RESEARCH ARTICLE

Seroprevalence of *Toxoplasma gondii* Immunoglobulin G and Immunoglobulin M Antibodies among Leukemia Patients in Erbil City-Kurdistan Region/Iraq by Enzyme-linked Immunosorbent Assay Technique

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ABSTRACT

The current study was revealed the seroprevalence of *Toxoplasma gondii* immunoglobulin (Ig)G and IgM among different types of leukemia (Acute lymphocytic leukemia, acute myeloid leukemia, Hodgkin lymphoma [HL], multiple myeloma [MM], non-HL [NHL], and chronic myeloid leukemia) from male and female patients in different age groups (18–≥48 years) using indirect enzyme-linked immunosorbent assay. The results of 140 cases of different types of leukemia showed the highest *T. gondii* IgG (44.44%) among MM leukemia whereas *T. gondii* IgM highest (12.5%) among NHL leukemia. Significant ($P \leq 0.01$) differences between *T. gondii* antibodies and age group were recorded, that increased with the age of Leukemia patients. Hence, it could be concluded that multiple myeloid leukemia is strong immunocompromised and high significant ($P \leq 0.01$) with toxoplasmosis.

Keywords: Enzyme-linked immunosorbent assay; Immunoglobulin G; Immunoglobulin M; Leukemia; *Toxoplasma gondii*

INTRODUCTION

Toxoplasma gondii, is one of the most common parasitic infections of man and other warm-blooded animals. It is estimated one-third of humanity has been exposed to this parasite. *T. gondii* seems to be nearly ubiquitous geographically and isolated from a variety of climatic regions on every continent surveyed (Dubey, 2010). Toxoplasmosis causes blindness, mental retardation, abortion, and stillbirth in congenitally infected children and devastating disease in immunocompromised individuals (Hill and Dubey, 2002; Makiani et al., 2012).

Toxoplasmosis in patients who are immunocompromised by virtue of underlying leukemia disease has received relatively little attention (Gharavi et al., 2017). Leukemia is a hematological malignancy that arises when something goes wrong in the regulation of the division or the life span of a blood cell (Rathee et al., 2014).

Multiple myeloma (MM) is a plasma cell dyscrasia that accounts for almost 10% of all hematologic malignancies and leading to production of nonfunctional intact

immunoglobulins (Igs) (Korbet and Schwartz, 2006; Gerecke et al., 2016). Acute lymphocytic leukemia (ALL) is a malignancy that begins from the early form of white blood cells called lymphocytes in the bone marrow (Bala et al., 2016) while acute myeloid leukemia (AML) is a blood cancer which characterized by the infiltration of proliferative, clonal and abnormally differentiated cells of the hematopoietic system it comes as a consequence of arrested myeloid differentiation (Dohner et al., 2015; Aziz et al., 2017). While chronic myeloid leukemia described as a hematologic malignancy that can affect patients of all ages (Tapela et al., 2016).

Hodgkin lymphoma (HL) is one of the most curable pediatric and adult cancers, with long-term survival rates (Mauz-Körholz et al., 2015; Karimi et al., 2016) whereas Non-Hodgkin's lymphomas (NHLs) represent a heterogeneous group of malignancies that arise from the lymphoid system which is sub-divided into B, T and Null cell categories (Nogai et al., 2011).

Diagnosis of *T. gondii* from cancer patients commonly used by enzyme-linked immunosorbent assay (ELISA)

technique as a standard serologically while different types of polymerase chain reaction (PCR) techniques are used as advance techniques for diagnostic of toxoplasmosis (Aburet et al., 2000).

MATERIALS AND METHODS

Sample Collection

A total of 140 human serum samples were obtained from the Nanakali Hospital-Erbil/Kurdistan region of Iraq. Blood samples were collected by taken 5 ml of venous blood from cancer patients. The sera were separated by centrifuge at 3000 rpm for 5 min then stored with in Eppendorf tube at -20°C till used for the serological testing process. The research and sample collection were proofed by Nankaly teaching hospital and Board of Medical Ethics in Erbil Health Technical College then the question rate form was filling out.

ELISA IgG and IgM

All the serum samples collected were tested for *T. gondii* IgG and IgM antibodies, using commercial indirect ELISA kit (catalog #TOXOG01 for IgG and #TOXOG02, Bioactive- diagnostica, Germany). Human serum samples were processed following manufactory's instruction. Briefly, serum specimens were prepared by mixing 10 μl of serum with 1000 (1:100) μl of sample diluents. Then, 100 μl of each diluted sample was added to the micro plate. Both negative and positive controls were also included. Micro titer plates were incubated at 37°C temperature for 30 min, then removed liquid from all wells, rinsed, and flicked microtiter wells 4 times by washing buffer. 100 μl of horseradish peroxidase-conjugated anti-human IgG was added and incubated at 37°C temperature for 30 min. The microplate was washed 4 times with washing solution and blotted onto paper towels. 100 μl of Tetramethylbenzidine was added to each well and incubated at room temperature for 15 min. Then, a 100 μl of Stop Reagent was added to each well. Samples were read by ELISA reader at 450 nm with subtracting the blank value. The results were interpreted as positive, equivocal, and negative by determining the Ig index. IgG and IgM index values <0.90 , $0.90-0.99$, and ≥ 1.0 were considered for negative, equivocal, and positive, respectively.

Data Analysis

Chi-square (or Fisher is exact) with the Graphpad prism v.5.01 Package (GraphPad Software, Inc. USA) was used to analyze differences of *T. gondii* IgG and IgM among all types of leukemia patients with demographic study in the tested groups. $P < 0.05$ was considered statistically significant.

RESULTS

In this study, the overall seroprevalence was (22.85% and 8.57%) for *T. gondii* IgG and IgM, respectively, among total types of leukemia [Figures 1 and 2]. While the distribution of *T. gondii* among types of leukemia was 44.44%, 23.33%, 20%, 18.18%, and 12.5% for MM, ALL, HL, AML, and NHL respectively [Figure 1]. The highest of *T. gondii* IgG was 44.44% and the lowest was 12.5% for MM and NHL respectively [Figure 1].

Regarding to IgM the seroprevalence of *T. gondii* was 12.5%, 11.11%, 10%, 9.09%, and 6.66% for NHL, MM, HL, AML, and ALL respectively [Figure 2]. While the high seropositivity of IgM was 12.5% and the lowest was 6.66% for NHL and ALL, respectively [Figure 2].

Regarding to age groups, the seropositivity of *T. gondii* IgG was 29.16%, 24.13%, and 18.18% for age grouped (≥ 48), (18–27) and (28–37), respectively [Figure 3]. While the high seroprevalence of *T. gondii* IgG was 29.16% and the lowest was 18.18% for age grouped (≥ 48) and (28–37), respectively [Figure 3].

Regarding to age groups, the seropositivity of *T. gondii* IgM was 10.34%, 9.09%, and 8.33% for age groups (18–27), (28–37), and (≥ 48), respectively [Figure 4]. While the high seroprevalence of *T. gondii* IgM was (10.35%) and the lowest (8.33%) for age groups (18–27) and (≥ 48), respectively [Figure 4].

According to gender, the seropositivity of *T. gondii* IgG was 25.92% and 20.93% for female and male, respectively [Figure 5]. Regarding to gender, the seropositivity of *T. gondii* IgM was 11.11% and 6.97% for female and male respectively [Figure 6]. According to occupation, the seropositivity of *T. gondii* IgG was 23.07% and 22.22% for home makers and employee respectively [Figure 7]. Regarding to occupation, the seropositivity of *T. gondii* IgM were 9.61% and 5.55% for homemakers and employee, respectively [Figure 8].

Regarding to educational status [Figure 9] seropositivity of *T. gondii* IgG was 26.08% and 16.66% for illiterate and educated status, respectively. Regarding to educational status, the seropositivity of *T. gondii* IgM was 8.69% and 8.33% for illiterate and educated, respectively [Figure 10]. According to residency, the seropositivity of *T. gondii* IgG was 27.27% and 18.91% for urban and rural, respectively [Figure 11].

According to residency, the seropositivity of *T. gondii* IgM was 12.12% and 5.4% for urban and rural, respectively [Figure 12]. Regarding to marital status, the seropositivity of *T. gondii* IgG was 25% and 21.42% for single and married, respectively [Figure 13]. Regarding to marital status, the

Figure 1: Seropositivity of *Toxoplasma gondii* immunoglobulin G Ab among different types of leukemia. There were significant differences ($P=0.001$) between types of leukemia and *Toxoplasma gondii*

Figure 2: Seropositivity of *Toxoplasma gondii* immunoglobulin M Ab among different types of leukemia. There were significant differences ($P=0.0076$) between types of leukemia and toxoplasmosis

Figure 3: Seropositivity of *Toxoplasma gondii* immunoglobulin G Ab among total leukemia patient-related with age group ($n=140$). There were significant differences ($P=0.005$) between age groups with *Toxoplasma gondii*

seropositivity of *T. gondii* IgM was 9.52% and 7.14% for married and single, respectively [Figure 14].

DISCUSSION

One of the most critical issues in leukemia is infectious diseases which may lead the patient succumbs to sudden death. The active infection may alter the normal immune

Figure 4: Seropositivity of *Toxoplasma gondii* immunoglobulin M Ab among total leukemia patient-related with age group ($n=140$). There were significant differences ($P=0.022$) between age groups with *Toxoplasma gondii*

response of the host. Granulocytes and macrophages play a main role in immune surveillance in innate immune system (Gharavi et al., 2017).

In current study observed that leukemia patients most susceptibility to be infected with *T. gondii* before and after treated because immunocompromised individuals are the main risk groups to be infected with toxoplasmosis (Flegr 2007; Yolken et al., 2009). Immunocompromised patients,

Figure 5: Seropositivity of *Toxoplasma gondii* immunoglobulin G Ab among total leukemia patient-related with gender group ($n=140$). There were significant differences ($P=0.0398$) between gender groups with *Toxoplasma gondii*

Figure 9: Seropositivity of *Toxoplasma gondii* immunoglobulin G Ab among total leukemia patient-related with educational status ($n=140$). There were significant differences ($P=0.0317$) between educational status with *Toxoplasma gondii*

Figure 6: Seropositivity of *Toxoplasma gondii* immunoglobulin M Ab among total leukemia patient-related with gender group ($n=140$). There were non-significant differences ($P>0.05$) between gender groups with *Toxoplasma gondii*

Figure 10: Seropositivity of *Toxoplasma gondii* immunoglobulin M Ab among total leukemia patient-related with educational status ($n=140$). There were non-significant differences ($P>0.05$) between educational status with *Toxoplasma gondii*

Figure 7: Seropositivity of *Toxoplasma gondii* immunoglobulin G Ab among total leukemia patient-related with occupation group ($n=140$). There were significant differences ($P=0.0358$) between occupation groups with *Toxoplasma gondii*

Figure 11: Seropositivity of *Toxoplasma gondii* immunoglobulin G Ab among total leukemia patient-related with residency group ($n=140$). There were significant differences ($P=0.0397$) between residency groups with *Toxoplasma gondii*

Figure 8: Seropositivity of *Toxoplasma gondii* immunoglobulin M Ab among total leukemia patient-related with occupation group ($n=140$). There were non-significant differences ($P>0.05$) between occupation groups with *Toxoplasma gondii*

Figure 12: Seropositivity of *Toxoplasma gondii* immunoglobulin M Ab among total leukemia patient-related with residency group ($n=140$). There were non-significant differences ($P=0.0317$) between residency groups with *Toxoplasma gondii*

Figure 13: Seropositivity of *Toxoplasma gondii* immunoglobulin G Ab among total leukemia patient-related with marital status (n=140). There were significant differences ($P=0.0385$) between marital status with *Toxoplasma gondii*

Figure 14: Seropositivity of *Toxoplasma gondii* immunoglobulin M Ab among total leukemia patient-related with marital status (n=140). There were non-significant differences ($P>0.05$) between marital status with *Toxoplasma gondii*

with cell-mediated immunity deficiency who are at risk for severe and life-threatening *T. gondii* infection, so early diagnosis and proper treatment of the patient may reduce the complications of infection and promote better due to the differences in location and age of the patients examined (Al-Mukhtar and Al-Najjar, 2009).

In this study, immunocompromised patients were found more frequently infected with *T. gondii* and revealed high seropositivity of IgG and IgM antibodies which induced by significant differences between the prevalence of infection. In this study high significant differences between *T. gondii* IgG and IgM antibodies 22.85% and 8.57%, respectively among different types of leukemia particularly MM and NHL ($P < 0.05$), this explains that MM leading to the production of nonfunctional intact Igs, causing severe immunodeficiency, (Gharavi et al., 2017). This study is in agreement with the study of Iran who recorded 5.9% and 56.4% for *T. gondii* IgG and IgM antibodies, respectively among leukemia patients (Gharavi et al., 2017). The current study disagrees with the study in Basra-Iraq (Al-Mukhtar and Al-Najjar, 2009), who conducted that the rate of infection with *T. gondii* was 48% for IgG. The seroprevalence variation of toxoplasmosis is different from the geographical area and age of patients. This finding is confirmed by the study in China who reported that

T. gondii infection appears to be associated with increased leukemia susceptibility (Huang et al., 2016). Regarding to international references, the study in Turkey (Yazar et al., 2004) reported that *T. gondii* IgG and IgM by ELISA were 63.0% and 6.5% respectively among neoplasia patients which is agreement with our finding.

Finally, in this study, some of demographic factors such as occupation, gender, residency, educational status, and marital status are considered as a strong relation with Toxoplasmosis ($P < 0.05$). During our review for literature between Toxoplasmosis and types of leukemia all papers supported to significant differences between acute and chronic infection of *T. gondii* with leukemia.

CONCLUSION

In this study revealed high seropositivity of IgG among MM, while significant relations observed between seroprevalence of *T. gondii* IgG and IgM with some demographic factors of cancer patients. For diagnosis of *T. gondii* they needed further study among cancer patients such as different types of PCR, Western blot, and gene probe. Hence, it is urgent issue for future about genotyping and virulent strains of *T. gondii* among types of Leukemia patients it will be the next attempt.

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