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Toxoplasma antibodies, *Toxoplasma*-DNA and Antibodies of HCV and HIV in Patients with Hemodialysis in Beni-Suef, Egypt

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Abstract

GLOBALLY, *Toxoplasma gondii* (*T. gondii*) infections are frequent in both humans and animals, and toxoplasmosis is among the most prevalent parasitic zoonoses. Hemodialysis (HD) is a potentiating factor for impaired cellular immunity, making patients more susceptible to the opportunistic pathogen *Toxoplasma gondii* and blood-transmitted viruses. To identify the sero-occurrence of Toxoplasmosis, HCV and HIV and the molecular-occurrence of *Toxoplasma* within the sera of patients undergoing HD. A single serum sample was collected from 75 HD patients. Serum samples were searched for anti-*Toxoplasma* IgG and IgM, HCV and HIV antibodies (Abs) using ELISA and *Toxoplasma* DNA using PCR assay. The results of this study indicated that *Toxoplasma* was detected by IgG, IgM and PCR (61.3%, 22.7%, and 1.3%, respectively). HCV Abs was detected in 14.6% of cases, co-infection with *Toxoplasma* (Abs) in 8 cases (10.6%), while HIV Ab wasn't detected in any patients by ELISA. This investigation concluded that seropositive *Toxoplasma* and HCV are frequent in HD patients with a very low prevalence of *Toxoplasma* DNA and regular checks for *Toxoplasma* and HCV seroconversion is desirable in these patients. A two-steps diagnostic immune-molecular approach in one single serum sample for detection of Toxoplasma-DNA in seropositive patients can rule out acute or active toxoplasmosis and avoid unnecessary treatment.

Keywords: Toxoplasma *gondii*, Ig G, Ig M, PCR, ELISA, HCV, HIV.

Introduction

Toxoplasma gondii is a parasitic obligate intracellular protozoan parasite that causes zoonotic infections all over the world. It has a usual clonal population of three virulent human and animal genotypes; I, II and III [1, 2]. *T. gondii* has various intermediate hosts: humans, livestock animals (sheep, goat, camels, cattle and buffaloes), other warm-blooded animals [3-6] and cats act as the definitive (final) host) [7]. Patients with immunocompromising illnesses, such as HIV/AIDS, cancer, and organ transplant recipients who have a compromised immune system, are at risk of dying

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from toxoplasmosis [8, 9]. The impaired cell-mediated immunity because of lymphopenia and dysfunction of the cluster of differentiation 4 (CD4+) T cells, where the counts diminished to < 200 cells/µl significantly linked to high Toxoplasmosis rate [10]. The majority of Toxoplasma infections are asymptomatic in the acute phase, except for cervical lymphadenopathy [11]. As a result, clinical signs alone cannot be used to diagnose toxoplasmosis in healthy blood donors; further parasitological or serological and immunological tests must be used to confirm the diagnosis [12].

Patients undergoing hemodialysis (HD) and suffering chronic kidney diseases (CKDs) are more susceptible to parasitic infections especially toxoplasmosis due to impaired defense immune mechanisms [13]. Renal failure impairs cellular immunity and causes deffient in immunity as lymphopenia [14, 15]. Cellular immunity is essential for defense against intracellular protozoa and viruses infected cells [16]. The prevalence of Toxoplasma low avidity antibodies in patient serum raises the possibility of toxoplasmosis transmission to seronegative recipients via blood transfusion so, *Toxoplasma* screening is important before donation of blood [17]. When hemodialysis patients are compared to healthy controls, they have a significant seroprevalence of *T. gondii* infection. As a result, regular screening programmes for *T. gondii* infection are recommended as a normal clinical treatment for hemodialysis patients [18, 19]. During Hemodialysis (HD), the association with the artificial membrane during dialysis promotes self-death of T cells, resulting in the downturn of monocytes and neutrophils phagocytic extent plus producing unusual cytokines that promotes inflammations [20, 21].

The hepatitis C virus (HCV) and human immunodeficiency virus (HIV) are overlapping viruses sharing routes of transmission [22, 23]. In acute viremia of HCV, NK cells are activated resulting in interferon-gamma (IFN-γ) release affecting virus-infected hepatocyte promotes killing cytotoxicity [24]. HIV attacks **CD4+ T cells** and **monocytes** causing a diminished CD4+ count that sooner makes patients immunocompromised [25]. The investigated prevalence of antibodies against *Toxoplasma gondii* was associated with the presence of hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) infections in pregnant women [26]. *Toxoplasma gondii* infections can cause major difficulties in HIV-positive pregnant women, including miscarriage, stillbirth, and birth abnormalities, as well as promote or exacerbate HCV and HIV vertical transmission from mother to child [27]. Toxoplasmosis is a serious public health concern among HIV-positive people because it causes physical and psychological difficulties due to the loss of CD4 cells, decreased cytokine production, and impaired cytotoxic T-lymphocyte activity, which leads to the reactivation of latent infection [28].

It is crucial to evaluate Toxoplasmosis among this category of patients whose immune state is impaired by the chronicity of the renal disease and are susceptible to complications of *Toxoplasma* or reactivation of the previous infection. Thus, *Toxoplasma* antibodies and DNA products were used to detect infection. In addition to detection of both HCV and HIV antibodies.

Material and Methods

Study population and design

Cross-sectional, hospital-based study was performed including 75 patients undergoing regular hemodialysis at Hemodialysis Center at Beni-Suef University. This research was held from August to December 2022. Patients' characteristics demographic data were recorded. Consents were taken before sample collection from patients.

Samples Collection and processing

Blood samples from the population undergoing haemodialysis were collected. Sera were separated then divided into aliquots and kept frozen at -20 °C for the subsequent assays.

Anti-Toxoplasma IgG and Ig M using ELISA

DRG *Toxoplasma* IgG ELISA EIA-1798 and DRG *Toxoplasma* IgM ELISA EIA-1799 kits (DRG Instruments GmbH, Germany) were used to detect anti-Toxoplasma IgG and IgM in sera of patients. Procedures were done as mentioned by kits and according to the method described by Barakat *et al.* [29].

Detection of HCV and HIV antibodies

HCV and HIV antibodies were detected using HCV ELISA (PRECHEK HCV ELISA test kit, Korea) and HIV ELISA (PRECHEK HIV ELISA test kit, Korea). Assay and interpretation were done as mentioned by the kits' manual according to procedures of Elfadaly *et al.* [30].

Molecular detection of Toxoplasma DNA

Quick-gDNA™ Miniprep Kit was used for genomic DNA extraction from serum samples as instructed by kits [31]. The extracted DNA products were amplified using *Toxoplasma gondii* 529 bp-RE (repetitive elements) primers and PCR reaction conditions that were described by Shaapan *et al.* [32].

Statistical analysis

Collected data were arranged and entered using Statistical Package for Social Science, (SPSS) software version 26. Descriptive data were computerized for categorical data using frequency. Quantitative data were expressed by the mean and

standard deviation (mean± SD) according to the procedures of Elfadaly *et al.* [33].

Results

Anti-*Toxoplasma* IgG and IgM were detected in 61.3% and 22.7%, respectively in patients undergoing HD. Among seropositive patients, *Toxoplasma* DNA was detected in only one case (Fig. 1).

Antibodies for HCV Ab was detected in 14.6% of cases, while HIV Ab wasn't detected in any patients by ELISA (Table 1) and the Mean of Ig G and Ig M titers were 1.71 and 1.38, respectively (Table 2). The majority of patients were female 62.6%, mean age was 50.73± 12.85 (Table 3).

Discussion

Toxoplasmosis is frequently spread among variable hosts. The two major highly vulnerable populations to *Toxoplasma* infection, the fetus and the immunocompromised people including those who are suffering from CKDs [34].

In the current study, *Toxoplasma* IgG (61.3%) and IgM (22.7%) were highly prevalent in the serum of patients undergoing HD. In spite high seroprevalence of toxoplasmosis among patients undergoing HD, *Toxoplasma* DNA was detected by PCR in only one IgG positive case (1.3%, respectively). In *Toxoplasma* reactivation, there is not always changes in the level of antibodies and serodiagnosis of active *Toxoplasma* infection is unreliable. This only PCR positive case indicates active infection. Where IgG positive referring to an old infection and IgM was negative supposing that, at the time of the study, it was too recent to detect the acquired new infection by IgM since it takes one week or more by IgM to appear in the blood. Its peak reached by one to three months later or reactivation of *Toxoplasma* infection where IgM may not appear [35, 36].

Undetectable PCR products among the other samples despite the positive IgM antibodies' levels can be explained by the presence of previous exposure and patients were immune as IgM remained elevated in chronic *Toxoplasma* infection. In addition, the possibility of false-positive IgM antibodies cannot be ruled out. In a similar study, Nahnoush *et al.* [37] reported anti-*Toxoplasma* IgG and IgM, and *Toxoplasma* DNA in 60%, 14.6%, 2%, respectively among HD patients. Nominating to the high-risk meeting these patients with exposure to active toxoplasmosis. They mentioned that DNA product for *Toxoplasma* was detected in two cases with seropositive IgG and one case with seropositive IgM. In contrast, Saki *et al.* [38], reported four cases of *Toxoplasma* positive DNA with only IgM positivity in the four cases.

Our results revealed that HCV antibody was detected in 14.6% of HD patients, co-positivity of *Toxoplasma* and HCV antibodies was reported in 8 cases (10.6%) while HIV antibody wasn't detected in all patients by ELISA. Coinfection of *Toxoplasma* and HCV was reported by other studies [39, 40, 41, 42]. Both the protozoan *Toxoplasma* and HCV need strong cellular immunity for better prognosis [43], a condition lost within HD patients with CKDs, making these patients more vulnerable to a bad outcome.

The problem meeting patients with hemodialysis is the shortage of essential cells sharing in cellular immunity, T cells, NK, macrophages, a situation that can't be improved with hemodialysis [44]. On the other side, we have a resistant intracellular protozoan that needs strong cellular immunity for defense. A bit of bad luck meets hemodialysis patients exposed to Toxoplasmosis and co-infected with HCV.

Different studies reported high seropositive anti-*Toxoplasma* IgG antibodies, 80% [45], 76.5% [46], 60% 27, 56.7% [47] and 33.3% 1 in patients undergoing hemodialysis. Differences in levels of antibodies in between hemodialytic populations are related to differences in the environmental conditions that favours the spread of infection in some areas and personal factors of consumption of contaminated food.

HIV in patients with HD is proportional morbidities, even it shortens the survival of HD patients and rapid reaches end-stage KDs [48]. It is a risk agent for death in HD [49]. Our studied population were negative for HIV Ab by ELISA. Regardless, hemodialysis needs constant long visits/weeks of staying at hospitals and medical centres, in some-extension the need for a blood transfusion by these patients increases the risk of exposure to blood transmitted viruses and protozoa.

Conclusions

Based on our results, in hemodialysis patients we recommend a two-step diagnostic approach in one serum sample from the single patient visit for detection of *Toxoplasma*-DNA in seropositive patients, can rule out acute or active toxoplasmosis and sero-negative patients to rule-in acute toxoplasmosis also avoid unnecessary treatment and development of drug resistance. Debited diseases especially renal failure and HD promotes subside of cell-mediated immunity that is vital in the defense of the intracellular *Toxoplasma*, virus HCV and HIV. Hence, *Toxoplasma* and HCV sero-conversion should be regularly investigated.

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Conflicts of interest

The authors declare that they have no conflict of interest or financial or otherwise.

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Ethical considerations

Patients preceded taking serum samples and collection of data where the Medical Research Ethics Committee-National Research Centre, Egypt under registration number 1-2, approved the work ethically /0-2-1.2022

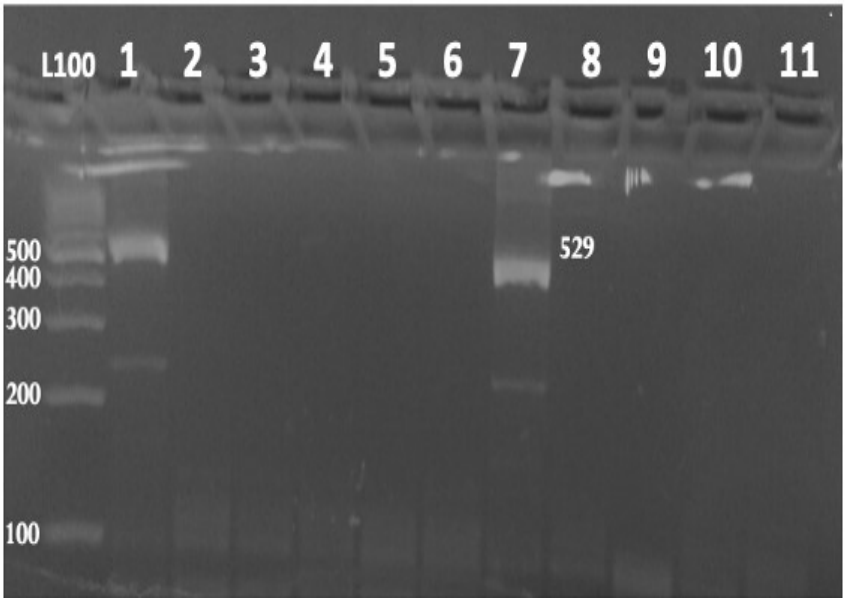

Fig. 1 Stained agarose gel exhibiting the PCR product triggering *Toxoplasma gondii* 529 bp-RE; Footnote: L100 (ladder 100bp), lane 1: positive control, lane 2: negative control, lanes 3-6 & 8-11: negative samples, lane 7: positive sample (529 bp).

TABLE 1. Toxoplasmosis by immune-molecular assays, HCV and HIV serology

	Toxoplasma gondii				HCV ab	HIV ab
	+ve Ig G	+ve Ig M	+ve Ig G +ve Ig M	+ve PCR	+ve	+ve
Positive	46 (61.3%)	17 (22.7%)	15 (20%)	1 (1.3%)	11 (14.6%)	0 (0%)
Negative	29 (38.6%)	58 (77.3%)	60 (80%)	74 (98.6%)	64 (85.3%)	75 (100%)

TABLE 2. Mean of Ig G and Ig M titers

	Mean	±SD
Ig G titer	1.71	.619
Ig M titer	1.38	.309
The Studied population age	50.73	12.85

TABLE 3. Distribution of age and sex among the studied population

Variables		n (%)
Sex	**Male**	28 (37.3)
	Female	47 (62.6)
Age group (years old)		
>30-40		2 (2.7)
>40-50		12 (16)
>50-60		16 (21.3)
>60-70		20 (26.7)
>70-80		25 (33.3)

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الأجسام المضادة للتوكسوبلازما والحمض النووي للتوكسوبلازما والأجسام المضادة لفيروس التهاب الكبد الوبائي وفيروس نقص المناعة البشرية في مرضى غسيل الكلى في بني سويف، مصر

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الملخص

تنتشر عدوى التوكسوبلازما (داء المقوسات) على الصعيد العالمي، في كل من البشر والحيوانات، ويعد داء المقوسات من بين الأمراض المشتركة الطفيلية الأكثر انتشارًا. ويعد غسيل الكلى عاملاً محفزًا لضعف المناعة الخلوية، مما يجعل المرضى أكثر عرضة للإصابة بمسببات الأمراض الانتهازية مثل التوكسوبلازما والفيروسات المنقولة بالدم. والتعرف على التواجد المصلي لداء المقوسات وفيروس التهاب الكبد الوبائى C وفيروس نقص المناعة البشرية والتواجد الجزيئي لمرض التوكسوبلازما داخل أمصال المرضى الذين يخضعون للغسيل الكلى تم جمع عينة مصل واحدة من 75 مريضا يجرى لهم غسيل الكلى. وتم البحث في عينات المصل عن الأجسام المضادة لـ التوكسوبلازما (IgM وIgG) وعن الأجسام المضادة لـ وفيروس التهاب الكبد الوبائي C وفيروس نقص المناعة البشرية (HIV وHCV) باستخدام اختبار الإليزا (ELISA) و تم تعيين والتواجد الجزيئي لمرض التوكسوبلازما (Toxoplasma DNA) باستخدام اختبار تفاعل البلمرة المتسلسل (PCR). وأشارت نتائج هذه الدراسة إلى أن التوكسوبلازما تم اكتشافها بواسطة تعيين الأجسام المضادة والحمض النووى (IgG وIgM وPCR) بنسب (61.3%، 22.7%، و1.3% على التوالي).وتم اكتشاف فيروس التهاب الكبد الوبائي (HCV Ab) في 14.6% من الحالات، وكانت العدوى المتزامنة لفيروس الكبد الوبائي مع التوكسوبلازما في 8 حالات (10.6%)، في حين لم يتم اكتشاف فيروس نقص المناعة البشرية (HIV Ab) في أي مريض بواسطة ELISA. خلص هذا البحث إلى أن التوكسوبلازما وفيروس التهاب الكبد C شائعان في مرضى الغسيل الكلوي مع معدل انتشار منخفض جدًا للحمض النووي للتوكسوبلازما ، ولذلك يفضل إجراء فحص منتظم للأمصال ضد مرض التوكسوبلازما وفيروس التهاب الكبد الوبائي في هؤلاء المرضى الذين يعانون من إجراء الغسيل الكلوي. وإن اتباع طريقة تعيين الإصابة للتوكسوبلازما في عينة مصل واحدة للكشف عن الحمض النووي في المرضى إيجابيي المصل للأجسام المضادة للطفيل ، يمكننا أن نتجنب نقل داء المقوسات الحاد أو النشط لمرضى الغسيل الكلوي ويتجنب العلاج غير الضروري لهم ضد التكسوبلازما.

الكلمات الدالة: التوكسوبلازما، الأجسام المضادة، البلمرة المتسلسل، الإليزا، فيروس نقص المناعة البشرية، فيروس الكبد الوبائي.