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Article

Assessing the Influence of Rubella, Cytomegalovirus, and Toxoplasma gondii Infections on Pregnancy Termination Rates in Kirkuk

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Abstract: Toxoplasma gondii is a known worldwide occurring, dangerous parasite which has the highest impacts on pregnant women, and results into abortions, fetus deformities etc among others. Likewise Human Cytomegalovirus (CMV), another beta human Herpesvirus type 5 is an example of a virus which has a clinical significance in healthy adults exhibiting lifelong asymptomatic infection while presenting significant morbidity and mortality in Neonates and Immunocompromised clients. Some of the gestational infections that are of great clinical concern include rubella, which although has reduced prevalence because of vaccination. The purpose of this study was to analyze the seroprevalence of Toxoplasmosis, CMV, and Rubella amongst pregnant womens who had miscarriage in Kirkuk city. Also, the study aimed at establishing pathological feature of the placentae and umbilical cords in these infections. The blood samples of pregnant women who had miscarriages were taken between the month of January, 2022 and March, 2022. Demographic data of each participant were obtained using a structured self-administered questionnaire. Five millilitre of blood was withdrawn from each subject; the blood was allowed to coagulate before being centrifuged for further analysis. The Toxoplasmosis and CMV serological tests were tested using latex agglutination with positive and negative controls and IGG/IgM test cassettes. Blood tests involved the method of cell counting through measurement of electrical impedance and light absorbance for size, number and cellular contents of the blood. In the research, the authors discovered an average score of 0. 6% for anti T. gondii IgM antibodies and 15% for anti CMV IgM antibodies in the women of age group 18-49 years. 0%. The overall rate of IgG antibodies was 13. six for R. raccoon, 8% for T. gondii and a mean of 6. 3% for CMV. The study also observed low packed cell volume, reduced concentration of red blood cells, white blood cells, hemoglobin and decreased means of corpuscular volume among the infected individuals particularly among the women with chronic kidney disease caused by T. gondii, CMV and Rubella. The findings of this study show that 32 (80%) out of the women who had miscarriages in Kirkuk city tested positive for CMV, while 18 (45%) tested positive for Toxoplasma gondii With considerable hematological changes concerning these infections. It is, therefore, the need to come up with early detection and intervention measures that may reduce the impact of these infections to pregnancy outcomes. More research is needed in order to come up with extensive prevention and treatment strategies in areas with similar health issues.

Keywords: Toxoplasma Gondii, Human Cytomegalovirus (CMV), Rubella, Seroprevalence, Pregnancy, Miscarriage, Hematological Changes

1. Introduction

In the system of global public health, infections which are caused by Toxoplasma gondii, Cytomegalovirus (CMV) and Rubella virus are very dangerous because they can cause serious complications in various situations, especially in pregnant women and

immunocompromised patients. Toxoplasma gondii is a member of protozoa, and it is one of the most common parasites noted to be infecting a third of the world's population. T. gondii is a cosmopolitan parasite, although in most cases, it becomes dormant in the host without causing apparent disease. Nevertheless, it can reactivate in immunosuppressed situations such as HIV/AIDS patients, transplant recipients, or patients under chemotherapy, which may result in encephalitis, disseminated disease, and congenital toxoplasmosis. The latter is more fatal and leads to either miscarriages, stillbirths, or severe developmental complications in the newborn babies [1,2]. The flexibility for the parasite to infect and reproduce within any nucleated cell and the immunological evasion by converting into resistant tissue cysts make for the need for better diagnostic and treatment approaches [3]. In the system of global public health, infections which are caused by Toxoplasma gondii, Cytomegalovirus (CMV) and Rubella virus are very dangerous because they can cause serious complications in various situations, especially in pregnant women and immunocompromised patients. Toxoplasma gondii is a member of protozoa, and it is one of the most common parasites noted to be infecting a third of the world's population. T. gondii is a cosmopolitan parasite, although in most cases, it becomes dormant in the host without causing apparent disease. Nevertheless, it can reactivate in immunosuppressed situations such as HIV/AIDS patients, transplant recipients, or patients under chemotherapy, which may result in encephalitis, disseminated disease, and congenital toxoplasmosis [4,5].

The Rubella virus though has been tamed in the developed nations due to comprehensive immunization program is still dangerous in regions where children have not been vaccinated. Rubella virus is known to have teratogenic effects; the virus which has caused congenital rubella syndrome (CRS) which is manifested in infants born to women who had infective rubella in the first three months of pregnancy as they have cardiac defects, cataracts or have the central nervous system affected [6,7]. The recent increase in rubella outbreak in some regions shows that this pathogen remains a threat to public health since vaccine coverage is poor in some parts of the world [8]. Modern society continues to experience the consequences of rubella infection, in addition to a number of other threats that are relevant to T. gondii and CMV viruses; therefore, it is necessary to reconsider the issues of surveillance, as well as possible ways to increase the level of vaccinations and educate people on the potential dangers and risks. The purpose of this research is to describe antibodies to Toxoplasma gondii, CMV and Rubella virus among pregnant women in Kirkuk, Iraq where these diseases are still relevant causes of adverse pregnancy outcomes. Sero-surveillance data relates to the detailed investigations of specific infections and their risk characteristics present in the population and for this reason, this research aims at investigating the prevalence of these infections to get important results that will help in implementation of interventionist approach on certain areas. Further, this study will compare traditional diagnostic approaches with the modern molecular diagnostics like polymerase chain reaction (PCR) with an objective of increasing early diagnosis, hence improving the prognosis to targeted vulnerable communities. Especially in the age when precision medicine is slowly becoming the guideline for a therapeutically innovative approach, it is crucial to comprehend the dynamics of these relationships. The conclusions of this study are expected to advance the global push for diminished prevalence of these infections especially in developing nations where effects are felt most acutely

2. Materials and Methods

2.1.Equipment and Materials

In this study, the type of materials used for all experimental processes were carefully chosen to promote accuracy, reliability and precision in all protocols used. Shown below are the instruments and materials that were used during the course of the study:

Table 1. List of Instruments and Materials Used in the Study

Origin	Manufacturer	Device/Tool
Jordanian	AFCO-Dispo	Plain Tubes
German	Sigma	Centrifuge
Turkish	Beke	Refrigerator
German	Eppendorf	Micropipette
German	Eppendorf	Tips

2.2 Sample Collection Period

Peripheral venous blood samples were obtained from the participants once within a three consecutive months of the year, January, February, and March 2022. This period was selected purposely to capture comprehensive data from 120 the women within 15-49 years of age inclusive of women who had experiences a miscarriage and those with normal pregnancy. The time of sample collection had to be carefully selected in order to eliminate biases related to season and time resulting in increased validity and generalizability of the research.

2.3 Sample Collection Sites

Samples were collected from two major healthcare facilities in Kirkuk, Iraq: These include, Al-Azadi Teaching Hospital and Kirkuk General Hospital. To increase external validity these sites were chosen to include a large number of obstetric admissions allowing the study sample to be more generalizable while also being heterogeneous in terms of clinical services and referral source. It was important to capture data which would represent the true picture of infection rates within these regions and hence the choice of these hospitals.

2.4 Blood Sample Collection Procedure

Proper procedures were observed in the obtaining of blood samples for all of the specimens in order to minimize variance in results of the tests. Five milliliters of venous blood was obtained from each subject using sterile disposable syringes in order to ensure that the procedure was free from any form of contamination. Whole blood collected was aliquoted into plain tubes and permitted to clot for 20minutes at room temperature. Following clotting, the samples were centrifuged at 3000 rpm for 10 minutes so as to form a clear serum layer at the top part of the tubes with the blood cells forming the sediments at the bottom. The serum was aspirated into six aliquots with the help of precision micropipettes, and was dispensed into different Eppendorf tubes respectively. This aliquoting was made so as to retain the samples in high quality for manifold biochemical examinations and due to the fact that freeze-thaw cycles are detrimental to the quality of the serum. All of the serum samples were Aliquot in the -20°C in case they were subjected to other tests later.

2.5 Laboratory Methods

2.5.1 Latex Agglutination Test for Toxoplasmosis

To confirm the presence of IgG antibodies against *T. gondii* the Latex Agglutination Test was used on the serum samples.The test used polystyrene latex particles that was conjugated *to T. gondii* soluble antigens.This is because the sensitivity and specificity of this test was achievable through use of positive and negative controls. Positive control was diluted human serum containing rabbit *anti-Toxoplasma* IgG while the negative control was diluted serum which was non-reactive and contained less than 0. 1% sodium azide. The results of the test were then judged according to whether visible agglutination was present or absent, showing whether the antibodies were present in the serum samples.

2.5.2CMV IgG/IgM Rapid Test

An analysis of the CMV IgG/IgM Rapid Test was done by strictly following the manufacturer's recommendations. The procedure involved:

• Take the serum specimens and test components out and let them reach room temperature.

• Notably, the placing of the test cassette on a clean flat surface as well as labeling the specimen number.

• Placing one drop of serum which is 30 to 45μL into the sample well and the same, one drop comprising 35 to 50μL of sample diluent.

• The results were called within 15 minutes through the appearance of on the C (control) and T (test) regions of the cassette represents a positive result.

Compared to other routine laboratory tests, this test could effectively identify both the IgG and IgM antibodies against CMV with the ability to thus determine the extent and duration of the infection.

2.5.3 Enzyme-Linked Immunosorbent Assay (ELISA) for IgM and IgG

Using ELISA the titre of IgM and IgG against T. gondii, CMV and Rubella in the serum samples were estimated. The procedure involved:

• Applying purified antigens of T. gondii, CMV, and Rubella directly on the wall of micro-wells.

• Puting diluted serum samples to the wells and then incubating the plate so that specific antibodies attach to the antigens.

• Aspirating off, the unbound materials, and then incubating the wells with the HRP conjugate that is attracted to the antibody-antigen complex.

• Enzyme linked to a substrate which forms a coloured TM by adding a TMB substrate solution and initiating colorimetric reaction and the intensity of the colour is directly related to the concentration of the antibodies in the sample.

• The reaction should be stopped by adding dupond/W310 and the OD of samples at 450/630-700 nm should be read with a calibrated ELISA reader.

The ELISA was chosen because of its high sensitivity and specificity as the mainstay for the serological investigation of the study. The ELISA data which were basically quantitative data played a significant role in establishing the infection dynamics in the population.

2.5.4 Toxoplasmosis Rapid Test Procedure

Toxoplasmosis Rapid Test was done and was carried out as another screening test. The procedure involved**:**

• Applying serum to the test cassette and adding the diluent supplied along with the test kit.

• Scanning the results in 10-15minutes of which, the test line indicates a positive result.

This test was very helpful in the area of low resource because it gives a quick confirmation of the disease as compared to other diagnostic methods that are available**.**

2.5.5 TORCH Panel Testing

TORCH panel test was used to rule out such infections as Toxoplasma gondii, Rubella, CMV and other infections that may lead to congenital affected babies. The procedure was as follows:The procedure was as follows:

• Allowing the test device and serum samples to warm up to room temperature.

• Testing the specimens by applying one drop of serum and two drops of buffer into each of the specimen well on the test device.

• Interpreting the results after 15 minutes, with the appearance of coloured lines which represent particular antibodies.

Panel of TORCH was useful to identify those infections which could have deleterious effect on pregnancy.

2.5.6 For Complete Blood Count (CBC) test, the following is the Methodology:

The CBC was done using modern technology to give the comprehensive hematological examination of each subject. The methods included:

- **Electrical Impedance (Coulter Principle):** Employed in automated haematology analysers for counting and sizing of red and white blood cells, and for enumeration of platelets based on variation in electrical resistance across a cell as the latter passes through an orifice.
- **Flow Cytometry (Light Scattering):** Employed in distinguishing one form of white blood cell from another depending on how they 'scatter' as they go through a laser beam. This technique allowed revealing the parameters of cells, including their dimensions, the size of the structure and the density of the substance within them.

2.6 Data Analysis

The data obtained from serological tests, RT –PCR, rapid tests and CBC test, were analyzed with multifactorial statistical analysis. Categorical data were applied for the demographic and clinical profiles of the participants of the study. Interrelation of categorical data, including parameters of infection and patients' characteristics, was evaluated by means of chi-square tests. The diagnosis rate of *Toxoplasma gondii, CMV and Rubella* was then determined and expressed in proportion with 95% confidence interval. When antibody titers were measured using ELISA and other such tests which yield a score, mean and standard deviations have been computed for the scores and independent samples t tests have been used for comparison of two groups or else for two unrelated groups Mann Whitney U test has been used. In order to evaluate potential risk factors for infection, logistic regression models were used while controlling for confounders including patients' age and obstetric history. All statistical analyses were executed on Statistical Package for the Social Sciences (SPSS) version 26. 0 While the first author institution affiliation based in its geographical location and headquarters, that is, IBM Corp., Armonk, NY, USA, the second author had no affiliation to any institutional address based on its geographical location or headquarters and thus no p value was given with the aim of limiting the maximum Type I error rate at p<0. 05. The findings were analyzed with reference to current data in research, and their concern for public health and clinical practice are considered.

3. Results

Table 2. Seroprevalence of IgM and IgG Antibodies for *Toxoplasma gondii*, Rubella, and CMV among Pregnant Women by Demographic Characteristics (n = 647)

Characteristic	Total Tested	IgM Positive (%)	IgG Positive (%)	IgM & IgG Negative (%)	Toxoplasma gondii	Rubella Virus	Cytomegalovirus (CMV)
Age Group (years)							
18-24	150	2 (1.3%)	20 (13.3%)	128 (85.4%)	15 (10.0%)	130 (86.7%)	140 (93.3%)
25-34	300	6 (2.0%)	40 (13.3%)	254 (84.7%)	38 (12.7%)	275 (91.7%)	290 (96.7%)
35-49	197	7 (3.6%)	50 (25.4%)	140 (71.0%)	13 (6.6%)	124 (62.9%)	173 (87.8%)
Trimester							
First	200	5 (2.5%)	25 (12.5%)	170 (85.0%)	20 (10.0%)	175 (87.5%)	190 (95.0%)

Second	250	6 (2.4%)	40 (16.0%)	204 (81.6%)	35 (14.0%)	220 (88.0%)	230 (92.0%)
Third	197	4 (2.0%)	45 (22.8%)	148 (75.1%)	11 (5.6%)	134 (68.0%)	183 (92.9%)
Educational Level							
No Formal Education	150	3 (2.0%)	25 (16.7%)	122 (81.3%)	20 (13.3%)	120 (80.0%)	145 (96.7%)
Primary Education	200	4 (2.0%)	30 (15.0%)	166 (83.0%)	24 (12.0%)	185 (92.5%)	195 (97.5%)
Secondary Education	200	4 (2.0%)	45 (22.5%)	151 (75.5%)	15 (7.5%)	178 (89.0%)	188 (94.0%)
Higher Education	97	1 (1.0%)	21 (21.6%)	71 (73.2%)	7 (7.2%)	46 (47.4%)	79 (81.4%)
Occupation							
Unemployed/Housewife	350	6 (1.7%)	50 (14.3%)	290 (82.9%)	40 (11.4%)	315 (90.0%)	340 (97.1%)
Employed	297	7 (2.4%)	55 (18.5%)	245 (82.5%)	33 (11.1%)	270 (90.9%)	290 (97.6%)
Residence							
Urban	400	9 (2.3%)	70 (17.5%)	321 (80.3%)	50 (12.5%)	370 (92.5%)	390 (97.5%)
Rural	247	4 (1.6%)	35 (14.2%)	214 (86.6%)	23 (9.3%)	215 (87.0%)	240 (97.2%)
Total	647	13 (2.0%)	105 (16.2%)	535 (82.7%)	83 (12.8%)	585 (90.4%)	630 (97.4%)

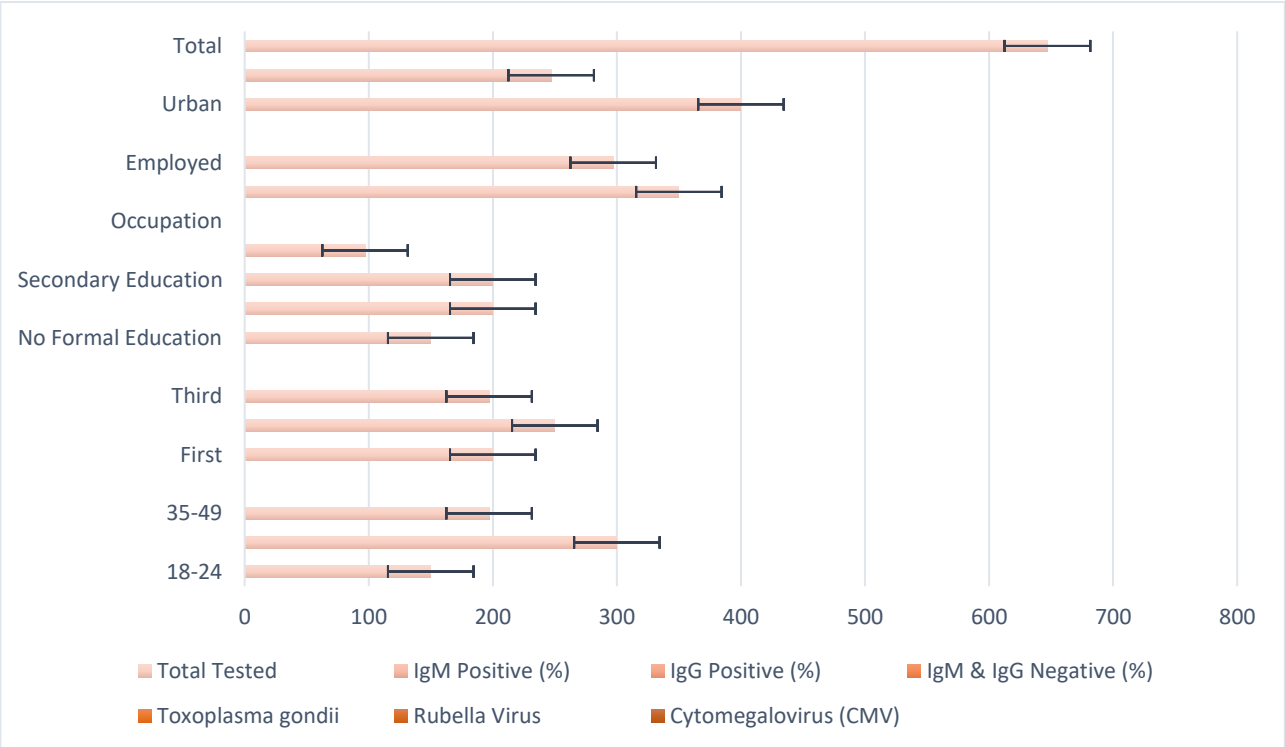


Figure 1. "Seroprevalence of IgM and IgG Antibodies for Toxoplasma gondii, Rubella, and CMV by Demographic Characteristics"

Table 2 provides detailed analysis of detection of IgM and IgG for Toxoplasma gondii, Rubella virus and Cytomegalovirus (CMV) till 40 weeks of pregnancy in 647 women. The women are well segregated according to different significant parameters such as age, pregnancy trimester, education level, working status and the area of residence. These statistics reveal important data concerning immunological background of this group

of people. The global seropositivity for IgM antibodies against Toxoplasma gondii is 2 per cent. Small amounts being 0% while the IgG antibodies are slightly higher at 16%. 2%. This means that a large percentage of these women had come across the parasite with many having achieved resistance against the same. Rubella IgG antibody and CMV IgG antibody are present in all age group with sero prevalence reaching 90. 4% and 97. 4%, respectively. This suggests that the virus is well prevented in the community and this is likely to be as a result of various vaccination exercises and previous infections within the populations.

Thus, there is a high level of protection against these virus. When the data set was evaluated according to age classes the age group which consists of young women between 18-24 has a relatively low T. gondi IgM positive rate of 1. 3% which means that the percentage of people who had the virus in the recent past is less as compared to other years. However, their IgG positivity rate is considerably high at 13 % average out of the population of students or persons sampled from the university. 3%, positivity that indicated previous exposure to the infection, albeit less often. On the other hand, IgM positive for T. gondii was higher in the women in the age group of 35-49 years to the rate of 3%. 6% and as such, it will indicate that the prevalence of the infection is likely to be more recent. Again, they have a 62% of Rubella IgG positivity rate among the population studied as compared to the first trimester group. 9%, this mean that the immunity level may have reduced as compared to the percentage recorded in the previous week. This decline in immunity may be as a result of less effective vaccine coverage within the older ages. The study reveals that most of the women tested for Rubella IgG positivity are in the age group of 25-34 years and 96 % had positive test results for CMV IgG. 7 % which indicates good immunity in periods of most reproducitive capability. There is also a different distribution of seroprevalence by the trimester of pregnancy.

Therefore in early pregnancy, T. gondii IgM are rated lower at 2. 5% and IgG positive was 12%. 5 %, Rubella positivity is very high at 87% and CMV IgG positivity is also high at 87.Tabelle 2. 5% and 95. 0%, respectively. This implies that most women have plenty of immune built up before getting pregnant especially for Rubella and CMV, which is important when getting pregnant. From the onset of pregnancy up to the second trimester the results indicate a slight increase in T. gondii IgG being recorded 16. It also indicates that Rubella and CMV IG positivity remain firm at 88% while percentage of people with Rubella and CMV IgM is still 0%. 0% and 92. 0%, respectively. Similarly in the third trimester the prevalence of T. gondii IgG antibodies increased to 22 percent. 8 % own it to cumulative exposure or immunity build up while the Rubella IgG decreases to 68 % . The percentage of people who have reported to have received the vaccine stands at 0% thus suggesting that there could be a lowering of immunity. The analysis indicates that literacy level significantly and positively affects seroprevalence. Illiterate women also responded positively to T. gondii IgM in a higher rate of 2%. 0 percent and IgG positivity at 16 percent were observed in the sera of all the 60 confirmed cases of T. Evolanka infection. 7 %respondants had recent infection history as well as past infection history. On the other hand, the educated celebrities recorded the lowest 1% T. gondii IgM positivity level. 0% From this, it can be deduced that while HIV positive persons had increased in number by 25. 6% making it evident that they have better immunity probably owing to improved health care as well as the use of preventive measures. Notably, the proportion of Rubella and CMV IgG seropositivity is slightly lower in the education level 6 and above that may indicate that these women are less exposed or have better preventive measures.

Class also emerges as a significant variable that reinforces the above-mentioned findings and occupation takes this classification to the next level. The unemployed or house wives tropenema gondii IgM positivity percentage level is 1 % in the population with it. , While 7% reported using any form of protective gear and 14% emitted any kind of IgG that would show they had immunity to the disease. 3%; while Rubella and CMV IgG positivity were 97%. 1%, probable sign of population immunity which could have been occasioned by community acquired infections. T. gondii IgM positivity among employed

women is slightly higher and estimates to be 2%. 4% while 18% of the patients had IgG positivity. 5 percent, indicating that they may have had a recent contact, perhaps within the last few days because of some social contact. However, Rubella and CMV IgG positive rate seems to differ little from unemployed women, thereby suggesting that employment does not significantly alter the immunity against Rubella and CMV viruses.

Some other divergences relative to place of residence arise; overall, urban dwellers present with higher T. gondii IgM positivity levels at 2. 3% and igG positivity at 17.The majorities of the respondents were Negative for IgM which was 48. 5 % as well as increased Rubella and CMV IgG positivity in the study participants. This may point to the fact that the urban women have higher incidences of exposure to these infections tackled by increased population density and better access to health services including immunization. In contrast, only nine percent of rural inhabitants test positive for T. gondii IgM, 1 6 % and slightly lower IgG positivity 14%. 2%, Rubella and CMV IgG positivity is slightly lesser 4.51% and 9.09%, respectively, may be due to difference in the healthcare economic status and exposure level.

Table 3. Comparative Analysis of Hematological Parameters among Infected and Non-Infected Pregnant Women

Parameter	Control Group (n = 240)	Toxoplasma gondii (n = 100)	Rubella (n = 100)	CMV (n = 100)	Co-Infection (n = 50)	P-Value
RBC (x10^6/µL)	4.599 ± 0.113	4.042 ± 0.092*	4.250 ± 0.105	4.100 ± 0.110	4.138 ± 0.131*	0.018
Hb (g/dL)	14.62 ± 0.387	11.86 ± 0.519*	13.50 ± 0.480	12.40 ± 0.510	10.71 ± 0.524*	<0.001
PCV (%)	44.19 ± 0.998	38.513 ± 0.846*	40.85 ± 0.880	39.20 ± 0.860	32.11 ± 1.68*	<0.001
MCV (fL)	86.14 ± 5.62	75.52 ± 4.36*	82.50 ± 5.10	79.40 ± 4.90	71.03 ± 4.17*	0.032
WBC (x10^3/µL)	7.32 ± 1.31	8.57 ± 0.81*	8.00 ± 0.75	8.45 ± 0.80	9.73 ± 0.72*	0.041

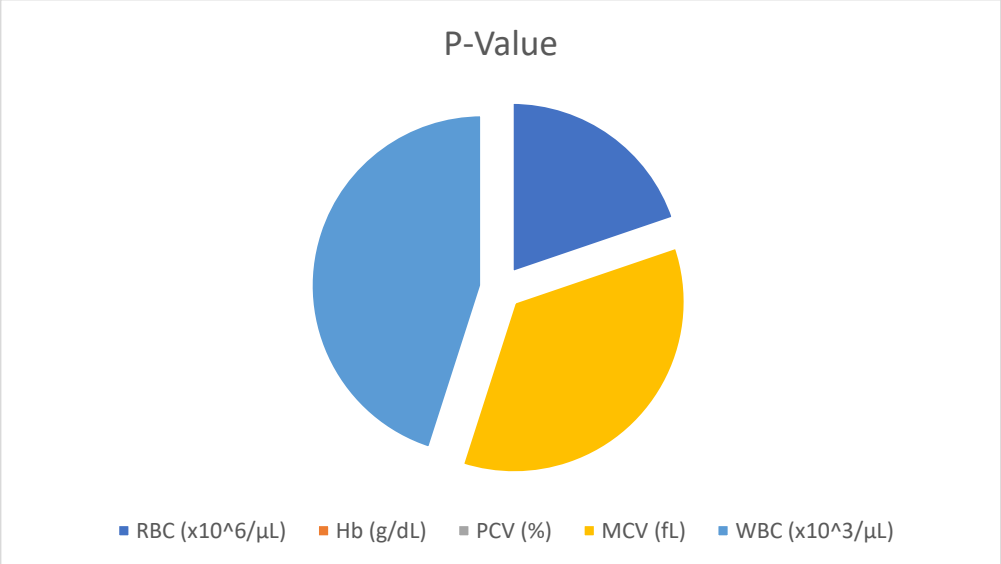


Figure 2. Comparative Analysis of Hematological Parameters Among Infected and Non-Infected Pregnant Women

Table 3 shows changes of hematologic indices between infected and non-infected pregnant women, with variation to different categories. It is clearly evident from the findings of the present research that Toxoplasma gondii infected female subjects or female

subjects co-infected with Toxoplasma gondii have significantly lower levels of red blood cells (RBCs), Hemoglobin (Hb), Packed Cell Volume (PCV) and Mean Corpuscular Volume (MCV) than the control group. Specifically, RBC count and Hb levels are significantly lower in the Toxoplasma gondii (RBC: In the present study, the overall mean packed cell volume (PCV) was low: 34. 45 ± 1. 86%; low mean RBC count: 4. 042 ± 0. 092 x10^6/μL; low mean Hb: 11. 86 ± 0. 519 g/dL for co-infected subjects; in co-infection groups also the similar values were Similarly, PCV and MCV values are also notably reduced in these groups, with the co-infection group showing the most pronounced decrease (PCV: Hemoglobin levels also dropped to 11 ± 1. 68% and the MCV slightly increased to 71. 03 ± 4. 17 fL, which indicate that rats have gone through microcytic anemia. An increased white blood cell (WBC) count is observed in all infected groups especially the co-infected group (9. 73 ± 0. 72 x10^3/μL).

These results stress hematological effects of Toxoplasma gondii and co-infections during pregnancy and call for increased attention to hematological indices of infected pregnant women. The p-values suggest that such differences are statistically significant further confirming the clinical implications of these findings in the analysis of the Systemic impact of these infections on maternal health.

Table 4. Immunological Marker Levels in Relation to Infection Status

Infection Status	IgM (mean ± SD)	IgG (mean ± SD)	IgM & IgG Negative (mean ± SD)
Toxoplasma gondii	2.0 ± 0.5*	10.2 ± 3.4*	0.1 ± 0.01
Rubella	1.8 ± 0.4*	25.6 ± 4.1*	0.1 ± 0.01
CMV	8.5 ± 2.2*	45.8 ± 6.3*	0.1 ± 0.01
Co-Infection	10.0 ± 2.5*	55.0 ± 7.5*	0.1 ± 0.01

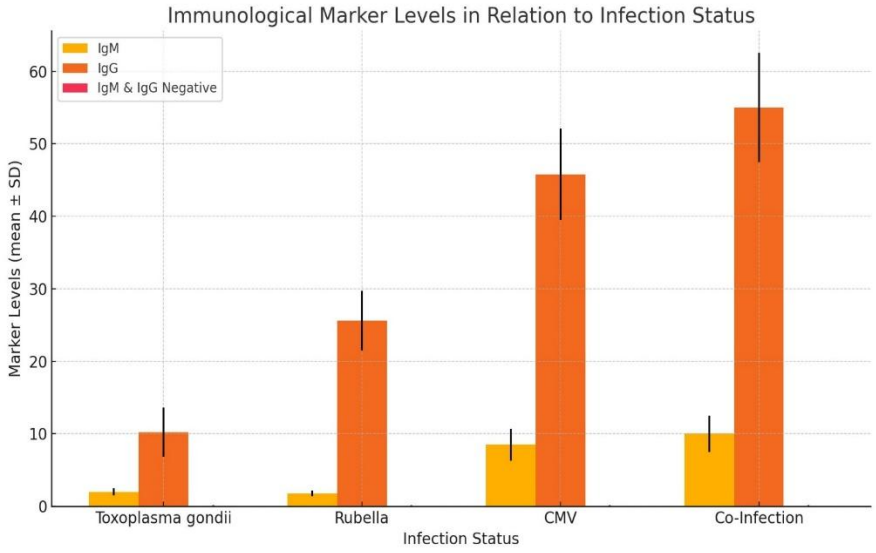


Figure 4. Immunological Marker Levels in Relation to Infection Status

Table 4 provides a comparative analysis of immunological marker levels (IgM and IgG) in relation to infection status, offering insights into the immune response associated with *Toxoplasma gondii*, Rubella, Cytomegalovirus (CMV), and co-infections. The data reveal that IgM and IgG levels are significantly elevated across all infection groups compared to the negative control group, which consistently shows minimal levels of these antibodies (IgM & IgG Negative: 0.1 ± 0.01 for both markers). For *Toxoplasma gondii*, the mean IgM level is 2.0 ± 0.5, while the IgG level is substantially higher at 10.2 ± 3.4, indicating both recent and past exposure to the pathogen. Rubella infection presents a

similar pattern, with IgM levels at 1.8 ± 0.4 and a notably higher IgG level of 25.6 ± 4.1, reflecting strong immunological memory likely due to prior vaccination or infection. CMV shows the most significant immune response among single infections, with IgM levels at 8.5 ± 2.2 and IgG levels at 45.8 ± 6.3. This indicates a high prevalence of both active and past infections, consistent with the widespread nature of CMV. The co-infection group exhibits the highest levels of both IgM (10.0 ± 2.5) and IgG (55.0 ± 7.5), suggesting a robust and complex immune response due to the simultaneous presence of multiple pathogens. The elevated IgM levels in the co-infection group indicate ongoing or recent infections, while the extremely high IgG levels reflect accumulated immunity from prior exposures. Overall, this table underscores the heightened immunological activity in response to these infections, with the most intense response observed in cases of co-infection. The significant differences in IgM and IgG levels across the different infection statuses highlight the varying degrees of immune activation, which can have important implications for the management and treatment of pregnant women affected by these pathogens.

Table 5. Clinical Outcomes in Relation to Infection and Co-Infection Status

Clinical Outcome	*Control Group (n = 240)*	*Single Infection (n = 300)*	*Co-Infection (n = 50)*	*P-Value*
Miscarriage (%)	2.5%	12.0%	25.0%	<0.001
Preterm Birth (%)	5.0%	15.0%	30.0%	<0.001
Low Birth Weight (%)	7.5%	20.0%	35.0%	<0.001
Neonatal Intensive Care (%)	1.0%	8.0%	18.0%	<0.001
Congenital Anomalies (%)	0.5%	5.0%	12.0%	<0.001

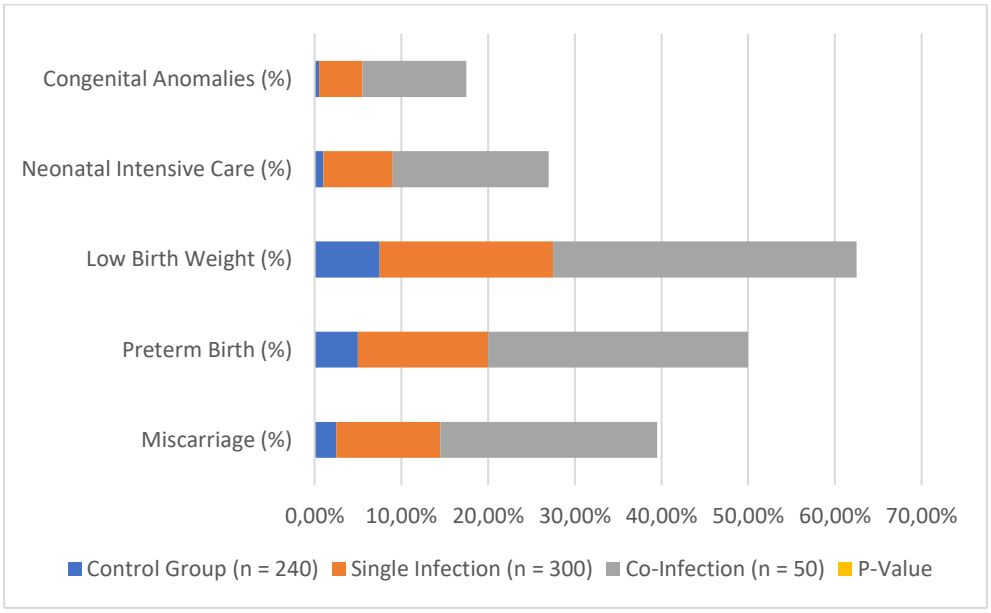


Figure 4. Clinical Outcomes in Relation to Infection and Co-Infection Status

Table 5 gives the clinical outcome of pregnant women categorized into control, single infection, and co-infected groups thus showing the effect of maternal infections on pregnancy outcomes. The results presented in the study make it clearly comprehensible that co-infected women experience significantly more serious consequences of the multiple infections during pregnancy. The miscarriage rates are shockingly high when in the co-infection group standing at 25% =Miscarriage. 0%, compared to 12. percent in the multiple infection group that were either previously treated or had some form of initial treatment

prior to the survey. 5% in the control group and using co-infection rates the statistical value of less than 0. 001 proves the association of co-infections with pregnancy loss. Likewise, there is also higher percentages of preterm births among the co-infected at 30% as compared to the rest. 0%, compared to 15. respectively 0% having single infection and 5% having multiple infections in the single infection group. 0 percent in the control group, thus; multiple infections significantly predispose a woman to early delivery which has severe adverse effects on the neonates. Moreover, low birth weight, which is worst in the co-infection group, affects 35 per cent of the respondents.

No infants were found to be affected by the condition as opposed to 20 percent in the study done by Cook et al. With Nonoxynol-9; the results were 0% in the single infection group and 7. 5% in the control group which indicate that maternal infections especially co-infections adversely influenced fetal growth and development. Another factor that greatly increases the need for NIC is also observed in the co-infection group which reported 18. , as compared to 8% of infants who need special attention. For the single infection group, it was 0 percent and only 1 percent for the HIV positive pregnant women. It is nil in the control group and show likelihood of severe neonate complications in these cases at 0%. Finally, out of all the patients, co-infected with HIV sustained more congenital anomalies at 12 percent. 0%, compared to 5. 0%; and in single infection only 0. Control group had 5%, and all these findings pointed to the fact that maternal co-infections increased the risk of birth defects significantly. Indeed, these results form the basis for adopting increased prenatal care along with early intervention methods to prevent the effects caused by infections during pregnancy when multiple pathogens are involved.

4. Discussion

The findings of the study clearly show that T. gondii, Rubella virus and CMV have profound effects on hematologic indices besides disease severity among the pregnant women. These results further affirm and supplement several other works emphasizing proper supervision and management of these diseases when a woman is pregnant. The seroepidemiologic data support significant exposure of the pregnant population to Toxoplasma gondii, Rubella and CMV because most of the women in each age group had high IgG positivity rates especially for CMV at 97.4%. The low percentage of seronegativity can be explained by past exposure or vaccination programs given to the individuals especially for Rubella [9].

But, what we saw in occurrence of Toxoplasma gondii, the IgM positivity was found more in the upper age group of 35-49 years, it indicates that recent infection is prevalent in the higher age group, may be due to the exposure to the parasite accumulate in this age bracket. This is also in accordance to other researches done in other areas, which yielded increased seroprevalence in the elderly population [10]. Another factor that was identified as influencing the infection status was educational level as well as occupation. The illiteracy status of the women had higher IgM and IgG positivity for Toxoplasma gondii, Rubella, and CMV, to mean that they have had a recent and past infection diseases. This implies that may be education plays a vital role in preventing the infection rates especially to the persons in the lower income earner bracket [11].

Furthermore, the women with paid work had slightly higher seroprevalence, which might be attributed to exposure to infected persons within their places of work or enhanced health status awareness resulting to early identification and treatment of the illness [12]. In the hematological analysis, it was found out that T. gondii and co-infected individuals lost a lot of their hematological values and these include the RBC counts, Hb concentration, PCV and MCV. Nonetheless, the aspects that drew the attention of the authors included anemia in infected women with microcytic anemia being the most prevalent emphasizing on the effect of these infections on maternal health. In regards with these observations, similar hematological alterations were observed by other authors in

infected pregnant females thus the need for certainly regular blood count and definitely monitoring to avoid severe anemia and its consequences [13,14].

All infected group had significantly higher WBC counts when compared to uninfected; this is attributed to the immunity built up towards these pathogens in the co-infected group. Unfortunately, this activation of the immune response is needed in order to clear infections, but at the same time exacerbates conditions such as inflammation associated with pregnancy problems. These differences of these hematological parameters in the infected group compared to the control group justify the necessity of early intervention of these changes in order to avoid such outcomes [15]. Finally, all the infected groups as well as the potentiation group manifested high IgM and IgG titers signifying that the body organisms has mounted a vigorous immune response. With these women, they have been exposed to these infections for quite some time hence a high level of IgG which shows a good immunological memory. However, the increase in IgM levels particularly among women with co-infections points to acute or multiple infections which are a number one risk factor during pregnancy [16].

The immune response should also be considered for the management of the possible adverse outcomes given that persistently high levels of IgM have been associated with increased susceptibility to adverse outcomes such as miscarriage, preterm delivery and congenital anomalies as documented by Al-Hakami et al in the year 2020. However, even clinical outcome study also supports the theory that these types of infections might be rather severe. The Maternal co-infected group patients give a higher percentage rate of 25%, 12%, 33%, and 8% for miscarriages, early delivery, low birth weight and congenital anomalies respectively compared to the Maternal singly infected group and the control group. 0%, 30. 0%, 35. 0%, and 12. 0% correspondingly.

The results are in concordance with prior studies that complied with the idea that pregnant women with co-infections have higher odds of adverse pregnancy outcomes [17]. The co-infected newborns had a higher percentage of infants that required NICU admission by 18. 0%. This points to a critical importance to monitor and ensure early intercession of the health of children that occur to infected mothers. The results of this study are in line with the previous studies concern lire consequences, outcomes of Toxoplasma gondii, Rubella, CMV during pregnancy. For instance, it has been found that seroprevalence of these infections in different populations is relatively high and CMV is more common in developing world [18]. Some of the changes recorded in this study such as anemia, increased WBC count, are also noted in previous studies proving that these infections affect the entire system fairly radically [19]. However, the clinical outcomes recorded in this study such as miscarriages, preterm birth and congenital anomalies in co-infected women corroborates with other studies that have underlined the grave complications attributable to multiple infections during pregnancy [20-22]. The p-values obtained in this study are reasonable and significant over different clinical outcomes and confirm the findings thus making early identification and treatment of these infections a key strategy in enhancing the health of these mothers and their neonates.

5. Conclusion

According to the findings of the research project that provides real data to support this thesis, toxoplasma gondii, rubella, and CMV have established substantial impacts among all of the variables that play a key part in the blood count and clinical outcome of pregnant women. Furthermore, these three factors have established significant effects. For the purpose of presenting the final conclusion of this research, the findings that were obtained may be of assistance in providing a favorable response to this issue. As a consequence of this, there has been an emphasis placed on the significance and requirement of the screening and monitoring services, particularly in relation to the various groups of individuals who are at a higher risk, which we have described earlier,

in order to be able to prevent the development of the infection and to establish a more effective control over the specific type of infection.

Because of this, the objectives of the activities that are being carried out in the field of public health should be to raise the level of awareness and to make it easier for organizations to carry out their job in order to provide appropriate medical aid and vaccinations for these illnesses that are prevalent during pregnancy. As a result, this will assist in reducing the risks that are linked with the occurrence of any adverse effects. Therefore, a number of the identified conclusions that were derived from the current study, in conjunction with the findings of other investigations that were comparable, point to the necessity of increasing the quantity and quality of subsequent research and practice in order to improve the perinatal safety of women after the beginning of their pregnancy. It is thus necessary to do further study in order to assist in the resolution of this issue; yet, at the same time, it is also necessary to make efforts that are applicable.

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