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Determination the Specificity of IgG Antibodies for Specific Antigen of Toxoplasma gondii and Rubella Virus among Breast Cancer Female

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ABSTRACT: Toxoplasma gondii and rubella virus are significant health concerns among breast cancer female women. Therefore, it is crucial to determine the prevalence of using specificity tests of Toxoplasma IgG and Rubella IgG to different Toxoplasma and Rubella antigens, the primary goal of this study is to ascertain the duration of T. gondii infection and rubella virus infection among women with breast cancer. Data and sample collection for the study began on September 1, 2025, and ended in January 2026 in order to examine the prevalence of toxoplasmosis and the rubella virus in 110 cases of breast cancer. The female breast cancer patients' avidity of antibodies for particular Toxoplasma antigens was assessed using a line immunological assay after their Toxo-IgG seroprevalence was assessed using the percentage of female breast cancer patients who were Toxo IgG + Rubella IgG- seropositive was 25 (23%). The frequencies for Toxoplasma ROP1C, MIC3, GRA7, GRA8, p30, MAG1, GRA1, and rSAG1 antigens were 3 (12%), 3 (12%), 19 (76%), 9 (36%), 24 (96%), 16 (64%), 8 (32%), and 10 (40%) positive. The rates of high avidity were greater than those of low and intermediate avidity when taking into account Toxo-IgG's specificity for these antigens. Therefore, P30 antigen had the greatest rate of high specificity at 96%. Additionally, the avidity test was used to establish the specificity of the identified specific Rubella-IgG seropositive females with (Toxo IgG-Rubella IgG+) and the different Rubella antigens (E1, E1-E2, Protein c, and E2). Among the 16 females with Rubella-IgG seropositive breast cancer, the frequencies of Rubella IgG against these antigens (Toxo IgG-Rubella IgG+) were 16 (16%), 3 (19%), 4 (25%), and 2 (13). The high percentages of particular Toxo-IgG seropositive against different Toxoplasma antigens are determined to be P30. Additionally, among women with breast cancer, the rates of particular Rubella-IgG seropositive against different Rubella antigens are E1 seropositive.

1. INTRODUCTION

Whenever many T. gondii pathogens infect an organism at the same time, it can result in compound infections, which may impact the severity and replication of the disease. These infections frequently make diagnosis and therapy more difficult, especially when there are rubella virus antigens present that could obstruct intracellular interactions. [1]. 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 [2]. Antibodies, antigens, cytokines, interleukins, and other elements of the immune response are all evaluated by serological assays. IgM is the first immunoglobulin produced in response to the initial T. gondii infection; it is a short-term reaction that can continue for weeks or years, but IgG arrives approximately two weeks after infection and can stay in the body for a considerably longer period of time [3]. For a precise diagnosis, T.

T. gondii antibody detection is crucial. The *Toxoplasma gondii* parasite is known to produce the proteins indicated (ROP1c, MIC3, GRA7, GRA8, p30, MAG1, GRA1, and rSAG1). They serve as antigens in infection confirmation testing, especially when identifying and evaluating IgG antibody specificity. In order to precisely assess the immune response and distinguish between acute and chronic infections, these proteins are generated in high-frequency, pure forms and mounted on test strips [4]. The IgG specificity test was created to help differentiate between illnesses that were acquired lately and those that were acquired in the past. The goal of this test is to measure the avidity (functional affinity) of toxoplasma-specific IgG antibodies. The newly generated antibodies usually have a low average affinity after being exposed to an antigen [5]. Although the precise connection between *T. gondii* and breast cancer is still unclear, it has been suggested that *T. gondii* is essential for the maturation of dendritic cells, which in turn activate CD8+ T lymphocytes that can eradicate the cancerous cells [6]. Additionally, attenuated *T. gondii* has shown a lot of promise as a novel immunotherapy for the treatment of breast cancer; however, more research is required to fully realize its potential and elucidate the precise mechanisms involved [7]. Furthermore, research conducted on animals has demonstrated that *T. gondii* is capable of effectively preventing the growth and spread of breast cancers [4]. Some research suggests that a history of febrile infectious childhood disorders [8], such as rubella, may be associated with a lower risk of non-breast malignancies, but not specifically breast cancer. [9, 10]. The three primary structural proteins that make up rubella virus antigens are nucleocapsid protein C, glycoproteins E1 and E2. E2 is a membrane-spanning glycoprotein, whereas E1 is the primary envelope antigen and hemagglutinin in charge of receptor binding. Transport and immunity require the heterodimer complex (E1-E2) that these proteins form [11]. A history of childhood viral disorders, including rubella, may reduce the incidence of non-breast malignancies, according to research [12]. The level of antibody binding is reflected in IgG specificity to rubella virus antigens [13]; low specificity indicates a recent infection (within the last few months), whereas high specificity suggests a previous infection or immunization. By determining whether IgG antibodies are newly generated (low specificity) or mature (high specificity), this test is essential in the early stages of pregnancy to rule out acute rubella infection, which carries a significant risk for fetal abnormalities [14]. The immune system of the body creates IgG antibodies that bind to the virus's antigens poorly (low specificity) when you initially become infected. The antibodies develop over time as the infection worsens, becoming more specific and attaching to the antigens with considerably greater strength (high specificity) [15].

MATERIALS AND METHODS

Collection of blood sample

The study conducted at oncology and Hematology Specialized center in Kirkuk city, between 1 of September 2025 to end on January 2026. 110 patients with breast cancer had their blood drawn, and 80 controls were part of the control group. After gathering the samples and letting them sit at room temperature for half an hour in sterile gel tubes, the serum was separated by centrifuging them for five minutes at 3000 rpm. Until needed, the samples were stored at -20 °C in an eppendorf tube (11). Serum examined for seroprevalence of Anti-toxoplasma IgG and Anti-Rubella IgG by using ECLIA Technology then Recomeline IgG immunoassay are used to determine the specificity of IgG for *Toxoplasma* Antigens (Recomeline) and recomblot immunoassay are used for evaluate the specificity of IgG to specific Antigens of Rubella virus (E1/E2/C, E1_E2). Statistical Analysis: The Statistical Package for the Social Sciences (SPSS, version 27, IBM) was used to perform statistical analyses. The Chi-square test was used to compare categorical variables at $P \leq 0.05$.

Ethical Approval and Consent

The NTU Institutional Review Board authorized this study on November 18, 2025 (Approval No. 13459). Prior to participation, each subject provided written informed permission. Results A total 110 breast cancer females whose were examined for seroprevalence of specific Toxo-IgG and Rubella IgG by using Electrochemiluminescence immunoassay (ECLIA). The seroprevalence of Toxo IgG+Rubella IgG- was 25(23%), Toxo IgG - Rubella IgG+ was 16(15%), Toxo IgG + Rubella IgG+ was 4(4%), as well as Toxo IgG - Rubella IgG- was 63(58%) among breast cancer females. While the seroprevalence among 80 control (without breast cancer females) the seroprevalence of Toxo IgG+Rubella IgG- was 6(8%), Toxo IgG - Rubella IgG+ was 10(13%), Toxo IgG + Rubella IgG+ was 2(2%), as well as Toxo IgG - Rubella IgG- was 62(77%) was 5 (5.0 %), From statistical analysis it has been found that all two factors of contacts show Significant differences in seroprevalence of specific Toxo-IgG and Rubella IgG distribution in general, P value =0.01, as shown in Table 1.

TABLE 1: The seroprevalence of specific Toxo-IgG and Rubella IgG among breast cancer females and control by using avidity test

Results	Breast Cancer Females		Control (Healthy Females)	
	No.	(%)	No.	(%)
Toxo IgG+Rubella IgG-	25	23	6	8
Toxo IgG - Rubella IgG+	16	15	10	13

Toxo IgG + Rubella IgG+	4	4	2	2
Toxo IgG - Rubella IgG-	63	58	62	77
Total	110		80	
X ² =2.4		P value =0.01 Significant different		

Regarding the specificity of the identified specific Toxo-IgG against the different Toxoplasma antigens (ROP1C, MIC3, GRA7, GRA8, p30, MAG1, GRA1, rSAG1). Among the 25 females with Toxo-IgG seropositive breast cancer, the rates of Toxo-IgG against these antigens (Toxo IgG + Rubella IgG-) were 3 (12%), 3 (12%), 19 (76%), 9 (36%), 24 (96%), 16 (64%), 8 (32%), and 10 (40%) seropositive for Toxoplasma ROP1C, MIC3, GRA7, GRA8, p30, MAG1, GRA1, and rSAG1. In comparative the specificity of Toxoplasma IgG against Toxoplasma Antigens among seropositive control groups (healthy female), the rates of Toxo-IgG against these antigens were 2 (33%), 2 (33%), 3 (50%), 6 (100%), 1 (17%), 1 (17%), and 1 (17%) seropositive for Toxoplasma ROP1C, MIC3, GRA7, GRA8, p30, MAG1, GRA1, and rSAG1 antigens, as indicated in Table 2.

TABLE 2. The rates of specific Toxo-IgG seropositive against various *Toxoplasma* antigens (Toxo IgG+Rubella IgG-) among breast cancer females by using avidity test

<i>T.gondii</i> antigens	Toxo –IgG seropositive of females with (Toxo IgG+Rubella IgG-)			
	Breast Cancer Females (25)		Control (Healthy Females) (6)	
	No.	%	No.	%
ROP1c	3	12	2	33
MIC3	3	12	2	33
GRA7	19	76	2	33
GRA8	9	36	3	50
P30	24	96	6	100
MAG1	16	64	1	17
GRA1	8	32	1	17
rSAG1	10	40	1	17
$\chi^2 = 4.2$	P value = 0.02		Highly significant	

In terms of the specificity of the identified Toxo-IgG seropositive females within (Toxo IgG + Rubella IgG+) to different used Toxoplasma antigens, the highest rates of specificity were 75% for P30 antigen in females with breast cancer; while, the highest rates of specificity were 100% for P30 antigen in Control (Healthy Females), which also reported P=0.001 highly significant, as indicated in Table 3.

TABLE 3. The specificity of Toxo-IgG (Toxo IgG+Rubella IgG+) seropositive to various *Toxoplasma* antigens.

<i>T.gondii</i> antigens	Toxo –IgG seropositive of females with (Toxo IgG+Rubella IgG+)			
	Breast Cancer Females (4)		Control (Healthy Females) (2)	
	No.	%	No.	%
ROP1c	1	25	1	50
MIC3	1	25	1	50
GRA7	2	50	1	50
GRA8	1	25	1	50
P30	3	75	2	100

MAG1	1	25	1	50
GRA1	1	25	1	50
rSAG1	1	25	1	50
$\chi^2= 82.1$	P value =0.001			Highly significant

Regarding the specificity of the determined specific Rubella –IgG within of females with (Toxo IgG-Rubella IgG+) the various Rubella antigens (E1, E1-E2, Protein c and E2) was identified separately by using Recomblot Technique. The rates of Rubella IgG against these antigens (Toxo IgG-Rubella IgG+) among the total 16 Rubella -IgG seropositive breast cancer females were 16 (16%), 3 (19%), 4(25%), and 2(13) respectively. As well as the rates of Rubella IgG against these antigens (Toxo IgG-Rubella IgG+) among the total 10 Rubella -IgG seropositive Control (Healthy Females) were 10(100%), 3 (30%), 2(20%), and 3(30) respectively, also reported P= 0.01 significant different as shown in Table 4.

TABLE 4. The rates of specific Rubella –IgG seropositive of females with (Toxo IgG-Rubella IgG+) among breast cancer females by using avidity test

Rubella antigens	Rubella –IgG seropositive of females with (Toxo IgG-Rubella IgG+)			
	Breast Cancer Females (16)		Control (Healthy Females) (10)	
	No.	%	No.	%
E1	16	100	10	100
E1-E2	3	19	3	30
Protein c	4	25	2	20
E2	2	13	3	30
$\chi^2= 4.08$	P value = 0.01			significant different

Regarding the specificity of the determined specific Rubella –IgG seropositive of females with (Toxo IgG+Rubella IgG+) the various Rubella antigens (E1, E1-E2, Protein c and E2) was identified separately by using avidity test. The rates of Rubella IgG against these antigens (Toxo IgG+Rubella IgG+) among the total 4 Rubella -IgG seropositive breast cancer females were 3(75%), 1(25%), 1(25%), and 1(25) respectively. As well as the rates of Rubella IgG against these antigens (Toxo IgG+ Rubella IgG+) among the total 2 Rubella -IgG seropositive Control (Healthy Females) were 2(100%), 2(100%), 1(50%), and 1(50) respectively, also reported P= 0.02 significant different as shown in Table 5.

TABLE 5. The rates of specific Rubella –IgG seropositive of females with (Toxo IgG+Rubella IgG+) among breast cancer females by using avidity test

Rubella antigens	Rubella –IgG seropositive of females with (Toxo IgG+Rubella IgG+)			
	Breast Cancer Females (4)		Control (Healthy Females) (2)	
	No.	%	No.	%
E1	3	75	2	100
E1-E2	1	25	2	100
Protein c	1	25	1	50
E2	1	25	1	50

4. DISCUSSION

T. gondii is one of the many infections that cancer patients can contract. According to the findings of a meta-analysis, cancer patients had a threefold increased incidence of toxoplasmosis compared to healthy individuals (16). The current study revealed that among females with breast cancer, the rates of Toxo IgG + Rubella IgG- were 25 (23%), Toxo IgG - Rubella IgG+ was 16 (15%), Toxo IgG + Rubella IgG+ was 4 (4%), and Toxo IgG - Rubella IgG- was 63 (58%) due to a persistent latent infection that was previously acquired and is associated with elevated IgG levels (17). Recombinant antigens (ROP1c, MIC3, GRA7, GRA8, p30, MAG1, GRA1, and rSAG1) are used in RecomLine Toxoplasma IgG immunoblot to measure IgG specificity. Research has shown that high specificity rates (e.g., 96% for P30) are predictive of infections older than three to six months. High avidity denotes a chronic infection, whereas low specificity indicates a recent infection (less than four months). Low or high specificity is the basis for the Toxo IgG specificity test's primary usage (18). While high specificity indicates a low probability of intrauterine transmission during the first trimester, low specificity indicates an increased risk of transmission. IgG specificity results that are intermediate or moderate have been thought to be challenging to interpret for risk assessment purposes (19). These days, scientists think that intermediate/moderate Toxo IgG avidity needs to be interpreted cautiously and further research with other commercial Toxo IgG avidity is necessary. The findings demonstrate a distinct variation in the immune response to components of *Toxoplasma gondii* in breast cancer patients. GRA7 (76%) and p30 (96%) had the greatest IgG positive rates, suggesting a robust immune response to these antigens. A poorer response or overlap with a subsequent infection may be indicated by the lower frequencies of ROP1C and MIC3 (both 12%) (20). since high antibody concentrations indicate a prior infection, analysis utilizing the avidity adhesion test aids in distinguishing between acute and chronic infection. These findings demonstrate *Toxoplasma gondii*'s ability to cause cancer, especially through long-lasting immunological reactions (21). It has been shown that *T. gondii* can lessen the risk of breast tumors by controlling the signaling pathway of breast cancer, hence inhibiting the growth and metastasis of malignant cells, albeit the exact method is unknown. IgG is first created after an infection, although its binding affinity is initially lower (low avidity). The immune system's antibody receptor genes gradually mature over the course of weeks or months, increasing their capacity to bind to the antigen and producing stronger specificity.

Thus, Low specificity, very recent infection High specificity Older/persistent infection. The avidity data (high specificity vs. low specificity) for P30 (MAG1) provide compelling evidence of older/established exposure to *Toxoplasma* in the group with breast cancer. 75% of females with breast cancer (P30) have high avidity. 100% high avidity in controls and healthy females (P30) $P \geq 0.001$ (referred to as "highly significant") indicates statistical significance. This pattern indicates that the immune response to the P30 antigen is primarily of the high- specificity (more mature/older infection) kind, which is consistent with chronic or earlier exposure and less likely recent infection. The distribution of P30 avidity differs significantly between healthy controls and breast cancer patients, with controls exhibiting a greater percentage of high- specificity responses. In females who tested positive for Toxo-IgG, the avidity assay for P30 (MAG1) revealed primarily high-avidity responses in both groups (22). Compared to 100% of healthy controls, 75% of breast cancerpatients showed strong specificity. There was a statistically significant difference ($P = 0.001$). Using the same specificity percentages (low/high) for the other antigens you measured (ROP1C, MIC3, GRA7, GRA8, MAG1, GRA1, and rSAG1), I will assist you in writing a coherent paragraph summarizing all antigens consistently including comparisons and implications). These findings indicate that *Toxoplasma* exposure associated with the P30 antigen is primarily of older/chronic nature (high-specificity IgG). Rubella IgG specificity was measured against the E1, E1-E2, Protein C, and E2 rubella antigens in females with (Toxo IgG -Rubella IgG+). Breast cancer patients and healthy controls had different distributions of avidity-defined rubella IgG, with the breast cancer group exhibiting reduced avidity-associated patterns in antigen-specific rates. Overall, these differences were statistically significant ($P=0.01$), indicating that females with breast cancer in the (Toxo IgG -Rubella IgG+) category had a different rubella immune recognition profile. These results could be explained by variations in the time of rubella exposure or the kinetics of IgG maturation between the two groups, since IgG avidity often indicates the maturation/age of the humoral response (23). IgG avidity testing was used to assess Rubella-specific IgG against individual Rubella antigens (E1, E1-E2, Protein C, and E2) in the subgroup of females with (Toxo IgG + Rubella IgG+). Variations in the period of Rubella exposure within this subgroup may be reflected in the distribution of avidity categories across antigens, which showed antigen-dependent changes in the strength of Rubella-specific IgG binding. The percentages of Rubella-IgG avidity positivity against the antigens were reported as E1: 3 (75%), E1-E2: 1 (25%), Protein C: 1 (25%), and E2: 1 (25%) in females with breast cancer (total = 4n Rubella-IgG seropositive within the subgroup). The comparable rates were E1: 2 (100%), E1-E2: 2 (100%), Protein C: 1 (50%), and E2: 1 (50%) in the healthy control females (total = 2n). Overall, there was a statistically significant difference ($P=0.02$) in the between-group comparison for Rubella-specific antigen avidity patterns. According to the findings, two individuals tested positive for Rubella IgG, and one also tested positive for *Toxoplasma* IgG, indicating pre-existing immunity to both toxoplasmosis. This implies that some people may have dual immunity to significant infectious agents during breast cancer and that prior rubella infection is widespread in the group (24).

CONCLUSION

The high rate of particular Toxo-IgG seropositive against different Toxoplasma antigens were determined for P30 antibody. Additionally, among women with breast cancer, the were highest rates of particular Rubella-IgG seropositive against different Rubella antigens are E1 antigen.

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Conflicts of Interest

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