

Infections with *Toxoplasma gondii* in malignant brain tumor patients in Mosul city, Iraq

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ABSTRACT

Background: toxoplasmosis is considered as a risk factor for many diseases, either by contributing in pathogenesis, or by causing further complications as an opportunistic infection in immune compromised patients; this study is to investigate the incidence of toxoplasmosis in a group of immune compromised patients, i.e. malignant brain tumor patients.

Method: the subjects enrolled in this study were 40 patients with diagnosed malignant brain tumor and 80 apparently healthy individuals as blood donors, blood samples collected, separated and tested by ELISA method for *T. gondii* IgG and IgM.

Results: the study showed that 6 out of 40 (15%) of patients were positive for IgG, and 2 (5%) of patients were positive for IgM; while 28 out of 80 (35%) of normal individuals were positive for IgG, and only 1 (1.25%) were positive for IgM.

Conclusion: prevalence of toxoplasmosis in patient is lower than in normal individuals, but there is a still risk factor for those patients in case they received donated blood.

Keywords: brain tumor, Blood donors, cancer, ELISA, *T. gondii*, toxoplasmosis.

INTRODUCTION

T. gondii is indeed a typical parasite which is found worldwide and often at very high prevalence (Robert-Gangneux & Dardé 2012). About half of the human population are asymptomatic carriers. The rapid multiplication of the invading tachyzoite stage, leads to a mild to subclinical phase (Kasper et al 2005). Recovery is associated with parasite sequestration into cysts containing bradyzoites located particularly within skeletal and heart muscle and in the central nervous system and remaining latent for life. (Mehlhorn 2008)

Patients with malignant brain tumors are on radio- and chemotherapy after diagnosis, and they are immune compromised due to the psychological and physical stress they exposed to.

So, these patients are more vulnerable and at higher risk for opportunistic infections including *T. gondii* by either new infection or by activation of an old latent infection.

Toxoplasmosis is caused by the intracellular sporozoan *T. gondii*. The infection could be congenital or acquired. The life cycle of *T. gondii* is similar to that of coccidian parasites and its taxonomic status is now considered to be a coccidian parasite related to the genus *Isospora*. Reproduction of the organism is by binary fission. Toxoplasma trophozoites and cysts characterize acute and chronic infections respectively, but cysts may form early in the acute stage and trophozoites may remain active for years in some chronic infections. (Lucas & Gilles 2003)

Toxoplasmosis has a worldwide distribution. In the tropics it is probably commoner than is generally realized. Many studies had been conducted in Iraq about the incidence and prevalence of *T. gondii* in different groups of population. Starting with a study conducted by Al-Jebouri and his colleagues in 2013, in Al-Hawija and Al-Baiji Districts in the middle of Iraq, the study subjects were 285 women (150 from Al-Hawija and 80 from Al-Baiji subjects) with average number of abortions (1 - 3) during 2009; the method of screening was measurement of IgM antibodies by ELISA method and were conducted in College of Medicine, University of Tikrit; Al-Jebouri and his colleagues found that more than 40% of women were infected with toxoplasmosis. (Al-Jebouri et al 2013).

Another study carried out by Bakre and his colleagues on 93 schizophrenic patients and compared with 93 normal individuals of Hawler population (Erbil north of Iraq) in 2015, the toxoplasmosis was detected by ELISA technique and the study showed that 39 schizophrenic patients (i.e. 41.9%) were exposed to toxoplasma infection in comparison to 5 (i.e. 5.3%) of normal individuals (30 IgG and 9 IgM of schizophrenic compared to 4 IgG and 1 IgM of control). (Bakre et al 2015).

A study published at the same year, i.e. 2015 by AlSaadawi and Alkhaledin Al-Muthanna province (south of Iraq) which was conducted on 92 patients with renal infection. They used On Site ToxoIgG/IgM rapid test – Cassette method and ELISA technique for detection of IgG and IgM antibodies in patients serum. Their results showed that 12 patients (13%) were positive for IgG test and only one patient (~1%) was IgM positive. (AlSaadawi & Alkhaled 2015).

A more recent study conducted in Baghdad by Khalil and his team in 2016 on 800 apparently healthy individuals who were tested for IgM in different techniques and found that 29 women out of 135 women who suffered from repeated abortions were positive for toxoplasma IgM (21.5%) by ELISA technique; and found 217 patients out of 800 (27.1%) patients were positive for toxoplasmosis by LATEX method. (Khalil et al 2016). While there is an older study conducted by me in Mosul city in 2011 on 90 apparently healthy blood donors and found that about 3% of them were positive for IgM antibodies (i.e. active toxoplasmosis). (Al-Dabbagh 2011)

Aim: to study the prevalence of *T. gondii* exposure and active infection in brain tumor patients and blood donor persons in Mosul city- Iraq.

SUBJECTS, MATERIALS AND METHODS

A totally 120 subjects were enrolled in this study and categorized as 40 patients with malignant brain tumor who were receiving chemotherapy in Mosul oncology and nuclear medicine hospital, and 80 apparently healthy blood donors who visited Mosul city central blood bank for blood donation.

Blood samples were taken from these individuals as 5 ml from each, either by plastic disposable syringe from brain tumor patients, or from the blood pack collected from blood donors and placed in plain tubes with labels, then centrifuged at 3000 rpm for serum separation, the serum placed in test tubes and kept in refrigeration at -10 C.

After sampling, the serum samples were tested for IgG and IgM antibodies of *T. gondii* by using ELISA technique and commercially available kits, the method used in serum testing is as provided with kits protocol catalogue.

The ELISA test was done by serum dilution 1:40, enzyme conjugate addition, TMB reagent then stop solution is added, read at 450 nm in ELISA reader for both *T. gondii* IgM and IgG. The results were calculated for each determination by referring the value to the cut-off value reading of the used kits.

The data presented as percentage, Z-test was used to test proportion from zero, $p < 0.05$ was considered significant.

RESULTS

The test results of patients and control shows the following data:

- 1- Number of positive IgM results in blood donors was only one out of eighty, while in brain tumors was two out of forty.
- 2- Number of positive IgG results in blood donors was twenty eight out of eighty, while in brain tumors was six out of forty.

Table 1: prevalence of *T. gondii* IgM in blood donors and brain tumor patients.

| Category | Total | Positive (%) | Negative (%) |
|--------------|-------|--------------|--------------|
| Blood donors | 80 | 1 (1.25) | 79 (98.75) |
| Brain tumor | 40 | 2 (5) | 38 (95) |

The test results showed that there is only 1 person among blood donor group who was positive for *T. gondii* IgM, which represent 1.25%.

While there is 2 patients of brain tumor group which showed positive result for *T. gondii* IgM which represent 5% of this group.

Table 2: prevalence of *T. gondii*IgG in blood donors and brain tumor patients

| Category | Total | Positive (%) | Negative (%) |
|--------------|-------|--------------|--------------|
| Blood donors | 80 | 28 (35) | 52 (65) |
| Brain tumor | 40 | 6 (15) | 34 (85) |

Test result showed that there is 28 person in blood donor group who were positive for *T. gondii*IgG which represent 35%; while there were 6 patients who showed positive results for *T. gondii*IgG which represents 15%.

Table 3: comparison of *T. gondii*IgG levels in Brain tumor patients and blood donors

| Group | IgG (mean \pm SD) | P value |
|--------------------|---------------------|---------|
| Brain Tumor (n=40) | 0.15 \pm 0.36 | 0.0218 |
| Blood donor (n=80) | 0.35 \pm 0.48 | |

Significant difference in IgG levels between brain tumor group and blood donor group in which higher levels in IgG was found in blood donor group

DISCUSSION

It is well known that the incidence of most of parasitic infection is highly correlated with hygienic level of the community (CDC 2013), this might explain the high incidence of *T. gondii* in Iraq and the drastic increase in this incidence levels over the past decade.

***T. gondii*IgG:** the results of this study was incomparable to that conducted by Bakre on schizophrenic patients and normal volunteers as control were founded that the normal individuals are with low incidence of toxoplasma in Erbil city (only 5.3%), in comparison to patients with schizophrenia, in which, toxoplasmosis is considered as a risk factor now (Hamidinejat et al 2010), the low incidence in normal individuals could be attributed to higher hygienic standard in comparison to other localities in the country.(Bakre et al 2015).

The study of AlSaadawi showed a lower incidence of toxoplasmosis infection in the study sample (only 13%) in comparison to this study (35%), and that could be attributed to the types of patients enrolled in AlSaadawi's study. (AlSaadawi & Alkhaled 2015).

The study conducted by Khalil in Baghdad city showed a close results to this study, were found that 27.1% of the relatively large sample enrolled in their study were exposed to toxoplasmosis infection, comparing to this study which showed that 35% of the sample were exposed. (Khalil et al 2016).

***T. gondii*IgM:** The result of this study showed that only 1.25% of apparently healthy blood donors are exposed to toxoplasmosis, and that is in contrary with Al-Jebouri study in Al-Hawija and Al-Baiji Districts which found about 40% of the samples were positive for active toxoplasmosis. (Al-Jebouri et al 2013). This could be explained that Aljubory study were conducted on pregnant women (usually immune compromised), while the blood donors are healthy men (immune competent), also such results may also compatible with the higher percentage of active toxoplasmosis in brain tumor patients which show about 5% of them with active toxoplasmosis which is higher than normal individuals and mostly due to immune compromise due to disease or therapy.

The result of this study is also comparable to that of AlSaadawi result which showed that about only 1% of their sample showed active toxoplasmosis which is in agreement with the results of this study which showed only 1.25% of normal individuals are with active toxoplasmosis. but still disagree with the results of Khalil's study which showed that about 27% of the investigated samples were positive for active toxoplasmosis.

Finally this study is relatively in agreement with a previous study conducted on same kind of population (blood donors) in 2011 in Mosul city which showed that 3% of blood donor samples were positive with active toxoplasmosis compared to this study samples that showed only 1.25% with active toxoplasmosis, this variance could be explained by the relatively small sample number enrolled in both studies.

CONCLUSION

This study showed that there is a relatively low prevalence of *T. gondii* infection among malignant brain tumor patients but a high prevalence in apparently healthy individuals and those people donating blood and that could be a risk factor in spreading toxoplasmosis specially in immune compromised patients.

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