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Seroprevalence of *Toxoplasma gondii* and *Rubella* virus IgG Antibodies among breast cancer women in Kirkuk City

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***Abstract*— One of the most prevalent illnesses in the globe is toxoplasmosis. It affects a significant percentage of people worldwide. It affects all warm-blooded species, including humans, and is known to be asymptomatic. The second most prevalent TORCH infections are rubella, which is brought on by the rubella virus (RV), and the parasite *T. gondii*. Infection risk is highest in patients with compromised immune systems, such as those with breast cancer. This study aimed to evaluate seroprevalence of *T. gondii* and rubella infection among in breast cancer female. This study included (160) female breast cancer patient and control groups, Data and sample collection started from 1 of September 2025 to Ended on January 2026, this study was conducted at Kirkuk specialized center for oncology and Hematology in Kirkuk city. The blood samples collected from female breast cancer patients at different ages. The blood drawing mechanism included obtaining the patient's consent initially, and drew 5 ml of venous blood after sterilizing the patient's arm. The blood was placed in a test tube with a yellow cap, and the gel tube was then separated from it by means of a centrifuge. After the serum was separated Regarding the blood, the serum was distributed into two Ependorf tubes for the purpose of storing and freezing it for using serological test by IgG antibodies to *Toxoplasma gondii* and *Rubella virus* in human serum can be quantitatively determined in vitro using immunoassay. The Cobas e 411 immunoassay analyzers are designed to employ the electrochemiluminescence immunoassay "ECLIA.". *T.gondii* IgG antibodies showed a significant difference (P-value = 0.001) between patients with breast cancer and control groups. Patients with breast cancer had significantly different levels of rubella (IgG) antibodies than control individuals (P-value = 0.001). Additionally, the rate of co-infection with *T. gondii* (IgG) and Rubella (IgG) in patients with breast cancer A substantial change was observed (P-value = 0.002).**

***Keywords*— IgG antibodies, *Toxoplasma gondii*, *Rubella virus*, seroprevalence, breast cancer.**

I. INTRODUCTION

Toxoplasma gondii (*T. gondii*), an intracellular tissue protozoan parasite that may infect both humans and animals. *T. gondii* is the causative agent of toxoplasmosis, an infection with high prevalence worldwide (1). There is only one species, *T. gondii*, in the genus *Toxoplasma*, and maybe the widest host range of other protozoans. *T. gondii* was recognized in tissues of a congenitally infected infant, and when it was found to cause abortions in sheep in 1957, it is veterinary significance became well established (2). While toxoplasmosis causes a minor illness or an asymptomatic infection, *T. gondii* infections in people are typically asymptomatic. Congenital infection with the parasite Toxoplasma can devastate a child's health (3). The parasite's capacity to infect all animals has allowed it to produce a host-specific clinical response. Toxoplasmosis causes major complications in immunocompromised individuals, while it is asymptomatic in more resilient individuals (4). To diagnose toxoplasmosis in humans, a combination of biochemical, serological, histological, or molecular methods is employed. The clinical symptoms of toxoplasmosis are non-specific and cannot accurately differentiate it from other infectious diseases. In fact, *T. gondii* infection can exhibit similarities to other infectious diseases (5). The IgG avidity test was developed to aid in distinguishing between previous and recently acquired infections. This test focuses on determining the avidity (functional affinity) of IgG antibodies that are specific to *toxoplasma*. Typically, following exposure to an antigen, the newly formed antibodies exhibit relatively low average affinity (6). The exact relationship between *T. gondii* and breast cancer has not been fully understood until now; however, it has been reported that *T. gondii* plays a key role in maturing the dendritic cells which activate the CD8+ T cells that able to eliminate the malignant cells (7).Also, the attenuated *T. gondii* has demonstrated great promise as a creative immunotherapy for breast cancer treatment; further investigations are needed to exploit its full potential and clarify the specific mechanisms involved. Moreover, the experimental investigation on animals has revealed that *T. gondii* possesses an effective ability to inhibit the development and metastasis of breast tumors (8). Although the whole mechanism is unclear, it has been demonstrated that *T. gondii* can reduce the threat of breast tumors by regulating the signaling pathway of breast cancer, thus suppressing malignant cell development and metastasis (9). The Rubella virus is an enveloped, positive-stranded RNA virus that is a member of the Matonaviridae family and genus Rubivirus (10). As the second and third of just three

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members of the genus Rubivirus, Ruhugu and Rustrela viruses joined the Rubella virus. The rubella virus has a diameter of 60–70 nm and is generally spherical in shape. It is made up of a pleomorphic nucleocapsid with a 9,762 nucleotide single-stranded, positive-sense RNA genome. Three structural proteins make up the virus: two in the envelope (E1 and E2) and one in the core (capsid or C protein) that surrounds the RNA (11). The rubella cellular receptor is still unknown. The glycoproteins that make up the envelope proteins, E1 and E2, are heterodimers that extend from the virus to create surface spikes that are 6 to 8 nm in diameter. Neutralizing and antigenic epitopes are linked to E1, which seems to be the predominant surface molecule. The virus has a single serotype (12). Some research suggests that a history of febrile infectious childhood diseases like rubella may be associated with a lower risk of non-breast cancers, though not breast cancer specifically (13). The main connection is that the MMR (measles, mumps, rubella) vaccine, which contains rubella, is considered a potential oncolytic therapy against certain cancers, and that a weakened immune system due to cancer makes individuals vulnerable to measles infections. Some studies have shown that viruses in the MMR vaccine, including the rubella component, may have anti-cancer properties by causing cell cycle arrest and being toxic to certain cancer cells, suggesting the MMR vaccine could be studied for cancer treatment (14). Among the existing serologic tests, enzyme linked immunosorbent assays (ELISA) are most frequently used to assess Rubella-specific IgG and IgM because to their high sensitivity, high specificity, technical simplicity, speed, and low cost (15-16).

II. Materials and methods

A. Collection of blood samples:

Blood samples were taken from 160 breast cancer patients who visited oncology and Hematology in Kirkuk city, between 1 of September 2025 to Ended on January 2026. An additional 80 persons were included in a control group who were aged between (30 and more than 60), Marital status (married, unmarried), Patients receiving chemotherapy were included, A closed- end questionary was used to collect out data from the participants appendix. They were asked about their age, residency, contact with animal or cats , family history with breast cancer and History of rubella vaccination. drew 5 ml of venous blood after sterilizing the patient‘s arm, Conditions such as recurrent miscarriages, hepatitis, HIV, and other types of cancer such as uterine and ovarian cancer were excluded the samples were collected and allowed to remain at room temperature for 30 minutes in sterile gel tubes, then centrifuged for five minutes at 3000 rpm to isolate the serum. The samples were placed in an eppendorf tube and kept at -20 °C until required (11).

B. Detection of T.gondii (IgG): antibody and Rubella (IgG) antibody use cobas E 411 immunoassay analyzers.

Test principle

The sandwich principle. The experiment takes eighteen minutes in total. The first incubation consists of six microliters of material, a biotinylated recombinant T. gondii-specific antigen, and a monoclonal anti-Rubella antibody recombinant antigen tagged with a ruthenium complex. Second incubation: Following the addition of streptavidin-coated microparticles, biotin and streptavidin interact to bind the complex to the solid phase. The reaction mixture is aspirated into the measuring cell, where the microparticles are magnetically captured onto the electrode's surface. After that, unbound materials are eliminated using ProCell II M. A photomultiplier is used to monitor the chemiluminescent emission that is produced when a voltage is applied to the electrode.

▪ A calibration curve, which is specially produced using 2-point calibration, and a master curve obtained through the Cobas link are used to determine the results.

C. Statistical Analysis:

Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS, version 27, IBM). Categorical variables were compared using based on the p-value at P≤ 0.05.

III. Ethical Approval and Consent

This study was approved by the NTU Institutional Review Board (Approval No 13459 on 18/11/2025). Written informed consent was obtained from all participants prior to enrollment.

IV. Results

The results showed that 36 (22.5%)of the breast cancer patients were infected with *T.gondii* (IgG), and 124(77.5%) without *T.gondi* (IgG), based on the serological analysis with the cobas E 411 immunoassay analyzers, reporting a significant differences (P-value = 0.001). As well as results showed that 10 (12.5%) of the control persons were infected with *T.gondii* (IgG), and 70(87.5%) without *T.gondi* (IgG), reporting a significant differences (P-value = 0.001) as listed in Table 1.

TABLE **1.** *T.gondii* IgG antibodies among patients with breast cancer compared with control persons.

Case	*T.gondii* IgG IU/ML	No. (%)	Mean ± Sd	P-value
Patients with breast cancer	**Positive**	36 **(22.5%)**	218.70± 96.2	0.001
	Negative	**124(77.5%)**	0.58 ±0.021	
Control	**Positive**	10 **(12.5%)**	326.17± 180.5	0.001
	Negative	70**(87.5%)**	0.43 ±0.061	
Total: Patients (160)			**Total: Control (80)**	

The results showed that 39(24.3%) of the breast cancer patients were infected with Rubella (IgG) antibodies, and 121(75.7%) without Rubella (IgG) antibodies, based on the serological analysis with the cobas E 411 immunoassay analyzers, reporting a significant differences (P-value =

0.001). As well as results showed that 21(26.2%) of the control persons were infected with Rubella (IgG), and 59(73.7%) without Rubella (IgG), reporting a significant differences (P-value = 0.001), as well as between the patient groups and the control group, the results showed a significant difference of 0.04 as listed in Table 2.

TABLE **2.** Rubella (IgG) antibodies among patients with breast cancer compared with control persons

Case	**Rubella (IgG) IU/ML**	**No. (%)**	**Mean ± Sd**	**P-value**
Patients with breast cancer	**Positive**	39(24.3%)	218.99± 98.78	0.001
	Negative	121(75.7%)	.078 ±0.26	
Control	**Positive**	21(26.2%)	219.13± 193.72	0.001
	Negative	59(73.7%)	.015 ±0.086	
P-value = 0.04				
Total: Patients (160)			**Total: Control (80)**	

The results showed that patients with breast cancer who were aged between (41 and 50) had the greatest risk of having a toxoplasmosis infection (33.3%), followed by patients who were aged between (51-60) had a toxoplasmosis infection (25%), while patients who were aged between (30-40) and (>60) had the lowest infection rates (19.4% and 22.3%). The findings revealed a non-significant variation (P-value= 0. 3) in age between patients with breast cancer and *T. gondii* infections as listed in Table 3.

TABLE **3.** Age of breast cancer witht *T.gondii* (IgG)

Age(Years)	No. Patients With *T.Gondii* (IgG)	(%)	P-value
30-40	7	19.4	**0. 3** *P≤ 0.05 significant variation
41-50	12	33.3	
51-60	9	25	
>60	8	22.3	
Total	36	100	

The results showed that patients with breast cancer who were aged between(41-50) had the greatest risk of having a Rubella (IgG) infection (38%), followed by patients who were aged between (>60, 51-60 and 30-40) had a Rubella (IgG) (23%, 21% and 18%), respectively. The findings revealed a non-significant variation (P-value= 0.7) in age between patients with breast cancer and Rubella (IgG) infections as listed in Table 4.

TABLE **4.** Age of breast cancer with Rubella (IgG)

Age(Years)	No. Patients With Rubella (IgG)	(%)	P-value
30-40	7	18	**0. 7** *P≤ 0.05 significant variation
41-50	15	38	
51-60	8	21	
>60	9	23	
Total	39	100	

There were a significant difference (P-value = 0.001) based on the histologic grading of breast cancer patients with *T.gondii* (IgG) with grade 2 which have 17 (47.2%), where the result showed a high rate of breast cancer patients with *Rubella* (IgG) 18 (40%) in grade 2 compared with grade 1 and grade 3, which have 8 (22.2%) and 11 (30.6%), respectively breast cancer patients with *T.gondii* (IgG). As well as breast cancer patients with *Rubella* (IgG) in grade 1 and grade 3 recorded that 9 (30%) and 12 (30%), respectively.

Further Co- infecting high rate of breast cancer patients with grade 2 recorded that 3 (50%) while grade 1 and 3, which have 1 (16.6%) and 2 (33.4%), chi-square X^2= 2.28, respectively as shown in Table 5.

TABLE 5 **.** Histologic grading of breast cancer patients with Co- infection with *T.g ondii* (IgG)+ Rubella (IgG)

Grades	No. Patients With *T.gondii* (IgG) (%)	No. Patients With Rubella (IgG) (%)	Co- infection
Grade 1: Low Grade	8 (22.2%)	9 (30%)	1 (16.6%)
Grade 2: Moderate Grade	17 (47.2%)	18 (40%)	3 (50%)
Grade 3: High Grade	11 (30.6%)	12 (30%)	2 (33.4%)
Total	**36 (100%)**	**39 (100%)**	**6 (100%)**
P-value	0.001		
chi-square X^2	2.28		

V. DISCUSSION

According to this study, there are differences in the seroprevalence rates of toxoplasmosis and rubella co-infection between the age groups of patients with breast cancer. This could be explained by immune system deterioration and infection exposure brought on by unfavorable living circumstances brought on by the conflict. According to certain research, the seroprevalence of toxoplasmosis rose between the ages of 41 and 50. This was described as a reflection of a person's higher lifetime risk of co-infection with *T. gondii* (IgG) and Rubella (IgG). Chemotherapy-exposed cancer patients are known to experience immunological suppression, which facilitates the activation of parasites to spread throughout the patient's body (17), and the primary determinant of toxoplasmosis infection is the host's immune system (18). In contrast to a study conducted in Thi-Qar province, Iraq (19), which reported high seroprevalence among cancer patients undergoing Grade 3 (37.63%) compared with those in Grade

2, the previous study found that toxoplasmosis has a slightly higher seroprevalence among cancer patients receiving chemotherapy in Grade 2 than those not receiving chemotherapy. This distinction results from the way chemotherapy inhibits the immune system in cancer patients. (20). and the host's immunological condition is the main risk factor for toxoplasmosis infection. The prior study found that toxoplasmosis has a slightly higher seroprevalence among cancer patients receiving chemotherapy than those not receiving chemotherapy, in contrast to a study carried out in Thi-Qar province, Iraq (21), which reported a high seroprevalence of toxoplasmosis among cancer patients undergoing Grade 3 (37.63%) compared to Grade 2. This difference arises from the way cancer patients' immune systems are suppressed by chemotherapy.

The results of Al-Tameemi study that was carried out in Basrah Province, Iraq, to determine the prevalence of *T. gondii* infection was revealed a high incidence of *T. gondii* infection among patients in the 41–50 age group (22). Furthermore, the results of a previous study Assim that examined 90 cancer patient samples from Baghdad, Iraq, in order to determine the prevalence of toxoplasmosis infection among cancer patients and its findings demonstrated that among breast cancer patients, the age range of 31 to 40 years old had the highest *T. gondii* infection, which is inconsistent with the present study (23).

The present results showed that were a high incidence of *T. gondii* in grade II among breast cancer patients (59.15%) compared with grade I and grade III (7.04%) (33.8%), respectively. Due to the tumor burden and systemic effects of cancer, patients with advanced breast cancer (such as those in stage III) may have a weakened immune response. Breast cancer patients may be more susceptible to opportunistic diseases like toxoplasmosis (24).

The results of this study are dissimilar to those of another study in Guangzhou, China, which recorded a higher infection rate of toxoplasmosis and Rubella infection among breast cancer patients in stage II (50.6%) compared to other stages (25).

This study is not without limitations, such as the apparent lack of avidity testing, determination of possible correlations between *T. gondii* seroprevalence and Rubella infection and many socio-demographic variables in the women studied. Additionally, the current seroprevalence results were based on samples of Iraq women of **breast cancer** and may not be representative for the whole country. Furthermore, to examine the immunological condition of the Iraq female population with *T. gondii* and Rubella infection, it is necessary to conduct additional large-scale and multi-center screening studies in which IgG avidity testing, immunoblotting, and PCR is performed. The same is true for estimating the risk of congenital infection in newborns.

VI. Conclusion

The findings indicate that toxoplasmosis is significantly more common in patients with breast cancer. For instance, co-infection and rubella (IgG) are more common in patients with moderately developed malignancies (Grade II). These findings further emphasize the need for additional investigation into the role of co-infection with T. gondii and Rubella in the development of breast cancer.

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Conflicts of Interest

The authors declare no conflict of interest.

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