

## RESEARCH ARTICLE

# The Association of Toxoplasmosis and the Levels of IL-10 and IL-12 in Women with Breast Cancer

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Received: 08th March, 2021; Revised: 16th April, 2021; Accepted: 09th May, 2021; Available Online: 25th June, 2021

## ABSTRACT

The current study conducted during the period from October 2019 to April 2020 in the Department of Biology/ Faculty of Education for Girls/ University of Kufa, which aims to investigate the association of infection with the *Toxoplasma gondii* on the concentration of IL-10 and IL-12 in women with breast cancer in Al Najaf Governorate. The study included 49 serum samples belonging to women with breast cancer and 30 serum samples for healthy women who represented the control group. In both groups, the women ranged between (30–70) years. IgG and IgM antibodies to *T. gondii* parasite and IL-10, IL-12 levels were tested for all serum samples using ELISA technique. The results showed that the highest percentage was for IgG-positive samples, which amounted to 57.14%, while the lowest percentage was for IgM- positive samples and IgM & IgG - positive samples that reached 24.5%. The results also showed that the infection with *T. gondii* has a significant effect at the level  $P \leq 0.05$  in the rate of concentration of IL-10, IL-12, which was  $(359 \pm 28.3, 21 \pm 2.4)$  pg/mL respectively, compared to the control group that was  $(118 \pm 11.8, 8 \pm 1.04)$  pg/mL, respectively. The conclusion from this study that most infections with *T. gondii* parasite were chronic due to the high percentage of IgG-positive cases and that the parasites had a significant effect in stimulating the cellular immune response by increasing the level of IL-10 and IL-12 in women with breast cancer.

**Keywords:** Breast Cancer, IL-10, IL-12, *Toxoplasma gondii*.

International Journal of Drug Delivery Technology (2021); DOI: 10.25258/ijddt.11.2.4

**How to cite this article:** Kanash AK, Yousif JJ. The Association of Toxoplasmosis and the Levels of IL-10 and IL-12 in Women with Breast Cancer. International Journal of Drug Delivery Technology. 2021;11(2):265-268.

**Source of support:** Nil.

**Conflict of interest:** None

## INTRODUCTION

Toxoplasmosis is a common parasitic disease between humans and animals (Zoonotic disease) that is widespread caused by the *Toxoplasma gondii*, which is an obligatory intracellular protozoan parasite.<sup>1</sup> The life cycle of this parasite is complex, as it needs two types of the host, final hosts manifested of the Felidae family, especially cats, and intermediate hosts that include a large number of homeothermic animals that include humans and other types of mammals and birds.<sup>2</sup> Human infected by eating food contaminated with Oocyst located in the feces of infected cats or direct contact with these cats or eating meat containing tissue cysts and others not cooked well, drinking unpasteurized milk, and contaminated water well as contaminated fruits and vegetables. The infection is transmitted directly from the mother to the fetus through the transmission of the active phase (Tachyzoite) through the placenta and rarely through blood transfusions or the transfer of affected organs (transplantation).<sup>3,4</sup> The infection is asymptomatic in the host with a high immune efficiency<sup>5</sup> where the immune system of the host can stop the multiplication of the parasite and the formation of tissue cysts in most tissues of the body, which are highly concentrated in the central

nervous system, skeletal muscles, and cardiac muscles without the appearance of any symptoms in most cases.<sup>6</sup> The true seriousness of the disease appears in individuals with immunodeficiency diseases or immunosuppressants and pregnant women, as the infection can pass from mother to fetus and cause abortion or cause congenital disabilities in the fetus.<sup>7</sup> For cellular immunity, an effective role in resisting the *T. gondii* parasite as macrophages and the lymphocyte T cells on one hand and on the other hand cytokines, the essential elements contribute to the immune response. While the antibodies, especially the Immunoglobulin G (IgG) and Immunoglobulin M (IgM) playing an important role in the complement fixation necessary in the parasite-infected cells decomposition, also it has a major role in the diagnosis of Toxoplasmosis.<sup>8,9</sup>

Cancer is the leading cause of death worldwide<sup>10</sup> despite the exact diagnosis of this malignant tumor, but it is the most common cause of death associated with cancer.<sup>11</sup> Over the past two decades, there has been a clear increase in breast cancer rates.<sup>12</sup> It is the second most common type of cancer after lung cancer and is the most prevalent among the malignant diseases affecting women worldwide.<sup>13</sup>

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The epidemiological relationship between cancer and infections has been proven, as chronic inflammation contributes significantly to the development of cancer,<sup>14</sup> and the release of free radicals during inflammation can stimulate the accumulation of genetic mutations leading to the emergence of hyperplasia (cancer cells)<sup>15</sup>

A group of studies showed the relationship between parasitic infections and human cancers, where serological tests showed that *T. gondii* IgG and IgM, are associated with many cancerous diseases.<sup>16</sup>

This study investigates the association of infection with toxoplasmosis on IL-10 and IL-12 in women with breast cancer in the Al-Najaf governorate.

**MATERIALS AND METHODS**

The study was conducted from October 2019 until April 2020 in the Department of Biology, College of Education for Girls, University of Kufa, Kufa, Iraq. The study included 49 serum samples belonging to women with breast cancer and 30 serum samples for healthy women who represented the control group. In both groups, the women ranged between (30–70) years. According to the manufacturer’s instructions for the examination kits, igG and IgM antibodies to *T. gondii* parasite and IL-10, IL-12 levels were tested for all serum samples using ELISA technique.

**Statistical Analysis**

The results were statistically analyzed using the SPSS version 22, and the chi-square and t-test were used to indicate the significant differences between the study groups.<sup>17</sup>

**RESULTS AND DISCUSSION**

**Seroprevalence of *T.gondii* Antibodies IgG and IgM in Women with Cancer**

The results of the ELISA examination showed in Table 1 that the highest percentage reached 57.14% for IgG (+), while the lowest percentage was 24.5% for IgM (+) and IgM& IgG (+). The results of the statistical analysis through the chi-square test showed significant differences at P ≤0.05.

The current study results agreed with several studies in which the ELISA test was used to examine the sera of women with breast cancer, as observed.<sup>11</sup> The percentage of IgG (+) cases was 86.4% and IgM (+) 7.6%, as well as some study<sup>18</sup> where the percentage of IgG (+) cases was 77.50%, and there were no IgM (+) cases, also the results of some study<sup>19</sup> that the percentage of IgG (+) cases was 72.22% and there are no IgM (+) cases, and who found that the percentage of IgG (+) cases was 96.1% and IgM (+) 3.9%.<sup>20</sup>

The results of the present study were not consistent with previous studies, including one study.<sup>21</sup> in which the percentage of IgG-positive cases was 100% while there were no IgM-positive cases, and in some research results,<sup>22</sup> the percentage of positive cases of IgG was 13% and IgM 2%. The difference in the percentages of positive cases is due to the different types of samples, their numbers, climatic and environmental conditions, and eating habits.<sup>23</sup>

IgM-positive cases that are specific to the *T. gondii* are the beginning of the infection or acute infection after which the IgG antibody is formed, and the positive cases to the IgG specializing in the *T. gondii* mean that the infection has entered into the latent stage or the presence of a previous infection. Here these antibodies remain for long periods of up to a year or more, and that the rise of IgG antibodies in the sera of infected women can be attributed to the fact that the continued presence of IgG is one of the essential components of the humoral immune response in controlling the parasite and limiting its spread.<sup>24</sup>

The positive cases of IgG and IgM antibodies together mean that the infection is chronic, and the immune response to the parasite produces these antibodies.<sup>25</sup> Because the immune system in cancer patients is weak, the recurrence of the chronic form of toxoplasmosis and the activation of the tissue cysts of the *T.gondii* can be very high and dangerous.<sup>26,27</sup> Breast cancer or any other type of cancer makes patients more susceptible to toxoplasmosis, and in the case of a previous infection, the possibility of reactivating the latent infection will be high as a result of severe immune suppression, and at the same time, infection with toxoplasmosis allows cancer to be more fierce and aggressive.<sup>28,29</sup>

The results showed in Table 2 that the highest concentration of IL-10 and IL-12 was in women with breast cancer and toxoplasmosis, which reached (359 ± 28.3 and 21 ± 2.4) pg/mL, respectively, followed by women with only breast cancer, which reached (333 ± 22.2 and 7 ± 1.4) pg/mL, respectively, compared to the control that reached (118 ± 11.8 and 8 ± 1.04) pg/mL, respectively. Statistical analysis using the t-test showed that there were significant differences between study groups for IL-10, and for IL-12, there were significant differences between the group of women with breast cancer & toxoplasmosis and control. At the same time, there were no significant differences between women with breast cancer and the control group at P ≤0.05.

The immunological examinations showed an increase in the level of IL-10 in the sera of women with breast cancer compared to control. This is consistent with some previous

**Table 1:** Serological examination of *T.gondii* antibodies IgG and IgM in women with breast cancer

Antibodies	Breast cancer patients n = 49	
	No.	%
IgG(+)	28*	57.14
IgM(+)	12	24.5
IgG & IgM (+)	12	24.5

\*Significant differences at P ≤0.05

**Table 2:** Levels of IL-10 and IL-12 (pg/mL) in the control group and breast cancer patients according to Toxoplasmosis infection

Cytokines	Control Group M ± SD	Patients (+) M ± SD	Patients (-) M ± SD
IL-10	118 ± 111.8	*359 ± 28.3	333 ± 22.2
IL-12	8 ± 1.04	*21 ± 2.4	7 ± 1.4

\*Significant differences at P ≤0.05

researchers,<sup>30</sup> who found a strong relationship between IL-10 concentration and breast cancer where IL-10 levels in the sera of women with breast cancer were statistically higher than in non-infected women, indicating that high serum IL-10 concentration was closely related to breast cancer. Perhaps the reason for this is that breast cancer enhances IL-10 production by Th2 lymphocytes, as IL-10 it is an immunosuppressive cytokine that facilitates the development of cancer by supporting the tumor's escape from the immune response<sup>31</sup> by preventing the presentation of tumor antigen to CD8 T-lymphocytes by suppressing the expression of MHC class I and II.<sup>32</sup> The results also showed that infection with *T. gondii* parasite, affects the IL-10 level as mentioned in some previous studies,<sup>33,34</sup> which found that the IL-10 level was higher in women with toxoplasmosis compared to non-infected women. It may happen due to parasite's ability to enhance Th2 cells produced cytokines, including IL-10. As the IL-10 controlled the type of immune response by inhibiting the production of IL-6 and TNF- $\alpha$  and then, stimulating Pathway of the immune response of Th2 cells and stimulation of B- lymphocytes to form antibodies.

Nevertheless, IL-10 considered a powerful inhibitor of macrophage ability to kill bacteria inside cells, and microbes like *T. gondii*; therefore, the presence of *T. gondii* will lead to an increase in the IL-10 level.<sup>35-38</sup>

The results of immunological examinations showed that there were no statistically significant differences in the level of IL-12 in women with breast cancer and control, and this was consistent with several studies in which the ELISA test was used to estimate the level of IL-12, including some findings,<sup>39</sup> where the IL-12 level was found to decrease in the sera of women with breast cancer compared to control.

The previous researchers have mentioned no significant difference in levels of IL-12 among women with breast cancer and control, and there were no statistically significant differences in IL-12 levels among women with breast cancer and control.<sup>19</sup> Low IL-12 levels and high IL-10 levels in breast cancer patients indicate a deficiency in Th1 and Th2 responses that may contribute to disease progression.<sup>41</sup>

The results also showed that the infection of women with breast cancer *T. gondii* parasite affects the level of IL-12, where the results of the study showed that IL-12 levels were high in the sera of women with breast cancer and toxoplasmosis compared to control,<sup>19</sup>

Possibly, the reason for the high levels of IL-12 in the sera of women with toxoplasmosis compared to control is that the *T.gondii* parasite is a potent stimulator for dendritic cells (DC) to produce IL-12.<sup>42</sup> After a parasite infection occurs, infected cells produce toxic molecules such as NO and chemical attractants called chemokines that work to attract immune cells, including dendritic cells (DCs), which secrete IL-12.<sup>43</sup>

## CONCLUSION

From this study, most infections with *T.gondii* parasite were chronic due to the high percentage of IgG-positive cases and that the parasites had a significant effect in stimulating the

cellular immune response by increasing the level of IL-10 and IL-12 in women with breast cancer.

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