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Detection of Acute Toxoplasmosis: The Genitally Transmittable Infection

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Abstract

Objectives: Toxoplasma gondii is an obligate intracellular parasite that infects a broad range of warm- blooded animals including human. Tachyzoites of T.gondii invade the host cell, replicate and finally lead to the lysis of the cell. T. gondii is associated with congenital infection and it can cause encephalitis, or systemic infection in immunocompromised patients. It is important to know whether the infection is recently acquired or is chronic. Differentiation between acute and chronic infection has a dramatic impact, especially for the developing fetus. In this study, Toxoplasma gondii was detected in acute phase of infection in serum sample of a person who had been accidentally infected with tachyzoites of RH strain in the laboratory.

Materials and Methods: Anti- T.gondii IgG antibody was prepared by rabbit immunization with soluble antigen of tachyzoites of RH strain. Capture- ELISA, immunoblotting and PCR were performed in the laboratory.

Results: : Antigenemia and parasitemia was detected in serum sample of infected person by capture_ELISA, immunoblotting and PCR techniques respectively.

Conclusion: Acute T.gondii infection could be detectable in a short period of time in the sera of infected person.

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Introduction:

Toxoplasma gondii, the obligate intracellular parasite invades the host cell and leads to lysis of the cell (1). T.gondii infection is distributed worldwide with a different prevalence of 7.5 to 95% in different parts of the world (2). Toxoplasmosis is associated with congenital infection and can cause severe problems in the fetus (3).

In order to make the decision about the treatment of T.gondii infection, it should be known whether the infection is in acute or chronic phase (4). The diagnosis is based on serological tests with detection of specific IgG and IgM antibodies . These methods have poor efficiency especially in neonates and in immunocompromised patients. So detection of parasite or its components could define the acute toxoplasmosis (5). Recently the PCR method has been successfully used for detection of congenital toxoplasmosis and toxoplasmosis in immunocompromised patients (6).

In this study, antigenemia and parasitemia was detected in a person who was accidentally infected with tachyzoites of T. gondii, RH strain.

Material & Methods:

In a randomized clinical trial (RCT), 92 During a research project, one of the staff from Tehran University of Medical Sciences was accidentally infected with tachyzoites of T. gondii, RH strain by needle injection. She suffered from fever, headache and fatigue. Captur_ELISA and immunoblotting were performed for detection of antigenemia and PCR pereformed for detection of parasitemia in her serum.

Briefly, rabbits were injected with soluble antigen tachyzoites of T. gondii, RH strain. After booster injections they were bled and IgG was isolated from their sera by ammonium sulfate precipitation and ion-exchange column chromatography. For capture- ELISA, a part of isolated polyclonal antibody was conjugated with horse- radish peroxidase enzyme by means of periodate method. Microtiter plates were adsorbed with polyclonal rabbit- antiserum of T. gondii. After incubation, sample was added. The rabbit anti - T.gondii IgG conjugated with horse-radish peroxidase was added followed by choromogenic substrate ortho-phenylen diamidine (Sigma,USA). The reaction was stopped and the absorbance was read at 492 nm.

For immunoblotting, serum sample was electrophoresed using 10% separating gel and 3% stacking gel. After electrophoresis immunoblotting was performed and protein bands

were transferred on nitrocelloluse membrane (45m, Porablot, MN, Germany) over night. After saturation with skimmed milk 2.5%, anti T. gondii rabbit IgG was applied followed by peroxidase conjugated anti- rabbit IgG (Dako, Germany).The reaction was developed with the substrate, diamino benzidine (Sigma, USA).

Amplification of B1 gene was performed with two sets of primers

5'ATTGCCCGTCCAAACTGCAACAACTG and 5'TGGGTCTACGTCGATGGCATGACAAC.

Briefly 10µl of DNA product extraction was added to 10µl of PCR buffer, 5µl of 25 mM $MgCl_2$, 2µl of each 10 mM deoxynucleoside triphosphate, 20 pmol of each primer (Roche, Germany), 10 IU of Tag-DNA polymerase and 18 µl of distilled water. 35 cycles were run, and PCR product and DNA mol. wt marker (100 bp ladders, Biolab, England) were analyzed simultaneously by electrophpresis in an agarose 1%w/v gel (Merck, Germany).Amplified fragments were visualized under UV illumination after staining with ethidium bromide (Sigma, USA).

Results:

Antigenemia was detected in the serum by capture-ELISA. Immunoblotting showed positive result and the 30 kDa antigen band was detected in the patient's serum (Fig 1). PCR assay gave positive result too and the amplified 570 bp fragment was detected in her serum (Fig 2). Antigenemia was detectable after two month in the patient's serum by capture- ELISA. No positive result was seen in control serum.

Discussion:

Serologic diagnosis of toxoplasmosis is complicated by the high prevalence of Toxoplasma antibodies. Alternatively, detection of circulating antigens or cellular

components of the parasite may be much quicker (5,7).

The results obtained here, suggest that although antigenemia occurs in a short period of infection with T. gondii, it is detectable in serum of infected person by capture- ELISA and immunoblotting in this time. Also parasitemia is detectable during this acute phase of infection by PCR in the same serum.

Conclusion :

Acute toxoplasmosis could be detectable in a short period of time after T. gondii infection.

Conflicts of interest:

The authors declare no conflict of interest in this study

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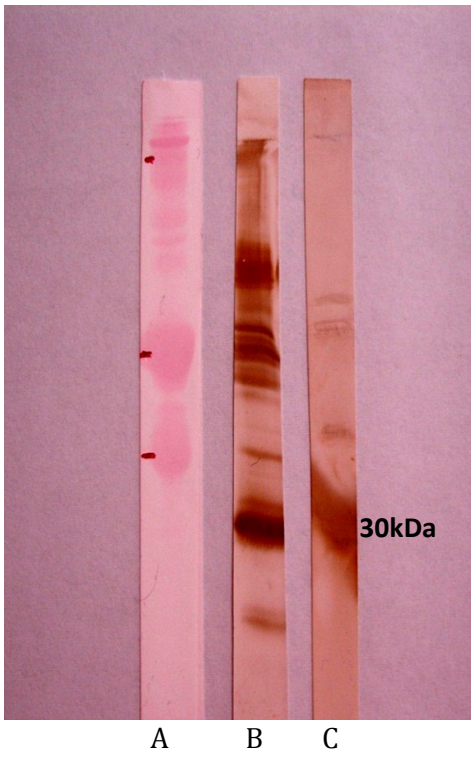

Figure1. Immunoblotting of serum from patient with acute toxoplasmosis **A**. Marker (albumin, ovalbumin, IgG), **B.** Toxoplasma antigen, **C.** patients' serum.

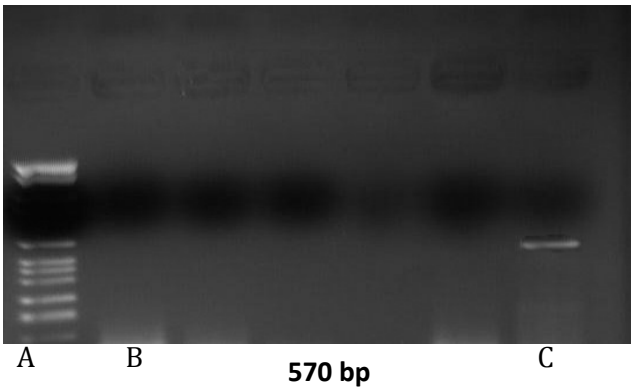

Figure2. DNA amplification of serum from patient with acute toxoplasmosis
A. Sigma marker (No.6), **B.** negative control, **C.** patients' serum

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