

## Immune status of health care personnel & post vaccination analysis of immunity against rubella in an eye hospital

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**Background & objectives:** Congenital rubella syndrome (CRS) accounts for a significant amount of mortality and morbidity in India. Rubella vaccination is not included in our national immunization programme. Occupational exposure of the health care personnel to rubella infection is well known. This study aims to assess the serological status of health care workers against rubella virus in Aravind Eye Care System, Madurai and to follow the immune response in the seronegative individuals after vaccination.

**Methods:** A total of 500 female and 81 male workers were enrolled in the study. Blood sample was collected for the analysis of rubella specific IgM and IgG antibodies. The seronegative individuals were vaccinated with monovalent rubella vaccine, RA 27/3. The post-vaccination samples were analysed for the antibody levels and their avidity using enzyme immunoassay.

**Results:** Of the 581 volunteers, 493 were seropositive with good protective immunity and 22 had both IgM and IgG antibodies. Sixty six volunteers (59 females and 7 males) were found to be seronegative to rubella. The seroconversion was observed in all the sixty vaccinated individuals, as seen by the appearance of anti-rubella IgG antibodies by fourth week, reaching the peak protective levels (>20 IU/ml) by third month. There was also a progressive increase in the avidity after vaccination.

**Interpretation & conclusion:** Nearly 11.4 per cent of the health care workers were found to be seronegative for rubella virus and after vaccination, these volunteers developed a good protective immunity, thereby reducing the risk of contracting the hospital based rubella infection. Therefore, rubella vaccination may be instituted in hospitals for the benefit of health care workers.

**Key words** Avidity - immunity - rubella - serosurvey - vaccination

Primary acquired rubella infection in the first three months of pregnancy can cause severe congenital malformations in the foetus and also delayed complications in later life, collectively known as congenital rubella syndrome (CRS)<sup>1,2</sup>. It was observed that 25 per cent of congenital cataracts in children in south India were due to maternal rubella, 50 per cent infants were found to have cardiovascular anomalies and 34.8 per cent infants attending the pediatric department of Aravind Eye Care System (AECS) had developmental and neurological defects<sup>3,4</sup>.

The outbreak of rubella in Europe and United States (1962-1965) highlighted the importance of developing rubella vaccine in 1969 to prevent the foetal infection and the consequent CRS<sup>5</sup>. In many developing countries including India, rubella vaccination is not a part of national immunization programme but is available through the private sector in the combined measles-mumps-rubella (MMR) vaccine<sup>5</sup>.

Occupational exposure of the health care personnel to rubella infection deserves special attention. Non-immune pregnant women are at a risk of contracting the infection from patients. Infected health care workers can also act as a potential vectors in the transmission of the virus<sup>6</sup>.

Rubella specific IgG begins to rise after the onset of rash, peaks at about 4 wk and lasts for life, serving as a long term marker for protective immunity<sup>7</sup>. Serosurvey studies among the hospital personnel from various countries showed that 2.4-36.3 per cent of the female health care workers were seronegative against rubella and were at the risk of acquiring infection<sup>6,8-12</sup>. In Songklanagarind University Hospital, Thailand, a total of 366 health care personnel (256 female and 110 male) were tested for rubella antibodies by haemagglutination inhibition (HAI) test, of which 36.3 per cent of females were found to be seronegative. Vaccination resulted in 100 per cent seroconversion<sup>11</sup>. In India, a hospital based rubella outbreak was reported in 1990 from St. John's Medical College and Hospital Campus, Bangalore, where 7 of the 12 female workers (medical students, nurse and staff) did not have rubella specific IgG

antibodies at the time of onset of illness<sup>13</sup>. In a study on seroprevalence of rubella involving 580 women including 80 of medical community at Amritsar district (Punjab), India, 20 per cent were found to be susceptible with risk of acquiring rubella infection<sup>14</sup>. Non-immune pregnant women are also susceptible for rubella infection from unimmunized men<sup>15</sup>. Nearly 20.9 per cent of susceptibility rate was reported among male health workers in Thailand<sup>11</sup>.

In 2002, a serosurvey study was conducted among the female health care workers of AECS, India and reported 15 per cent seronegativity<sup>16</sup>. The present investigation was designed to carry out serosurvey on the rubella immune status among both male and female health care workers in AECS, Madurai, to follow the immune status of the seronegative individuals after vaccination and also to determine the avidity index of the anti-rubella antibody.

### Material & Methods

*Study subjects:* The study was conducted between July 2004 and May 2005 among the newly joined health care workers (Table). They were given adequate background information of the study and those volunteered with a written consent were enrolled. A total of 500 female and 81 male health care personnel between 15-40 yr of age working in different departments of AECS, Madurai, were screened. The Institutional Review Board of AECS approved the study. Details of the information of marital status, immunization history, type and duration of employment were obtained for analysis using a well designed questionnaire.

*Collection of blood sample:* Two ml of blood specimen was collected from each participant. Serum was separated and stored as aliquots in -70°C. Samples to be used immediately were stored at -20°C.

*Detection of seronegativity:* Rubella specific IgM and IgG antibodies were detected by enzyme linked immunosorbent assay (ELISA) using the Euroimmun Kit (Medizinische Labordiagnostika AG, Lubeck) according to the manufacturer's instruction. The results were interpreted as seronegative if the ratio

of the extinction value was less than 0.8 and seropositive if more than 1.1. Samples with the ratio between 0.8 - 1.1 were considered equivocal and retested using a fresh blood sample. Validation of the kit was carried out using the Quality Control samples from Aravind Eye Hospital, India, and Health Protection Agency, London. All participants were duly informed of the results. The samples from those cases that were positive for both IgM and IgG antibodies (n =22) were collected again after 4 months for retesting with the Euroimmun kit.

*Vaccination of seronegative workers:* Sixty seronegative individuals (55 female and 5 male) were given a single dose of rubella vaccine and the married females were advised to avoid conception for three months following vaccination<sup>17</sup>. The RA 27/3 rubella vaccine (Serum Institute of India, Pune) was reconstituted with diluent supplied by the manufacturer and administered intramuscularly.

*Antibody titre after vaccination:* Blood samples from the vaccinated individuals were obtained at first and fourth week, third and sixth month, and their sera were analysed for IgM and IgG antibodies. The Visual Basic programme, developed by using the algorithm specified in the Euroimmun kit, was used for quantification of IgG antibodies in International Unit per ml (IU/ml).

*Avidity index:* Sera collected at the end of first, third and sixth month were analysed for avidity by

Euroimmun IgG Avidity Kit (Medizinische Labordiagnostika AG, Lubeck). The assay was based on protein denaturing avidity principle using urea as a protein denaturant. The samples were assigned as high avidity if the avidity index was > 60 per cent and low avidity if < 40 per cent. The avidity index between 40-60 per cent was considered as equivocal.

*Statistical analysis:* The data were double-entered in Epi Info, version 6.04 (Centers for Disease Control and Prevention, Atlanta, GA, USA and WHO, Geneva, Switzerland). Analysis and representation of the data was carried out using Stata, version 7.0 (Stata Corporation, College Station, TX, USA) software and Microsoft Excel. The data were tested using Mann Whitney Rank Sum test with a significance level of 0.05. Repeated measures of ANOVA has been employed for testing the differences in mean avidity index among three time periods.

## Results

*Anti-rubella antibody profile:* Of the 581 personnel tested, IgG anti-rubella antibody was observed in 493 and both IgM and IgG antibodies in 22 personnel. However, 66 were negative for both IgM and IgG antibodies. Seronegativity was high among the laboratory/research staff and physicians and lowest among housekeepers/caterers (Table). The proportion of seronegativity was 11.8 per cent among the female and 8.6 per cent among the male workers.

**Table.** Serological results of health care personnel (male and female) of Aravind Eye Care System

Personnel	No. examined (male & female)	IgG & IgM negative	IgG positive & IgM negative	IgG & IgM positive
Physicians	31 (27+4)	5	26	0
Laboratory/research staff	12 (2+10)	2	8	2
Administrators/patient co-ordinators	71 (25+46)	7	61	3
Nurse/counsellor	274 (8+266)	39	227	8
Housekeeper/catering	73 (0+73)	3	68	2
Medical instrument production/repair staff	79 (11+68)	7	66	6
Opticians/refraction	37 (4+33)	3	33	1
X-ray/photography	4 (4+0)	-	4	-
Total (%)	581	66 (11.4)	493 (84.8)	22 (3.8%)

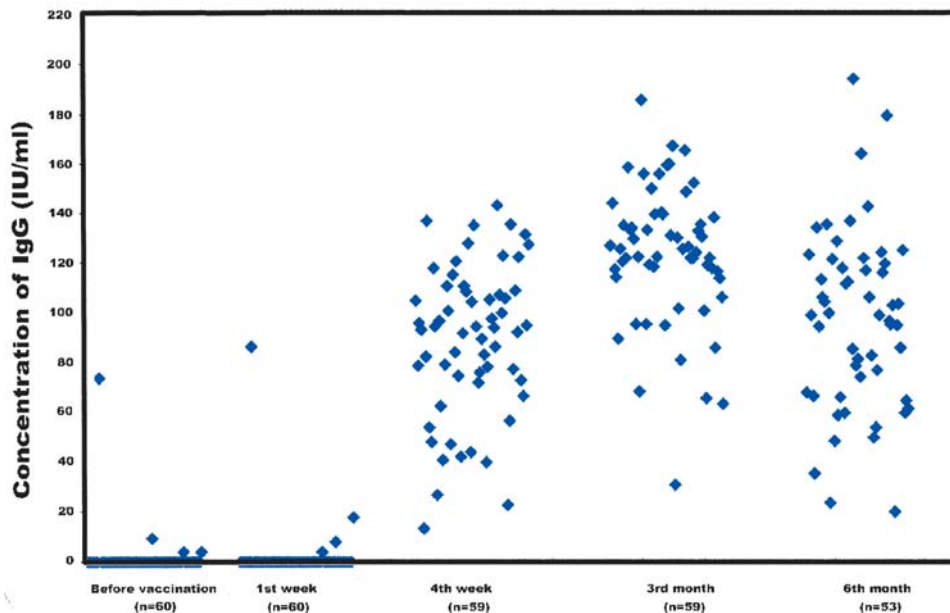


Fig. IgG antibody profile in the healthcare workers after immunization with RA 27/3 monovalent rubella vaccine.

**Rubella specific IgM antibody:** Twenty two samples were found to have both IgM and IgG antibodies for rubella. To determine whether there would be reduction in IgM positivity, samples were collected four months later from these workers. The presence of both anti-rubella IgM and IgG antibodies was confirmed in the second samples. There was no significant difference in the mean avidity indexes (mean  $\pm$  SD) between the first sample ( $79.8 \pm 10.81$ ) and the second ( $82.5 \pm 10.26$ ) sample.

**Immune status after vaccination:** Among the 60 seronegative individuals three samples showed low levels of anti-rubella IgG (4-9.6 IU/ml) and one a fairly high level (74.3 IU/ml) when the same samples were tested with Quantification assay. All 60 vaccinated volunteers were seropositive for IgG by fourth week and the level of IgG antibody was more than 20 IU/ml by third month, remaining at the same level by sixth month (Fig.). IgM antibodies were observed in 56 individuals by the fourth week and 15 individuals by sixth month after vaccination out of 60 tested. In other words, 28 per cent of vaccinated individuals remained positive for both anti-rubella IgG and IgM antibodies even six months after vaccination. Three out of four samples that showed IgG antibody before vaccination in the quantification assay were negative for IgM antibody after vaccination.

Avidity index analysis for IgG revealed that the levels of highly avid antibodies increased progressively after immunization from the first month to sixth month. A significant ( $P < 0.001$ ) difference in the mean avidity index (mean  $\pm$  SD) was observed among the fourth week ( $9.2 \pm 15.23$ ), third month ( $36.9 \pm 12.20$ ) and sixth month ( $58.2 \pm 9.25$ ) post vaccinated samples.

## Discussion

Rubella is an infectious disease affecting all age groups and both sexes<sup>18</sup>. Health care personnel especially those without protective anti-rubella antibodies are at high risk for rubella infection. To prevent infection in susceptible employees and to reduce the likelihood of nosocomial transmission to patients and non-immune health care workers, vaccination against rubella is necessary<sup>8,16</sup>. The present study among the health care workers in an eye hospital revealed 11.4 per cent to be of high risk group. The major proportion of seronegativity was observed between 15 and 19 yr of age among females and 25-29 yr age group among males. This may be a reflection of the population status for rubella immunity in this part of the country.

Serosurveys conducted in 45 developing countries in 1990<sup>17</sup> indicated that many women of

child bearing age living in developing countries remain at risk for developing CRS in their infants. In 2001, a serosurvey study conducted in New Delhi among young school girls of age 9-12 yr attending Paediatric outpatient department revealed 10 per cent of seronegativity and vaccination provided seroconversion completely<sup>19</sup>. In Amritsar district, Punjab, India seroprevalence of rubella was determined among 580 women by IgG antibody analysis and 71.3 per cent were found to be seropositive. This indicated that about one third of women were susceptible to rubella infection<sup>14</sup>. Similar to this 11.8 per cent of our study population also exhibited susceptibility in the child bearing age group.

Our study further revealed that 8.6 per cent of male health care personnel were seronegative, thus indicating the risk of transmission to the susceptible women<sup>15</sup>. In a serosurvey study conducted among the Nigerian and Swedish population, the rate of non-immunity was higher in females than males and the difference was not significant, possibly due to lower number of male participants<sup>12</sup>. The present study confirms the above findings.

The vaccine RA 27/3, administered to the seronegative individuals is known to be highly efficacious. In clinical trials done among 12 month and older age group, 95-100 per cent of seroconversion was observed by 21-28 days after vaccination. With vaccination at 9 months of age, more than 95 per cent of seroconversion was reported<sup>17</sup>. In Thailand, during the serosurvey of health care workers, the seronegative individuals were vaccinated by RA 27/3 Wistar vaccine and 100 per cent seroconversion was observed<sup>11</sup>. According to WHO, 10-12 IU/ml of IgG level is usually satisfactory for serosurveys in enzyme immunoassay<sup>7</sup>. In the present study, all the volunteers immunized with RA 27/3 developed protective immunity with the titre of more than 20 IU/ml and high avidity within three months after vaccination, thus confirming the above reports for India.

Rubella specific IgM antibodies were detectable starting from the day 5-10 after infection and rose rapidly around the day 20, proceeding to disappear

by the day 50-70<sup>20-22</sup>. In 1975, Pattison *et al*<sup>21</sup> observed the persistence of IgM antibody in 2 individuals out of 13, more than 10 months after acute rubella infection during the outbreak among the medical students and nurses in London. In the present study, persistence of IgM antibodies was also observed four months after the first test and further there was no change in the IgG avidity index between these two samples. Similarly, the presence of IgM antibodies even six months after vaccination was observed. However, no explanation could be provided.

We have demonstrated that the seronegative workers were not immunologically defective but were capable of producing good protective immunity. The avidity of rubella specific IgG antibody also established a progressive increase in the maturation of antibody from the first to sixth month after vaccination. A similar study with all these parameters needs to be conducted in different Indian populations.

It is evident from the present study that rubella virus infection is prevalent in our population. Women in childbearing age group without protective immunity are highly susceptible in acquiring infection. Since vaccination against rubella is not mandatory in our country, there is a need to formulate an effective rubella immunization programme especially for the women of child bearing age group. As the serological screening for anti-rubella IgG antibody is very expensive, WHO recommends the direct vaccination of all women in childbearing age group since there is no harm in vaccinating already immune individuals<sup>17</sup>. Therefore it may be recommended that vaccinating the health care workers against rubella at the beginning of their employment would help to protect health care personnel and also to prevent hospital based outbreaks.

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