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Original Article

Diagnosis of *Toxoplasma* Infection in Allogenic Pre HCTSP Patients Using Molecular Methods

Meysam Yusefi [1,2], Zahra Arab-Mazar [1,3], Shirzad Fallahi [4,5], Amirreza Javadi Mamaghani [6], Shahnaz Sali [1], Naeem Nikpour [7], Meisam Barati [8], Arian Karimi Rouzbahani [9,10] , *Farnaz Kheirandish [5,11]

1. *Infectious Diseases and Tropical Medicine Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
2. *Research Center of Tropical and Infectious Diseases, Kerman University of Medical Sciences, Kerman, Iran*
3. *Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
4. *Hepatitis Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran*
5. *Department of Medical Parasitology and Mycology, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran*
6. *Department of Parasitology and Mycology, Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
7. *Department of Hematology and Oncology, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
8. *Department of Cellular and Molecular Nutrition, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
9. *Student Research Committee, Lorestan University of Medical Sciences, Khorramabad, Iran*
10. *USERN Office, Lorestan University of Medical Sciences, Khorramabad, Iran*
11. *Razi Herbal Medicines Research Center, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran*

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***Correspondence Email:**
kheirandish81@yahoo.com

Abstract

Background: We aimed to estimate the incidence of *Toxoplasma* infection in *T. gondii*-seropositive patients under allogeneic hematopoietic stem cell transplantation (HSCT).

Methods: The present research was a prospective study on 54 whole blood samples of allogeneic HSCT recipients, who were referring to bone narrow transplantation centers affiliated to Shahid Beheshti University of Medical Sciences, Tehran, Iran in 2018. All patients were IgG positive against *T. gondii*.

Results: Overall, 54 *Toxoplasma* positive pre-HCTSP patients were enrolled. 53.7% (n= 29) were male, also 1.9% (n=1) had germ-line type of the disease. The Multiple myeloma patients had higher age in comparison with other disease, but pairwise comparison showed the difference of age between Multiple myeloma patients were statistically significant with Acute lymphoblastic leukemia, Acute myeloblastic leukemia and Huntington's disease ($P< 0.05$). The results of PCR assay showed 5.6% (n= 3) of the patients were infected with *Toxoplasma*.

Conclusion: PCR method has detected considerable incidence of *Toxoplasma* infection for monitoring HSCT recipients at risk for toxoplasmosis, and many patients who showed the incidence of toxoplasmosis had previous infections with the *Toxoplasma* parasite.

Introduction

Toxoplasmosis is a zoonotic disease that mostly affects humans and warm-blooded animals throughout the world. Overall, 500 million to 1 billion people are suffering from this chronic disease (1-4). Toxoplasmosis causes congenital, neurological, ocular, and mild or asymptomatic infections in humans (5, 6).

In hosts with a healthy immune system, the acute form of the disease is spontaneity improved without treatment, but in people with immune deficiency such as those with AIDS or people taking immunosuppressive drugs, there is a very high likelihood of reactivation of brain cysts and risk of host's death (3, 7-9).

Toxoplasma gondii infection causes a high mortality rate in the congenital infected infants (2, 10, 11). Disseminated toxoplasmosis is also an unpleasant but well-known complication after bone marrow transplant (BMT), reported in 0.1%-6% of allogeneic transplant recipients (12). Despite severe complications in AIDS patients infected with *T. gondii*, toxoplasmosis was already considered as a rare infection in hematopoietic stem cell transplants (HSCTs) recipients (12, 13). However, invasive diseases could be more common than what has been previously believed. For instance, in the earlier studies, the reactivation of *T. gondii* and mortality in allogeneic transplant patients has been reported 4% and 60%-90%, respectively (14, 15).

A prospective study conducted the reactivation of *T. gondii* in allogeneic HSCT recipients that were in chronic phase of toxoplasmosis. Three out of 24 patients were found positive PCR results after receiving immunosuppressive drugs, which indicated the reactivation of *T. gondii* in them (15).

About two-thirds of toxoplasmosis cases have been reported in allogenic pre HCTSP recipients. These results approve the major role of activating latent toxoplasmosis infection in these patients (16).

Many studies have noted the role of trimethoprim/sulfamethoxazole (TMP/SMX) to prevent the reactivation of latent opportunistic infection (17-19). However, since there is a likelihood of toxoplasmosis flare-ups prior to drug administration in people with positive serology, the role of the drug in the prevention of this disease is not entirely clear (16, 20).

Transplantation is a widely used treatment for replacing non-functioning organs and tissues with healthy tissues. The major histocompatibility complex (MHC) molecules are responsible for majority of strong transplant rejection reactions. Providing antigens by antigen-presenting cells, often MHC1 and MHC2, would help $TCD4^+$ and $TCD8^+$ in transplant rejection, respectively (21). T lymphocytes reject the transplant in two stages.

First, transplant tissue-based APCs express donor MHC molecules with the help of stimuli such as B7 that are present on Antigen Presenting Cells (APCs) and paired with CD28 on T cell. Second, the receptor dendritic cells invade the transplant tissue and provide the donor antigen to the receptor T cell, and finally, T lymphocytes enter the transplant rejection stage (21, 22). To prevent the transplant rejection for the recipient during the transplantation, the immunosuppressive drugs are prescribed. In bone marrow transplantation, in fact three types of cell such as adult T lymphocytes, hematopoietic stem cells, and lymphoid stem cells are transferred from donor to the recipient, which are the causes of post-transplant biological and clinical events (21, 23).

Because of receiving immunosuppressive drugs prior to transplantation, the number of $TCD4^+$ cells are significantly reduced compared to the control group. $TCD4^+$, which plays a key role in the fight against parasites entered the body, do not get back to normal, and during this period, the latent infection can be reactivated and cause complications (24).

Following immunosuppression, the level of interferon-gamma that is one of the major immune cytokines is reduced, and also the activity of macrophages and the number of $TCD4^+$ are decreased, and the opportunistic parasite *T. gondii* uses these conditions and become reactivated (25).

A study was carried out on 50 renal transplant recipients. Eight transplant recipients had latent *T. gondii*, and the parasite was activated caused by receiving immunosuppressive drugs and the symptoms of fever, lymphadenopathy and neurological syndrome were occurred (26).

The survey of lgM and its elevation represent a recent infection, but in those whose immune systems are defective due to the use of immunosuppressive drugs, the elevated IgM and even IgM might not be observed at all (27, 28). Therefore, the use of serological tests that evaluate antibodies is not sufficient alone (18, 29, 30).

In a study, the death caused by toxoplasmosis was reported in 62% of allogenic pre-HCTSP patients. In case the infection was diagnosed and cured in the early stages, this percentage can be declined (16). Since the reactivation of latent *T. gondii* in immunosuppressive drugs recipients can lead to unpleasant complications, therefore, the rapid and accurate diagnosis of toxoplasmosis greatly help the prevention and control of the disease, especially in those are at risk.

The use of the PCR method in the initial diagnosis of individuals infected with latent disease seems to be useful for preventive treatment (16, 31-34). In the present study diagnosis of *Toxoplasma* infection in allogenic pre HCTSP patients was done by PCR method.

Materials and Methods

Sample collection

The present research was a prospective study on 54 whole blood samples of allogeneic HSCT recipients, who were referring to bone narrow transplantation centers affiliated to Shahid Beheshti University of Medical Sciences, Tehran, Iran in 2018. All patients undergoing study were IgG positive against *T. gondii*. To confirm the recurrence of the disease, a PCR test was performed on all patients using specific *Toxoplasma* primers.

DNA extraction

The blood samples of the subjects were taken at rate of 5 ml, and after separating buffy coat DNA extraction was conducted using the Blood Genomic DNA Extraction kit (yekta tajhiz azma Co. Iran) according to factory protocol. The concentration of extracted DNA was measured by spectrophotometer.

PCR analyses for T. gondii

Specific primer pairs for replicating B1 gene of *T. gondii* consisted of forward primer (Tox1, 5-GGAACTGCATCCGTTCATGAG-3) and reverse primer (Tox2, 5-TCTTTAAAGCGTTCGTGGTC-3)(35). Expected PCR product of *T. gondii* was 194bp PCR method was done with a 25-µl reaction mixture containing: 12.5 µl of Master Mix (ampliqon, Denmark), 10 pmol of each forward and reverse primers, 10 ngr of DNA template. The PCR conditions consisted of 5 min at 94 °C, followed by 30 cycles of 94 °C for 60 sec, 55 °C for 60 sec 72 °C for 90 sec and finally for 5 min at 72 °C. *T. gondii* RH-strain genomic DNA and D.W were used as positive and negative controls, respectively.

Statistical analysis

The data analysis was done by SPSS software version 22 (IBM Corp., Armonk, NY, USA). Qualitative and quantitative data were presented as frequency (percent) and mean± SD, respectively. Data analysis was performed test for qualitative variables by Chi-Square. For quantitative variables Independent Sample *t* test and Analysis of variances (ANOVA) tests were conducted. *P*-value less than 0.05 were considered as significant.

Ethics approval

This study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.RETECH.REC.1396.1121).

Results

Overall, 54 serologically *Toxoplasma* positive pre-HCTSP patients were included. Moreover, 53.7% (n=29) were male, 1.9% (n=1) had germ-line type of the disease. The demographic characteristics of the study subjects are summarized in Table 1. The mean of age for the patients was 41.54± 13.24 year. 40.7% and 25.9% of the study population had Multiple myeloma and Huntington's disease, respectively. No significant differences were found between male and female subjects in terms of age and disease type.

Table 1: The data are presented as frequency (percent) and mean ± SD for qualitative and Quantitative variables, respectively. **ALL**: Acute lymphoblastic leukemia; **AML**: Acute myeloblastic leukemia; **HD**: Huntington disease; **MM**: Multiple myeloma; **NDH:** Nasu-Hakola disease

Variables		***Value***
Age		41.54±13.24
Sex	Male	29(53.7)
	Female	25(46.3)
Diseases	ALL	6(11.1)
Type	AML	9(16.7)
	Germ line	1(1.9)
	HD	14(25.9)
	MM	22(40.7)
	NDH	2(3.7)

Although, the distribution of disease type was not significant across gender groups, but germ line and Nasu-Hakola types only were found in female and male subjects, respectively (Table 2). Fig. 1 shows the mean age of the disease included in the current study. The Multiple myeloma patients had higher age in comparison with other disease, but pairwise comparison showed the difference of age between Multiple myeloma patients were significant with Acute lymphoblastic leukemia, Acute myeloblastic leukemia and Huntington's disease (P<0.05). The PCR assay showed 5.6% (n=3) of the serologically *Toxoplasma* positive patients infected with *Toxoplasma* parasite (Fig.2).

Table 2: the distribution of disease type across gender groups. P-value was reported based on Chi-Square Test. Data are presented as frequency (Percent). **ALL**: Acute lymphoblastic leukemia; **AML**: Acute myeloblastic leukemia; **HD**: Huntington disease; **MM**: Multiple myeloma; **NDH:** Nasu-Hakola disease

Variables		***The groups***		***P-value***
		Male	Female	
Diseases	ALL	3(10.3)	3(12)	0.682
Type	AML	5(17.2)	4(16)	
	Germ line	0(0)	1(4)	
	HD	8(27.6)	6(24)	
	MM	11(37.9)	11(44)	
	NDH	2(6.9)	0(0)	

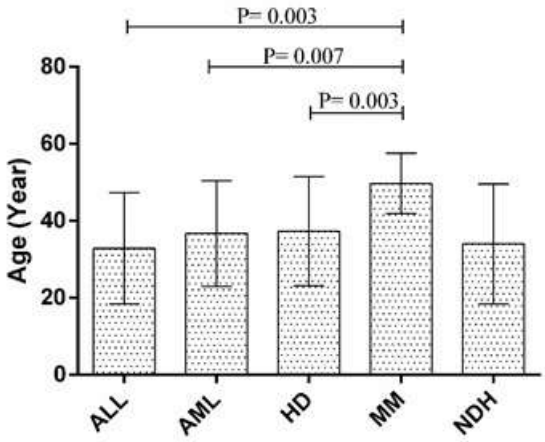

Fig. 1: the comparison of age variable between the disease types. Data are presented as Mean± SD. Data analysis was done by Independent Sample T test. ALL: Acute lymphoblastic leukemia; AML: acute myeloblastic leukemia; HD: Huntington's disease; MM: Multiple myeloma; NDH: Nasu-Hakola disease

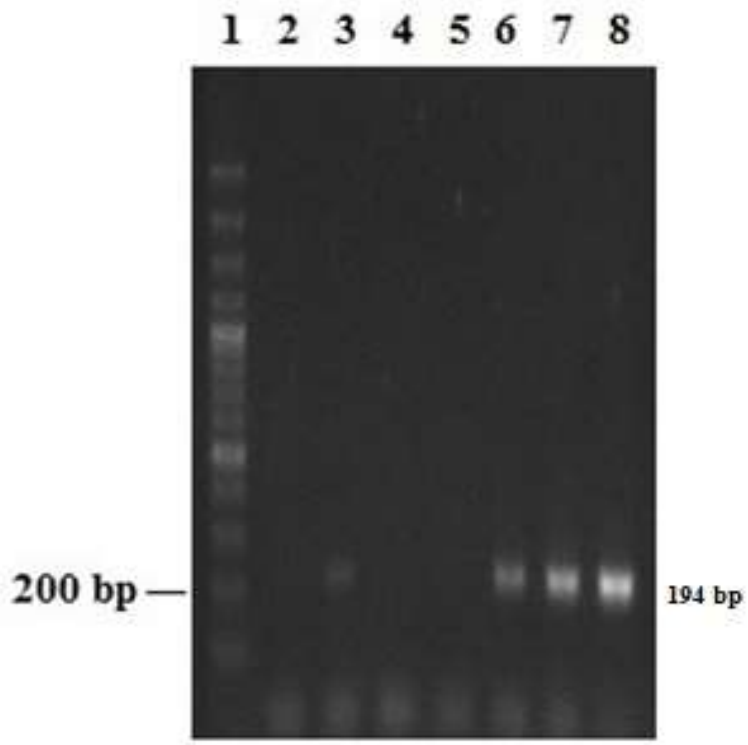

Fig. 2: PCR assay by targeting B1 gene in order to amplify T. gondii. (194 bp). Lanes: 1 100 bp DNA ladder marker. Lane 2 Negative control. Lane 3 Positive control. Lanes 4,5 Negative examples and Lanes 6-8 Positive examples

Discussion

The early diagnosis of the presence of opportunistic infections in HSCT recipients has been a priority for physicians (20). One of such infections is CMV infection, and a preventive treatment at early stages of infection considerably reduces the incidence and mortality rate (36).

Toxoplasmosis is also an opportunistic infection but there is a little information about this disease in these patients. In a study, *Toxoplasma* is reported in 57% of cardiac recipients, 20% of hepatic recipients, and less than 1% of renal transplant recipients (37). Toxoplasmosis has occurred in these patients as a result of activation of a secret infection or in those recently infected with the parasite (31, 38).

The parasite was reactivated in other non-transplanted diseases such as AIDS (39). In patients with immunosuppression, the reactivation of latent infection might not be associated with IgM response (37, 38, 40). In past, most cases were diagnosed only in dissection, because the histologic evidences of invasive toxoplasmosis to CNS or other organs were rarely obtained before death (20).

Diagnosis of *T. gondii* DNA in peripheral blood by PCR could be helpful in early diagnosis of cases of invasive disease (15, 41, 42). The PCR technique has been studied as a non-invasive diagnostic method of cerebral toxoplasmosis in patients with AIDS, and the sensitivity and its properties are reported to be 50%-65% and 95%-100% (39, 43-46). Overall, 41 cases of toxoplasmosis were diagnosed in 15 European transplant centers in patients underwent allogeneic hematopoietic stem cell transplants (HSCTs). In that research, toxoplasmosis in HSCT patients was a severe infection with a high mortality rate, which can prevent its consequences by PCR and earlier diagnosis (15).

Toxoplasma serology was investigated in 251 allogeneic HSCT recipients from 1998 to 2015. All recipients were examined in terms of serology prior to transplantation. The results showed 51 (20.3%) patients were *Toxoplasma* IgG positive. All patients received Trimethoprim/Sulfamethoxazole (TMP/SMX) twice a week. None of the patients had active toxoplasmosis after Allo-HSCt (47). The importance of activating latent infection in toxoplasmosis in these patients is approved, so that the *Toxoplasma*-related deaths in Allo pre HCTSP is reported to be 62%, and this percentage could be reduced if the infection is diagnosed and treated at early stages. In this paper, no clinical trials have been conducted to compare the effectiveness of relapse preventive drugs for *Toxoplasma* after HCT, but the role of TMP/SMX is noted.

The preventive treatment seems to be useful in early diagnosis using common PCR method (16). Toxoplasmosis generally occurs after HSCT, and PCR testing of peripheral blood samples could be a proper tool for performing preventive treatment from severe invasive disease in patients with toxoplasmosis. In this study, detection of *Toxoplasma* infection in HCT patients was performed by PCR.

The results showed indicating recurrence of the disease due to reactivation of *Toxoplasma* cysts due to decreased immune control. Therefore, it is suggested that toxoplasmosis infection be investigated in patients before transplantation.

Conclusion

PCR method has detected considerable incidence of infection for monitoring HSCT recipients at risk for toxoplasmosis, and many patients who showed the incidence of toxoplasmosis had previous infections with the *Toxoplasma* parasite. Accurate and timely detection of toxoplasmosis in immunocompromised patients and timely treatment of the disease can reduce disease reactivation.

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Conflict of interest

The authors declare that there is no conflict of interest.

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