Original Article

Seroprevalence of Toxoplasma Gondii among Thalassemia Patients and its Relation with Some Biochemical Parameters

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Abstract

Background: Thalassemia patients are at an increased risk of contracting opportunistic infections such as toxoplasmosis due to frequent blood transfusions and underlying immune deficiency. **Objectives:** This study aimed to determine the seroprevalence of Toxoplasma gondii among thalassemia patients compared to healthy controls and examine any relation with biochemical parameters like serum calcium and growth hormone levels. **Methods:** A total of 40 thalassemia anti-T. gondii IgG antibodies seropositive patient with 40 healthy subjects, were enrolled in the study. Serum analysis for anti- T. gondii IgG antibodies and growth hormone using ELISA. Genomic DNA extraction was performed on white blood cells and quantified by spectrophotometry. T. gondii infection was detected by real-time quantitative PCR (qPCR) based on amplification of a species-specific region of tachyzoite p30 gene using previously published primers. Serum levels of total calcium were estimated by colorimetric assay based on o-cresolphthalein complex method. ABO and Rh blood grouping, complete blood picture and other hematological analysis were performed based on standard methods. **Results:** Anti-T. gondii IgG antibodies were detected in 28% of thalassemia patients compared to only 5% of controls, indicating significantly higher exposure among thalassemia patients. Both serum calcium and growth hormones were also significantly lower among thalassemia patients. Further genomic analysis by qPCR revealed 72.5% of IgG positive thalassemia patients were positive for T. gondii DNA compared to 100% of IgG positive controls. **Conclusion:** The high susceptibility of thalassemia patients to T. gondii infection indicates the importance of screening donor blood to prevent transmission by frequent transfusions. Detailed investigations are warranted to elucidate the mechanisms behind disturbances in calcium homeostasis and growth hormone axis among thalassemia patients and any linkage with T. gondii co-infection.

Keywords: Thalassemia Major, Toxoplasma Gondii, Seroprevalence, qPCR, Growth Hormone, Calcium.

Introduction

Thalassemia syndromes comprise a heterogeneous group of inherited disorders characterized by reduced or absent production of normal adult hemoglobin (HbA) due to mutations in the alpha or beta globin chain encoding genes.[1-5] Beta thalassemia major, also known as Cooley's anemia, arises from mutations in HBB gene leading to severely impaired or absent production of beta globin chains.[6-8] The resultant alpha/beta globin chain imbalance precipitates ineffective erythropoiesis and chronic hemolytic anemia requiring lifelong blood transfusions and extensive iron chelation therapy for patient survival beyond the first decade of life.[9-11]

The repeated blood transfusions and secondary iron overload make thalassemia major patients susceptible to

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infections, especially those transmitted by the blood-borne route.[12-14] Additional factors like functional asplenia commonly occurring after second decade of life and hypersplenism further compromise their capacity to mount effective immune response.[15,16] The pro-oxidative state resulting from labile plasma iron also hampers lymphocyte and phagocyte function.[17] Such immune defects predispose thalassemia patients to infections by a range of viral, bacterial and protozoan pathogens.[18]

Toxoplasma gondii is an obligate intracellular protozoan parasite capable of infecting almost all mammals, including humans.[19] Although usually asymptomatic in immunocompetent individuals, T. gondii infections can lead to severe manifestations in the foetus following congenital transmission or immunocompromised patients where it can disseminate widely leading to necrotizing encephalitis, pneumonia or myocarditis.[20,21] Specific subgroups like thalassemia patients are especially vulnerable owing to their immunosuppressed state coupled with increased infection pressure from repeated blood transfusions.[21,22]

Data on prevalence rates of T. gondii infection among thalassemia patients remain relatively sparse with considerable variations across geographical regions. Reported seropositivity rates range from 5% among Brazilians to as high as 39.3% in some Iranian cohorts indicating substantial population-level differences.[22-24] The burden in many developing countries may be even higher considering their continually elevated infection rates.[25] The current study from Iraq helps address this gap through systematic documentation of exposure levels in addition to highlighting increased susceptibility of thalassemia major patients to acquire and potentially develop toxoplasmosis.

Beyond vulnerability to infections, patients with beta thalassemia major also frequently demonstrate abnormalities in endocrine functions which adversely impact their quality of life and survival.[26,27] High prevalence of complications like growth retardation, osteopenia, osteoporosis, vitamin D deficiency and hypogonadism have been reported with evidence of progressive decline with age.[28] Proposed mechanisms relate chronic anaemia and tissue hypoxia resulting in higher expression of TGF-beta along with increased oxidative stress from iron overload.[29,30] The interplay of immune dysregulation from recurring infections like toxoplasmosis may potentially also contribute by altering normal homeostasis. Elucidating such associations can provide opportunities for optimizing clinical monitoring protocols.

Materials and Methods

Study design and participants: This case-control study enrolled 143 thalassemia major patients registered at the Thalassemia Centre in Mosul (Iraq) between January 2021 and January 2022. After initial screening, 40 patients were selected based on presence of anti-T. gondii IgG antibodies. Additionally, 40 healthy individuals matched by age and gender were recruited as controls from subjects undergoing routine annual examinations at local health facilities in Mosul. Informed consent was obtained from all participants and the study was approved by the local institutional ethics review committee.

Sample Collection and Serological Analysis: About 5 ml venous blood was collected from each participant and transported to the laboratory under cold chain maintenance. Hemolysed or hyperlipidemic samples were discarded and replaced as needed. Serum was separated by centrifugation and stored at -20°C until further analysis. The serum samples were tested for anti- T. gondii IgG antibodies using a commercial ELISA kit (Anti-Toxoplasma IgG ELISA kit, Monobind, USA) as per manufacturer's instructions. This kit has a reported diagnostic sensitivity and specificity of >95%.

Molecular Analysis: Genomic DNA extraction was performed on white blood cells using commercially available columns (Addbio DNA/RNA extraction kit, Korea). Extracted DNA was quantified by spectrophotometry (Nano-Drop 2000, Thermo-Fisher Scientific, USA) and purity assessed by A260/A280 absorbance ratio. T. gondii infection was detected by real-time quantitative PCR (qPCR) based on amplification of a species-specific region of tachyzoite p30 gene using previously published primers.[31] Human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as internal control. qPCR was performed on a real-time thermal cycler (Eco Real-Time PCR System, Illumina, USA) using the following thermal profile (initial denaturation at 95°C for 5 min, followed by 40 cycles of 95°C for 10 sec, and 60°C for 30 sec). Result analysis was done by absolute quantification method using a standard curve and EcoStudy software (Illumina, USA).

Biochemical Analysis: Serum levels of total calcium were estimated by colorimetric assay using a commercial reagent kit (Giesse Diagnostics, Italy) based on o-cresolphthalein complex method. Serum growth hormone levels were determined by enzyme-linked immunosorbent assay using a commercially available kit (Biovision Inc, USA) as per manufacturer's protocol.

Haematological analysis: ABO and Rh blood grouping, complete blood picture and other hematological analysis were performed based on standard methods

Statistical Analysis: Statistical analysis was performed using SPSS version 27 (IBM Corp, USA). Continuous variables were presented as mean ± SD and comparisons were done by independent student's t-test. Categorical variables were expressed as percentages and analyzed by Chi-square test. P values <0.05 were considered statistically significant for all analyses.

Results

Demographic Profile: The study comprised 80 participants divided into thalassemia major patients (n=40) as cases and healthy controls (n=40). The age range was 10-26 years in control group with average 18 years. Similarly, patient age ranged from 10-24 years with mean of 17 years. Statistical analysis revealed no significant age difference between the groups (p>0.05). The gender distribution was 52.5% males and 47.5%

females among cases while control group had 55% females and 45% males.

Seroprevalence of Anti-Toxoplasma IgG: Out of 143 thalassemia patients screened, 28% (n=40) were seropositive for anti-T. gondii IgG antibodies. In stark contrast, only 5% (n=2) of healthy controls demonstrated prior exposure as indicated by positive serological test (Figure 1). The comparative seroprevalence was significantly higher ($p<0.01$) in thalassemia major patients likely owing to increased risk from more frequent blood transfusions coupled with an overall immunocompromised state predisposing them to infections like toxoplasmosis.

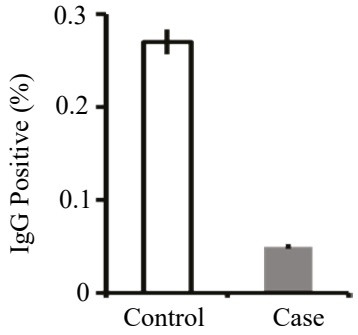

Figure 1: Comparative Anti-T. Gondii IgG Seroprevalence Rates.

Biochemical Parameters: Patients with thalassemia major had considerably lower levels of serum calcium (1.88 ± 0.15 mmol/L) compared to healthy control group (2.34 ± 0.19 mmol/L). The difference of ~20% drop in cases was statistically significant ($p<0.01$) (Figure 2).

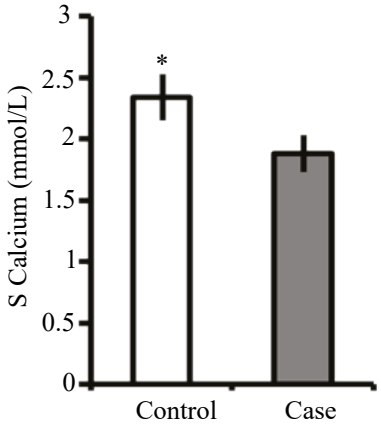

Figure 2: Serum Calcium Levels among Cases Vs. Controls. Data Expressed as Mean±SD, T-test Used to Compare the Two Group and * Indicates Significant Difference.

Similar trends were discernible in growth hormone levels measured among the two cohorts. Mean serum levels in patient group were measured at 8.52 ± 0.73 ng/mL compared to 12 ± 0.77 ng/mL in matched healthy individuals (Figure 3). The nearly 30% deficit indicates significant growth hormone deficiency prevailing among thalassemia major patients ($p<0.01$).

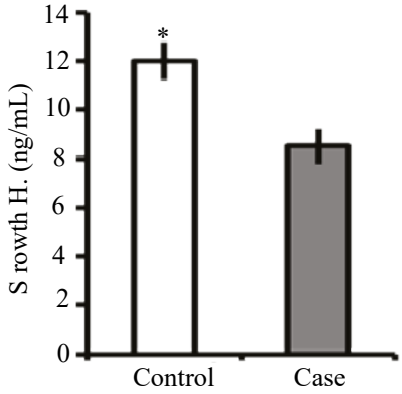

Figure 3: Growth Hormone Levels among Thalassemia Patients Vs. Controls. Data Expressed as Mean±SD, T-test Used to Compare the Two Group and * Indicates Significant Difference.

Correlative Analysis: Further analysis of age and serum ferritin levels across the different groups revealed a weak positive correlation of 5% between increasing age and rising ferritin concentrations (Table 1). This matches the known gradual accumulation of iron burden with multiple transfusions over time. Comparative evaluation by Duncan's test highlighted significantly elevated ferritin among thalassemia patients of both genders relative to controls while no major age variance was discernible across the cohorts. The markedly high iron overload likely contributes to the various metabolic and hormonal disturbances observed in these patients.

Table 1: Correlative Analysis of Age and Ferritin Levels.

Samples	Age (Mean ± SE)	Ferritin (Mean ± SE)
Control females	15.9 ± 2.523	544.5 ± 426
Control males	16.5 ± 2.181	244.1 ± 17.6045
Females	17.52 ± 1.606	2140 ± 336.501
Males	14.809 ± 1.745	4281.02 ± 678.451

T. gondii DNA PC: Molecular diagnostic testing by quantitative PCR targeting genomic parasite DNA was undertaken in all participants testing positive for anti-Toxoplasma IgG antibodies. This technique confirmed presence of circulating parasites in 72.5% (29 out of 40) of seropositive thalassemia patients indicating possibility of reactivated or suboptimally controlled infection. Comparatively, both seropositive healthy controls showed PCR evidence of asymptomatic parasitemia indicating effective immune regulation in immunocompetent individuals.

The elevated ferritin levels coupled with higher PCR detection rates of viable T. gondii among thalassemia patients relative to controls correlates with the known immune dysregulation and inability to effectively restrain infections in this cohort. The resulting unchecked proliferation likely also interacts with and alters normal calcium/bone homeostasis contributing to deficits in

both skeletal remodelling and growth hormone activity critical for development.

The measured haematological parameters has shown that almost all haematological parameters were significantly ($p<0.05$) reduced in case groups whether male or female compared to control apart from MCHC which was significantly elevated in case versus control groups in either sex, moreover, the reduction has remarkably reached to be close to lower limit of reference range or even lower than the lower limits. Noteworthy to mention that platelet level has significantly elevated in in male patients compared to control or female groups.

Table 2: Haematological Parameters of the Studied Sample Compared to Reference Ranges.

Measured Parameters	Female		Male		Reference Range
	Control	Case	Control	Case	
Haemoglobin (g/L)	134.4±7.9^	88±13	149±9.4*	87±10.5	120-155
Haematocrit (%)	40.6±2.3^	26±4	45±3*	26±2.3	36-46
RBC ($x10^{12}$/L)	4.3±0.3^	3±0.64	4.7±0.35*	3±0.3	3.8-4.8
MCV (fl)	95±3^	87±9.7	96±1.9*	89±6.3	80-100
MCH (pg)	31±1.2	29.3±4	31±1.2	30±2.4	27-32
MCHC (g/L)	329±6.3	335.5±12^	328±7.7	338±6.4	310-370
Platelets ($x10^3$/L)	225±46	279±108.5	211±37	359±222*	150-450
WBC ($x10^9$/L)	7.4±0.85	7.6±4	7.7±1	10.5±6.8	4-11

Data Expressed as mean±SD
^ indicate significant differences in female at $p<0.05$
* indicate significant differences in male at $p<0.05$

The distribution of blood groups in the studied samples has shown non-significant differences between groups apart from the variation in the distribution in the type of blood group which do reciprocally related to the normal distribution of ABO blood subtypes.

Table 3: Frequency of Blood Groups Subtypes in Studied Sample Correlative to Sex Distribution.

	Male (%)	Female (%)	Chi-square	P value
A (+/-)	15/1	14/2	0.4	0.55
B (+/-)	5/1	14/1	0.42	0.5
AB (+/-)	1/0	1/1	0.2	0.9
O (+/-)	20/3	19/2	0.3	0.08

Discussion

The seroprevalence of T. gondii infection among chronically transfused thalassemia patients in this Iraqi cohort was found to be 28%. This is comparable to rates between 5-39% documented across different geographical settings.[22,32,33] The statistically significant higher prevalence relative to just 5% among healthy controls matches trends reported previously.[34] Repeated blood transfusions coupled with an immunocompromised state render them highly susceptible to contract transfusional infections.

However, a subset of 72.5% thalassemia patients were positive for circulating T. gondii DNA by PCR indicating inability to effectively control parasite proliferation. In contrast, the immunocompetent controls displayed intact capability to prevent disease reactivation as evidenced by asymptomatic parasitemia.[35] Unregulated growth of tachyzoites can lead to systemic dissemination and multi-organ involvement with potentially fatal outcomes.[19] Thalassemia major patients, therefore, represent an extremely vulnerable cohort warranting screening of donor blood to mitigate transmission risks.[36] Furthermore, this subset may also benefit from heightened surveillance and empiric treatment guided by rising parasite DNA loads to prevent onset of severe toxoplasmosis.

Concurrently, significant derangements were discernible in both calcium metabolism and growth hormone activity among thalassemia patients. The multifactorial pathogenesis likely involves complex interplay between chronic tissue hypoxia, oxidative stress, vitamin D deficiency and iron overload mediated endocrine damage.[28,37] T. gondii is known to profoundly reprogram host cell signalling pathways concerned with essential processes like apoptosis, proliferation and cytokine production to ensure its intracellular survival and replication.[38] The potential downstream implications with regards to skeletal remodelling, bone loss and growth impairment merits deeper investigation.[39] Future studies can focus on characterizing specific pathway perturbations along with effects of anti-parasitic treatment on reversing such anomalous changes. Children are highly affected by these modulation in their endocrine dysfunction resulting in an impact on their bone growth and ultimately their length.[40]

The markedly elevated ferritin levels, with means of 4281 ng/mL and 2140 ng/mL among male and female patients respectively compared to only 244 and 545 ng/mL in matched healthy controls, provide clear quantitative evidence of the heavy iron overload in thalassemia patients that accumulates gradually with successive transfusions over years.[1-5]

Regardless of sex, haematological variables has clearly confirmed that patients with thalassemia reported reduced laboratory haematological parameters including hemoglobin, haematocrit, RBCs, MCV, and MCH. Conversely, MCHC elevated in both sex in case group. These findings agreed with a study conducted by Sari *et al.*[2], who have reported a reduced hematological parameters in thalassemia patients.[2]

Overall, thalassemia major patients across different geographical settings demonstrate consistently heightened risk of exposure to T. gondii infection attributable to repeated blood transfusions. A subset exhibits suboptimal immune control with viable, circulating parasites having implications for reactivation and disseminated disease. This silent parasitic infection could potentially carry the risk of biochemical and endocrine changes resulting in disturbed bone maturation and calcium hemostasis opening the research horizons for patients monitoring and future directions. Other limitations include small sample size and unicentre studies.

Conclusion

Thalassemia major represented by increased risk of infection by T. gondii due to continuous blood transfusions due to their genetic abnormalities which offsets blood cell synthesis. During blood transfusion viable parasite transfused from seronegative donor due to their apparently negative test results increasing the susceptibility of blood recipient to infectious parasites. Concurrent changes in blood biochemical and endocrine parameters might be related encouraging further study requirement to clarify the pathophysiological link between these disturbed parameters.

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