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Original Research Article

Prevalence and Molecular Detection of Toxoplasmosis in Babylon Province

Maani Seher Abid Al-Kafaji[1]*

[1]Department of Microbiology-College of Medicine – University of Babylon –Iraq

***Corresponding Author:** Maani Seher Abid Al-Kafaji
Department of Microbiology-College of Medicine – University of Babylon –Iraq

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Abstract: ***Background*:** Toxoplasma is a parasitic illness that befalls a person through the consumption of food of Watchman characteristically meats that have cysts of T. gondii or via contact with the feaces of infected cats or a pregnant woman to the fetus during pregnancy. This study was aimed at assessing the burden of toxoplasmosis in varying geographical locations and enhancing the detection of T. gondii. ***Material and Methods*:** A new study was conducted in Babylon province Thursday (Jan 1, 2023- May 15, 2023) in Teaching hospitals with a sample size of 261 pregnant women and their paired cord blood to be screened of toxoplasmosis. A conventional PCR analysis at a molecular level was conducted on seropositive samples. Such diagnostic tests as MRIs or CT scans were applied with a view to diagnose brain lesions in extreme situations. ***Results*:** Also, anti-toxoplasma antibodies were desired, as well as DNA extraction through the help of extraction kits. Among the samples of maternal blood (87) there were toxoplasmosis-positive cases. Out of 40 cord bloods 38 percent were positive to the anti-Toxoplasma IgG, 3 percent positive to IgM, (15. 3%). Molecular screening using PCR revealed that all the samples to be positive to B1 gene (100%), 20 of the samples contained the P30 gene (50%) and 10 samples contained the 18Rdna gene (25%). Seropositive Sera 1) a sample of cord blood was positive and therefore, one new-born was proven to have congenital toxoplasmosis. Therefore, core relationship between relative parasite transmission risk and neonatal seropositivity was nowhere to be found (chi-square > 0. 05). ***Conclusion*:** This study comes clear about the fact that congenital toxoplasmosis posed itself more in the rural settlements than in the urban establishment. In terms of mitigating the risk of disease, it implies screenings early in life, that is, when a child is born and additional testing.

Keywords: IGG, IGM, Toxoplasmosis, congenital, CT, MRI.

INTRODUCTION

Toxoplasmosis is a much more widespread disease which is caused by such a protozoan parasite as Toxoplasma gondii. The infection takes place in humans and general category of animals, the parasite life cycle takes two hosts first and final host (Fadel, 2024). A lot of the infections have no signs or symptoms, toxoplasmosis can have very serious signs or symptoms in immunocompromised patients and pregnant women, and thus is an important matter of concern to the general public (Fatmawati, *et al*., 2023). Toxoplasma gondii is basically named on an arc shape of the parasite and the parasite where it was first discovered. The reproductive cycle of parasite is sexual and asexual. Sexual phase transpires in only the intestines of feliniodefinitive host (Khattab, *et al*., 2022). The sexual reproduction results in oocyst that is released to intestine site of cat and excreted through cat feces. Oocyst stage may persist in the external environment and find its way into the water, food and the soil. The existence of toxoplasmosis is distributed all over the world, and there are great variations in seroprevalence at a regional level (Sardarian, *et al*., 2019). The infection rates depend on diet, climate, sanitation, and keeping of cats. To a certain extent, it can be seropositive in 90 percent of the population, meaning that people encountered the parasite before. Healthy significance of toxoplasmosis is that it is highly common and may lead to serious outcomes, especially when experienced by immunocompromised patients and pregnant women. Efficient primary health

care, continuing research, and education play an important role in regulating and limiting the effects of this illness globally (Dai, *et al*., 2019).

SPECIMENS AND MATERIAL

1. Sampling

So, the resent study was conducted at a Hospital Hilla teaching in babylon province, between the month of January to May 2023. 100 specimens were collected form pregnant women. (120) samples were collected form rural areas and (41) were form amniotic fluid. (Fig.1). All the (n=261) specimens that were collected on pregnant women (blood samples). Detection of IGG/IGM using rapid test to all specimens received by using sterile syringes under aseptic conditions of 3 ml of blood in pregnant women and umbilical cord blood after child birth.

2. DNA Isolation and Multiplying

Isolation of Isolated Toxoplasma gondii Genomic DNA done by using the Classical protocol of Presto Mini gDNA bacteria Kit (Geneaid, USA).

3. Primers

The primers were used in the present study and also made by the Macrogen company in Korea and made the working solution by diluting the primers in the stock (with the TE buffer) to have a concentration of ten picomoles / microliter before storing it in -20 C. All the oligonucleotide primers of all the genes mentioned in the current research were creations of previous researchers as clarified in table number one. The primer sequences of respective studied gene, its size in base pairs (bp) amplicon and respective reference is explained in table one. The (PCR) primers were used in examined subtypes of system in clinical isolates of Toxoplasma gondii.

Table 1: This Table includes the primer sequences for each studied gene

Primer	Sequences (5'-3')	PCR-Products(pb)	References
B1 Gene	5'-TCTTTAAAGCGTTCGTGGTC-3 5-TGCATAGGTTGCAGTCAGTCACG-3'	193	Colin *et al.*, 2000
P30 Gene	5'-TTGCCGCGCCCACACTGATC-3 5-CGCGACAAGCTGCGATAG-3	914	Colin *et al.*, 2000
Ribosomal Gene (18S rDNA)	5'-CCT- TGGCCGATAGGTCTAGG-3' 5'-TAGGCATTCGGGTTAAAGATTA- 3'	88	Colin *et al.*, 2000

RESULTS

Current research demonstrated the distribution of percentage regarding pregnant women samples by different regions as in Figure (1). The 46 percent of the pregnant women were rural, 38 percent urban and 16 percent of the samples amniotic fluid in the study.

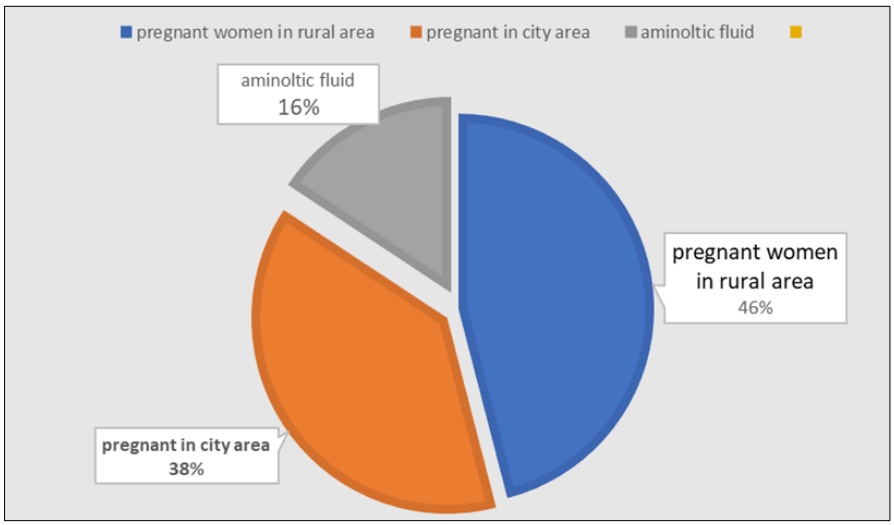

Figure 1: Infection rate with *toxoplasma gondii* among pregnant women in different area of present study

The figure (2) titled results shows that all the cord and blood of pregnant women were referred to immunological tests to detect toxoplasma gondii infection. The rapid test results shows that there are 160 samples only IgG Ab, 40 samples IgGAb and IgM Ab and 60 samples only IgM Ab. (1) IgG and IgM Abs were positive in the sample of amniotic fluid. The presence of Toxoplasma gondii was identified with the help of PCR and their screening was performed against the 18rDNA genes, B1 and P30. The results demonstrated that 40 samples (100%) were positive to the B1 gene, whilst having a PCR product equal to 193 bp. The study of P30 gene shows 50% (20/40) of the samples, showing PCR product of 914 bp. Also 25 % (10/40) of samples contained the 18rDNA gene with a PCR product of 88 bp.

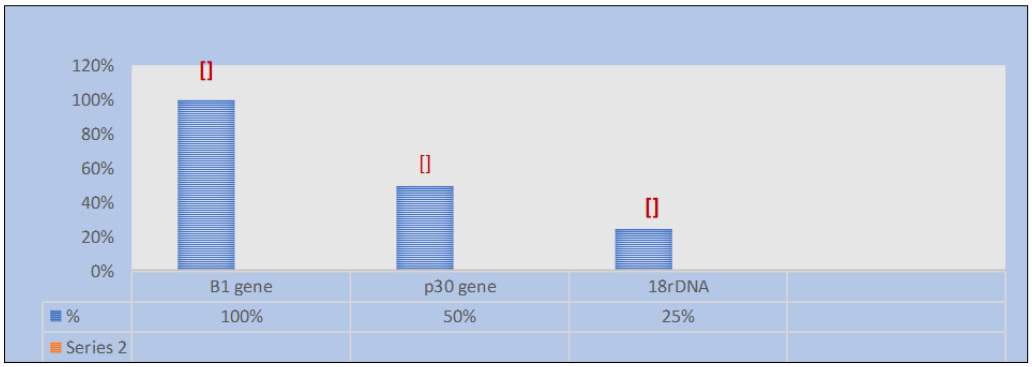

Figure 2: Distribution of different genes used in resent study

In current studies, the results of PCR were indicated in the form of figures (3,4,5). The B1 gene of the T. gondii DNA was used as primers in molecular method PCR. A 193bp species were the product of PCR in which the products was resolved 193 bp at 2 percent TBE agarose gel, ethidium bromide was used to stain and photo was taken under a UV illuminator. PCR procedures performed the cycle 40 times at 93 c for 10 sec, in doing the denaturation process, 57 c for 10 sec in doing the annealing process, respectively and 72 c for 30 sec as the final extension process. To prepare the PCR Blank you would replace the template DNA with sterile distilled water. PCR was carried out using DNA of tachyzoite and primers of the p30 gene specific sequence and the PCR products produced gave one large band of 914 base pairs as shown in Figure (4). They were resolved on 2% agarose/TBE gel and bands of DNA were observed after the fixing of the gel using ethidium-bromide and bands observed using UV light. The sequence of the reactions of PCR was performed using the below temperature profiles: these are denaturation step in which the temperature is set to 95 0 C and the complex is exposed to it within one minute, annealing step in which the temperature is set at 65 0 C and the extension cycle in which the conditions are set at 740 C and at this temperature the cycle is repeated 35 times and each time three minutes. The very same technique of preparing another sample was carried out with no additions of the template DNA; 50ml of distilled water was placed in the Centriix reaction mix. In a similar manner, PCR amplification of the T. gondii DNA by the 18rDNA gene produced one PCR product of 193 base pair as shown in Figure(5). The effectiveness of all the generated output signals was ascertained by means of 2 % agarose/TBE gels that were stained by means of ethidium bromide at an UV setting of low. The PCR has been explained as follows: the first one is the means of launching the DNA strands, 95 mean 10 minutes, the second is the repeated denaturation effected at 95-degree sensitivity, after 10 seconds, third is the annealing zone which requires 60-degree sensitivity on a 30 second sensibility whereafter the fourth effect is the extension which takes place at 74-degree, sensitivity after 1 minute and repeated 35 times. In order to minimize the chance of nonspecific binding of DNA, rather than using water, as a negative control, a no-template sample was used. The detection of PCR products B1 and P30 on the tissue samples was conducted by applying a 10ul B1 P30 amplification product and a loading buffer before laying on 1-2% TBE/agarose gels or ethidium bromide-stained gels after which they are viewed under the UV lamp. In addition, the gathering of the ribosomal samples of 20 microliter that incorporated the products of the gene multiplication was cast with 4per-TBE metaphor agarose gel or 8-10per-TBE polyacrylamide gel. A sample of a control molecule in a range of 50 to 1500 base pairs was also added in all these experiments.

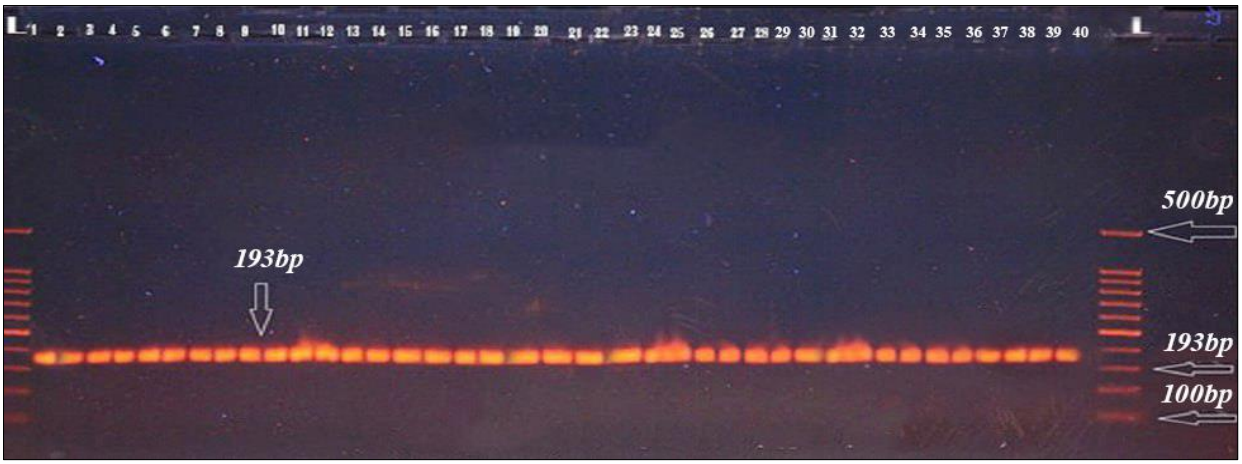

Figure 3: Amplification of 193-bp B1 PCR product from purified genomic DNA

Figure 4: Amplification of 914-bp P30PCR product from purified genomic DNA

Figure 5: Amplification of 88-bp 18 rDNA PCR product from purified genomic DNA

DISCUSSION

The authors of the current study conducted a research regarding the overall prevalence of Toxoplasma antibodies among pregnant women and cord blood samples and received the following results 33.3% and 15.3%, respectively. Out of the 261 cord blood specimens that were tested on rapid test, 40 were positive but only a single case showed IgG and IgM anti-Toxoplasma antibodies. Pinpointing of the presence of such serum-positive samples in molecular analyses of the molar samples established that 0.4 percent of the newborns were infected with congenital toxoplasmosis (CT) a rate that conforms to the estimated particle rate of congenital toxoplasmosis worldwide of about 0.15 percent of live births. Moreover, 46 percent of samples located in rural communities tested positive, 38 percent of the samples in urban regions tested positive, and 16 percent of the samples in the amniotic fluid tested positive. Genetic data showed B1 gene being present in 100 percent cases, P30 gene in 50 percent and 18S rDNA in 25 percent. The current results are comparable to those of (Azimpour-A. *et al*., 2021) and (Sağlam *et al*., 2022) since they also found similar rates of detection. Adding to that, the results obtained by the researchers (Issad *et al*., 2020] were also similar, as it was confirmed that the B1 gene was the most prevalent in all of the specimens. [Mahmood *et al*., 2021) however, posted different results contrary to what was found in our study. Nevertheless, similarly to the latter case, [Cong *et al*., 2020] concluded that the B1 gene was dominant in different types of specimens in case of T. gondii detection. Such findings present the importance of genetic markers, especially B1 gene, in successful identification of T. gondii regardless of the environment and specimen types. Their differences according to the results conducted by various studies state the necessity of a consistent genetic study to raise the stability of the detection of T. gondii. (More than other protozoan parasite, T. gondii has a wide range in distribution as argued in (Zhai, *et al*., 2023) and (Dubey,1997) and it has the characteristic of infecting human at any age since all its stages in the life cycle are infectious and are able to initiate infection due to its

ability to infect warm blooded animals. The knowledge of the genetic composition of this parasite is essential in understanding the pathogenesis mechanism, mode of transmission and possible targets of treatment and prevention (Kompalic, 2007) and (Mercier, 2010). B1 gene is important in the molecular point of detection, P30 in the point of interaction between the host and the parasite and 18rDNA in genetic characterization of T. gondii. The characterization of these genes tells us a lot about the biology and pathogenesis of T. gondii and is part of the work to understand and control the disease of toxoplasmosis (Scott 2020), (McGovernm2020).

CONCLUSION

The B1 PCR protocol is very effective and sensitive to detection of T. Gondii DNA and with it, T. gondii DNA in amniotic fluid has been detectable. This gives the direct indication of Toxoplasmosis in pregnant woman, can contribute to the treatment of congenital toxoplasmosis. This test protocol is very sensitive thus being an asset in early diagnosis and intervention that can work to the benefit of the vomiting pregnancy.

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