

Validity of a new immunochromatographic test in detection of *Toxoplasma gondii* in cancer patients

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Received: 3 October 2018 / Accepted: 24 November 2018 / Published online: 30 November 2018
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Abstract Screening of toxoplasmosis in cancer patients is mandatory especially before starting treatment to guard against life-threatening disseminated disease. Diagnosis of toxoplasmosis rely mainly on serology. The most widely used method for detection of anti-*Toxoplasma gondii* (*T. gondii*) antibodies is the enzyme linked immunosorbent assay (ELISA), being available and reliable. Immunochromatographic tests (ICT) attracted a lot of attention recently being one of the high quality, rapid and easy to perform tests. Available data comparing the performance of ICT versus ELISA techniques have yielded inconsistent results and none compared their performance among the immunocompromised cancer patients. Therefore, we designed this study to compare the performance of a new ICT (the OnSite Toxo IgG/IgM Combo Rapid test) and ELISA techniques for the detection of anti-*T. gondii* antibody as a tool for screening for toxoplasmosis among cancer patients in Cairo-Egypt. Among 180 cancer patients, a total of 110 patients (61.1%) were positive for anti-*T. gondii* antibodies by one or both methods. Agreement between both methods was found in 78.8% of the samples. By using ELISA technique as a gold standard test for the detection of anti-*T. gondii* antibodies, our results showed 87.5% specificity and 74% sensitivity of ICT technique. Moreover, our results proved that ICT is more sensitive in detecting lower level of antibodies than ELISA,

that makes it preferable as a screening test for the immunocompromised patients.

Keywords ELISA · ICT · *Toxoplasma gondii* · Cancer

Introduction

Toxoplasma gondii (*T. gondii*) is an Apicomplexan parasite causing the disease toxoplasmosis. This disease affects more than 2 billion persons worldwide (Harba and Afifi 2012). Therefore, this parasite recently has gained an increasing global attention. Following initial infection, *T. gondii* remains under the control of immune system in a dormant status and converts to the active form only in case of immunosuppression. This causes a disseminated disease with fatal outcome. Better diagnosis and consequent early intervention of this disease can be life saving in immunocompromised person such as cancer patients (Torgerson and Mastroiacovo 2013; Begeman et al. 2017). Different diagnostic methods are available; however, diagnosis is routinely based on serology (Pishkari et al. 2017).

Serologic screening has been demonstrated to be cost effective especially in developing countries with limited resources for testing and medications (Stillwaggon et al. 2011). Till now there is no screening program in Egypt for toxoplasmosis in cancer patients although recently demonstrated to be at high risk for acquiring the infection with life-threatening sequelae (Abdelmalek et al. 2018). The use of an inexpensive, easy-to-use test facilitates screening and consequently allows proper management and diminishes the health burden caused by this disease (Begeman et al. 2017).

In the last few years, development of immunochromatographic tests (ICT) to detect toxoplasmosis has gained

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attention between researchers worldwide. In many countries, researchers have tested different types of ICT as a potential alternative screening tool to the most commonly used diagnostic methods that require special settings with promising results (Wang et al. 2011; Terkawi et al. 2013; Mahinc et al. 2017; Begeman et al. 2017; Chapey et al. 2017). A very recent study showed that another modified ICT testing for *Toxoplasma* infection using finger pricks has an excellent performance with high sensitivity (100%) and specificity (100%) (Lykins et al. 2018).

In this report, we evaluated another new rapid ICT IgG–IgM test (the Onsite Toxo IgG/IgM Rapid Test, CTK Biotech, California, USA) available in the Egyptian market. However, in our study we used it for screening of toxoplasmosis in cancer patients. This test is less expensive than other tests (available at ~ 1 US\$); easy to perform; results are obtained within 15 min and requires no machinery or expertise. The specificity and sensitivity of this test were compared to ELISA being the Gold standard diagnostic test for screening of toxoplasmosis.

Patients and methods

Study design

This cross-sectional study was conducted in the Clinical Oncology department of Cairo University Hospitals after approval of the departmental scientific committee, to assess efficacy of the rapid onsite test in detection of *T. gondii* in cancer patients.

Study population

One hundred and eighty cancer patients with established diagnosis and under treatment were selected for this study; each participant provided one blood sample.

Sample collection

Samples were obtained from each participant, after obtaining verbal consent, by venipuncture into a pre-labeled EDTA tube and left to clot at room temperature then centrifuged at 2000 rpm for 5 min to separate the serum. The separated serum was transferred to 1.5 cm Eppendorf tubes labelled by a unique identification number and matched with patient's identification number in the study. Serum was kept frozen at -20° till performance of the tests.

Examination

We examined the serum for anti-*T. gondii* IgG and IgM by two techniques; the immunochromatography assay and the ELISA technique. For the ICT we used the (OnSite Toxo IgG/IgM Combo Rapid test[®] by CTK Biotech Inc, CA, USA) (Fig. 1). This is a lateral flow chromatographic immunoassay for the simultaneous detection and differentiation of IgG and IgM anti-*T. gondii* in human serum, plasma or whole blood. For the ELISA technique, we used the PRECHEK EIA kit provided by Precheck Bio, INC, CA, USA for the quantitative determination of IgG and IgM class antibodies against *T. gondii* in human serum or plasma (citrate) according to the manufacturer instructions. On the basis of cut values 0.355 and 0.072, patient samples were considered as reactive and non-reactive for anti-*T. gondii* IgG and IgM respectively.

Statistical analysis

Statistical analysis of the results was made by using IBM SPSS statistics version 20 for windows (IBM corporation). Categorical data was analyzed descriptively, and the Chi-square test was used to find the difference between the two variables of study (ICT and ELISA). The P value less than 0.05 was considered as significant.



Fig. 1 ICT cassette showing positive IgG/negative IgM (to the left) and negative IgG/negative IgM (to the right)

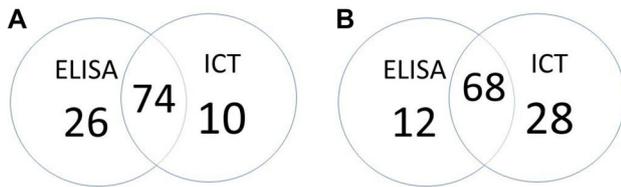


Fig. 2 Venn diagram demonstrating positive (a) and negative (b) cases of *T. gondii* detected by ELISA and ICT

Results

Among 180 cancer patients, a total of 110 patients (61.1%) were positive for anti-*T. gondii* antibodies IgG by one or both methods but none was positive for anti-*T. gondii* antibodies IgM. Agreement between both methods was found in 78.8% of the samples (74 positive and 68 negative).

One hundred patients (55.5%) showed positive results for *T. gondii* by ELISA-IgG. Out of them, 26 patients (26%) were negative by ICT. While among 80 seronegative patients tested by ELISA-IgG, 10 patients (12.5%) showed positive results for *T. gondii* by ICT. On the other hand, by ICT, 84 patients (46.6%) showed positive results for *T. gondii*, among them, 10 patients (11.9%) were negative by ELISA-IgG. Interestingly, all the ICT positive—ELISA negative samples had a very low titer by ELISA (less than 0.05). While among 96 seronegative patients tested by ICT, 26 patients (27%) showed positive results for *T. gondii* by ELISA-IgG with an overall 88% and 84% positive and negative predictive value for ICT respectively. This means that by using the ELISA technique as a gold standard test for the detection of anti-*T. gondii* antibodies, ICT showed 87.5% specificity and 74% sensitivity with an overall accuracy of 80%. The distribution of positive and negative results by both methods for detection of anti-*T. gondii* antibodies is shown in the Venn diagram (Fig. 2 and Table 1).

Discussion

Clinical symptoms of *T. gondii* infection cannot be diagnosed clinically due to the non-specific symptoms which makes resorting to laboratory diagnosis a must (Tenter et al. 2000). In the present study we tested the validity of the OnSite Toxo IgG/IgM Combo Rapid test[®] by CTK Biotech Inc, CA, USA which is commercially available in the Egyptian market at the price of approximately 1 USD. We tested its usefulness in term of specificity and sensitivity compared to ELISA which is the most widely used test and considered the Gold standard diagnostic test for detection of *T. gondii*; nevertheless, it is double priced. Our results showed a comparable sensitivity (74%) and specificity (87.5%) of the test under assessment in comparison to the reference technique. Moreover, our results proved that ICT is more sensitive in detecting lower level of antibodies than ELISA, that makes it more preferable as a screening test for the immunocompromised patients due to the possibility of insufficient antibodies production. This goes in agreement with a study conducted in USA that reported that the *Toxoplasma* ICT test properly diagnosed 21 positive sample with low IgG titers that couldn't be detected by other techniques (Begeman et al. 2017). The study also found the ICT test reliable when testing sera during seroconversion of 5 pregnant women (Begeman et al. 2017). Similarly, another study comparing ICT to the automated Architect, ICT detected 20 positive samples that were in the gray zone and 14 positive samples that were negative using the Architect system. These results were confirmed by Western Blot (Mahinc et al. 2017).

To the best of our knowledge, this is the first report comparing the OnSite Toxo IgG/IgM Combo Rapid test[®] by CTK Biotech Inc, CA, USA with ELISA. Nevertheless, previous studies compared other immunochromatographic assays to ELISA and other standard diagnostic approach. Begeman et al. (2017), studied the efficacy of another ICT: the point of care test for *Toxoplasma* by LDBIO Diagnostics, Lyon, France; which proved to be highly sensitive (100%) and specific (100%) in differentiation between positive and negative sera with known infection. The point

Table 1 Positive and negative cases of *T. gondii* detected by ELISA and the Onsite Toxo IgG/IgM Combo Rapid test[®] among cancer patients

	Positive cases by ELISA (n = 100)	Negative cases by ELISA (n = 80)	
Positive cases by ICT	74	10	Sensitivity ^a = 80%
			PPV = 88%
Negative cases by ICT	26	70	Specificity ^a = 87.5%
			NPV = 84%

PPV positive predictive value, NPV negative predictive value

^aSensitivity and specificity of ICT in comparison to ELISA

of care LDBIO test was validated in a large scale study in France and compared to the Architect automated chemiluminescence test that is widely used in France and gave an excellent performance with 97% sensitivity and 96% specificity (Chapey et al. 2017).

Conclusions

This study shows that *Toxoplasma* ICT IgG-IgM test has the potential for true clinical application as it is a simple, quick, cheap, easy to perform, and reliable test to diagnose *T. gondii* infection among cancer patients making screening feasible. This can be easily incorporated in their workup prior to starting and during treatment to monitor seroconversion, which is crucial for early intervention and hence could prevent serious complications.

Acknowledgements Authors would like to thank the lab personnel at the Clinical Oncology Department, Faculty of Medicine, Cairo University for their help in blood samples collection and serum separation.

Author's contribution Dr. RW designed the idea after careful search of databases, made examination of blood samples, recorded the results and wrote the manuscript. Dr. RA-M got the ethical approval for the study and consent of the patients, made statistical analysis of the results and revised the manuscript.

Compliance with ethical standards

Conflict of interest Authors certify that they have no financial, academic, commercial, political or personal associations that might pose a conflict of interest with the submitted article.

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