

# Seroprevalence of Rubella and Herpes Simplex Virus in Women with Miscarriage and Stillbirth in Zakho City, Kurdistan Region, Iraq: A Cross-Sectional Study

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## Abstract

**Background:** In pregnant women, primary infection with rubella and herpes virus may lead to serious complications, including abortion, stillbirth, and severe birth defect. The objective of the present study was to determine the prevalence of rubella and herpes IgG and IgM antibodies in pregnant women.

**Methods:** A cross-sectional study was conducted in Zakho city, Kurdistan, Iraq. A total of 200 women aged 18-45 years with a history of miscarriage and stillbirth were recruited. Samples were collected from Private Clinical Health Centre at Zakho City between January 2015 and December 2018. All samples were then tested by Enzyme Linked Fluorescent Assay (ELFA) (VIDAS instrument) for detection of rubella and herpes specific IgG and IgM classes of antibodies.

**Results:** Of the total study participants, 166 (83%) were seropositive for anti-rubella IgG antibodies while nine (4.5%) were positive for anti-rubella IgM antibodies. In addition, seroprevalence of IgG and IgM antibodies to Herpes simplex virus was observed in 154 (77%) and 10 (5%) subjects, respectively. As far as age is concerned, the highest seropositivity of specific IgG and IgM antibodies to Rubella and Herpes was found in participants younger than 30 years, but statistically not significant differences were found ( $P < 0.45$  and  $0.73$  for Rubella and  $P < 0.86$  and  $0.19$  for Herpes, respectively).

**Conclusion:** This study provided pilot data on Rubella and Herpes virus infections among women in Zakho city, Kurdistan, Iraq. Data from the present study showed a high anti-Rubella and Herpes IgG and a low IgM positivity in the recruited subjects. Screening women with miscarriage or stillbirth might be conducive to specifying the causes of abortion or stillbirth.

**Keywords:** Seroprevalence, Rubella, Herpes, Pregnant women, Zakho city

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## 1. Introduction

Primary or recurrent infections of Rubella and Herpes Simplex Virus (HSV) during pregnancy can entail wide ranges of clinical symptoms based on the stage of pregnancy. During the early stages of pregnancy, infections may result in serious congenital malformations, intrauterine growth retardation, spontaneous abortion, and even fetal death (1, 2).

In pregnant women, rubella infection can lead to serious consequences due to transplacental infection of the fetus, causing miscarriages, severe fetal defects or malformations and other serious complications such as encephalopathy and thrombocytopenia, known as rubella congenital syndrome (3). To a large extent, the diagnosis of rubella depends on antibody positivity. While anti-Rubella IgG seropositivity indicates a previous exposure or vaccination, IgM seropositivity shows acute or recent exposure to rubella infection (or reinfection) during pregnancy (3).

Rubella vaccination program has been implemented in several industrial countries, significantly reducing the incidence rate of the infection (4). However, in certain developing countries, the vaccine has not been applied as a part of their national immunization program; therefore, rubella has become one of the most frequent infectious diseases and epidemics in these countries. Rubella vaccination is now available at reasonable prices and guarantees high rates of protective efficiency. In developing countries, it appears that governments' commitment to introduce rubella vaccination has not been satisfactory (4).

Neonatal herpes is a common global infection caused by herpes simplex virus type (HSV-1) or type 2 (HSV-2) (5). Neonatal herpes is acquired at the time of vaginal delivery rather than early in gestation; therefore, it is a disease that can be prevented. Between 60-80% of women delivering infants with neonatal herpes infection have no clinical signs, symptoms, or history of diseases (6). Several studies have proposed that the

risk of neonatal herpes infections tends to be higher in pregnant women with seropositivity for both HSV-1 and 2 (7). There is a 50% risk of neonatal infection in mothers who acquire a new HSV infection near the time of delivery (7). Neonatal infection presents itself as a serious central nervous system infection or an infection distributed through different organs such as liver, brain, and lungs (8).

Our previous study reported that the seroprevalence of CMV and *Toxoplasma* IgM antibodies was very low in women with a history of miscarriage in Zakho city, Kurdistan region, Iraq (9). It seems likely that these infections are not the main causes of miscarriage/stillbirth in the studied region. The prevalence rate of rubella and herpes virus in women with a history of miscarriage and stillbirth is not documented in the region. Accordingly, the aim of the current study was to evaluate the seroprevalence of rubella virus, HSV-II IgG, and IgM in pregnant women in Zakho city, Kurdistan Region, Iraq.

## 2. Methods

### *Study Population and Sample Size*

A cross-sectional study was conducted in Zakho city, Kurdistan, Iraq. Blood samples were taken from pregnant women with a history of miscarriage/ still birth of unknown causes attending private medical health centers. From 2015 to 2019, a total of 200 serum samples aged 18-46 years were collected and assessed to detect specific IgM and IgG antibodies against rubella and herpes virus. The inclusion criteria were women with a history of miscarriage/ stillbirth and agreement to participate in the study. Patients who did not agree to participate in the study were excluded.

### *Blood Collection and Handling*

Using a plain tube, 5 mL of blood was taken aseptically from each subject to test for anti-rubella and herpes IgM and IgG antibodies. Blood was allowed to clot for an hour at room temperature and centrifuged for 10 minutes at 3000 rpm; the serum was then separated, collected in sterile storage vials, and stored at -20°C until analysis.

### *Laboratory Analysis and Interpretation of Results*

Rubella and Herpes specific IgM and IgG antibodies were detected using an Enzyme Linked Fluorescent Assay (ELFA) as per the manufacturer's instructions

(VIDAS, BioMerieux SA, Marcy l'Etoile, France). This test is based on the two-step enzyme immunoassay sandwich method with a final fluorescent detection ELFA. The fluorescence was measured at a wavelength of 450 nm; the intensity of the fluorescence was proportional to the concentration of antibodies present in the sample. Anti-Rubella IgG antibody titres  $\geq 10$  IU/mL and anti-Rubella IgM antibodies titres  $\geq 1.1$  IU/mL antibody were considered positive. Anti-herpes IgG and IgM antibodies titres  $\geq 1.1$  IU/ml were considered positive.

### *Statistical Analysis*

The results of this study were analysed by GraphPad Prism software package, version 8. The seropositivity of IgG and IgM antibody was calculated by Chi-Square test (or Fisher exact test). P values of less than 0.05 were considered as statistically significant.

## 3. Results

A total of 200 women, aged 18-46 years, were enrolled in the present study. All participants were from Zakho city and its districts, including Bedar, Batifa, Darkar, and Hizawa. Table 1 shows the seropositivity of specific IgG and IgM antibodies against Rubella and Herpes virus in pregnant women. The overall seroprevalence of Rubella IgG antibodies was 166 (83%) of the total study participants; only nine (4.5%), on the other hand, were seropositive for rubella IgM antibodies. However, the seropositivity of IgG antibodies against Herpes simplex virus was found in 154 (77%) of the subjects while only 10 (5%) were seropositive for anti-Herpes IgM antibodies. In addition, 80 (40%) of the total participants were seropositive for specific IgG antibodies against both rubella and herpes virus; however, only four (2%) cases showed seropositivity for specific IgM antibodies against both infections.

According to the results, the highest seropositivity of anti-rubella IgG antibodies 96 (85%) was observed in women of  $\leq 30$  years old and 70 (80.5%) were seen in women of  $\geq 30$  years old (Table 2). Additionally, three (3.4%) of the IgM-positive participants were  $\leq 30$  years and six (5.3%) of the IgM positive pregnant women were  $\geq 30$  years old. Taken together, there was no significant association between age groups and the prevalence of Rubella infection (Table 2).

In terms of herpes simplex virus, the highest seropositivity of specific IgG antibodies against herpes virus was observed in the age group of  $\leq 30$  years 88

**Table 1:** Serological detection of anti-rubella and herpes IgG and IgM in the 200 recruited women in Zakho city, Kurdistan region, Iraq

Pathogens	Antibody	No. of Positive cases (%)	No. of Negative cases (%)
Rubella virus	IgG	166 (83)	34 (17)
	IgM	9 (4.5)	191 (95.5)
Herpes virus	IgG	154 (77)	46 (23)
	IgM	10 (5)	190 (95)

**Table 2:** Seropositivity of Rubella and Herpes virus among the 200 recruited women related to age group

Pathogen	Type of antibody	Age (Year)		*P value
		≤30 (n=113)	≥30 (n=87)	
		No (%)	No (%)	
Rubella	IgG	96 (85)	70 (80.5)	0.45
	IgM	6 (5.3)	3 (3.4)	0.73
Herpes	IgG	88 (77.8)	66 (75.8)	0.86
	IgM	8 (7.1)	2 (2.3)	0.19

\*The data was statistically analyzed using Chi-Square test (or Fisher exact test). P values <0.05 were considered as statistically significant.

(77.8%) and was 66 (75.8%) in women aged ≥30 years (Table 2). Furthermore, two (2.3%) of the anti-herpes IgM positive pregnant women were ≥30 years and eight (7.1%) of the IgM positive were ≤30 years. Overall, no significant differences were found between age groups and the prevalence of Herpes infection (Table 2).

#### 4. Discussion

Viral infections in pregnancy cause maternal and fetal morbidity and mortality (10). Rubella and HSV-2 infections have been reported to entail serious complications during pregnancy, including miscarriage, stillbirth, and reproductive failure (1-3). There is very limited data on the occurrence of the infections in Zakho city, Kurdistan Region, Iraq. Rubella and herpes specific IgM and IgG antibodies are crucial to studying the seroprevalence of rubella and herpes in pregnant women (11, 12). Therefore, the purpose of this study was to assess the seroprevalence of rubella and herpes specific IgG and IgM antibodies in women with a history of miscarriage and stillbirth. To our knowledge, these are the first published results of sero-epidemiology associated with rubella and herpes virus infection among pregnant women in Zakho city.

In the present study, the overall seroprevalence of rubella IgG and IgM among the recruited sample was 83% and 4.5%, respectively. These results are in line with the previous studies performed in Kurdistan Region, Iraq (13, 14). The present results are also consistent with a study reported in Iran where the seropositivity of rubella IgG and IgM antibodies in women with miscarriage was detected in 75.3% and 1.2% (15). In another study conducted in Sudan, it was shown that

72% and 3.4% of women were seropositive for anti-rubella IgG and IgM antibody, respectively (16).

In the present study; however, the seropositivity rate of IgG antibody to rubella virus in pregnant women was lower than that found in several studies reported in other countries such as Turkey (17, 18), Italy (19), and Australia (20). Such variations in the seropositivity of rubella IgG antibodies in these countries could be attributed to the differences in the endemicity of rubella virus, and/or the presence or absence of rubella vaccination in their national immunization programs. These differences may further be ascribed to the variations in the studied population, variations in the reference range of the used tests and the laboratory techniques employed in the studies.

In the current study, there was no statistically significant association between rubella antibody positivity and age; however, high anti-herpes IgG and IgM antibodies positivity was found in subjects aged < 30 years, a finding consistent with the results of other studies (13, 15, 20, 21). Another study reported that maternal age was significantly associated with rubella infection among pregnant women (22).

Neonatal infection with Herpes is acquired in utero from maternal infection; this infection is relatively severe and is usually related to high morbidity and mortality (23). In the present study, the seroprevalence rates of herpes IgG and IgM antibodies among the recruited women were 77.8% and 7.1%, respectively. This result is, to some extent, similar to a previous study performed in Kurdistan Region, Iraq (13). They found that the seropositivity of herpes IgG and IgM

antibodies among pregnant women was 52.2% and 4.3%, respectively. The data of the present study was higher than the study conducted in India in terms of both IgG and IgM antibodies against herpes infection (24). In this study, the seroprevalence rates of herpes IgG and IgM antibodies were 33.58 % and 3.6 %, respectively (24). In another study conducted in Turkey, the seropositivity of IgG antibodies to HSV was 8.2% in asymptomatic pregnant women (25).

In the current study, age was not significantly associated with herpes infections. However, the highest seropositivity of herpes antibodies was found in subjects younger than 30 years. This finding is in accordance with the data of several studies reporting that young women were at higher risk of herpes infection (21, 26, 27). Furthermore, Al-Saeed and co-workers found that herpes seropositivity was significantly higher in women less than 30 years old (13). The present results are also similar to the findings of Al-Taie who reported that most of the cases (57%) with high delivery risk factors were found between 21-23 years old in Mosul province. Iraq (28). The optimum childbearing age is considered to be 18-30 years.

## 5. Conclusion

In conclusion, our study provided an insight into the prevalence of Rubella and Herpes virus infections in women with a history of miscarriage or stillbirth in Zakho city, Kurdistan, Iraq. Results indicated high anti-Rubella and Herpes IgG and a low IgM positivity in the recruited subjects. Screening women with miscarriage or stillbirth might be helpful to determining the causes. Further studies with larger sample sizes are also required to investigate the relationship between these infections and pregnancy outcomes.

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## Ethical Approval

This study was approved by the local scientific and ethics committee of the college of Medicine, University of Zakho. Written informed consent was obtained from all participants prior to sample collection.

**Conflict of Interest:** The authors declared no conflict of interest.

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