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Original Article

Clinical Relevance of IgG Avidity Testing for Identifying Acute Rubella Infection in Women of Reproductive Age

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Abstract

Background: Rubella virus infections during pregnancy are known to have considerable teratogenic effects. Maternal infection can lead to congenital rubella syndrome (CRS), a condition with lifelong complications. In fact, individuals affected by CRS often show higher rates of diabetes, osteoporosis, and thyroid dysfunctions in later life. This study aims to highlight the importance of distinguishing recent from past rubella infections in the earliest stages of pregnancy.

Methods: A total of 212 pregnant women were included in the study. They were in the second to fourth months of gestation and were considered at risk of rubella virus infection. At the beginning of pregnancy, serum samples were collected, and anti-rubella IgG and IgM levels were measured using a standard ELISA kit. IgG avidity was assessed using the method proposed by Hedman et al, following the avidity index (AI) protocol with the Euroimmun diagnostic kit.

Results: Among the 162 serum samples analyzed during the first trimester, participants were categorized into 3 distinct groups based on their serological profile. Group A consisted of 100 individuals (61.7%) who tested positive for both IgG and IgM. Group B included 8 individuals (4.9%) who were IgG positive but IgM negative. Group C comprised 54 individuals (33.3%) who tested negative for IgG but positive for IgM. This categorization offered a clearer understanding of immune status and potential recent infection.

Conclusion: The findings of this study highlight the practical value of IgG avidity testing in early pregnancy. By enabling clinicians to more accurately differentiate between acute and past infections, the test plays a critical role in supporting timely medical decisions, potentially helping to prevent complications associated with rubella in unborn children.

Keywords: Rubella virus, Congenital rubella syndrome, IgG avidity, Early pregnancy, Serological diagnosis

Introduction

Rubella virus, taxonomically belonging to the Togaviridae family and the sole representative of the *Rubivirus* genus, is known for causing a generally mild self-limiting illness in children and adults.[1,2] Transmission of postnatal rubella occurs primarily through direct exposure to nasopharyngeal secretions. While these infections typically resolve without complication in the general population, the consequences of primary maternal infection during gestation are profoundly more serious. Notably, maternal rubella infection during the first trimester, especially within the first eight weeks, poses an exceptionally high teratogenic risk, increasing the probability of fetal damage. Despite the asymptomatic or subclinical nature of rubella infection in pregnant women, the virus exerts significant embryopathic effects through mechanisms that likely involve cytopathogenic interference with mitotic activity and the induction of apoptosis at critical junctures in embryonic cell proliferation and tissue differentiation. The transmission of the virus to the fetus is facilitated in the viremic phase of maternal infection, during which the placenta becomes the main target for viral invasion and dissemination.[3,4] Rubella outbreaks typically occur in late winter to early spring, underscoring its seasonal epidemiological patterns.[4] Acute maternal infections occurring in early pregnancy can lead to fetal demise or result in congenital anomalies, involving the central nervous system, cardiovascular structures, auditory system, and ocular development. The spectrum of congenital rubella syndrome (CRS) is broad and permanent in nature, often encompassing conditions such as sensorineural deafness,

congenital heart disease, cataracts, glaucoma, pigmentary retinopathy, developmental delays, and endocrinopathies, including diabetes mellitus, thyroid dysfunctions, and osteoporosis in later life.[5] The main clinical features of rubella virus infection include a transient maculopapular rash primarily localized to the cephalothoracic region, transient fever, lymphadenopathy, and arthralgia.[5] Alarmingly, despite the longstanding availability of effective vaccination, only 57% of global health systems have integrated rubella immunization into national programs, leaving a considerable portion of the world's population especially in developing nations vulnerable to CRS, with an estimated global incidence exceeding 100000 cases annually.[1] Diagnosis of rubella infection in pregnancy relies fundamentally on serological assessments, particularly the detection of specific IgM and IgG antibodies.[6] These tests aid in the classification of infection stages (primary, reinfection, or past immunity) by assessing variations in antibody titers, isotypic distributions, and binding characteristics namely affinity and avidity.[7] Technological advances in IgG avidity assays now allow accurate discrimination between recent primary infections and reinfections using a single serum sample.[6] Avidity reflects the cumulative strength of multivalent antibody-antigen interactions, whereas affinity denotes the binding strength between individual antigenic epitopes and corresponding antibody paratopes. Avidity maturation is known to increase after exposure through natural infection or immunization.[8] Traditionally, the co-detection of rubella-specific IgM and compatible clinical findings serves as the standard method for diagnosing acute rubella. However, the mere presence of IgM is not unequivocally diagnostic due to potential confounding factors such as persistent IgM responses (lasting 8–12 weeks) and serological cross-reactivity during reinfections. In this context, researchers can examine the subclass profile of rubella-specific immunoglobulins (notably IgG1 and IgG3) in both primary and recurrent infections, although such analyses remain insufficiently reliable for definitive differentiation.[9] Thomas and Morgan-Capner proposed a refined enzyme-linked immunosorbent assay (ELISA) incorporating antiglobulin reagents to assess the functional avidity of rubella-specific IgG subclasses [10]. Earlier experimental protocols developed by Inouye et al in 1984 and subsequently by Rousseau and Hedman utilized protein denaturants such as guanidine hydrochloride and urea, respectively, to demonstrate that reduced IgG avidity is indicative of recent primary infection, thereby establishing its utility as a discriminative tool.[11,12] Throughout the 1980s, considerable advancements were made in ELISA methodologies to enhance the practical application and diagnostic reliability of avidity-based serological testing.[13] Despite the global availability of efficacious rubella vaccines for over 4 decades,[14,15] CRS continues to represent a significant public health challenge, particularly in resource-limited regions.[16] Effective prevention hinges on robust immunization programs and comprehensive antenatal screening strategies. The present investigation underscores the clinical necessity and public health value of accurately distinguishing rubella infections during early pregnancy, with specific emphasis on the diagnostic refinement of rubella virus infection in the first trimester.

Materials and Methods

Patients

A total of 212 pregnant women who were in the second to fourth months of their pregnancy period and were at risk of rubella virus infection were recruited. This study was carried out at the Department of Infectious Diseases of Sina Hospital and the Special Clinic of Tabriz University of Medical Science. Women's medical records were gathered within a four-year period (October 2012 to October 2016). To diagnose the active infection, they were later referred to the Medical Diagnostic Laboratory.

ELISA Test

Anti-rubella IgG and IgM levels were measured at the beginning of pregnancy using an ELISA kit (Vircell Microbiology Company). The measurement of anti-rubella IgG was quantitative but the measurement of anti-rubella IgM was achieved through the computation of the cut-off point to report an index describing the IgM amount.

Avidity Test

This assay was performed according to the avidity-index (AI) method developed by Hedman et al using the Euroimmun kit as follows. Briefly, 100 µL of each patient's diluted serum was added to rubella-antigen-coated microplates. In the next step, urea solution (8M) as an elution agent was added to microplates containing the antigen-antibody complex. To wash off excess antibody, the washing buffer was applied. Then, labeled anti-IgG antibody was added to the microplates. The plate was covered with foil and incubated in a moist atmosphere for 30 minutes at 37 °C, and after another round of washing, the substrate was dispensed into each well. In the final step, sulfuric acid was added to stop the reaction. The absorbance was measured at 450 nm against the differential wavelength of 600 nm. Absorbance readings from plates washed in elution agent (EA) were used to calculate an avidity index (Al) as follows:

$$AI = (OD \text{ of the sample treated with Urea} - OD \text{ of the blank}) / (OD \text{ of the sample treated without Urea}) \times 100$$

Statistical Analysis

The data obtained from this study were analyzed using descriptive statistical methods. The diagnostic values were calculated using SPSS version 14.0 (IBM, USA).

Results

Our results from the analysis of serum samples of 162

pregnant women during the first 4 months of pregnancy were categorized into three groups: Group A (100 cases; IgG+, IgM+, 61.7%), group B (8 cases; IgG+, IgM-, 4.9%), and group C, (54 cases; IgG-, IgM+, 33.3%) (Tables 1 and 2). Our data are shown in Figures 1, 2, and 3.

Discussion

In the present investigation, 8 individuals were excluded from group B due to negative Rubella-specific IgG results, which made it impossible to perform the avidity assay on their serum samples. All participants assigned to group C tested negative for Rubella-specific IgM antibodies and simultaneously exhibited high-avidity IgG responses, strongly indicating a resolved or inactive infection with no current risk to fetal development. A fundamental serological indicator for diagnosing rubella infection is the observation of seroconversion, characterized by negative Rubella-IgG levels before conception and positive titers detected during gestation. However, in many developing societies, including ours, routine pre-pregnancy screening for rubella immunity is not a standard practice, which elevates the importance of IgG avidity testing as a diagnostic tool during pregnancy. According to our findings, 32.7% of the pregnant women demonstrated negative IgM and positive IgG antibodies. Notably, most of these individuals had high IgG avidity indices, suggesting previous exposure and immune memory formation before conception and implying a relatively high background immunity to rubella in the general population. Conversely, 61.7% of the sampled pregnant women tested positive for both IgG and IgM, suggesting recent or ongoing exposure. Within this subgroup, two cases (1.2%) exhibited low avidity IgG, confirming acute primary infection during pregnancy, a condition that significantly increases the risk of CRS. The main clinical consequences for the fetus may include congenital cataracts, sensorineural deafness, microcephaly, and neurodevelopmental delays, all of which contribute to long-term societal and economic

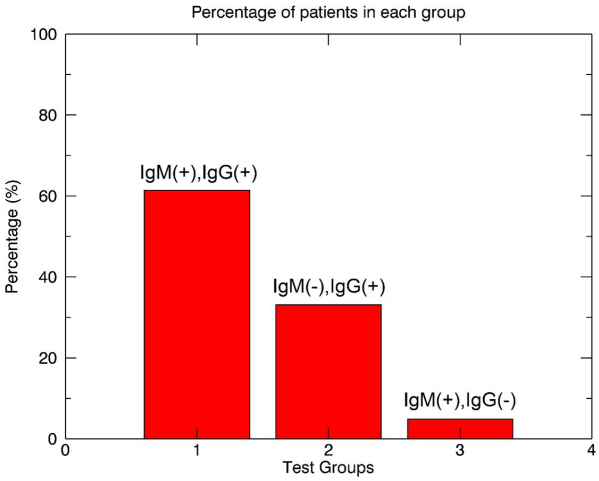

Figure 1. Percentage of Patients in each Group

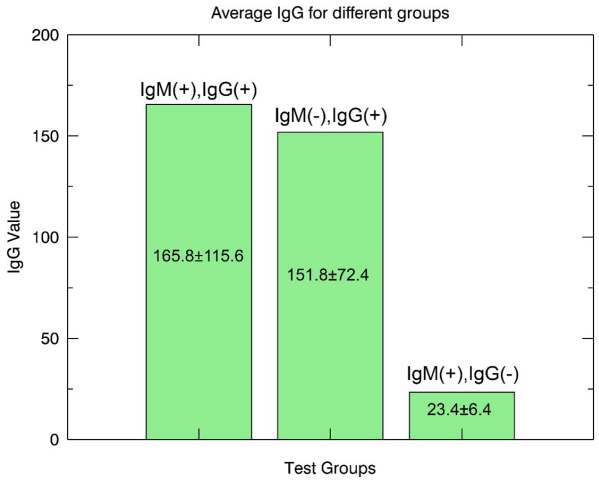

Figure 2. Mean IgG Level in Different Groups

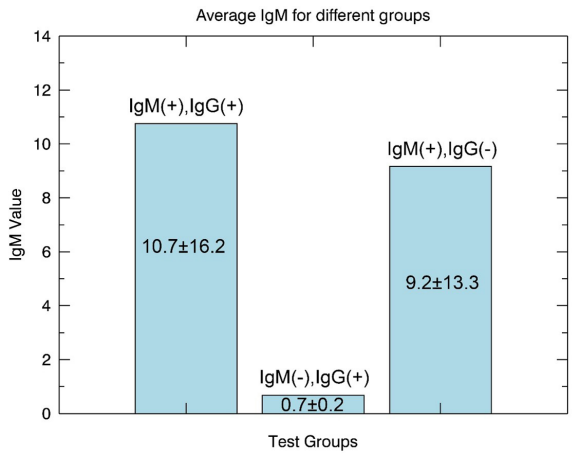

Figure 3. Mean IgM Level in Different Groups

Table 1. Anti-rubella IgG, IgM, and IgG Avidity in the Sera of Pregnant Women

Avidity*	Group A	Group B	Group C	Total
	IgM(+), IgG(+) N (%)	IgM(+), IgG(-) N (%)	IgM(-), IgG(+) N (%)	
Low	2 (1.2)	0 (0)	0 (0)	2 (1.2)
High	96 (59.2)	8 (4.9)	53 (32.7)	157 (96.9)
Borderline	2 (1.2)	0 (0)	1 (0.6)	3 (1.9)
Total	100 (61.7)	8 (4.9)	54 (33.3)	162 (100)

Table 2. The Mean Values of IgG, IgM, and IgG Avidity in the Sera of Early Pregnant Women

	Mean IgM	SD IgM	Mean IgG	SD IgG	Number of patients	Percentage of patients
IgM(+), IgG(+)	10.75	16.18	165.58	115.58	100	61.73
IgM(-), IgG(+)	0.68	0.23	151.80	72.45	54	33.33
IgM(+), IgG(-)	9.16	13.26	23.41	6.44	8	4.94

In this study, avidity was categorized into three groups: low avidity (<40), borderline avidity (≤60 and≥40), and high avidity (>60).

burden due to lifelong care requirements and impaired quality of life. Emerging research has highlighted the value of including IgG avidity testing as a complementary tool for distinguishing recent infections from reinfections or persistent antibody responses. This is especially crucial in settings where nucleic acid-based diagnostics (such as RT-PCR) may not be financially or logistically feasible. Furthermore, recent studies emphasize the public health significance of rubella surveillance. In Nigeria, seroprevalence surveys report rubella antibody positivity rates ranging from 16.3% in North-central regions to over 76% in South-western states.[17,18] These data point to regional disparities in immunity and call for tailored vaccination strategies. In our study, avidity was interpreted using thresholds validated in prior investigations, including research on Toxoplasma gondii, where similar categorization methods have been applied for reliable distinction between acute and past infections. Additional contemporary evidence supports that avidity maturation can be affected by individual immune status, with delayed avidity maturation observed in immunocompromised populations. Therefore, avidity testing should always be interpreted in conjunction with clinical findings and other serological markers.

Conclusion

Although direct viral detection methods such as viral isolation or nucleic acid amplification remain the gold standard for confirming viral infection, these techniques are not always accessible or practical in low-resource settings. In these contexts, IgG avidity testing serves as a valuable cost-effective serological tool for distinguishing primary rubella infections from past exposures or reinfections. Its application is essential for the accurate management of pregnant women to minimize the incidence of CRS. Implementing routine avidity testing in prenatal care protocols, especially in settings without universal vaccination coverage, can enhance early diagnosis and intervention, thereby reducing the likelihood of fetal complications and optimizing maternal-fetal health outcomes.

Authors' Contribution

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Competing Interests

The authors declare that they have no conflict of interests.

Ethical Approval

The study protocol was approved by the Ethics Committee of Tabriz University of Medical Science (No. 92-62). Informed consent was obtained from all patients prior to the study.

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