STUDY OF SEROEPIDEMIOLOGY OF RUBELLA IN SCHOOL GIRLS

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CERTIFICATE

Certified that this dissertation entitled **"STUDY OF SEROEPIDEMIOLOGY OF RUBELLA IN SCHOOL GIRLS"** is a bonafide work done by DR.S.GIRIDHAR, M.D. Post graduate student of Paediatric Medicine, **INSTITUTE OF CHILD HEALTH AND HOSPITAL FOR CHILDREN, EGMORE, CHENNAI - 600 008,** attached to Madras Medical College, during the academic year 2003-2006.

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DECLARATION

I declare that this dissertation entitled **"SEROEPIDEMIOLOGY OF RUBELLA IN SCHOOLGIRLS"** has been conducted by me at the Institute of Child Health and Hospital for Children, under the guidance and supervision of my unit chief **Prof. R. Duraisamy M.D.,D.C.H.** It is submitted in part of fulfillment of the award of the degree of M.D. (Paediatrics) for the February 2006 examination to be held under the Tamil Nadu Dr.M.G.R Medical University, Chennai. This has not been submitted previously by me for the award of any degree or diploma from any other university.

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CONTENTS

INTRODUCTION

The International Classification of Diseases classifies rubella as two diseases: Rubella (ICD-9 056; ICD-10 B06) and Congenital Rubella Syndrome (ICD-9 771.0; ICD-10 P35.0) (World health organization 1993, Benenson 1995).

HISTORY OF RUBELLA

The earliest description of rubella dates back to the 1700's when two German physicians De Bergen in 1752 and Orlow in 1758 described the clinical manifestations of the disease. At that time the clinical manifestations were considered to be a variant of measles and scarlet fever and was called the "third Disease". It was not until 1814, that the illness was described as a distinct entity for the first time by another German physician George de Maton who called it "RÔTHELN"(1). Because of the strong German connection the disease was also called "German measles" or "Three-day measles" due to the similarity of the illness to measles. In 1866 the disease was renamed "Rubella" by Henry meaning "Little Red" in Latin (2).

In 1914 Hess postulated a viral etiology based on his work with monkeys. Hiro and Tasaka in 1938 documented the viral etiology by passing the disease to children using filtered nasal washings from acute cases (2). The illness was merely considered a mild illness of children and adults with a prodrome of cold like symptoms. When Australia faced an epidemic of Rubella in 1940-41, it was even debated that the illness was due to a mutant strain of the virus as many adults were infected and there was high incidence

of associated arthritis and arthralgia. After that epidemic in 1941, Dr Norman Gregg a senior ophthalmologist reported an “out break” of unusual type of cataract where all the layers except the outermost layer of the lens were affected, with other associated “defects” comprising of a variety of heart defects predominantly patent ductus arteriosus. He presented that “The remarkable similarity of the opacities in the lens, the frequency of an accompanying affection of the heart and the widespread geographical incidence of the cases suggested there was some common factor in the production of the diseased condition, and suggested it was the result of some constitutional condition of toxic or infective nature rather than of a purely development defect” and he added that “maternal rubella infection in early pregnancy was the cause of the babies ‘defects’ ”. The recognition of association of rubella to these birth defects by Dr. Gregg is considered remarkable.

In 1962 rubella virus was first isolated by Parkman and Weller and soon this was followed by the development of the vaccine, which was licensed in 1969, and once the human diploid cell cultured RA 27/3 strain vaccine was licensed for use, all other vaccines strains were discontinued.

THE VIRUS

The Rubella Virus (RV) is a human-specific, non-arthropod borne, lipid-enveloped, positive-sense, single-stranded RNA virus. It is the only member of the genus *Rubivirus* in the *Togaviridae* family. The mature RV virion is a round or ovoid particle approximately 60 nm in diameter. The RV virion contains a RNA genome enclosed in an icosahedral capsid composed of

protein C (33kDa). Surrounding this nucleo-capsid is a lipid bilayer, in which viral glycoproteins E1 (58 kDa) and E2 (42 to 47 kDa) are embedded (3). The protein C, glycoproteins E1 and E2 are the three structural proteins and there are two non-structural proteins NS1 and NS2. RV is relatively unstable and is rapidly inactivated by 70% alcohol, ethylene oxide, formalin, ether, acetone, chloroform, free chlorine, deoxycholate, beta-propiolactone, ultraviolet light, extreme pH (<6.8 or >8.1), heat (>56°C), and cold (from -10 to -20°C). It is resistant to thiomersal and is stable at temperatures of -60°C or less.

The virus replication cycle is confined to the cytoplasm of the infected cells. RV considered non-cytopathogenic to most mammalian cells, induces a cytopathogenic effect only in continuous cell lines such as RK13 (rabbit kidney) and Vero. Immunofluourescense is used to identify the presence of the virus in culture. The humoral immune response to RV is predominantly to the E1 glycoprotein and persists indefinitely after infection (4).

E1 has 6 distinct antigenic determinants, 4 associated with haemagglutination and 2 with neutralization. Though sequencing studies have recognized two genotypes; one from Europe, North America and Japan and another identified from isolates in India and China, RV is antigenically stable and hence does not pose a problem for serological diagnosis or for vaccination (5).

There is only one serotype of the rubella virus (6). Humans are the only known natural hosts for the RV. The lack of animal models to reproduce the cytopathic effects of rubella virus has hindered significant research opportunities and understanding of the teratogenic properties of the virus.

Hence, unlike the situation for most human teratogens, animal models of CRS are not particularly useful and have not contributed much to the understanding of the pathogenesis of the defects. However, in contrast to the situation for other human teratogens, there is good histopathology of infected abortuses, and these have provided valuable information on the pathogenesis of the abnormalities (7).

EPIDEMIOLOGY

OCCURRENCE

Rubella occurs worldwide.

RESERVOIR

Rubella is a human disease. There is no known animal reservoir. Although infants with CRS may shed rubella virus for an extended period, a true carrier state has not been described.

TRANSMISSION

Rubella is spread from person-to-person via airborne transmission or droplets shed from the respiratory secretions of infected persons. There is no evidence of insect transmission. Rubella may be transmitted by subclinical or asymptomatic cases (up to 50% of all rubella virus infections).

TEMPORAL PATTERN

In temperate areas, incidence is usually highest in late winter–early spring. Epidemics occur every 5-9 years. However, the extent and periodicity

of rubella epidemics is highly variable in both developed and developing countries. The reasons for this are not known. Before the introduction of large-scale rubella vaccination, the average age at which children were infected varied between 6-12 years in industrialized areas and 2-8 years in urban areas of developing countries.

COMMUNICABILITY

Rubella is only moderately contagious. The disease is most contagious when the rash is erupting, but virus may be shed from 7 days before to 5–7 days or more after rash onset.

Infants with CRS shed large quantities of virus from body secretions for up to one year and can therefore transmit rubella to persons caring for them who are susceptible to the disease.

RUBELLA

Rubella is a common cause of maculopapular rash illness with fever during childhood. In the industrialized world where the routine vaccination against rubella is in place, the occurrence of rubella infection is noted predominately in adolescents and young adults; but in India where rubella vaccination is not part of the national immunization programme, the disease is prevalent across all age groups from early childhood through adolescence to adulthood, though the pre-school children are relatively spared.

A history of exposure may not be present. The incubation period is usually 14 days with a range of 12 days to 23 days. The illness in childhood is

usually without prodromal symptoms unlike in adults who may experience a 1–5 day prodrome before the onset of rash. The signs and symptoms include

1. Eye pain on lateral and upward eye movement (particularly troublesome complaint)
2. Conjunctivitis
3. Sore throat
4. Headache
5. General body aches and malaise
6. Low-grade fever
7. Chills
8. Anorexia
9. Nausea
10. Tender post-auricular, occipital and posterior cervical lymphadenopathy is characteristic and precedes the rash by 5-10 days.
11. Forchheimer sign (an enanthem observed in 20% of patients with rubella during the prodromal period; can be present in some patients during the initial phase of the exanthem; consists of pinpoint or larger petechiae that usually occur on the soft palate)
12. Arthralgia or arthritis, more common in women than men, may occur in up to 70% of adult women with rubella.

13. Rare complications include thrombocytopenic purpura and encephalitis.

The exanthema (rash) of rubella consists of a discrete rose pink maculopapular rash ranging from 1-4 mm. Rash in adults may be quite pruritic. The synonym “3-day measles” derives its name from the typical course of rubella exanthema that starts initially on the face and neck and spreads centrifugally to the trunk and extremities within 24 hours. It then begins to fade on the face on the second day and disappears throughout the body by the end of the third day. The clinical diagnosis of rubella is unreliable, as it is one of many diseases causing maculopapular rash with fever. The differential diagnosis includes

1. Measles,
2. Dengue,
3. Parvovirus B19,
4. Human herpes virus 6,
5. Coxsackie,
6. Echovirus,
7. Ross River,
8. Chikungunya,
9. Entero and adenoviruses,
10. Streptococcus group A (beta hemolytic).

Measles is most frequently associated with cough, coryza, and conjunctivitis, though these are relatively nonspecific symptoms common to a number of viral infections. Joint symptoms are seen in up to 60% of adult

women with rubella, but joint symptoms are also frequent with parvovirus B19 infection and with dengue and other arboviral diseases. Post-auricular lymphadenopathy is associated with rubella and roseola infantum (usually seen in children < 4 years); thus, the differential diagnosis of rubella remains difficult in young children. For these reasons, confirmation of rubella is not possible without laboratory testing (8).

CONGENITAL RUBELLA INFECTION (CRI) AND CONGENITAL RUBELLA SYNDROME (CRS)

Rubella virus infection imparts a public health concern only when pregnant women and women of the childbearing age contract the disease, because of the teratogenic potential of the rubella virus (9).

Congenital Rubella Infection (CRI) encompasses all outcomes of intrauterine rubella infection including abortion, stillbirth, congenital defects noticed soon after birth or that which develops as a late-manifestation referred to as Congenital Rubella Syndrome (CRS) and asymptomatic rubella infection.

When a pregnant woman contracts the disease, the average risk to the fetus all through the pregnancy is 45%. In the first trimester there is almost 81% chance of the fetus being infected. The rate drops to 54% for weeks 13 to 16 and the lowest risk period is between 23-26 weeks at 25%. During the last 10 weeks the rate of infection rises again to be 60% between 31 and 36 weeks and it was 100% beyond 36 weeks of gestation (10).

Rubella embryopathy almost exclusively results from first trimester maternal infection (11) and is greatest in the first 8 weeks of pregnancy.

Cardiac and eye defects are more likely to result when maternal infection is acquired during the first 8 weeks of pregnancy (i.e.) during the critical phase of organogenesis whereas retinopathy and hearing defects are more evenly distributed throughout the first 16 to 20 weeks of gestation. After the first trimester, the virus is isolated infrequently from the neonates, probably because fetal immune mechanisms can be activated and infection can be terminated.

Following intrauterine infection in early pregnancy the virus persists throughout the gestation. Virus can also be recovered from nasopharyngeal secretions, urine, stools and CSF from survivors. However, by the age of 3 months the proportion-excreting virus declines to 50-60% and by 1 year, 10%.

MAIN CLINICAL MANIFESTATIONS OF CONGENITAL RUBELLA (12,13)

The clinical manifestations of CRS can be transient, developmental or permanent

CATEGORY	SPECIFIC MANIFESTATION
GENERAL	Fetal loss (spontaneous abortion and stillbirth) Low birth weight
AUDITORY SYSTEM	Sensorineural deafness Central auditory deafness Speech defects
CARDIOVASCULAR SYSTEM	Patent ductus arteriosus Pulmonary stenosis Coarctation of aorta Ventricular septal defects Complex congenital heart disease Myocarditis

OCULAR SYSTEM	Pigmented retinopathy Cataracts: pearly, dense, nuclear 50% bilateral, very often with retinopathy Microphthalmos Cloudy cornea Glaucoma
HEMATOLOGICAL	Thrombocytopenia with or without purpura Hemolytic anaemia Altered blood group expression
CENTRAL NERVOUS SYSTEM	Meningoencephalitis Microecphaly Intracranial calcifications Electro encephalographic abnormalities Mental retardation Behavior disorders Autism Chronic progressive panencephalitis
SKIN	Blue berry muffin spots Chronic rubelliform rash Dermatoglyphic abnormalities
IMMUNOLOGICAL	Hypogammaglobulinaemia Lymphadenopathy Thymic hypoplasia
ENDOCRINE	Insulin dependent Diabetes Mellitus Hyperthyroidism or hypothyroidism Growth hormone deficiency
GENITOURINARY	Cryptorchidism Polycystic kidney disease
LIVER	Hepatosplenomegaly Jaundice Hepatitis
LUNGS	Interstitial pneumonia
BONE	Radiographic lucencies Large anterior fontanelle Micrognathia

IMMUNE RESPONSE IN RUBELLA

The humoral immune response to rubella infection has been well studied. Rubella specific IgM is diagnostic of acute infection; IgM usually appears within four days after onset of the rash and can persist up to 4-12 weeks. Rubella-specific IgG is a long-term marker of previous rubella infection; IgG begins to rise after the onset of the rash, peaks about four weeks later, and generally lasts for life (14).

The natural infection with wild virus was shown to induce more vigorous immune response than a vaccine induced response (15). Serology remains the method of choice for diagnosis of rubella infections and for determination of susceptibility.

The serum immune response in CRS differs from that seen in rubella (and from many other viral diseases). At birth, the serum of an infant with CRS contains maternally derived rubella-specific IgG antibodies as well as IgG and IgM antibodies synthesized by the fetus. Maternal rubella-specific IgG is also found in normal infants born to women who are immune to rubella. Therefore, rubella-specific IgM is used to diagnose congenital rubella infection in infants. In infants with CRS, rubella-specific IgM can be detected in nearly 100% at age 0-5 months; about 60% at age 6-12 months; and 40% at age 12-18 months; IgM is rarely detected after age 18 months (16).

DIAGNOSIS OF RUBELLA AND CONGENITAL RUBELLA SYNDROME

Either one of the following is necessary for diagnosis of Rubella or CRS

- Demonstration of a rubella-specific IgM antibody
- Demonstration of infant IgG rubella antibody level that persists at a higher level and for a longer time than expected from passive transfer of maternal antibody (i.e., rubella titre that does not drop at the expected rate of a twofold dilution per month)
- Isolation of rubella virus, which can be obtained from nasal, blood, throat, urine, or cerebrospinal fluid specimens (best results come from throat swabs)
- Detection of virus by RT-PCR can be used to detect the presence of rubella virus after growth in tissue culture or directly in clinical specimens.

In 1998 the World Health Organization (WHO) Department of Vaccines and Biologicals, in collaboration with WHO regional offices and with specialists from the WHO Programme for the Prevention of Blindness and Deafness, developed standard case definitions for Rubella and CRS to be used for surveillance (17,18,19)

RUBELLA

a. *Suspected rubella case*: A suspected rubella case is any patient of any age in whom a health worker suspects rubella. A health worker should suspect rubella when a patient presents with fever, maculopapular rash, and one or more of the following: cervical adenopathy, suboccipital adenopathy, postauricular adenopathy, or arthralgia/arthritis.

b. *Clinically or Laboratory confirmed rubella case*: A laboratory-confirmed rubella case is a suspected case with a positive blood test for rubella-specific IgM. The blood specimen should be obtained within 28 days after the onset of rash.

c. *Epidemiologically confirmed rubella case*: An epidemiologically confirmed rubella case is a patient who meets the suspected case definition and is epidemiologically linked to a laboratory confirmed case.

CONGENITAL RUBELLA SYNDROME

a. *Suspected CRS case:*

A suspected case is any infant less than one year of age in whom

1. There is a maternal history of suspected or confirmed rubella during pregnancy.

2. When the infant presents with heart disease, and/or suspicion of deafness, and/or one or more of the following eye signs:

- White pupil (cataract);
- Diminished vision;
- Pendular movement of the eyes (Nystagmus);
- Squint;
- Small eye ball (Microphthalmia);
- Large eyeball (congenital glaucoma).

b. *Clinically confirmed CRS case:*

A clinically-confirmed case is one in which a qualified physician detects two of the complications in group *(a)* OR one from group *(a)* and one from group *(b):*

(a) Cataract(s) and/or congenital glaucoma; congenital heart disease; loss of hearing; pigmentary retinopathy.

(b) Purpura; hepatosplenomegaly; microcephaly; developmental delay; meningoencephalitis; radiolucent bone disease; jaundice with onset within 24 hours after birth.

c. *Laboratory-confirmed CRS case:*

A laboratory-confirmed CRS case is an infant with a positive blood test for rubella specific IgM who has clinically-confirmed CRS.

d. *Congenital rubella infection (CRI):*

An infant with a positive blood test for rubella IgM who does not have clinically confirmed CRS is classified as having congenital rubella infection (CRI).

RUBELLA VACCINE

The first vaccine was developed in the early 60's (HPV77.DE5 and Cendehill) and was licensed for use in 1969. In 1979 the HPV77.DE5 strain was replaced with RA27/3 and Cendehill is no longer available. Vaccine is available, either as single antigen vaccine or combined with measles vaccine (MR), mumps vaccine or measles and mumps vaccine (MMR). Most of the currently licensed vaccines are based on the live, attenuated RA 27/3 strain of rubella virus, propagated in human diploid cells. The RA27/3 vaccine is highly stable at –70°C. When stored at 4°C, its potency is maintained for at least five years. The vaccine should be stored at 2–8°C and protected from light. Each dose of this vaccine, which is given by the subcutaneous route, contains a defined number of active virus particles (>1000 TCID 50). Other attenuated rubella vaccine strains, such as the Matsuba, DCRB 19, Takahashi, Matsuura and TO-336 strains are used primarily in Japan; the BRD-2 strain is used in China.

Rubella vaccine is usually administered at age 12–15 months, but can also be administered to children as young as nine months of age. In most countries, the vaccine is given as MR or MMR, and the age of administration is chosen based on the appropriate age for measles vaccination. It may also be administered to older children, adolescents, students, childcare personnel, health care workers, military personnel and adult men in contact with women of childbearing age. Rubella vaccination should be avoided in pregnancy because of the theoretical (but never demonstrated) teratogenic risk. Consequently, there is no need to screen women for pregnancy before rubella

vaccination. If pregnancy is being planned, then an interval of one month should be observed after rubella immunization. Rubella vaccination during pregnancy is not an indication for abortion. Although the virus is excreted by vaccinated, it is not transmitted to susceptible contacts.

Persons with a history of anaphylactic reaction to neomycin or an anaphylactic reaction after a previous dose of rubella vaccine should not receive the vaccination. Rubella vaccines should not be given to persons suffering from advanced immunodeficiency including congenital immune disorders, malignancies and immunosuppressive therapy. However, asymptomatic HIV-positive persons can be immunized. Children with malignant disease or who have had a bone marrow transplant should be immunized against rubella six months after immunosuppressant treatment is stopped. Vaccination should be postponed if the potential vaccinee has a serious illness. Persons with active tuberculosis should not be vaccinated until treatment has been established. Rubella antibodies present in blood products may interfere with rubella vaccination. Therefore, persons who received blood products should wait at least three months before vaccination and if possible, blood products should be avoided for up to two weeks post-vaccination.

Generally, the adverse events following vaccination with the RA27/3 rubella vaccine are mild, particularly in children. Most of the available data on adverse events are for the MMR combination. Common adverse events include pain, redness and induration at the site of injection. Low-grade fever and rash, lymphadenopathy, myalgia and paraesthesia are commonly reported. Joint symptoms tend to be rare in children (0%–3%) and in men, but are common

among vaccinated adolescent and adult females; they include arthralgias (25%) and arthritis (10%) that usually last from a few days to two weeks. These transient reactions seem to occur in non-immune individuals only, for whom the vaccine is important. Thus, fear of unjustified side effects should not prevent vaccination of women with uncertain rubella immune status. As there is no harm in vaccinating already immune individuals, serological testing before immunization is not necessary. Although concerns have been raised that rubella vaccination of adult women might occasionally lead to chronic arthritis, large epidemiological studies have not supported a role for rubella vaccine in chronic joint disease. Thrombocytopenia is rare and has been reported in less than 1 case per 30,000 doses administered. Anaphylactic reactions are rare after RA27/3 vaccines.

The RA27/3 vaccine is highly efficacious. In clinical trials 95%–100% of susceptible persons aged 12 months and older developed rubella antibodies by 21–28 days after vaccination. Vaccination even at nine months of age results in seroconversion rates of more than 95%. Vaccine-induced immunity is generally assumed to be lifelong, although rubella antibodies may fall below detectable levels.

SEROSURVEILLANCE OF RUBELLA AND CRS (17)

The WHO has issued guidelines for surveillance of rubella and CRS. For countries that wish to assess whether to add rubella vaccine to their national immunization programme, baseline information on the disease burden due to CRS may be helpful.

There are several options for assessing the disease burden due to CRS:

1. Carry out CRS surveillance for at least two years, either nationwide or in selected urban and rural populations where there are at least 200,000 births per year.

2. When a rubella outbreak is detected, conduct investigations, including laboratory tests, of a small number of suspected rubella cases per month (perhaps 5 to 10 investigations per month). All febrile rash illnesses in pregnancy should be investigated. If rubella cases are reported in individuals > 15 years of age, active surveillance should be conducted until nine months after the end of the outbreak to identify suspected CRS cases in infants 0-11 months of age.

3. Conduct antenatal serosurveys to assess the proportion of women at risk for rubella infection in pregnancy.

4. Where resources permit, conduct a community-based serological survey to estimate the potential CRS disease burden and the potential impact of different rubella vaccination strategies.

5. Conduct serosurveys among women of childbearing age, which indirectly reflects the proportion of pregnant women at risk.

Because the public health burden of rubella relates to the risk of infection of pregnant women, which in turn may cause CRS in their offspring, many countries have conducted serosurveys to determine the proportion of

women of childbearing age who are susceptible to rubella as it indicates the potential risk for rubella infection in pregnant women.

A single cross-sectional survey of IgG seroprevalence in women of childbearing age is of limited usefulness in demonstrating disease burden. Although a high level (e.g. >20%) of susceptibility is likely to indicate a high risk of CRS in that population, a low level of susceptibility cannot be taken to mean no risk of CRS. Even when susceptibility levels in women are below 10%, CRS can occur (20,21). Therefore serological surveys are of most use to monitor trends in the proportion of adult women who are susceptible, in particular in countries, which have introduced rubella vaccination for women of childbearing age. In special situations when financial and technical resources permit, a country can consider conducting an age-stratified serosurvey for rubella. However, this will be a major research study that requires the participation of a virologist whose laboratory is prepared to conduct large numbers of serological tests; one or more epidemiologists to design the study; staff to carry out the fieldwork; and a mathematical modeler experienced in studies of communicable diseases to analyze the results. This type of survey can provide point estimates (with confidence intervals) of the proportion susceptible to rubella for each age group surveyed. Such data, in conjunction with mathematical modeling, can be used to estimate the average age at rubella infection and to predict the effect of different immunization strategies on the incidence of CRS over different periods of time (22,23).

INTRODUCTION OF RUBELLA VACCINATION INTO THE NATIONAL PROGRAMME

Once the baseline information on disease burden is available, strategies for introduction of rubella vaccination into the national immunization programme should be implemented.

The immunization of boys and girls aged 1 year (childhood immunization) aims to protect women of childbearing age from exposure to the rubella virus by interrupting its transmission (24,25). This can lead to a rapid reduction in cases of congenital rubella and extension of the interepidemic period, but if vaccination coverage is low there is concern that this strategy may increase the incidence of rubella in susceptible adolescents and adults, thus increasing the incidence of congenital rubella. It has been estimated that in developed countries this could happen in the long term if immunization uptake was lower than 50%-60%, with wide oscillations in the incidence of congenital rubella in the medium term (24,26-29). This shift in the proportion of susceptibles in older age groups can result in more cases of CRS than in the prevaccination period. Consequently, it is essential that childhood vaccination programmes achieve and maintain high levels of coverage. All countries undertaking rubella elimination should ensure that women of childbearing age are immune and that routine coverage in children is sustained >80%.

In contrast, when immunization is targeted at adolescent girls or women of childbearing age (selective immunization), the epidemiology of rubella is largely unaffected since most infections occur before the age at immunization.

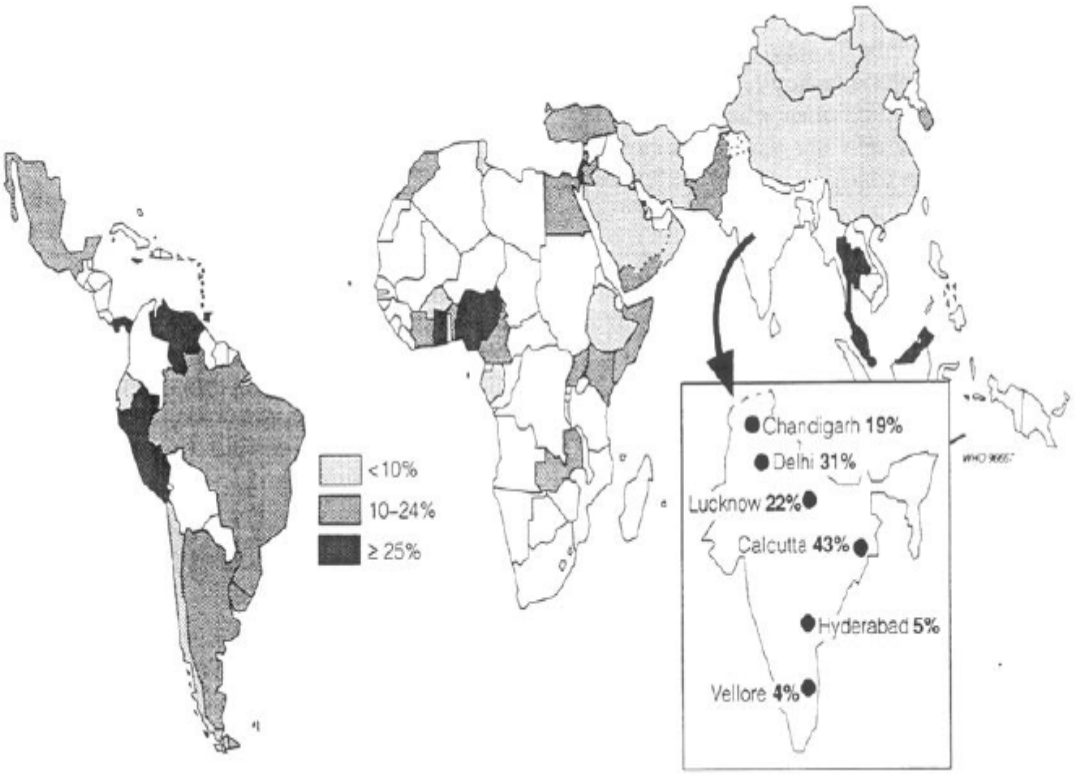
With such an approach, the incidence of CRS declines linearly with the level of coverage. However, elimination of CRS cannot be achieved with this strategy, in part because it would require every susceptible woman to be effectively immunized (30). Several countries have adopted a combined vaccination strategy because of its advantages (31).

Countries wishing to adopt the selective immunization strategy should identify their target population. The precise target population addressed will depend on the susceptibility profile, cultural acceptability and operational feasibility. The most rapid impact would be achieved by mass campaigns for women of childbearing age (and men preferably). For increased impact even men should be vaccinated. In non-vaccinated individuals, susceptibility or immunity to rubella can be ascertained only by serological tests. However, serological testing is expensive and operationally impractical, and as there is no harm in vaccinating already immune individuals, serological screening for susceptibility is not recommended before rubella vaccination.

REVIEW OF LITERATURE

Rubella IgG serosurveys among women of childbearing age indicate the potential risk for rubella infection in pregnant women.

A review conducted by WHO in 1997 identified over 45 seroprevalence studies of rubella in developing countries conducted on women of child bearing age (32). The proportion of women who were seronegative to rubella was less than 10% in 13 countries (29%), 10—24% in 20 countries (44%), and at least 25% in 12 (27%) countries, worldwide.



A comprehensive review of literature revealed 16 rubella serosurveys among Indian women. All the studies carried out in the 1990's and subsequently involved the use of Rubella IgG ELISA assays, whereas earlier studies utilized Haemagglutination Inhibition assays.

All the serosurveys revealed susceptibility ranging from 5% to 45%, reflecting the large size of the country and the pattern of rubella virus in circulation.

Study year and reference	**Location**	**Test**	**Group age (in years)**	**Sample size**	**Percent negative**
1970 (33)	Urban Delhi	HAI	Women 15-34	137	15
1970 (33)	Rural Delhi	HAI	Women 15-34	124	28
1973 (34)	Calcutta	HAI	Women 15-25	176	43
1973 (35)	Chandigarh	HAI	Women 16-40	325	19
1978-79 (36)	Lucknow	HAI	Women antenatal	300	22
1982 (37)	Kerala	HAI	Women antenatal	536	25.9
1984 (38)	Vellore	HAI	Women antenatal	132	4
1989 (39)	Delhi	HAI	Women antenatal	603	31
1990-91 (40)	Vellore	ELISA	Women antenatal	931	11
1991 (41)	Hyderabad	ELISA	Women antenatal	274	5
1995 (42)	Delhi	HAI	Women 10-40	200	45
1997-98 (40)	Vellore	ELISA	Women antenatal	765	13
2000 (43)	Delhi	ELISA	Girls 9-12	140	10
2002 (44)	Madurai Coimbatore Tirunelveli	ELISA	Women 18-40	1000	12 15 21
2003 (45)	Amritsar	ELISA	Women 10-45	580	31.2

2005 (46)	Delhi	ELISA, HAI	Women antenatal	5022	17
2003 (47)	Srilanka	ELISA	Women antenatal	620	24
1987-88 (48)	Pakistan	ELISA	Women antenatal	2000	16
2001 (49)	Iran	ELISA	Women antenatal	255	4
2002 (50)	Taiwan	ELISA	Women antenatal	1087	23

ELISA = Enzyme Linked Immuno Sorbent Assay

HAI = Haemagglutination Inhibition test

The largest study thus far in India was the serosurvey done at the National Institute of Communicable Diseases, Delhi, by *Gandhoke L et al* between 1988 and 2002. The study showed that 83% of normal antenatal women and 86% of antenatal women with bad obstetric history were immune. Immunity status among antenatal women from 1988 onwards showed a steady rise. While in late 1980s it varied from 49 to 72.33%, there was a steady increase in 1990s till the new millennium where it was 87 to 92 % (46). This study did not analyze the seroprevalence in girls who were between 10 and 15 years.

Other studies from the Indian subcontinental countries like Pakistan and Srilanka have estimated the seronegativity in antenatal women to be 16% and 24% respectively (47,48). In Iran the susceptible population was about 4% (49) and in Taiwan about 23% (50).

INDIAN SEROSURVEYS IN THE PREFERTILE AGE GROUP:

There have been a few Indian studies that assessed the seroprevalence of Rubella in schoolgirls.

Sangita Yadav et al in 2000 serosurveyed 140 healthy girls aged 9-12. Ten percent of the girls surveyed were found to be seronegative. Following vaccination the seronegative girls seroconverted and geometric mean titre (GMT) of rubella antibodies rose in those girls who were already seropositive (43).

Singla N et al in 2003 studied 580 women out of which 200 were in the prefertile age group (10-15 years). There was an increasing trend in seropositivity from 64% in the prepubertal age to the maximum incidence of 77.2% in the age group 26-35 years (peak fertility age). This was followed by a conspicuous decline to 59.3% beyond 35 years. A decline in the immune status with rising socioeconomic status was also observed (45).

Bhaskaram P et al in 1991 serosurveyed 139 children aged 1-15 years for Rubella IgG antibodies by ELISA. Children between 1 and 5 years showed the lowest seropositivity of 69.2%, which gradually increased to reach near 95% levels by 15 years (41).

Yadav S et al in 1995 serosurveyed 40 girls in the prefertile age group 1-5 years and 160 females of child-bearing age. 55% of the prefertile girls were seropositive for Rubella IgG. There was a gradual increase in the immunity status, with peak incidence of 66% between 30 to 34 years of age.

Females of low socioeconomic status showed higher incidence of immunity (63%) compared to social class I (40%) (42).

Seth P et al in their study on seroepidemiology of rubella infection in female subjects of Delhi and its surrounding villages in 1971, showed that 76.7% of the urban population and 64.3% of the rural population of girls between 10-14 were seropositive for Rubella IgG antibodies. In both urban and the rural population the seropositivity increased with age to reach a maximum between 25-34 years. But GMT of rubella IgG antibodies declined with increasing age and the antibody levels were 5-6 fold reduced at 25-35 years when compared to 10-14 years (33).

M.S Chakraborthy et al in their seroepidemiological study of rubella in Calcutta, done in 1976, showed that the seroprevalence in children of both sex at 11-15 years was 54.38%. There was no significant difference in sex distribution of positive sera. They had also shown a rising trend in seropositivity with increasing age. But GMT of rubella IgG antibodies did not vary significantly with increasing age. A study by the same author in 1973 had showed that the incidence of seropositivity to rubella was 53.14% in female subjects in the age group 12-25 years (34).

Pal et al had conducted a serological investigation of rubella virus infection in and around Chandigarh in 1974. They had demonstrated a seropositivity of 62% in boys and girls between 10 and 15 years. They had also demonstrated a rise in the GMT of antibodies from 6 months to 15 years. Thereafter the titre showed a steady decline to reach a nadir at 35-40 years (35).

An overview of these studies fails to establish any specific trend in seroprevalence of rubella in India, probably reflecting high seropositivity during outbreaks of rubella and low seroprevalnce during quiescent interepidemic intervals. Despite problems with the data, these estimates lend further support to the assertion that rubella is an under appreciated problem in our country, with no official data to appreciate the disease burden.

STUDY JUSTIFICATION

The public health importance of rubella relates to the teratogenic effects when rubella infection is acquired in the early months of pregnancy.

The endemicity of rubella has been well established in India. However, no official data is available regarding the prevalence of acquired and congenital rubella infection as it is not a notifiable disease. About 50% of children acquire rubella antibodies by the age of 5 years and 80 to 90% become immune by 15 years. Studies from India and abroad have found that 10-20% women in child bearing age are susceptible to rubella. Between 6-12% of babies born with congenital malformations or infections have serological evidence of rubella. These studies highlight the existence of rubella leading to fetal malformation and wastage.

Despite a safe and effective vaccine being available for more than a decade in India, so far there has been no clear-cut policy regarding rubella immunization of children either at 15 months or young girls at 9-12 years.Therefore the need for routine immunization to control rubella has not been duly recognized so far. But in a significant deviation from the National Immunization Schedule, the government of Tamil Nadu launched a pilot project in five districts to administer the measles, mumps and rubella (MMR) vaccine to children and the rubella vaccine to adolescent girls. The new immunization schedule was launched in one block each in Theni, Vellore, Tiruvannamalai, Cuddalore and Perambalur districts. Conceptualised under the World Bank-assisted ICDS project and executed by the Public Health

department, the vaccine (0.5 ml) will be administered through subcutaneous injection at anganwadi centres and schools.

For a developing nation, like India, to take informed decisions on the incorporation of vaccines into the national programme, data on the burden of the disease as well as feasibility and likely impact of implementing different vaccination strategies needs to be assessed.

It was therefore considered worthwhile to study the rubella seroprevalence rates in schoolgirls and to analyze the influence of variables like age, socioeconomic status, previous history of immunization, previous history of exposure to rubella, nutritional status and onset of menarche, on seroprevalence. The age group 10-15 years has been chosen as it represents the age that the vaccination strategy is likely to target. The seroprevalence in this age group also represents the likely seroprevalence in women who enter childbearing age. Thus an indirect estimate of CRS burden in the community can be arrived at.

AIM OF THE STUDY

- To assess the overall seroprevalence of rubella in schoolgirls aged between 10 and 15 years.

- To assess the influence of variables like age, socioeconomic class, immunization status, exposure to exanthematous illness, nutritional status and onset of menarche, on the seroprevalence of rubella antibodies at that age.

MATERIALS AND METHODS

STUDY DESIGN

The study conducted was a cross sectional survey.

STUDY PLACE

The study was conducted at three schools in Chennai city.

1. Rani Meyyammai Girls Higher Secondary School
2. Bharath Dass Matriculation Higher Secondary School
3. Vanavani Matriculation Higher Secondary School

The schools were chosen so as to include children from all socioeconomic strata. These three schools served a large and diverse population of the South of Chennai.

STUDY PERIOD

The study was conducted over a 1-year period from July 2004 to August 2005.

STUDY POPULATION

Inclusion Criteria:

All girl children aged between 10 and 15 years were included in the study, subject to availability of consent.

Exclusion Criteria:

Nil

SAMPLE SIZE

The Sample size for the study was calculated based on the following considerations:

Estimated seropositivity in girls between 10 and 15 years: 15%

Confidence interval = 95%

α Error = 0.05

β Error = 0.2

Precision = 5%

Calculated Sample size = 196.

SAMPLING TECHNIQUE

Stratified random sampling

MANOUVRE

After obtaining necessary permission from the respective school heads, the nature of the study and its implications were thoroughly explained to the children during the school assembly and a notice containing the same was dispatched to their parents. Those girls who had consented for the study were enrolled. We thus enrolled 196 schoolgirls between 10 and 15 years in the study.

The girls were then made to fill a detailed questionnaire (appendix 1) which included details about their age; residence; per capita income, education

and occupation of the parents; past history of exposure to any exanthematous illness akin to Rubella or immunization with MMR/Rubella vaccine.

Subsequently a general health awareness camp was conducted in their respective schools. In this camp the girls were screened clinically for any illness/morbidity and appropriate medical advice was given to them. The girls were also advised on genital hygiene and reproductive health. The height in meters and the weight in kilograms of the cases were also recorded. A note was also made of the age of onset of menarche in the questionnaire. At the end of the camp, blood was drawn for the study.

Only girls with a documented evidence of immunization with MMR/Rubella, like a vaccination record, school record or a medical record, were considered to be immunized. An undocumented history alone was a not considered relevant.

The study required documentation of any past history of fever associated with skin rashes. Excluded from this parameter was the diagnosis of chicken pox, which generally had a classical mode of presentation. A history of any other febrile illness with skin rashes, available, was noted. As per the WHO definition of suspected Rubella, associated findings like lymphadenopathy and arthalgia/arthritis was also noted.

Socioeconomic stratification of the subjects was done as per modified Kuppuswamy's Socioeconomic Status Scale (51,52). In the modified scale, the educational and occupational criteria remain the same. To modify the economic criteria, the All India Average Consumer Price Index for Industrial

Workers (CPI-IW) was noted for the current year (Indian Labour Journal, published by Labour Bureau, Government of India, Delhi). The conversion factor between the CPI-IW for 1976 (the year when Kuppuswamy's scale was proposed) and the current year is then determined.

Conversion factor = CPI-IW for current year / 60.04.

Subsequently, all the income groups in the Kuppuswamy's scale are multiplied with the conversion factor to get the appropriate income groups for the year under study. The CPI-IW for the year 2005 as on June 2005 was 529 and the conversion factor determined was 8.81. This gave a modified income scale and a revised Kuppuswamy's Socioeconomic Status Scale that incorporated these modifications was used in our study.

SOCIOECONOMIC STATUS SCALE OF KUPPUSWAMY (INCLUSIVE OF REVISIONS)

(A) Education Score

1.	Professional or Honours	7
2.	Graduate or Post-Graduate	6
3.	Intermediate or Post-High-School Diploma	5
4.	High School Certificate	4
5.	Middle School Certificate	3
6.	Primary School or literate	2
7.	Illiterate	1

(B) Occupation Score

1.	Profession	10
2.	Semi-Profession	6
3.	Clerical, Shop-owner, Farmer	5
4.	Skilled worker	4
5.	Semi-skilled worker	3
6.	Unskilled worker	2
7.	Unemployed	1

(C) Modified Family Income Per Month (In Rs.) for 2005

1.	> 17600	12
2.	8800 - 17599	10
3	6600 - 8799	6
4.	4400 - 6599	4
5.	2650 - 4399	3
6.	901 - 2649	2
7.	<900	1

Total Score		**Socioeconomic Class**
26-29		Upper (I)
16-25	Middle	Upper Middle (II)
11-15		Lower Middle (III)
5-10	Lower	Upper Lower (IV)
<5		Lower (V)

SAMPLE COLLECTION

With strict sterile precautions 3 ml of venous blood was taken from all subjects of the study group, in a sterile graduated plastic tube. Sera from the collected samples were separated and stored at –20°C before being transported in an appropriate cold box to the Virology Laboratory at King's Institute of Preventive Medicine, Guindy, Chennai for analysis. All the sera collected were analyzed for the presence of rubella specific IgG antibodies using ELISA.

In the Virology Lab of Kings Institute of Preventive Medicine, IgG ELISA was done using a kit procured from Equipar, an Italian manufacturer of biological products. The sample analysis was done as per the manufacturer's recommendations.

Principle of the assay:

Microplates are coated with purified and inactivated rubella antigens that in the first incubation capture specifically anti-virus antibodies if present in the sample. After washing out the other components of the sample, specific anti-rubella antibodies are detected with a goat anti-human IgG antibody, conjugated with peroxidase (HRP). The intensity of the color, generated by the enzyme on the substrate/chromogen mixture in the last incubation, is proportional to the content of anti-rubella antibodies in the sample. Results are calculated by means of a standard curve calibrated on the WHO standard, providing a quantitative determination of Rubella-specific IgG.

Values are expressed in IU/ml.

Reactive sample (Positive test): >15 IU/ml,

Equivocal: 10 – 15 IU/ ml and

Non-reactive (Negative Test): < 10 IU/ ml.

STATISTICAL ANALYSIS

As already mentioned, this study aimed at analyzing the seropositivity for rubella IgG in schoolgirls between 10 and 15 years and the variables that have an influence on the seropositivity. The proportion of outcome measures were calculated as percentages.

Data were analysed using *SPSS,* version 13, to calculate mean, standard deviation and chi-squared values. A value of $P < 0.05$ was considered statistically significant.

OBSERVATIONS

A total of 196 schoolgirls who had consented for the study were included and the following observations were made

Table - 1

Variable		Frequency	Percentage
Age	10	31	15.8
	11	21	10.7
	12	25	12.8
	13	26	13.3
	14	60	30.6
	15	33	16.8
Socioeconomic class	1	10	5.1
	2	50	25.5
	3	68	34.7
	4	65	33.2
	5	3	1.5
Past immunization	None	157	80.1
	MMR	22	11.2
	Rubella	17	8.7
Past history of exanthematous Illness	No	144	73.5
	Yes	52	26.5

Table 1 gives the frequency distribution of the variables studied. All the ages had a fairly equal representation of cases except for girls at 14 years who represented 30.6% of the study population. A random selection of cases and the availability of consent are the reasons for the disproportionate representation of these girls when compared to other groups.

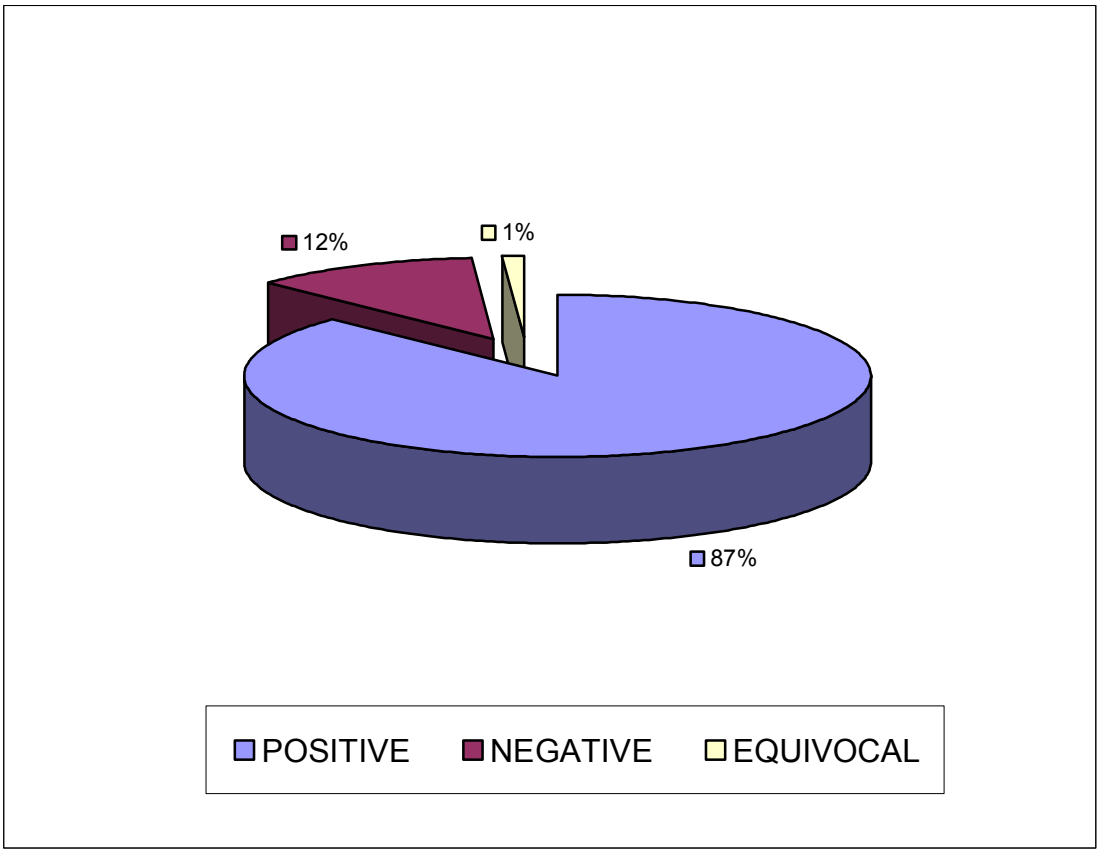
The frequency distribution of cases as per Kuppuswamy's socioeconomic status scale shows that classes 2, 3 and 4 constituted 25.5, 34.7 and 33.2 percent of the cases respectively. Classes 1 and 5 were the least represented, constituting 5.1 and 1.5 percent of the cases. Majority of girls belonging to class 1 had not consented for the study resulting in their poor representation. There were very few girls belonging to social class 5 in the schools studied.

The frequency distribution of cases based on previous immunization with MMR or Rubella is also tabulated. A total of 22 cases (11.2%) had received MMR and 17 cases (8.7%) had received rubella vaccination prior to the study. The rest of the cases (157 cases – 80.1%) had neither received MMR nor rubella vaccine previously. Seventeen school girls between 14 and 15 years belonging to Rani Meyammai school, had received rubella vaccination during a vaccination drive conducted by Lions Club, Chennai, two years prior to this study.

A past history of fever with rash was present in 52 cases (26.5%) as given in table 1. Seven girls among them gave a history compatible with the WHO definition of Suspected rubella. None of them had been rubella confirmed.

ANALYSIS OF DATA

Considering all age groups Rubella IgG seropositivity was found to be 87.2%(171 cases) in our study. Twenty-three cases (11.7%) were Rubella IgG negative and in 2 cases (1%) results obtained were equivocal.



SEROPREVALENCE AND GEOMETRIC MEAN TITRE IN THE IMMUNIZED SCHOOLGIRLS

TABLE - 2

Immunization	Result of Test			Total
	Positive	Negative	Equivocal	
None	132	23	2	157
MMR	22	0	0	22
Rubella	17	0	0	17
Total	171	23	2	196

TABLE - 3

Variable	Number	Seropositive (Percentage)	P
Unimmunized	157	84.1	0.018
Immunized	39	100	

Table 2 gives the distribution of seropositivity among immunized and unimmunized; and all the 39 vaccinated cases were seropositive (100%). Seronegativity and equivocal results were seen only among the unimmunized group. Twenty-two cases (11.2%) had received MMR and 17 cases (8.7%) had received rubella vaccination. Both set of girls who had received either MMR or rubella vaccine were seropositive. The difference in seropositivity among the immunized and unimmunized population as shown in Table 3 was statistically significant ($P < 0.05$).

TABLE - 4

Years elapsed since MMR vaccination	Number	GMT	S.D	P Value
3	7	240	1	0.000
4	7	202	2	
6	4	141	2	
9	3	48	2	
10	1	40	-	

GMT = Geometric mean titre in IU/ml S.D = Standard deviation

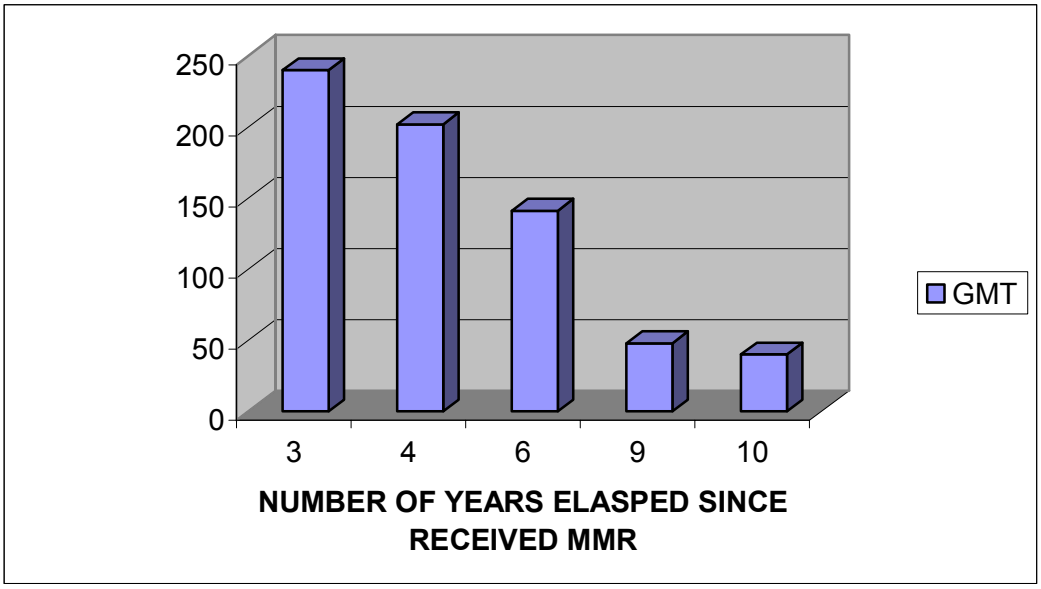


Table 4 gives the Geometric Mean Titre (GMT) of Rubella IgG antibodies in girls who had received MMR vaccination in ascending order of numbers of years elapsed since vaccination. Out of the 22 girls who had received MMR, 7 each had been vaccinated 3 and 4 years back; 4 had been vaccinated 6 years back, 3 nine years back and 1 ten years back. An analysis of the table shows that the GMT was the highest in girls who had received the vaccine 3 years back (240) and the least in girls who had received MMR 10

years back. The trend is better shown by the bar diagram which clearly reveals that the GMT of antibodies was highest in those who had received the vaccine 3 years back, decreasing with numbers of years that had elapsed since vaccination, to reach a nadir in girls who had been vaccinated 10 years back. The difference in GMT was also statistically significant ($p<0.05$).

TABLE - 5

Vaccination	Number	GMT	S.D	P-Value
MMR	22	146	2	0.008
RUBELLA	17	279	2	

GMT = Geometric mean titre in IU/ml S.D = Standard deviation

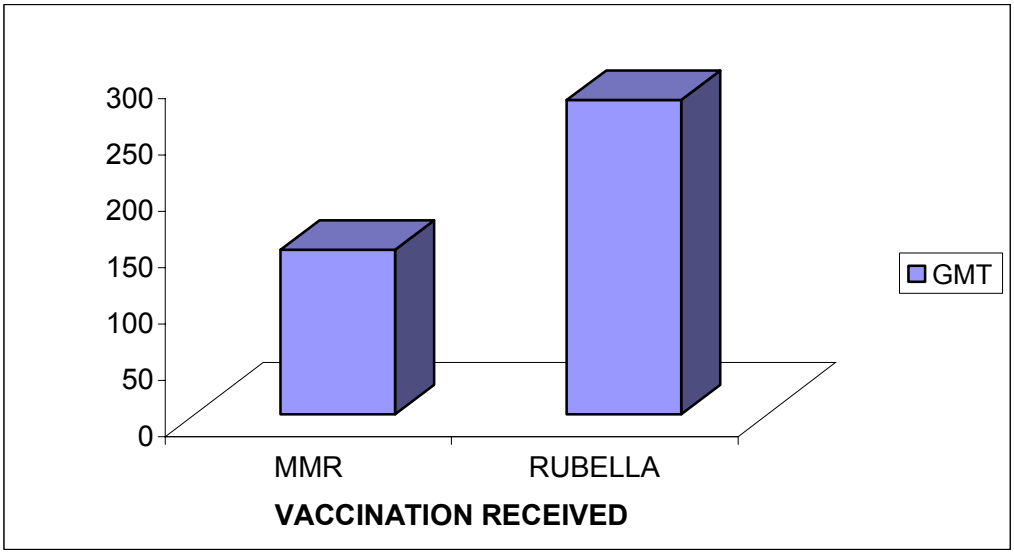


Table 5 gives the GMT in girls who had received MMR and rubella vaccination. An analysis of the table reveals that the GMT of Rubella IgG antibodies was 146 IU/ml in girls who had previously received MMR against GMT of 279 IU/ml in girls who had received rubella vaccination previously. The difference in titer was also statistically significant ($p<0.05$).

TABLE - 6

Age	Unimmunized		Immunized		P-Value
	GMT	S.D.	GMT	S.D	
10	87	6	196	2	0.04
11	111	7	100	4	0.94
12	97	4	135	3	0.61
13	81	6	84	3	0.98
14	49	5	260	2	0.02
15	59	4	287	2	0.001

GMT = Geometric mean titre in IU/ml S.D = Standard deviation

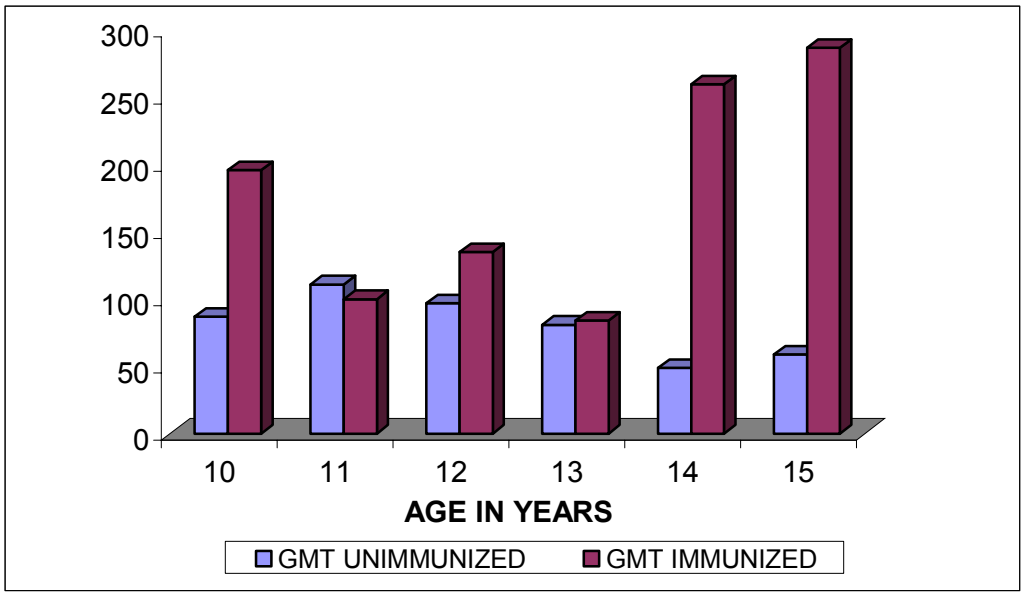


Table 6 gives the GMT at different ages in both the immunized and unimmunized population. An analysis of the table shows that the difference in GMT at 10 years, 14 years and 15 years was statistically significant between the two populations whereas there was no significant difference in the GMT between the two populations at 11, 12 and 13 years.

SEROPREVALENCE AND GEOMETRIC MEAN TITRE IN THE UNIMMUNIZED SCHOOLGIRLS

Out of the 157 schoolgirls who had neither received MMR nor Rubella, 132(84%) were seropositive, 23(15%) were negative and 2(1%) were equivocal.

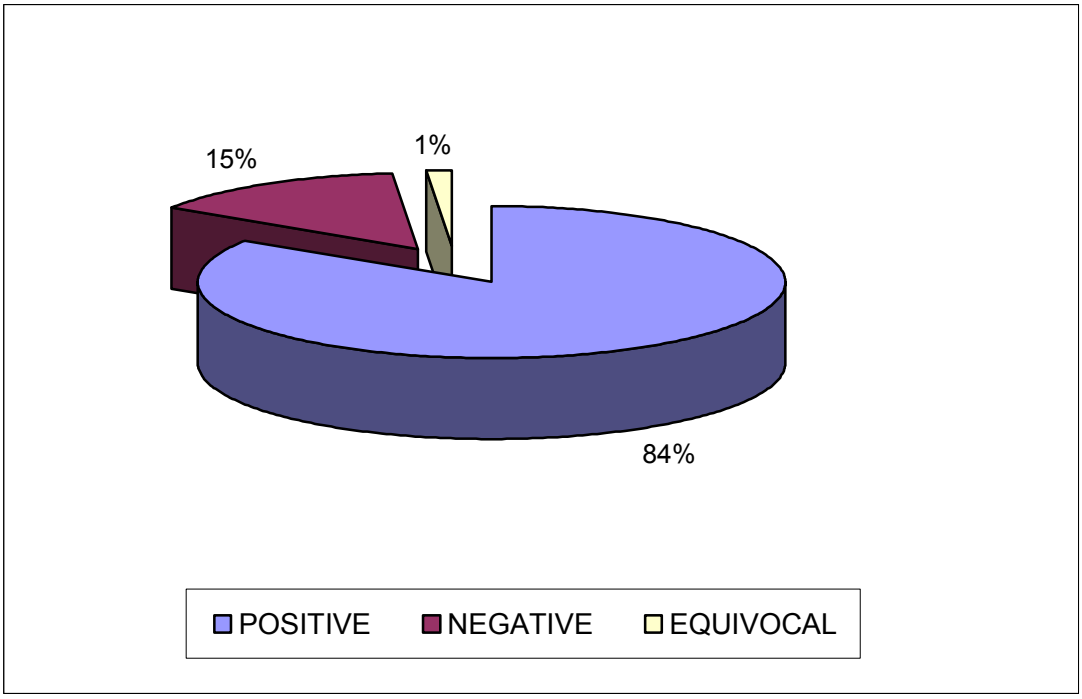


Table 7 gives the distribution of geometric mean titres(GMT) and the seropositivity for the different ages and socioeconomic classes in the unimmunized population. The GMT and seropositivity in girls who had a past history of exanthemous illness is also given in the table. The GMT of socioeconomic classes 1 and 5 have excluded due to the insignificant numbers in them.

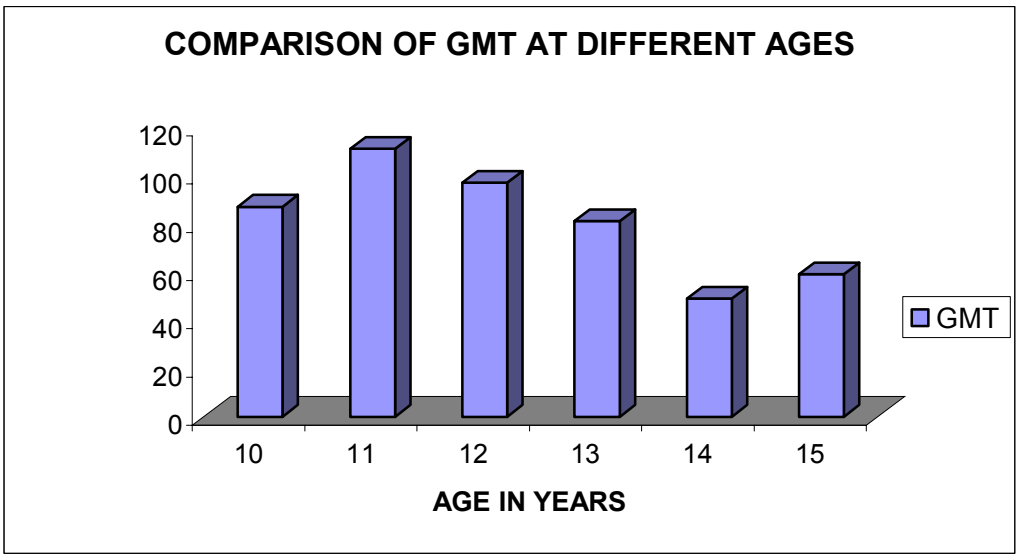
TABLE - 7

Variable		N	GMT	Negative	Seropositive Percent
Age in years	10	21	87	2	90.5
	11	19	111	3	84.2
	12	18	97	3	83.3
	13	23	81	2	87.0
	14	55	49	11	78.2
	15	21	59	2	90.5
SE class	1	1	-	Nil	100
	2	36	47	6	77.8
	3	58	61	7	82.8
	4	59	101	4	88.1
	5	3	-	Nil	100
Past H/o exanthem	Yes	43	72	6	81.6
	No	114	59	7	90.7

N = Number
GMT = Geometric mean titre in IU/ml
SE = Socioeconomic class as per modified Kuppuswamy's scale

1. AGE

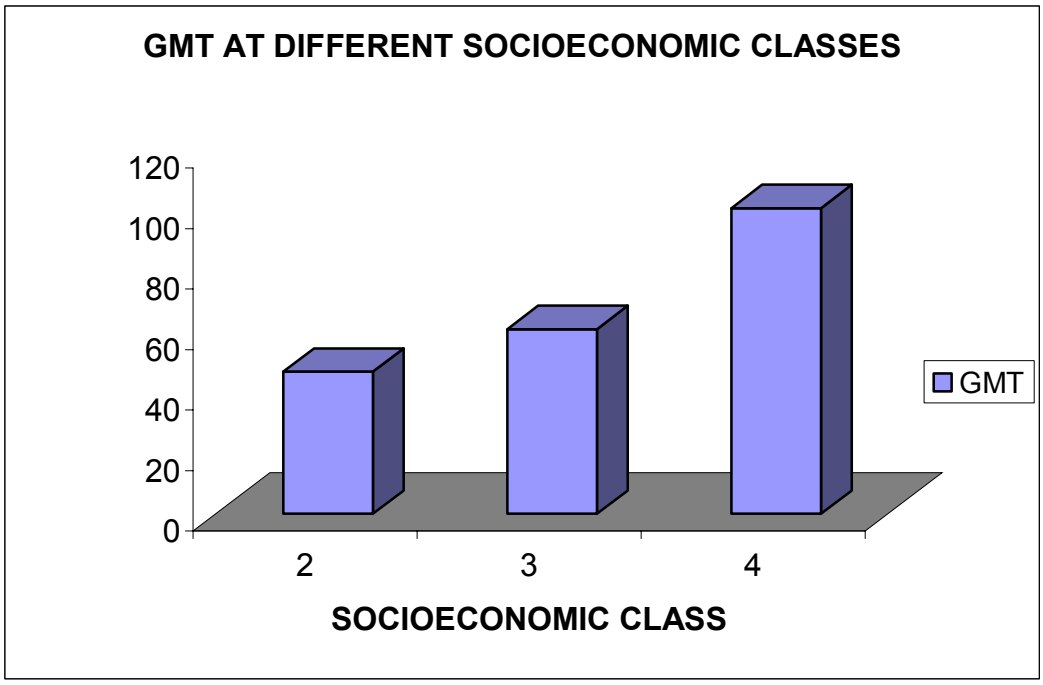
The seropositivity at different ages varies from a maximum of 90.5% at 10 years to a minimum of 78.2% at 14 years. The difference in seropositivity at different ages is not statistically significant (P>0.05).



The GMT in the unimmunized population shows a peak of 111 IU/ml at 11th year. A gradual decline is seen from the 11th year onwards upto the 14th year when the nadir is reached only to rise again at 15 years. The difference in GMT at 11 years is statistically significant from the GMT at 14 years.

2. KUPPUSWAMY'S SOCIOECONOMIC CLASS.

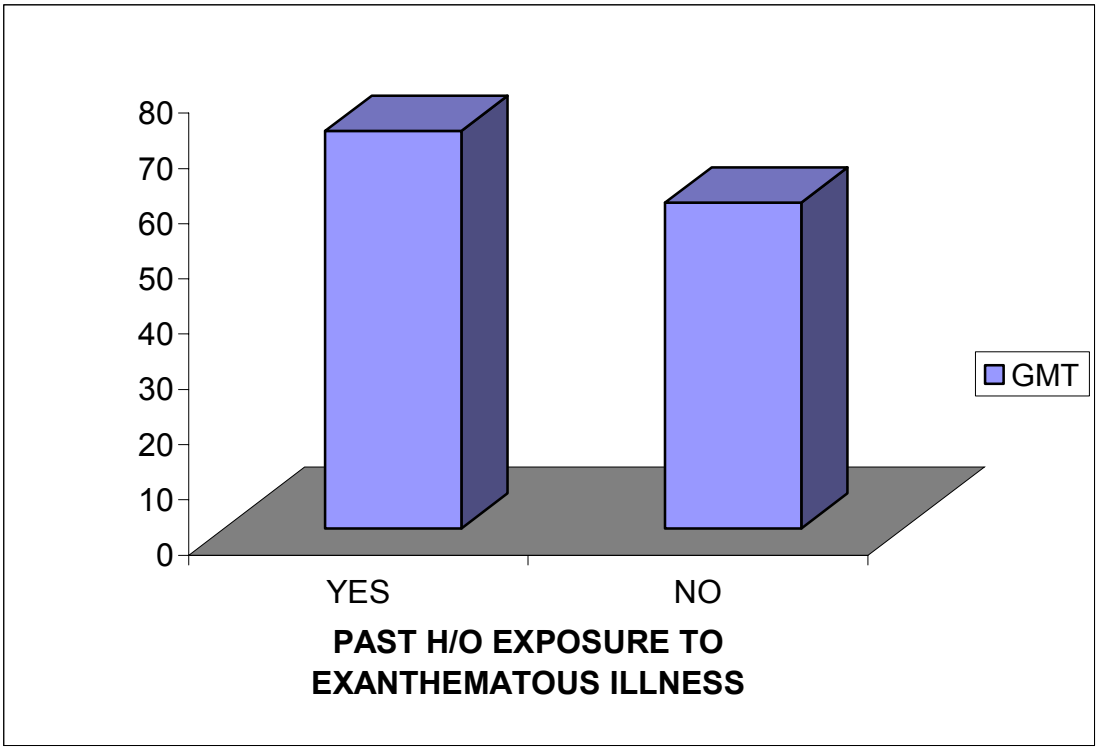
The seropositivity is maximal in the class 4 (88.1%), if classes 1 and 5 are excluded. Also there is an increase seen in seropositivity from classes 2 to 4 but this is not statistically significant.



An analysis of GMT of the different socioeconomic classes shows that there is a definite rise in GMT from 47 IU/ml for class 2, to 61 IU/ml for class 3, to 101 IU/ml for class 4. The difference in GMT among the different classes was also statistically significant.

3. PAST H/O EXANTHEMATOUS ILLNESS

The seropositivity in girls who gave a past history of exposure to exanthemous illness was 81.6%. All the seven girls who gave a history compatible with the WHO definition of suspicious rubella were seropositive (100%). There is no statistical significance in the seropositivity among those who had a past history of exanthematous illness with those who did not have.



An analysis of GMT in the girls who had given a past history of exanthematous illness also showed that it was not significantly higher ($p>0.05$) than the GMT in girls who did not give one.

4. BMI AND MENARCHE:

An analysis of BMI and seropositivity revealed that there was no significant correlation between the BMI and seropositivity in the study population as a whole and in the unimmunized population. (2-tailed significance of 0.220 for the entire study population and 0.316 for the unimmunized girls by Pearsons correlation).

An analysis of correlation between seropositivity and onset of menarche was also done. Here too there was no statistical significance ($P>0.05$ for population as a whole and the unimmunized population)

DISCUSSION

Rubella is a common worldwide infection; its importance in public health relates to the risk of malformations when primary infection occurs during pregnancy.

Interest in the burden of disease and rubella vaccination policies has increased recently for a number of reasons.

- Even though rubella outbreaks leading to CRS have not been documented in India, outbreaks in different parts of the world like in Panama in the mid-1980s, and in Oman and Sri Lanka in the 1990s have highlighted the importance of rubella.

- Measles vaccine coverage of infants is now >80% in many parts of India; thus effective rubella control programmes are feasible.

- MMR vaccine is distributed in the private sector. A recent publication provides details of an increase in CRS incidence in Greece that may have resulted from the misuse of rubella vaccination strategies (53). Rubella vaccine was introduced in Greece in 1975, mainly as MMR vaccine provided for children in the private sector, and coverage remained consistently below 50% in the 1980s. The proportion of women of childbearing age susceptible to rubella gradually increased. In 1993, the country experienced a large rubella outbreak with 69% of cases in persons >15 years of age. Sadly, 25 CRS cases occurred, and this is thought to be the largest CRS epidemic in Greece since 1950.

Vynnycky E et al also highlighted the danger of unmonitored immunization in the private sector (54).

There is thus a need to review the principles and practice of control of rubella and CRS.

Two approaches are recommended for CRS prevention – prevention of CRS only (through immunization of adolescent girls and/or women of childbearing age), and elimination of rubella as well as CRS (through universal vaccination of infants with/without mass campaigns, surveillance, and assuring immunity in women of childbearing age). Decisions on which approach is taken should be based on the level of susceptibility in women of childbearing age, the burden of disease due to CRS, strength of basic immunization programme as indicated by routine measles coverage, infrastructure and resources for child and adult immunization programmes, assurance of injection safety, and other disease priorities.

As CRS is not yet a notifiable disease in our country, data on CRS is scarce and the exact prevalence of CRS is not yet known. Because of the difficulty in conducting population-based studies of CRS incidence, many studies have estimated the proportion of defects such as congenital malformations, blindness or deafness caused by CRS, rather than the rate per 1000 live births. Extrapolating from these studies, rubella has been linked to the etiology of 26% of cataracts, 7-12% of congenital malformations and upto 29% of sensorineural hearing deficits in infants in India (55-58). Unpublished studies done in our own hospital have demonstrated 14% seropositivity for rubella among suspected CRS cases.

When data on CRS is scarce, assessment of disease burden can be made with the help of serosurveys in pregnant women and women of child bearing age. Because of the difficulty in clinical diagnosis, serological tests have become the mainstay of diagnosis of acquired Rubella.

In this study, serological analysis for IgG antibodies was done by ELISA. ELISA scores over Haemagglutination-Inhibition test (HAI) in its ability to detect low levels of rubella antibody that are undetected by HAI. HAI is also a labour-intensive test associated with both false positive and false negative results (59). A value of <10 IU/ml was taken as the threshold for negative serology and a value between 10 and 15 IU/ml as equivocal, as per the recommendations of the kit manufacturer. But recently a few studies have questioned these values. In 1997 *Matter et al* in his study on the serum levels of rubella virus antibodies indicating immunity, observed that, limiting the threshold for immunity as <15 IU/mL entails the risk of withholding rubella vaccination from susceptible persons and that only a subject having an anti-Rubella IgG concentration higher than 20 IU/ml is immunologically protected (60). Nevertheless the recommendations of the kit manufacturer have been followed, in the absence of any consensus statements.

There is considerable variation in the prevalence of rubella antibodies among women. European women have relatively higher prevalence of rubella immunity (93.2%) as compared to women of African (86.7%) and Asian origin (78.4%) (32). In India as reported earlier in the literature review, the figure ranges between 54% and 95%. The findings in this study of 87.2% fall within this range.

This study included a sizeable number of girls who had previously received MMR vaccine or rubella vaccine at various ages. This makes the study unique and different from all the other studies done in the Indian population so far. This study included 22 girls who had received MMR vaccine and 17 girls who had received rubella vaccine. All the girls who had been vaccinated previously were seropositive at the time of the study in contrast to only 84.1% of the unimmunized girls who were found to be seropositive. The GMT of Rubella antibodies at certain age groups among immunized girls was also significantly higher than in the unimmunized population at the corresponding age.

In India, MMR and rubella vaccine are manufactured by the Serum Institute of India in Pune. Rubella vaccine was first introduced in India in 1992 and MMR subsequently in 1993. Various studies from around the world have clearly demonstrated the superior efficacy of MMR and rubella vaccines but studies from India, on their efficacy, are scarce. *Yadav et al* in 2003 evaluated the efficacy of MMR vaccine at 9 & 15 months of age. A total of 240 normal children, 120 each in the age group 9-10 and 15-18 months had been enrolled for the study. Of the 120 infants in the age group of 9-10 months, 102 (85%) were seronegative for measles and 96 (80%) were seronegative for both mumps and rubella before vaccination. Following MMR vaccination 92% were seropositive for measles, 100% for mumps and 98% for rubella. In the age group of 15-18 months, of the 120 children, 67 (56%) were seronegative for measles, 84 (70%) for mumps and 86 (71.6%) for rubella before vaccination. After MMR vaccination, seropositivity of 92, 96 and 94 percent was observed for measles, mumps and rubella, respectively. The rise in the

pre- and post-immunization geometrical mean titre was significant ($P < 0.05$) for each component of the vaccine in both the age groups. They had concluded that MMR vaccine could be offered safely and with equal efficacy to children at 9 and 15 months of age (61).

Bhargava et al in 1995 studied the immunogenicity and reactogenicity of indigenously produced MMR vaccine in India. Studies were done on 89 children already immunized for measles, between 15 to 24 months of age. : IgG positivity 4 weeks after immunization rose from 75% to 100% for measles, from 12% to 92% for mumps, and from 13% to 99% for rubella. Only mild side effects including pain and swelling in 37 (4.3%) cases, mild fever in 51 (5.9%) cases, cough in 40 (4.6%) cases and a transient rash in 7 (0.8%) cases were observed (62).

In this study the 22 girls who had received MMR had done so at different ages. All of them were seropositive for rubella IgG, but the GMT showed a gradual decrease with the number of lapsed years since vaccination. This difference was also statistically significant.

Primary vaccine failure is known to occur in 2-5% of RA27/3 vaccine recipients, and a second rubella vaccination results in seroconversion in most cases (63-67). Antibodies have been found in 99.2% of schoolchildren after two doses of rubella vaccine, compared to 94.6% after one dose (68,69,70). *Davidkin et al* in their 15 year follow up study on the duration of rubella immunity induced by MMR vaccination in Finland had observed that upto 31% of children who had received MMR at 14-18 months were seronegative compared to 9% and 0% in girls who had received MMR at 6 years and 11-13

years respectively (71). *Picen Garces et al* in their study on immunity to rubella in vaccinated children showed that 8.7% of children between 5 and 7 years, who had received MMR at 15 months were seronegative. They also concluded that a high percentage of MMR vaccinated children showed minimal or undetectable levels of antibodies and thus merited a second dose of MMR to boost their immunity status (72).

Thus a significant waning of rubella antibody titer in girls who had previous received MMR at 1 year of age, as shown in this study, could indicate a need for booster vaccination with either MMR at 4-6 years or with rubella vaccine at 10-15 years. This inference is also seconded by the observation in this study that GMT was significantly higher in those girls who had earlier received rubella vaccine when compared with those who had previously received MMR. *Sangita Yadav et al* in 2000 had also observed that following rubella vaccination the previously seronegative girls seroconverted and geometric mean titre of Rubella antibodies rose in those girls who were already seropositive (43).

Inspite of the non-inclusion of MMR in the immunization schedule in our country, it had gained widespread usage in the private sector. The Indian Academy of Paediatrics recommends a dose of MMR in its schedule to all children at 12-15 months and presses for its inclusion in the national immunization schedule. Usage of MMR in the private sector has made it accessible to the elite section of the society but because of prohibitive costs and unavailability in government hospitals, it still remains out of reach of the

common man. This situation is also reflected in this study with only girls belonging to socioeconomic classes 1 and 2 having received MMR.

After the implementation of the pilot project of rubella vaccination in schoolgirls by the Govt of Tamil Nadu, there has been resurgence in the interest in rubella. Many non-governmental organizations have taken to rubella vaccination drives in schoolgirls. This has resulted in the vaccination of schoolgirls from lower socioeconomic classes. The 17 girls in our study, who had received Rubella vaccine, had done so during one such drive.

In the absence of previous immunization a number of other variables are known to affect the seropositivity. Socioeconomic status was reported to influence seropositivity by a number of Indian studies with a higher seropositivity seen among the lower socioeconomic classes (42,45). But the findings in this study had failed to demonstrate any significant change in seropositivity among socioeconomic classes. But what was significant was the increase seen in the GMT of Rubella antibodies in lower socioeconomic classes. The increased GMT in the lower socioeconomic classes could reflect the problem of overcrowding, adverse living condition, poor hygiene and environmental conditions, that results in easy transmission of infection from one individual to another.

A past history of exanthematous illness was present in 52 girls (26.4%). The seropositivity in girls who gave a history of exposure to exanthematous illness was also not significantly different from those who had not given such a history. This only confirms the fact that Rubella is very difficult to diagnose clinically. Many studies have reported that a positive history of rubella

infection is substantially less likely to correctly predict rubella immunity than is a positive history of vaccination; therefore a history of infection is not adequate for determining susceptibility (69,73,74,75).

This study also analyses the influence, if any, of the nutritional status of the subject and the onset of menarche with seropositivity of rubella but fails to establish any significant correlation.

ECONOMIC IMPACT OF CRS AND THE NEED FOR RUBELLA VACCINATION

Vaccines are important preventive medicines for primary health care, and are a critical component of a nation's health security. Although international agencies such as the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) promote global immunization drives and policies, the success of an immunization programme in any country depends more upon local realities and national policies. This is particularly true for a huge and diverse developing country such as India, with its population of more than 1 billion people, and 25 million new births every year.

Case series studies have showed that approximately 70% of patients with CRS had cardiac abnormalities, 60% had low birth weight, 60% had hearing loss and 45% had cataracts (76). Since rubella vaccine adds to the cost of immunization, and additional efforts are required to ensure that women of childbearing age are protected, concerns about the cost–benefit and cost–effectiveness of rubella vaccination assume considerable importance. These concerns may be heightened because many of the benefits of rubella

vaccination of children occur after adulthood has been reached, rather than relatively quickly, as with measles or polio vaccines. Furthermore, the elimination of rubella may require the vaccination of adolescent and adult males as well as females in order to ensure that transmission is interrupted.

Cost of treating a case of CRS is exorbitant (77). *Kommu & Chase* (78) estimated that the lifetime cost for treating a child with CRS in Barbados would be approximately US$ 50,000 and that lifetime costs of treating CRS cases from 1997 to 2012 in the absence of rubella immunization would exceed US$ 5.2 million. In Guyana, *Kandola* (79) estimated that the lifetime cost for treating a case of CRS would be US$ 63,990. Extropolating these data for a country like ours could be misleading; nonetheless, managing a case of CRS in India is not expected to cost any less. In addition to medical costs, many of the complications of CRS prevent people afflicted with the disease from entering the workforce, resulting in a significant loss of productivity to society. The medical costs could include the expenses for hospital visits and diagnostic investigations, the purchase of pharmaceuticals, special schooling, the surgical repair of congenital heart defects, the removal of cataracts, fitting hearing aids for deafness, so on and so forth. Indirect costs may include the loss of lifetime earning potential, attributable, for example, to irreversible blindness, congenital deafness, intractable seizures, mental retardation and/or premature death, and the loss of the potential wages of children's carers. It is difficult to make intercountry comparisons in this respect because the costs of health care and the monetary value of lost human productivity vary widely among countries.

The average life expectancy of an Indian is 64.35 years. In addition the 2004 estimate of Gross Domestic Product (GDP) per worker in India was $3,100. Estimating an average working lifetime of 54.35 years (assuming a child begins to work at 14 years of age), India would stand to lose US$ 1,68,485 in productivity for each person infected with CRS, assuming they would not enter the work force. The previously mentioned costs address only the financial burden to society of CRS children. The emotional costs to parents and society, while immeasurable, are significant and must also be considered.

In 1996, UNICEF discounted the price of vaccines for the developing countries. The prices were US$0.15 (monovalent rubella vaccine), US$ 0.55—0.59 (MR vaccine), US$ 0.72—0.95 (MMR vaccine) *(J. Gilmartin, personal communication, 1996).*

Using this estimate, one can calculate the cost of preventing one case of CRS by using the following equation:

(100 000 live births/CRS incidence per 100 000 live births) × (cost of vaccine/dose).

In this equation, the "(100 000 live births/CRS incidence per 100 000 live births)" term gives the number of uninfected infants born for each infant born infected with CRS. This value is equivalent to the number of uninfected mothers per infected mother, and thus, the number of mothers that would need to be immunized in order to prevent the birth of one CRS infant. The incidence of CRS in India is not exactly known. Data on the percentage of persons in

different age groups who are susceptible to rubella can be used in mathematical models to estimate the incidence of CRS.

In 1999 *Cutts et al* published a paper "Modelling the incidence of CRS in developing countries" in the International Journal of Epidemiology (80). They calculated the mean CRS incidence in India to be about 123 per 100,000 live births (range 44 to 275 per 100,000), from the seroprevalence of rubella in girls at 13 years. The wide range suggested was because of inter-regional variation in seroprevalence of rubella.

If we were to apply these estimates to the above equation, the cost of preventing one case of CRS would be

100,000 live births / 123 × US $.15 = US$ 121.95

Thus the cost of preventing one case of CRS in India would be about US$ 121.95. This figure would amount to about Rs.5246.

Although these calculations are based on a number of assumptions and are in no way foolproof, the gross difference between cost of treating one case of CRS and the cost of preventing one case of CRS by vaccination, as suggested by the above calculations strongly suggests that a national rubella vaccination programme in India could be cost-effective. Non-vaccine costs have not been included, since a rubella immunization programme could easily be added to existing programmes for DPT, polio, BCG, and measles. This would require only a marginal increase in cost, as the personnel and supplies for vaccine administration in India are already funded.

A WHO bulletin on "Economic analysis of Rubella and Rubella Vaccine" in 2002 reported that all the cost benefit studies of rubella vaccination in developing countries indicate an excess of benefits comparable to those estimated for the use of Hepatitis B vaccine or Haemophilus influenzae type b (Hib) vaccine in these countries (77). The economic data given in this bulletin supports the inclusion of rubella vaccine in the immunization programmes of both developing and developed countries and indicate economic benefits comparable to those derived from the use of Hepatitis B vaccine and Hib vaccine.

Although the estimates of CRS may be lower than the annual number of deaths from neonatal tetanus or pertussis, the life long disability associated with CRS presents a major burden to the individual, family and society. Rubella vaccine is more expensive per dose than Tetanus Toxoid or DPT vaccine but the administration costs are lower since only one dose is required and combined vaccination with measles and mumps can prevent extra cost of injection. Rubella vaccination is included in the national immunization programmme of a number of countries and territories of the world. According to a survey of the member countries in the World Health Organization, the number of countries that have incorporated rubella-containing vaccine into their routine national immunization programmes increased from 65 (33%) in 1996 to 110 (57%) in 2003. The vaccines are highly protective and without significant adverse effects. Caring for CRS cases is costly in all countries. All cost-benefit studies of rubella vaccination, in developing and developed countries, have demonstrated that the benefits outweigh the costs and that rubella vaccination is economically justified, particularly when combined with

measles vaccine (all of these studies have been conducted in countries with coverage > 80%). Large-scale rubella vaccination during the last decade has drastically reduced or practically eliminated rubella and CRS in many developed and in some developing countries.

Data from Tamil Nadu shows that the school going girl population is about 18.98 lakhs in the age group 11-14 with an enrollment percentage of 91.15%. At 14-16 years school going girl population dips to 11.98 lakhs with also a dip in enrollment percentage to 70.12% (Source: 2004 statistics – Department of School Education, Tamil Nadu). This sharp fall in school going population has been attributed to various factors like attainment of menarche in girls at about 13 years and the stoppage of the government sponsored free mandatory education for children at 14 years. Also familial pressures for supplementation of income results in more school dropouts. Therefore vaccinating all school girls at 13 years before a dramatic dip in attendance rates occur, would be ideal.

SUMMARY AND CONCLUSION

- The overall seropositivity for Rubella in schoolgirls between 10 and 15 years was 87.2%.
- 100 percent seropositivity was seen among girls who had received either MMR or Rubella previously.
- Seropositivity of 84% was seen in the unimmunized
- Difference in seropositivity among the immunized and the unimmunized girls was statistically significant.
- Difference in GMT among the immunized and unimmunized girls at different age groups was also statistically significant.
- The difference in seropositivity in the unimmunized schools belonging to different ages and socioeconomic classes was not statistically significant but the difference in GMT was found to be significant.
- Past history of exposure to exanthematous illnesses, nutritional status and onset of menarche did not influence seropositivity.
- The GMT showed a statistically significant decline with the number of years since vaccination with MMR.
- The difference in GMT among the girls who had received MMR and Rubella vaccines was statistically significant

RECOMMENDATIONS

All countries should assess their rubella situation and, if appropriate, make plans for the introduction of rubella vaccination. Although detailed surveillance and cost-benefit studies are not needed in every country before implementing rubella vaccination, the choice of policy in this regard requires some baseline information on the susceptibility profile of women of childbearing age (e.g. through serological studies of women). Findings in this study strongly advocate introduction of Rubella Vaccination into the national immunization programme targeting girls less than 13 years before a significant drop out in school attendance occurs.

ANNEXURE 1 - PROFORMA

1. Name: ---------- Ht:

2. School studying ---------- Wt:

3. Age (in months): ----------

4. Address: ----------

5.

Variable	Father	Mother	Member 1	Member 2	Member 3
Name					
Education					
Occupation					
Income					

6. History of prior vaccination with MMR/Rubella vaccine:

	MMR	No of doses	Rubella	No of doses
History				
When given				

7. Past history of exanthematous fever?

Yes	No

8. Whether attained menarche?

Yes	No

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