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# **Molecular and immunological Diagnosis of *Toxoplasma gondii* in Pregnant and Abortive Women in Al- Muthanna province**

**A thesis**

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## Summary

Primary maternal infection with toxoplasmosis during gestation and its transmission to the fetus continue to be the cause of tragic yet preventable disease in offspring.

During the period from January 2013 to March 2013, a total of 150 blood samples were taken from women who had abortion and from healthy women and normal pregnancy as a control in the AL-Samawa maternal and Children teaching hospital in AL-Muthanna province, southern of Iraq. These samples were tested for detection of anti *Toxoplasma gondii* antibodies.

The present study determined the prevalence of toxoplasmosis among the women by using latex agglutination test (LAT) and enzyme linked immunosorbent assay (ELISA) test, and Q-real time polymerase chain reaction were used in an attempt to diagnose toxoplasmosis in the blood of abortive and pregnant women.

One hundred fifty women were included in this study with a history of single or repeated abortion that was referred by physicians to detect anti-*Toxoplasma* antibodies. The study included one hundred women with spontaneous abortion and 25 women with normal pregnancy and 25 healthy women were used as a control.

Blood samples from all groups of women were tested for specific anti-*Toxoplasma* IgM and IgG antibodies by an enzyme-linked immunosorbent assay (ELISA) and for detection of *B1* gene and HLA alleles of *Toxoplasma* by real-time PCR (qRT-PCR). A total of 100 clinically suspected cases of toxoplasmosis and 50 healthy women were involved in this study. One hundred (100%) of these suspected cases were diagnosed firstly as toxoplasmosis by LAT. Out of these LAT positive cases, only fifty nine (62%) were positive by ELISA (IgG & IgM) which considered as a

confirmed toxoplasmosis cases. Twelve (12%) of abortive women had IgM<sup>+</sup>, (42%) had IgG<sup>+</sup>, (5%) had both IgM<sup>+</sup> and IgG<sup>+</sup>, and (3%) of a normal pregnancy had IgG<sup>+</sup> antibodies.

qRT-PCR test in blood of pregnant and abortive women has advantages in detection of recent or active toxoplasmosis. The *B1* gene was detected from *T.gondii* in the blood of abortive women, 17 (17.7%) of abortive women were identified to be positive for qRT-PCR analysis in comparison with normal pregnancy. While 79 (82.29%) of abortive women, and control group were revealed negative results. Remarkably, 5 (41.67%) of abortive women with IgM<sup>+</sup> were RT-PCR positive. They represent recent infection. In addition, 10 (23.81%) of abortive women with IgG<sup>+</sup>, 2 (40%) of abortive women with IgM & IgG were RT-PCR positive. They represent active infections due to reactivate of latent toxoplasmosis. The results of the present study indicate that molecular diagnoses are highly sensitive, strong and specific methods to detect *T. gondii*, while, the serological methods do not give a real perception of the disease.

Moreover, our work studying the associations between Human Leukocyte Antigen (HLA) (i.e. human major histocompatibility complex (MHC) genes and susceptibility to infections with toxoplasmosis have been described. The present study showed the percentage of the HLA-DQA1 and HLA-DQB1 alleles between the groups, there were decreased percentage of the HLA-DQA1\*0302 and HLA-DQB1\*0501 alleles in the patient group compared to the control group (37.5% vs 62.5%), (16.7% vs 70.8%) respectively, but the percentage of the HLA-DRB1\*1302 explained increased in patients with compared to the with control (66.7% vs 37.5%) respectively.