ORIGINAL ARTICLE

SEROPREVALENCE OF RUBELLA IgG AND IgM ANTIBODIES IN INFANTS SUSPECTED OF HAVING RUBELLA INFECTION

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This study was conducted to assess clinical and laboratory screening for suspected congenital rubella syndrome (CRS) and to evaluate the efficacy of reporting of CRS cases at the Children's Hospital and the Institute of Child Health. A total of 100 infants suspected of having rubella infection were included in the study. Rubella titer was used as an indicator. Enzyme linked immunosorbent assay was performed to detect rubella specific IgM and IgG. The data was analyzed on infants' birth weight, infants' clinical characteristics and laboratory evaluation. The study shows that out of 100 Infants, only three (3%) met the definition for confirmed rubella whereas thirty-four patients were positive for Rubella IgG only. Ninety-seven other children had clinical presentation that met the definition for a probable case but when they were tested for Rubella specific IgM antibodies, the tests were negative. Rubella IgG positive patients were further categorized on the basis of their age; 33 (97%) infants were of less than six months and only 1 (3%) infant was of more than six months. Average age of the patients was 2.2 months (SD±1.55). The common clinical presentations in rubella positive patients (n=3) were failure to thrive (100%), cataract (67%), patent ductus arteriosus (67%), microcephaly (67%), intracranial calcification (33%), buphthalamus (33%), and hepatosplenomegaly (67%). With regard to hematological abnormalities in the three rubella confirmed cases, three had anemia (100%), two infants had thrombocytopenia (67%), and only one infant had leucopenia (33.3%). The relatively high rate of susceptibility indicated a risk of a rubella outbreak, and the resulting. Congenital rubella syndrome is an under-recognized public health problem in Pakistan and can be reduced by vaccinating all seronegative women. There is an urgent need for the collection of appropriate data to estimate the cost effectiveness of a potential Rubella Control Programme.

INTRODUCTION

Rubella is a major public health problem which is usually a mild rash illness in children and adults. However, its seriousness and public health importance stems from the ability of rubella virus to cross the placental barrier and infect foetal tissue, which may result in congenital rubella syndrome (CRS).[1,2]

Rubella is an infectious disease affecting all ages and sexes.[2] Epidemiological surveys in the world indicate that immunity level to rubella virus in different communities is related to the age, socioeconomic status, climate, as well as population size and density. Rubella occurs worldwide with a seasonal distribution. The peak incidence of infection is in late winter or early spring.[1-3]

The mechanism by which rubella virus causes foetal damage is not well understood. The possible mechanism is direct viral damage of infected cells. The most devastating consequences of natural rubella infection during pregnancy are abortion, stillbirth, and foetal malformation that arises from maternal infection during the first trimester of pregnancy.[4] The clinical features of the congenital rubella syndrome may be categorized as transient, developmental and permanent.[5]

Transient clinical features include thrombocytopenic purpura, hepatoslenomegaly and haemolytic anaemia. These abnormalities are present during the first few weeks of life and are not associated with permanent sequelae. Transient bone lesions occur in 20% of congenitally infected infants. Twenty five percent have a meningoencephalitis, that may or not leave neurological sequelae. Jaundice is also commonly present.[2,5]

Developmental clinical features include sensorineural deafness, mental retardation, and insulin-dependent diabetes (IDDM). Developmental defects may take months before they become apparent but persist permanently. Congenital rubella remains the most common cause of congenital deafness in developed countries. Rubella deafness may be unilateral or bilateral and varies considerably in severity. IDDM is actually a common manifestation of CRS (up to 20%). However onset may be delayed till adolescence or adulthood and autoimmune mechanisms may be involved. Between 3-12 months some infants develop a rubbelliform rash, persistent diarrhoea and pneumonitis, which are referred to as "late onset disease". This carries a high mortality risk.[3,5]

Permanent clinical features include heart defects (patent ductus, VSD, pulmonary valve stenosis), eye defects (retinopathy, cataract, microopthalmia, glaucoma, severe myopia), CNS defects (microcephaly, psychomotor retardation).[5] In general, affected organs are hypoplastic, in part due to the reduction in total number of cells.[4]

Immunity, conferred by clinical or subclinical rubella infection, provides lifetime protection against another episode of the disease.[6] Reinfection in the presence of rubella-specific antibody, even with low titers, is very rare.[7] There is no true carrier state of rubella but infants infected with rubella before birth often shed the virus for as long as 12 months after birth, or, rarely, longer.[1]

The RA27/3 rubella vaccine was licensed for use in USA in 1979 and is uptil now is the only vaccine available.[9] Vaccination results in IgG antibody production in more than 98% of vaccine recipients, and a single dose confer long-term immunity against clinical and asymptomatic infection in more than 90% of vaccinated persons.[9,10] The first vaccine dose is routinely given at 12 to 15 months of age, usually in combination with the measles and mumps vaccines referred to as MMR, to decrease the cost and number of injections needed.[8] MMR vaccine is also available in Pakistan but the cost of this vaccine is relatively high. If we can provide these vaccinations in childhood then one can prevent the spread of diseases and often the death of small children due to these diseases. In short, there is no antiviral therapy. The effect of specific immunoglobulin is uncertain.[8-10] Vaccine provides about 95% protection but cannot be given during pregnancy for rubella exposure.[9]

Laboratory tests that can be performed for the diagnosis of rubella infection are viral cell cultures, enzyme linked immunosorbent assay, neutralization test, latex agglutination, hemagglutination inhibition test, immunofluorescent assay, Western blott, complement fixation, polymerase chain reaction, and Immunoblot.[1] ELISA is commonly used for the detection of rubella specific antibodies both of IgG and IgM isotypes. For patients with a clinical diagnosis of CRS, the ELISA may be used to confirm CRS. For most congenitally infected infants, IgM is detectable from birth to 1 month of age. The percentage of infants who are IgM positive declines over the first year of life, until at 1 year most infants are negative. In CRS patients, the IgG response increases gradually over the first 9 months, whereas the maternal IgG titer declines.[6,7]

In Pakistan, little data is available regarding the laboratory proven cases of congenital rubella. The objectives of this study therefore were to determine the Rubella IgG and IgM levels in the serum of infants suspected of having rubella infection, to find the number of confirmed cases of rubella registered during one-year period and to study their common clinical presentations and complications.

MATERIALS AND METHODS

This study was conducted in the Department of Immunology at the Institute of Child Health and Children Hospital, Lahore, Pakistan during the period of January 2004 to December 2004. The study includes a total of 100 infants of both sexes, suspected of having rubella infection with the age between one day to one year. An informed consent was obtained from parents of all patients. For data collection a proforma was developed. The clinical diagnosis was made according to the set of symptoms and findings at the physical examination done by the physicians.

Enzyme-Linked Immunosorbent Assay test was performed to detect rubella specific IgG and IgM antibodies. One hundred infants who were suspected of having rubella infection were tested for rubella IgM and IgG antibodies. All samples were processed according to the manufacturer's instructions. 3-4ml blood was drawn aseptically, 1ml was added to EDTA vial for complete blood picture and the rest was allowed to clot. Serum specimens were separated from the blood and stored at -20°C till assayed. Absorbance of controls and test specimens at the wavelength of 450 and 620-630 nm was measured. Positive as well as negative controls were used with each batch. Furthermore, haematological abnormalities were also studied. Statistical analysis was performed. Univariate analysis was done as mean values and standard deviations are given.

RESULTS

A total of 100 infants were the subject of this study their ages range was one month to one year with

mean age 2.2 months (SD±1.55). The results so obtained have been summarized in table 1-3 and in figure 1-2.

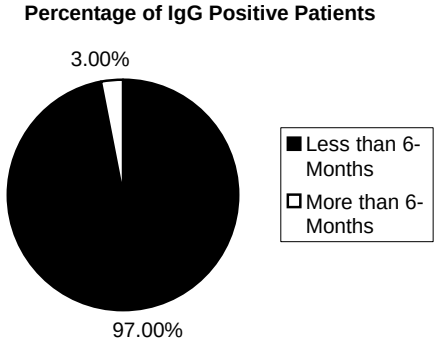

Fig. 1: Classification of Rubella IgG positive patients on the basis of their age.

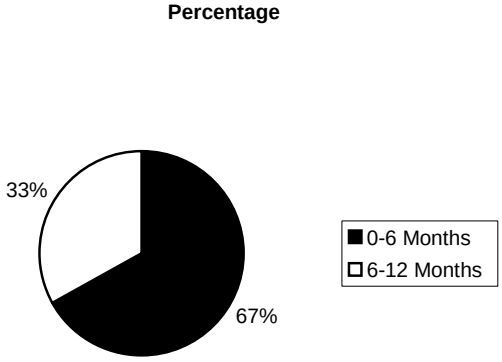

Fig. 2: Distribution of age in three infants with confirmed rubella infection.

Of the total 100 infants, 63(63%) were negative for both rubella IgG and IgM antibodies. Out of them, 39 (61.9%) were male and 24 (38.1%) were females. Of the remaining, 34 rubella IgG positive patients, more males (73.50%) were affected than females (26.50%) (table 1). Thirty-four (34%) patients who were found to be positive for rubella IgG antibody were further categorized on the basis of their age; 33 (97%) infants were less than six months and only 1(3%) infant was more than six months of age (Figure 1).

Three cases out of 100 were proven cases of rubella with both rubella IgG and rubella IgM positive. Two (67%) infants were females and 1 (33%) was male (table 1). In the case of these rubella positive patients, two were below 6 months (67%) and only one rubella positive patient was more than 6 months (33%). Age distribution is given in figure 2.

The common clinical presentations and complications in rubella positive patients (n=3) were failure to thrive 3 (100%), cataract 2 (67%), patent ductus arteriosus 2 (67%), microcephaly 2 (67%), intracranial calcification 1 (33%), buphthalamus 1 (33%), and hepatosplenomegaly 2 (67%) (table 2).

Of the three rubella confirmed cases, 100% had anaemia 67% had thrombocytopaenia, and only one infant had leucopaenia (33%) (table 3). The mean haemoglobin level of the infants, positive for rubella was 7.8g/dl (SD±0.76) and the mean TLC level of the infants, positive for rubella was 12.16 x 10^9/l (SD±7.97).

Table 1: *Distribution of patients suspected of having rubella infection according to sex (n=100)*

S. No.	Status of Rubella specific antibodies	Male Patients		Female Patients	
		n	%	n	%
1	IgM and IgG Negative (n=63)	39	61.90	24	38.10
2	IgG Positive (n=34)	25	73.50	9	26.50
3	IgM and IgG Positive (n=3)	1	33	2	67

DISCUSSION

Rubella is a mild exanthematous, and moderately contagious disease caused by rubella virus that occurs worldwide with a seasonal distri-bution.[1] Rubella virus can act as a teratogen, inducing Congenital Rubella Syndrome when spread from mother to foetus especially in the first trimester of preg-nancy.[2,10] The study was undertaken to determine the seroprevalence of rubella antibodies and the factors related to rubella infection in infants, suspected of having rubella infection. Rubella outbreak occurred in 2002 in the Lombardy region of northern Italy. From 13 maternal cases of rubella infection, congenital rubella infection was diagnosed in three foetuses and three newborns. Of the three infected foetuses, one was aborted and two died in utero, while of the three infected newborns, two were born with severe disease and one was subclinically infected.[1]

In studies from other parts of the world different incidences of congenital rubella infection have been reported. After the epidemic of 1993,

1993, the incidence of rubella in Greece decreased sharply, but in 1999, there was an-other epidemic of smaller magnitude. Four confirmed cases of CRS were recorded after the epidemic of 1999 (corresponding to 4.0 per 100,000 population) and none in 1995-1999 and 2001-2003.[12]

CRS incidence in Japan was determined to be 0.2–8.1 cases/ 100,000 live births per year in epidemic years and 0.1–0.7 in non-epidemic years, respectively. In the last 4 years, the number of CRS cases remarkably decreased to one–three cases per year. This decrease is thought to be because the immunization law was revised in 1994 for changing the focus of rubella immunization from junior high school girls to infants of both sexes.[13]

Table 2: *Common complications in confirmed rubella patients (n=3)*

	Complications	Number of cases (n)	Percentage (%)
General	Failure to thrive	3	100
Head	Microcephaly	2	67
	Intracranial calcification	1	33
Eyes	Cataract	2	67
	Buphthalamus	1	33
Cardiovascular System	Patent ductus arteriosus	2	67
Abdomen	Hepatosplenomegaly	2	67

Table 3: *Hematological parameters in confirmed rubella patients (n=3)*

Hematological parameters	Number of cases	%
Anemia Hb (<12g/dl)	3	100
Thrombocytopenia Platelet count (<150x10^9/l)	2	67
Leucopenia TLC (<12x10^9/l)	1	33

As compared to above reports of Greece and Japan, in the present study, the incidence of rubella was very high (3 cases/100 live births in one year, 2004, only in a single hospital of Pakistan). This may be because in Pakistan, cost of MMR vaccine is relatively high. Furthermore in many cases, women of childbearing age, living in rural and remote areas have very little access to immunization. Thus majority of women of childbearing age remain unvaccinated and susceptible to rubella infection.

In Brazil in 2003, confirmatory serum tests for rubella (IgM and IgG) were run by microparticle enzyme immunoassay. Serum samples from 55 neonates and 52 mothers were received, and 16 were positive. In 19 cases, the infant's IgG levels were interpreted by the pediatrician as being of maternal origin and were not followed. From these cases, three mothers received rubella vaccine before pregnancy.[6]

In Pakistan little data is available regarding the prevalence of congenital rubella infection. In this study, serological diagnosis of rubella in infants was demonstrated by the presence of rubella virus specific IgM in serum, which showed that during the one year study period three cases (3%) were positive. Furthermore, 34 (34%) infants were found to be positive for IgG antibodies only. Rubella IgG positive patients were further categorized on the basis of their age; 33 (97%) infants were of less than six months and only 1 (3%) infant was of more than six months. Confirmation of the diagnosis based solely on the presence of rubella IgG was difficult. No doubt, detection of rubella IgG by ELISA forms the basis of seroprevalence of rubella IgG antibodies but the test does not discriminate between maternally induced immunity and the infection acquired during early gestation. Only when IgG antiviral antibodies persist beyond 4-6 months of age in an infant, one can assume active infection.

In this study, only infants were included. In the case of rubella positive patients, two were less than 6 months (67%) and only one rubella positive patient was above than 6 months (33%). The clinical diagnosis was made according to the set of symptoms and findings at the physical examination performed by the physicians. The common clinical presentations in rubella positive patients (n=3) were failure to thrive 3 (100%), cataract 2 (67%), patent ductus arteriosus 2 (67%), microcephaly 2 (67%), intracranial calcification 1 (33%), buphthalamus 1 (33%), and hepatosplenomegaly 2 (67%).

Failure to thrive is a description applied to children whose current weight or rate of weight gain is significantly below according to their age and sex.[14] In this study, physical, mental and social skills of rubella positive patients were delayed. The term microcephaly simply means "small head".[15] This study also showed that CRS is a frequent cause of congenital microcephaly. Congenital rubella infection is a common cause of cataract.[16] In the present study, two rubella confirmed infants, were born with cataracts in both eyes but these cataracts were so small that they did not affect vision. In one rubella confirmed infant, glaucoma was manifested as buphthalamus. Intracranial calcification is a condition in which calcium and sometimes iron deposits on the wall of blood vessels at various sites of brain tissues (choroids plexus basal ganglia and pineal gland) or in abnormal pathological tissues.[17] In the present study, cranial ultrasonography showed intracranial calcification only in one (33%) infant with congenital rubella but the patients did not show hearing loss. The patients were a few months old, so one could not judge mental retardation but the failure to achieve developmental milestones was suggestive of mental retardation.

The combination of a patent ductus arteriosus with pulmonary stenosis is a typical manifestation of rubella infection in the heart.[18] Similarly, in this study, an echocardiogram was performed on infants' who were suspected of having rubella. Of the three, rubella confirmed infants, two (67%) showed PDA as a commonest clinical feature with pulmonary stenosis as mentioned above.

The most common clinical presentations in infants with IgM antibodies to Rubella virus were bilateral congenital cataract and hepatosplenomegaly, which prompted the clinicians to request assay for rubella antibodies.

Haematological abnormalities are common in rubella infection.[19,20] Leucopaenia is frequently found at the height of the illness and there may be an increase in plasma cells.[19] In the present study, of the three rubella confirmed cases, 67% had thrombocytopaenia, 67% had anaemia and only one infant had leucopaenia (33%) along with other clinical manifestations.

As a **conclusion**, this study shows that substantial proportion of pregnant women is susceptible to rubella infection. The rubella infection can cause multiorgan lesions, such as structural defects of the cardiovascular system, ophthalmologic defects, impairment of neurological system, and of the liver. The observed frequency of antibodies of rubella virus in infants is higher than those reported from other countries of the world. So it is recommended to reinforce procedures of vaccination for all women of childbearing age, routinely carry out tests for detection of antibodies against rubella like protocol of premarital study. This can be done by the inclusion of rubella vaccination in EPI program especially for girls. In view of the high prevalence reported in the present study, health education is necessary for the awareness of women regarding the lethal effects of rubella infection during pregnancy and the resulting CRS.

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