0.03), whereas the lowest concentration of IgM(COI) in females (0.300 ± 0.04) in Beta-thalassemia patients with Toxoplasma, and show the highest concentration of IgM(COI) in males (0.296 ± 0.01), whereas the lowest concentration of IgM(COI) in females (0.291 ± 0.02) in Beta-thalassemia patients without Toxoplasma.

DISCUSSION

According to residence area, the highest rate of male falls in rural areas (75%), whereas the highest rate of female falls in urban areas (66.67%), the lowest infection rate revealed in males in the urban areas (25%), compared with females that fall in a rural area (33.3%), from the above, there is no significant difference between the two groups ²⁶.

Three age groups show the highest rate in males (50%) in (10-15)years in patients with Toxoplasma, (50%) in (16-20) years in patients without Toxoplasma, and (25%) in (21-25) years in patients with Toxoplasma, whereas the highest rate in females (58.33%) in (10-15)years in patients without Toxoplasma, and (33.33%) in (16-20)years, and (16.67%) in (21-25)years in patients with Toxoplasma, from the above there is a significant difference between the three groups of age in the patients without Toxoplasma ²⁷.

Catscat presence shows the highest rate in males (12.5%) in patients with Toxoplasma and no cat presence (90.91%) in patients without Toxoplasma, whereas in females, the highest rate in cat presence (16.67%) and no cat presence (95.83%) in patients without Toxoplasma, from the above there is significant difference between cats presence and no cats presence in both the patients with and without Toxoplasma ²⁸.

The amount of antibody present in the sample is not determined by the magnitude of the detected result above the cutoff. Because of variances in assay and reagent procedures, anti-Toxoplasma IgM findings in a given specimen, as assessed by assays from various vendors, can vary (Meylan and Liesenfeld, 2015).

The IgM(COI) concentration shown in Cobas e411, there is one sample that gives a positive result with a reactive group (1.35COI), whereas all samples of IgM show non-reactive (Garnaud et al., 2020).

CONCLUSION

Hemoglobinopathies entail configurationally aberration in the globin proteins themselves; the dyad forms might partly cover, nonetheless, as a few forms which genesis anomaly in globin proteins (hemoglobinopathy) furthermore modifies their fabrication (thalassemia), hence, several thalassemias are hemoglobinopathies. Yet, the majority are patently not; either or both of these forms may well cause anemia.

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