East African Scholars Journal of Medical Sciences
Abbreviated Key Title: East African Scholars J Med Sci
ISSN 2617-4421 (Print) | ISSN 2617-7188 (Online) |
Published By East African Scholars Publisher, Kenya

OPEN ACCESS

Volume-2 | Issue-10| Oct -2019 | DOI: 10.36349/EASMS.2019.v02i10.036

Review Article

Rubella: Insights into Routs of Viral Infection, Pathogenicity, Diagnosis, Immune Responses, and Vaccination Programsss

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Abstract: **Background:** Rubella virus infects only humans and causes a disease called German measles or rubella. It infects pregnant women and leads to congenital rubella syndrome of the infant. There are about 100,000 cases of congenital rubella syndrome discovered per year. O**bjectives:** The aim of the present review is a high light on the structure of the virus, routs of viral infection, pathogenicity, symptoms, diagnosis, immune responses, and vaccination programs. Rubella virus consists of a single- positive stranded RNA virus. The harmful danger of infection occurs when infection to the fetus during the first trimester of pregnancy. After the virus penetrates the cell and uncoating, the plus-strand RNA is translated into several proteins. The replication and assembly occur in the cytoplasm, and the envelope is acquired from the outer membrane as the virion exits the cell. Virus transmission occurs by respiratory aerosols virus from person to another person. The virus replicates in the mucosal membranes of the upper respiratory tract after inhalation of infected droplets, later, it spreads to lymph nodes. Symptoms of rubella viral infection in children are including fine, distinct macules of a rubelliform erythematous rash, lymphadenopathy involving the posterior cervical and occipital nodes, low-grade fever, mild transient polyarthralgia, malaise and rarely encephalitis, thrombocytopenia and purpura are seen. Congenital rubella syndrome (CRS) causes ophthalmic (cataracts, glaucoma, chorioretinitis, and microphthalmia), cardiac, auditory (sensorineural deafness), craniofacial (microcephaly), complications hepatitis, Hepatosplenomegaly, and thrombocytopenia also resulted from damage of liver. CRS infant cases have severe mental delayed, development impairment, type1 diabetes, and thyroiditis can be lifelong complications. The specimens for the rubella diagnosis are nasopharyngeal secretions, oral fluids, throat swabs, and also by detection of antibodies from serum or oral fluids. The Antibodies IgM for rubella virus can be detected in biosamples within 2 to 5 days from starting of rash appearance. Rubella vaccine is highly effective and safe when used across a population. In 2015, 141 countries (72.7%) had established programs and a further 7 (3.6%) planned to implement immunization programs. Still, over 100,000 cases of CRS are recognized globally each year. It should be noted that a reservoir of rubella virus remains, with countries such as Vietnam, China, Poland, South Africa, Indonesia, and Romania reporting more than 2,000 infections in a single year since 2011. **Conclusion:** More studies on the rubella virus are recommended to gather more data for effective control measures and development of vaccine. Countries should consider strategies to add or improve surveillance for congenital rubella syndrome to better understand their burdens of disease and the associated costs.

Keywords: Rubella virus, routs of viral infection, pathogenicity, symptoms, diagnosis, immune responses, vaccination programs.

INTRODUCTION

Rubella is one of the most important pathogens around the world there are about 100,000 cases of congenital rubella syndrome discovered per year. Rubella virus was isolated in 1962 in the first time in cell culture (Weller, T.H., & Neva, F.A. 1963), it consists of a single-stranded positive sense RNA genome (Frey, T.K.1994). Rubella virus belongs to family Togaviridae and genus Rubivirus which is the sole member of this genus. The disease caused by the rubella virus is called German measles or rubella, most of the infections with rubella were self-limiting, but the harmful danger occurs when infection to the fetus during the first trimester of pregnancy.

Quick Response Code

Journal homepage:
http://www.easpublisher.com/easjms/

Article History
Received: 24.09.2019
Accepted: 05.10.2019
Published: 19.10.2019

Rubella virus is a spherical, enveloped, 40- to 80-nm, 9.6-kb, positive-sense, single-stranded RNA virus of the family Togaviridae. The genome of the virus is enclosed in a capsid formed of multiple copies of a capsid protein. There is lipid bilayer surrounding the nucleocapsid which embedding two viral envelope glycoproteins, E1 and E2. Hemagglutinin-containing spike-like projections are found on the outer surface of the virus has (Figure. 1). The molecular weights of the four structural polypeptides of virus are as follows: E1, 58,000; E2a, 47,000; E2b, 42,000; C polypeptide chain, 33,000. E1, E2a, and E2b are glycosylated and associated with the viral membrane. There are two nonstructural proteins, are involved in viral replication but are not immunogenic (p90 and p150). The E1 polypeptide, which is the largest one of the two glycoproteins and has the predominant immunogenic reactivity in individuals exposed to the virus through natural infection, congenital infection, and vaccination is also associated with the hemagglutinin function. The capsid protein, C, is associated with the 40S genomic RNA and it is nonglycosylated. There are two genotypes of the virus have been identified, but only one serotype that demonstrates no cross-reactivity with other viruses has been reported(Prasad, V. M. *et al.*, 2013; Oker-Blom, C. *et al.*,l 1983; Dimech, W .*et al.*,l 2016).

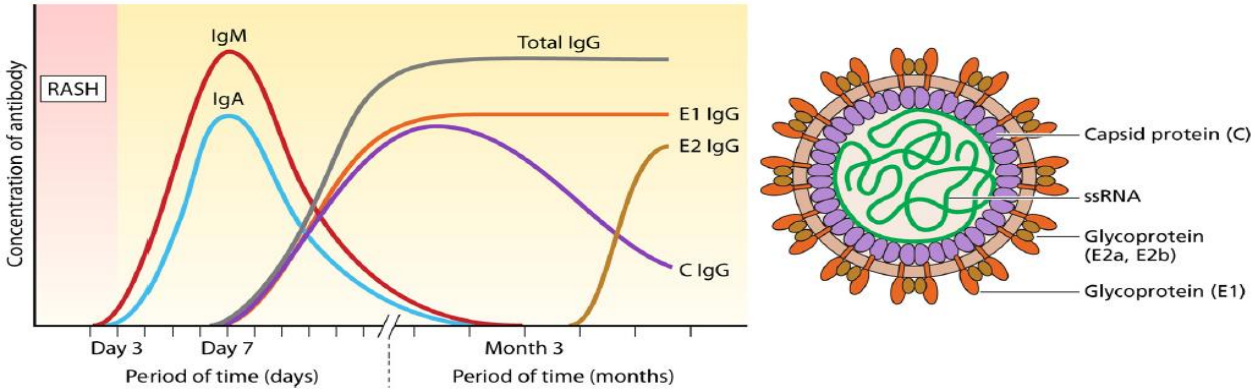

FIGURE (1)

A schematic diagram detailing the structure of the rubella virus, including the three immunogenic antigens; two envelopes (E1 and E2) antigens and a capsid (C) antigen, and single-stranded RNA (ssRNA). A plot of a normal immune response to rubella virus infections over time(Dimech, W. *et al.*, 2016) .

The knowledge of rubella virus replication is incomplete, thus the cycle based on the mode of replication of other togaviruses. After the virus penetrates the cell penetration of the cell and uncoating, the plus-strand RNA genome is translated into several nonstructural and structural proteins. Note the difference between togaviruses and poliovirus, which also has a plus-strand RNA genome but translates its RNA into a single large polyprotein, which is subsequently cleaved. One of the nonstructural rubella proteins is an RNA-dependent RNA polymerase, which replicates the genome first by making a minus-strand template and then, from that, plus-strand progeny. Both replication and assembly occur in the cytoplasm, and the envelope is acquired from the outer membrane as the virion exits the cell (Levinson, W. 2014).

ROUTS OF VIRAL INFECTION AND PATHOGENICITY

Rubella virus infects only humans, this unlike other togaviruses, the virus transmission occurs by respiratory aerosols virus from person to person. The virus replicates in the mucosal membranes of the upper respiratory tract after inhalation of infected droplets, later, it spreads to lymph nodes. The period of contagiously is approximately 5 to 7 days before and 3 to 5 days after the appearance of clinical symptoms. (Banatvala, J. E., & Brown, D. W. 2004).

Rubella viral infection in children causes mild disease with symptoms including fine, distinct macules of a rubelliform erythematous rash detected about 16 to 20 days post infection in which the rash starts on the face and spreads to the trunk and It is selflimiting within 48 hrs. Other nonspecific viral infection-like symptoms are common, including lymphadenopathy involving the posterior cervical and occipital nodes, low-grade fever, mild transient polyarthralgia and malaise. Rarely, more severe symptoms such as encephalitis, thrombocytopenia and purpura are seen (Anonymous.2011).

The major concern of rubella virus infection is the infection of pregnant women which leads to congenital rubella syndrome of the infant.(Lee, J. Y., & Bowden, D. S. 2000). The most dangerous effect, when the viral infection occurs in their first trimester of pregnancy, this results in approximately 90% of the fetuses being infected and 100% of those infected having congenital deformities, often resulting in miscarriage. The risk of congenital rubella syndrome (CRS) declines as pregnancy proceeds, with CRS rarely being associated with primary infection after 16 weeks of gestation(Anonymous. 2011) . CRS causes morbidity involving most major organs but particularly causes ophthalmic (cataracts, glaucoma, chorioretinitis, and

microphthalmia), cardiac, auditory (sensorineural deafness), craniofacial (microcephaly), complications hepatitis, Hepatosplenomegaly, and thrombocytopenia also resulted from damage of liver.(Plotkin, S. A. 2001).Many congenital rubella syndrome infant cases have severe mental delayed development impairment, type 1 diabetes and thyroiditis can be lifelong complications(Sullivan, E. M. *et al.,* 1999).

In the study of Nguyen *et al., 2015*. Which analyzed the pathological features of infection with rubella virus in human fetuses having congenital rubella syndrome. The results of this study indicated that the route of rubella virus infection was via the systemic organs of the human fetuses. This fact has been confirmed by immunohistochemistry and direct detection of viral RNA in multiple organs. This was also demonstrated by the detection of negative-stranded RNA of rubella virus, which indicates the replicative form of positive-stranded RNA virus in infected cells.

The liver of the embryo plays a very important role in hematopoiesis instead of bone marrow. The major liver histopathological change was observed in the study of Nguyen *et al., 2015*. Necrotizing and inflammatory changes in fetus liver suggesting viral hepatitis were present. However, obvious inflammatory changes caused by virus infection were mild in other organs from infected fetuses. In the CNS, no remarkable change suggesting viral encephalitis was found despite the virus infected the nerve cells in the cerebral cortex. This reason is not clear, but as one of the reasons, it might be due to differences in the response to the virus in the embryonic brain. The finding of viral antigen localized in epithelial cells of the glomerulus and proximal tubules in the kidney, this suggestion was proved by detection of the virus in urine. Furthermore, in this study rubella virus antigen can be detected in mononuclear cells from multiple organs, and rubella virus is distributed to the whole body by the circulating infected mononuclear cells. In fetal life, most of the mononuclear cells express CD34 antigen suggesting that they are hematopoietic stem cells produced mainly in the liver.

A cataract is one of the most important illnesses related to congenital rubella virus. In the study of Pham *et al., 2013*. on Vietnamese patients, all of 20 fetuses/newborns with congenital rubella infection presented with congenital cataract. In the pathological study of Nguyen *et al.,* 2015. which indicated the viral infection in the epithelial cells of the ciliary body and the lachrymal glands in the eye. The physiological function of the ciliary body is the production of aqueous humor (Goel, M. *et al.,* 2010). which is analogous to a blood surrogate for these avascular structures and provides oxygen and nutrition to the lens, removes excretory products of metabolism, transports neurotransmitters, stabilizes the ocular structure and contributes to the regulation of the homeostasis of these ocular tissues by the means of which is called aqueous circulation (Goel, M. 2010). If the viral infection affected the physiological function by the ciliary body or inhibited it, the aqueous circulation will not function smoothly and leads to physiological dysfunctions in lens and disorder such as a cataract. Since the importance of the ciliary body for lens function, it suggested rubella virus infection of the ciliary body might play an important role in cataractogenesis (Goel, M. 2010).

LABORATORY DIAGNOSIS OF RUBELLA

The specimens for the rubella diagnosis are nasopharyngeal secretions, oral fluids, throat swabs, and also by detection of antibodies from serum or oral fluids. The virus can be detected also in urine and cataract tissue. Urine, throat swab and oral fluids are the easy sources viral RNA, but the ease of obtaining oral fluids and throat swab makes these specimens the primary ones that are collected, detection of IgM and IgG from serum samples. Urine contamination is often the source of infection in congenital rubella syndrome (Bellini, W.J *et al.*,l 2011).

The collection time of samples is important in postnatal rubella. IgM antibodies for rubella are present in sera in only about 50% of infected cases on the day of rash, and on the fifth day after rash, most of have detectable IgM rubella antibodies. Most rubella cases are virus positive on the day of rash and may be positive from seven to ten days post rash. Congenital rubella syndrome and congenital rubella infection are positive for virus and IgM for months; therefore, timing is less critical for these patients. Dried blood spots and oral fluids, have been shown that were adequate for surveillance testing of rubella in either detection of virus or IgM (Centers for Disease Control & Prevention (CDCP). 2008). Real time -PCR technique for amplification of rubella RNA is now common. Assays that can reliably detect 3 to 10 copies of rubella RNA which is necessary because many diagnostic specimens have small amounts of virus RNA (Van Nguyen, T. *et al.,* 2013)

No cell type that reliably produces a cytopathic effect in a single passage of wild-type viruses. However, virus growth can now be identified in the absence of cytopathic effect using RT-PCR, immunocolorimetric assay and immunoflurscent assay to detect viral proteins or RNA (Maryland, *et al.*,l 2013). Sequencing of the rubella virus nucleic acid amplified directly from specimens or infected tissue culture cells can now provide useful information on vaccine versus wild-type viruses, on the likely origin of imported cases of rubella and CRS, and for the documentation of elimination (Abernathy, E.S *et al.*,l 2011 ; Organization, W.H. 2013). The sensitivity of the RT-PCR system used to generate sequencing templates from infected tissue culture cells is not critical, since the amount of rubella viral RNA in rubella virus infected

cells is higher than in clinical specimens. Detection of IgM for recently postnatal infection with rubella virus by either indirect IgM or IgM capture ELISA is the most common diagnostic test. If acute- and convalescent-phase sera are available, a four-fold rise in rubella virus-specific IgG (usually by ELISA) is also diagnostic for postnatal rubella infection. The same ELISAs may be used to confirm congenital rubella syndrome and congenital rubella infection(Bellini, W.J *et al.*, 2011).

Avidity tests have now been developed that are useful for suspect case classification in certain situations (e.g., the first serum sample was collected months after clinical symptoms). Low avidity anti-rubella IgG suggests recent infection. Avidity tests are not widely available and vary in performance (Mubareka, S. *et al.*, 2007).

Rubella virus specific IgM tests are laboratory tests which have largely been supporting surveillance for rubella and congenital rubella syndrome in control and elimination programs, this supported for some suspect cases by techniques by amplifying RNA of rubella virus(. Centers for Disease Control & Prevention (CDCP). 2008). However, with the WHA target of eliminating rubella in five of the six WHO regions in the next six years, control programs will move into developing countries, and laboratory testing algorithms supporting these programs are expected to change. Specifically, advanced molecular techniques and point-of-care diagnostics for rubella may be used(Warrener, L. *et al.*, 2011). Since the clinical symptoms of congenital rubella syndrome and postnatal rubella are dramatically different, there are significant differences in the immune responses of patients with these two diseases. These differences can be observed in Western blots, in which antibodies in sera from postnatal rubella patients often demonstrate different reactivity to congenital rubella syndrome patients (Katow, S& Sugiura, A. 1985).

Much of the rubella testing in many countries, is for immunity to rubella. There are slightly different criteria for rubella immunity that are recommended by various groups (most are 10 or 15 IU/ml). Commonly used tests (e.g., ELISA in the United States) are standardized to give positive results for 10 IU/ml(Skendzel, L. P. (1996) . Other tests (e.g., immunoprecipitation) detect rubella-specific antibodies, but have not been correlated with immunity. In some countries, monitoring of rubella vaccination programs by seroprevalence studies(Poethko-Müller, C., & Mankertz, A. 2012) . The hemagglutinin inhibition test was one of the standard antibodies tests for to rubella virus, and used for calibration of other assay methods. Neutralization tests for virus specific antibodies have advantages over other tests such as ELISA because of their ability to determine the biologic function of antibodies and can be also used with many virus strains. For some viruses (e.g., measles), the neutralization test has been developed as the standard assay for determinations of immunity (Pugachev, K. V *et al.*, 1997).

The plaque reduction neutralization test is used in case of necessity for a quantitative assessment of the neutralizing capacity of an antiserum. The essay follows a format common to many viruses. Such neutralization tests exist for laboratory-adapted rubella virus strains in several cell types, but an immunocolorimetric neutralization assay for rubella virus using a soluble substrate is a significant improvement over plaque development. The signal can be detected in three days instead of 6–11 days for plaques to develop, viewer subjectivity in plaque counting is eliminated, and wild-type viruses can be used because CPE is not required. Furthermore, the detection portion of the assay can be done using a microplate washer/dispenser, enhancing throughput by a factor of about three and reducing technician hands-on time by a factor of about six (Pugachev, K. V *et al.*, 1997).

Another study, about 2,500 sera were tittered by three technical staff in only four months using one automated machine, and a microplate washer/dispenser. More than 400 sera were tittered a second time. These repeated assays suggested a good degree of reproducibility, with person-to-person differences being more than 8 times higher than the observed within-assay variability. This compares favorably to the standardized measles PRN (Cohen, B. J. *et al.*, 2007) . The possibility of efficiently performing thousands of rubella neutralization titers opens the possibility of routinely using this neutralization assay for large studies, such as serosurveys.

Many postnatal rubella cases are asymptomatic and the individual defects found in congenital rubella syndrome are not specific for it. Thus, laboratories bear a considerable burden in rubella and congenital rubella syndrome diagnosis as in cases primary rubella virus infection which suspected for a pregnant woman, false negative and false positive results may lead to incorrect treatment decisions (Best, J.M *et al.*, 2002).

IMMUNE RESPONSES TO RUBELLA VIRAL INFECTION

Better understanding of cell-mediated immune responses to rubella virus would provide the basis for the development of safe and effective vaccines against rubella and would aid in analysis of the pathophysiology of congenital rubella syndrome (Chaye, H *et al.*, 1992) . The response of the immunity system to infection with rubella virus is typical of most viral infections, in which IgM rises first followed by IgG which more delayed than IgM. Class-switching recombination allows the selection of antibody isotopes best suited to eliminate the virus. The Antibodies IgM for rubella virus can be detected in biosamples within 2

to 5 days from starting of rash appearance and persists for 1 to 3 months (based on the used assay) (Wilson, K.M *et al.,* 2006; Vauloup-Fellous, C., & Grangeot-Keros, L.,2007). Persistence of anti-rubella virus IgM has been reported. Anti-rubella virus IgM may also be detected in reinfection and following polyclonal stimulation of the immune system The detection of IgM for rubella virus is the main method of diagnosis in case of acute rubella viral infection.(. Minakami, H. *et al.,* 2014).

RUBELLA VACCINATION PROGRAMS

Immunization with live attenuated rubella virus vaccine has the identified ability infection prevention and one of the most feared complications of congenital rubella syndrome. Rubella remains an important pathogen and public health concern around the world in spite of much progress has occurred. Recent rubella epidemic spreading of rubella in Japan in 2013 were more than 11,000 rubella cases occurring in the first half of this year and at least 13 congenital rubella syndrome cases occurring, this highlights the fact that a partial vaccination strategy leads to major outbreaks. 70% of the rubella cases in the Japanese outbreak occurred among males ages 20 to 39 years, indicating the weakness of an initial strategy that provided the rubella vaccine only to adolescent girls. Outbreaks of rubella were occurred in Romania and Poland in 2012 and predominantly affected males, this because the vaccination strategy in these countries focused on vaccination of females rather than males. For this reason, a global commitment to rubella control, elimination, and eventual eradication must be in place. Rubella vaccine is highly effective and safe when used across a population and, as a result, endemic rubella transmission has been interrupted in the Americas since 2009. Incomplete rubella vaccination programs result in continued disease transmission as evidenced by recent large outbreaks in Japan and elsewhere (Minakami, H.*et al.,* 2014; Paradowska-Stankiewicz, I. *et al.,* 2013; Janta, D.*et al.,* 2012; Böttiger, M. 1995).

Most licensed vaccines are based on the live attenuated RA 27/3 strain propagated in human diploid cells. Each dose contains a defined number of infectious units. The seroconversion rate after vaccination is expected to be greater than 95%. In December 2009, 130 of the 193 WHO member states had implemented a national immunization schedule. In 2015, 141 countries (72.7%) had established programs and a further 7 (3.6%) planned to implement immunization programs. Still, over 100,000 cases of CRS are recognized globally each year (Anonymous.2011).

Rubella vaccine was licensed for use in vaccination programs in Australia and France in 1970. At that time, only adolescent girls and non pregnant women were recommended to be vaccinated. In Australia, this protocol was replaced in 1989 with an MMR vaccination program aimed at infants 12 months of age irrespective of gender. Universal vaccination of adolescent boys and girls was introduced in 1993 (Francis, B.H. *et al.,* 1982). The current vaccination program targets boys and girls at both 12 months and 4 years of age. In France, measles-rubella vaccination of children was introduced in 1983 and the MMR vaccine was introduced in 1986. Since 2005, it has been recommended that all children receive two doses of MMR vaccine, at 12 and 24 months of age. It should be noted that a reservoir of rubella virus remains, with countries such as Vietnam, China, Poland, South Africa, Indonesia, and Romania reporting more than 2,000 infections in a single year since 2011. This situation is due to inadequate vaccination coverage or "conscientious objectors" to vaccination for religious or other reasons, as seen in the "bible belt" of the Netherlands or certain regions of the United States. The most common source of infection in countries with good vaccination coverage is through infected individuals traveling to and from regions where rubella is endemic for vacation, business, or immigration (Andrus, J.K.*et al.,* 2011; Goodson, J.L.*et al.,* 2011; Miller, E, *et al.,* 1982; Grillner, L.*et al.,* 1983) .

MEASLES VACCINE IN SAUDI ARABIA

Measles vaccination started in 1974 for children aged 1-9 years, it became a requirement for obtaining a birth certificate in 1982. The coverage rate increased from 8% in 1980 to 80% in 1984 and more than 90% in 1990. Although this was accompanied by a remarkable decrease in measles incidence, the overall impact of measles immunization was unsatisfactory. A follow-up study for measles maternal antibody showed that 33% of children at 6 months of age and 36% at 9 months of age were negative for measles maternal antibody. Assessment of seroconversion after Schwartz measles vaccination at 9 months showed that only 65% had a fourfold rise after immunization. After more than 10 years of using the Schwartz measles vaccine in Saudi Arabia, there was a need for change (Khalil, M.K.M *et al.,* 2005).

CONCLUSION

It should be recommended that serological and molecular screening tools should be used in surveillance for rubella virus especially in pregnant women in order to control rubella. More studies on the rubella virus are recommended to gather more data for effective control measures and development of vaccine. Countries should consider strategies to add or improve surveillance for congenital rubella syndrome to better understand their burdens of disease and the associated costs.

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