SEROPREVALENCE OF RUBELLA ANTIBODIES IN WOMEN OF REPRODUCTIVE AGE

By

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In
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Under the guidance of

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LIST OF ABBREVIATIONS USED

CRS	–	Congenital rubella syndrome
CRI	–	Congenital rubella infection
HI	–	Haemagglutination test
MMR	–	Measles, Mumps , Rubella
WHO	–	World Health Organization
RV	–	Rubella virus
IgG	–	Immunoglobulin G
IgM	–	Immunoglobulin M
RCV	–	Rubella containing vaccine
CMI	–	Cell Mediated immunity
RT-PCR	–	Real Time Polymerase chain reaction
NT	–	Neutralization Test
CPE	–	Cytopathic effect
ACIP	–	Advisory Committee On Immunization
CDC	–	Centers for Disease Control & Prevention
SEAR	–	South East Asia Region
ICMR	–	Indian council of Medical research
ORF	–	Open reading frames

ABSTRACT

Introduction: Rubella is a mild exanthematous disease of worldwide distribution. However there is risk of adverse pregnancy outcome & congenital defects in foetus when it infects susceptible pregnant women. The endemicity of rubella has been well established in India, still very few survey are done. Thus it is important to know proportion of women of childbearing age who are susceptible to rubella so as to know the risk of adverse pregnancy outcome.

Objective: To know the seroprevalence of Rubella antibodies in women of reproductive age group.

Materials and Methods: A total of 120 women of reproductive age group were selected randomly. About 2-3 ml of single blood sample was collected from selected women .Sera was separated and tested for IgG & IgM antibodies specific for rubella virus by ELISA.

Results: Overall prevalence of seropositivity of rubella IgG antibodies was 31.66% indicating they were immune for rubella infection. Seropositivity for IgM antibodies was found in one (0.83%) woman. Higher (40%) incidence of seropositivity for IgG antibodies was observed in women presenting with adverse pregnancy outcome than that of normal pregnancy outcome (29.1%). Rubella IgG seropositivity in age group of 16-25 year was 26.31% which increased to 40% in age group of 26-35 years.

Conclusions: Higher incidence of seropositivity observed in women presenting with adverse pregnancy outcomes suggests that Rubella could be a cause of repeated pregnancy wastage in these women. There is also considerable variation in the prevalence of rubella antibodies among women of child-bearing age, depending on

the socioeconomic strata and selection of study group. In our area substantial numbers of women reach childbearing age without acquiring natural immunity to Rubella. Hence serosurveillance of women of childbearing age should be continued in different area of country & immunization policy needs to be developed for these women to prevent adverse pregnancy outcome and control CRS.

Key words: Rubella, Congenital rubella syndrome, Seroprevalence, Women of child bearing age

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INTRODUCTION

Rubella is an acute febrile illness, which is caused by rubella virus, from Togavirus family genus Rubivirus. The disease is characterized by a rash and lymphadenopathy that affects children and young adults. It is the mildest of common viral exanthems. However, infection during early pregnancy may result in serious abnormalities of the foetus, including congenital malformations and mental retardation .[1]

Various maternal infections for eg Toxoplasma gondii, Rubella virus, Cytomegalovirus and Herpes simplex virus, transmissible in utero at various stages of gestation leads to unfavourable pregnancy outcome. Primary infections caused by them are the major causes for abortions, still births and congenital defects among foetuses of infected mother.[2] Among them rubella virus is most consistent in its harmful effects on foetus.The virus can be transmitted to foetus through the placenta and is capable of causing abortions, still births and serious congenital defects (Congenital Rubella Syndrome – CRS).If contracted during first trimester the risk of foetal infection is about 90% to suffer from CRS - blindness, hearing loss, heart diseases, psychomotor delay and mental retardation.[3]

The endemicity of rubella has been well established in India. Immunity to rubella among child bearing age group of women can indirectly hint at the risk of acquiring CRS. Recent data from Vellore show that 9.8 per cent of children in India, with suspected congenital infections had congenital rubella, as the cause. Thus it is important to know the proportion of the population susceptible to rubella especially in

women of reproductive age so as to know the risk of adverse pregnancy outcome.[4] As rubella infection presents atypically and is asymptomatic so clinical diagnosis is unreliable and serological tests having good sensitivity and specificity are of great value in diagnosis of rubella.[5]

The present study is undertaken to find out the role of rubella as a major foeto-pathogen associated with pregnancy wastage and thus to identify one of the preventable cause of fetal loss.

AIM AND OBJECTIVE

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To know the seroprevalence of Rubella in women of reproductive age.

REVIEW OF LITERATURE

History

Rubella was first described by 2 German physicians, De Bergan & Orlow, in the mid eighteenth century .At that time it was frequently known by the German name 'Roteln', and it was due to early interest of German physicians that the disease subsequently became to known as "german measles". Rubella was initially considered to be variant of measles or scarlet fever & was called as third disease.[6] The clinical difference between these diseases were recognized in nineteenth century & rubella was accepted as a distinct disease by an International Congress of Medicine in London in 1881.The disease received comparatively little attention, for infection was generally mild and severe complication were rare. However, infection during early pregnancy may result in serious abnormalities of the fetus, including congenital malformations and mental retardation. The consequences of rubella in utero are referred to as the CRS. [1]

In 1752 & 1758, clinical description confirmed by De Bergen & Orlow respectively.[7]

In 1814, George de Maton first suggested that rubella should be considered a disease distinct from both measles and scarlet fever.[7]

In 1866, Henry Veale, an English Royal Artillery surgeon, described an outbreak in India. He coined the name "rubella" (from the Latin word, meaning "little red").[8]

In 1914, Alfred Fabian Hess theorised that rubella was caused by a virus, based on work with monkeys.[9]

In 1938, Hiro and Tosaka confirmed this by passing the disease to children using filtered nasal washings from acute cases.[10]

In 1940, there was a widespread epidemic of rubella in Australia.[10]

In 1941, ophthalmologist Norman McAllister Gregg found 78 cases of congenital cataracts in infants, he published report, Congenital Cataract Following German Measles in the Mother.[7]

In 1962, the virus was isolated in tissue culture by two separate groups led by physicians Parkman and Weller.[7]

In 1962, pandemic of rubella started in Europe.[7]

In 1964-65, pandemic spread to United States & had an estimated 12.5 million rubella cases.[11]

In 1967, rubella virus shown to haemagglutinate. Haemagglutinate inhibition test (HAI) developed.[12]

In 1967, rubella virus first visualized by electron microscopy.[12]

In 1969 a live attenuated virus vaccine was licensed.[13]

In the early 1970s, a triple vaccine containing attenuated measles, mumps and rubella (MMR) viruses was introduced.[7]

In 1971, MMR licensed in USA.[7]

In 1988, UK policy augmented by offering MMR to preschool children of both sexes.[14]

In 1989–91, resurgence of rubella in USA.[14]

In 1996, in UK, schoolgirl vaccination discontinued but second dose of MMR introduced for children aged 4–5 years.[14]

In 2000, WHO organises first global meeting on rubella since 1984.[14]

In 2002, 123 (57%) of 212 of countries and territories include rubella vaccination in national immunisation programmes.[14]

In 2005, rubella declared no longer epidemic in United states.[15]

Epidemiology

Rubella although a mild viral illness, is of high public health importance owing to the teratogenic effects that can result from congenital rubella infection (CRI), leading to miscarriage, fetal death, or birth of an infant with CRS. The clinical spectrum of CRS includes ophthalmic, auditory, cardiac, and craniofacial defects. Worldwide, it is estimated that more than 100,000 infants are born with CRS each year. [16] According to the estimates based on a statistical model derived from the seroprevalence data from South East Asian Region (SEAR) during 2000-2009, 46,621 infants with CRS are born annually in SEAR alone.[17]

Rubella usually occurs in a seasonal pattern, with epidemics every 5–9 years major pandemics have occurred every 10 to 30 years.[18] However, the extent and periodicity of rubella epidemics is highly variable in both industrialized and developing countries.[19]

The highest risk of CRS is found in countries with high rates of susceptibility to rubella among women of childbearing age. These rates may vary considerably

among and within countries, mainly reflecting epidemiological and socioeconomic differences, and urban versus rural settings.[19]

The worldwide pandemic of rubella in 1962–1965 highlighted the importance of CRS. In the United States of America alone during 1964 and 1965 there were an estimated 11 000 fetal deaths and 20 000 infants born with CRS. This pandemic stimulated the development of rubella vaccines, with the first products licensed in 1969. Uptake of rubella vaccine in industrialized countries was high; however, rubella vaccine was not included in 1974 in the group of core antigens recommended for children in developing countries by the WHO Expanded Programme on Immunization. Thus, while rubella and CRS decreased markedly in the industrialized countries, endemic rubella and CRS continued to occur in much of the developing world. This situation received limited attention as a global public health issue until the mid-1990s.[16]

Since introduction of routine rubella vaccination programme in United states in 1969, number of rubella cases reported each year has dropped by >99 %.[15] However, occasional outbreak have led to elevated total number of cases in past few years. In 1990 & 1991, there were outbreaks of rubella in Amish communities in Ohio & Pennsylvania, which contributed to a total increase in congenital rubella cases in those 2 years.[20]

Before the introduction of rubella vaccine, the global incidence of CRS ranged from 0.8-4/1000 live births during rubella epidemics to about 0.1-0.2/1000 live births during endemic periods.[21] The WHO established goals to eliminate rubella and CRS in the WHO region of the Americas by 2010, and the WHO European region by 2015,

and in the WHO Western Pacific region for accelerated rubella control and CRS elimination by 2015. Sustained vaccination strategy enabled America to decrease rubella cases by 98%, from 1,35,947 in 1998 to 2,998 in 2006. Consequently, the CRS incidence had also decreased. The last confirmed case of CRS was delivered in Brazil on 26 August, 2009 and no new cases of CRS were reported from America in 2010. The Pan American Health Organization (PAHO) has confirm rubella and CRS elimination from the American region by 2012.[22]

Although rubella is no longer endemic in the united states it is still an illness with worldwide distribution, particularly in developing countries.[15]

BURDEN OF CONGENITAL RUBELLA SYNDROME IN DEVELOPING COUNTRIES

A WHO review carried out in 1996 revealed that 50 developing countries had already conducted substantial studies to assess their CRS disease burden, and more studies have been reported since then.[23] Special surveillance investigations in developing countries in Africa, the Americas, Asia, Eastern Europe, and the Eastern Mediterranean have documented incidence rates of CRS ranging from 0.4 to 4.3 per 1000 live births. These incidence rates are comparable to and in some cases higher than those seen in industrialized countries in the prevaccine era.[14,16]

Rubella immunoglobulin G (IgG) serosurveys among women of childbearing age indicate the potential risk for rubella infection in pregnant women. Serosurveys from 45 developing countries have shown a wide range of susceptibility: The proportion of rubella seronegative women was 25% or higher in 12 countries, 10%–24% in 20 countries, and below 10% in 13 countries. [23] These studies document that

many women of childbearing age living in developing countries remain at risk for having a child with CRS. Cutts and Vynnycky estimate that in 1996 there were 110 000 infants (95% confidence interval, 14 428 to 308 438) affected by CRS in developing countries (excluding the WHO European Region) that had not introduced rubella vaccine. [24]

A separate estimate for the WHO European Region suggests some 4 000 CRS cases occur annually in countries of that region that have not introduced rubella vaccine. While the western hemisphere continues to make huge strides in its endeavor to control CRS, 52% of the developing countries, including India, which account for two-third of the global birth cohort, are yet to incorporate the MMR vaccine in their national schedule. [25]

There are only few studies assessing the susceptibility of women in the reproductive age groups to rubella infection conducted in India.

SUSCEPTIBILITY OF NON-PREGNANT FEMALES IN REPRODUCTIVE AGE-GROUP TO RUBELLA

In a study conducted by Chandy et al from Vellore [25], records of 770 women aged ≥18years attending the departments of obstetrics and gynecology and reproductive medicine unit, were examined to assess the susceptibility to rubella. 12.5% of women in the reproductive age-group were seronegative for rubella. Women in the 19–23 and ≥35 years age-groups showed better levels of immunity to rubella (91%) than those in the 24–34 years age-group (85.5%).

In a study conducted by Singla et al from Amritsar[26], out of 580 subjects, there were 380 women in the reproductive age-group. The seroprevalence in women of age-groups 16-25y, 26-35y and 36-45y was 69.2%, 77.2% and 59.3% respectively. Overall, 28.7% of women in the reproductive age-group were susceptible to rubella infection. Out of the 380 women, 233 were pregnant and had a seropositivity of 67.8%; the seropositivity in the 147 non-pregnant women was 76.9% the difference was not statistically significant. Rubella seropositivity rates were also found to be higher in women of lower socioeconomic class (71.8%) than in women of upper class (55.9%) Analysis of antibody levels among rural and urban populations showed that seropositivity rate was much higher (76.6%) in urban women as compared to those residing in rural areas (58.1%).

In a study conducted by Yadav et al in Delhi [27], out of 162 females in the child-bearing age-group, 90 (56.2%) were seropositive for rubella. Nearly half of the females were susceptible to rubella infection. In this study urban females showed higher incidence of seropositivity (57%) as compared to rural females.

Seth P et al in their study from Delhi [25] tested 421 females aged 5-34y for rubella antibodies . Amongst the 220 women in reproductive age-group 12.7% were seronegative for rubella. The susceptibility in different age-groups was 5-9y: 52%, 10-14y: 29.5%, 15- 19y: 7.1%, 20-24y: 11.6%, 25-29y: 15.5%, and 30-34y: 15.4%.

SUSCEPTIBILITY TO RUBELLA IN PREGNANT FEMALES

Padmaja, et al. [28] in their study, assessed the seroprevalence to rubella among pregnant women. Out of 485 pregnant women attending the antenatal clinics of 3

government maternity hospitals in Thiruvananthapuram, Kerala, between 2003 and 2006, 283 women (65.7%) were IgG-positive.

In a retrospective study from a tertiary care hospital in Delhi [4] , case records of 305 pregnant women (73 of them had history of previous bad obstetric outcome: spontaneous abortion, premature labor or congenitally malformed or stillbirths) were assessed for immunity to rubella. 266 women (87.2%) had anti-rubella IgG. The age-wise prevalence of anti-rubella IgG was: 15-19y: 92.5%; 20-24y: 89.5%; 25-30y: 87%, and > 31y: 77.5%. The seropositivity rate among pregnant women aged 15- 19y was significantly higher than those aged > 31years. Seropositivity in those with previous bad obstetric outcome was 91.7% against 85.7% in women with normal obstetric performance. Only 3 women (0.98%) were positive for anti-rubella IgM.

Gandhoke, *et al.* [29] reported that about 14.6% of pregnant women in Delhi were susceptible rubella infection based on data collected between 1988 and 2002. Over 15 years, the susceptibility of pregnant women decreased from 51% in 1988 to 13% in 2002.The seroprevalence of rubella infection was higher in women with bad obstetric history (87%) compared to those with normal pregnancy outcome (83%).

In a prospective study from a tertiary hospital in Delhi[30], out of 100 pregnant women, 21 were seronegative for rubella.

Turbadkar, *et al.* [31] reported anti-rubella antibodies in 61.3% of pregnant women with bad obstetric history (BOH) in a prospective study in a tertiary hospital in Mumbai over 1 year. 26.8% of pregnant women with BOH had anti-rubella IgM antibodies.

In a study from Hyderabad by Bhaskaran et al [32], nearly 95% of pregnant women were seropositive for rubella, demonstrating high levels of immunity.

While in a study by Khare et al from Delhi [33] around the same time showed that only 50% of pregnant women had rubella antibodies.

Chaturvedi *et al.* [34] undertook a case control study wherein there were 144 pregnant women with bad obstetric outcome as cases and 50 pregnant women with normal obstetric history as controls. 12% of cases and 18% of controls were seronegative for rubella.

Classification

Rubella virus is an enveloped positive-strand RNA virus in the family *Togaviridae* .There are two genera that compose the *Togaviridae :Alphavirus,* which includes Sindbis & Semliki Forest viruses[10], & *Rubivirus,* whose sole member is RV.[10,35] Togavirus share a common genome organization and replication strategy, whereas alphaviruses employ animal reservoir & arthropod vectors for transmission , human appear to be the only natural host & reservoir for RV. Although only one serotype exists, there are atleast 10 genotypes of RV. There is no serologic cross reactivity between the alphaviruses & RV & only limited genome sequence similarity, predominantly within the nonstructural genes in regions that encode functional domains such as the polymerase and proteases activity.[10]

Morphology & Structure

RV is an enveloped virus with a 9.6-kb single-stranded, positive-sense RNA genome.[36] The mature RV virion is a round or ovoid particle approximately 60 nm in

diameter. The virion contains an electronlucent spherical core composed of multiple copies of the RV capsid protein and a single copy of the viral RNA genome. The RV core (30 nm in diameter) is surrounded by a host-derived lipid bilayer containing 5 to 6-nm-long spikes which project from the virion surface; the spikes are composed of the E2 and E1 glycoproteins.[7] The symmetry of the nucleocapsid has been difficult to establish because of its instability , but rotational analysis of thin section of rubella virions suggested that the core had a T= 3 icosahedral symmetry & 32 capsomers.[10]

Genome Structure[10]

The genome of RV is a single strand of RNA. This 40S genomic RNA is infectious, but the recovery of infectivity is poor. The genome is 9759 nucleotide in length excluding the 3'terminal poly(A) tail & is capped at 5' end. The cap is required for efficient translation as it serve as a ribosome recognition site. The base composition of the genome is A 14.9%, U 15.4%, G 30.8% & C 38.7%. The high G+C content of the genome has made sequence determination difficult. The genome is composed of 2 long open reading frames (ORFs) & has some features in common with the alphaviruses . The 5' proximal ORF is 6345 nucleotides in length & codes for the non structural proteins(NSP). The 3'proximal ORF is 3189 nucleotides in length & codes for structural proteins. The 2 ORFs are in the same translational frames & are separated by 123 nucleotides. The subgenomic RNA, which is capped, methylated & polyadenylated, is transcribed from the negative sense subgenomic RNA, for which the start site is nucleotide 6433 (U) . Genome – length c DNA clones have been produced & used to synthesize infectious RNA transcripts.

Sequences at the 5' & 3' ends of rubella virus RNA can form stable stem- loop structures. These structures are thought to be involved with virus replication . Recent work suggests that RV RNA interacts with host cell proteins. Cell proteins of 59 & 52Kda bind to the 5' stem –loop structure, these 2 proteins are related to the autoantigen La. The host cell protein that binds to the 3' stem-loop structure is autophosphorylated calreticulin, another putative autoantigen. It has been suggested that these autuantigen –RV-RNA complexes may play a role in the replication & pathogenesis of RV.

Replication[10]

RV enters cell by receptor mediated endocytosis . Membrane lipid molecules play an important role . Reproductive cycle takes place in cytoplasm.

Virion is internalized in a coated vesicle & transported to endosomal compartment. At the low pH in the endosome the C protein becomes lipid soluble which allow association of capsid with viral membrane to uncoat the viral RNA within viral envelope. Low pH also triggers a confirmational change in envelope glycoprotein & mediate fusion of viral membrane & endosomal membrane to allow release of viral RNA into cytoplasm.

The virion RNA is translated to produce 2115 amino acid polyprotein encoded by 5'proximal ORF. This polyprotein is proteolytically cleaved to give the non structural protein which may interact with host cell protein to replicate a negative sense genome RNA & subgenomic 24sRNA. The negative polarity RNA is present only in this form & function as a template for positive polarity RNA synthesis . The subgenomic RNA that is transcribed from this negative template is translated to

produce a polyprotein of 110 kda, which is cleaved by host cell proteases to produce 3 structural protein, 2 glycoprotein – E_1 & E_2, one capsid protein –C. E_1 & E_2 signal sequences remain attached to mature C & E_2 respectively. C protein forms a non covalently bonded dimer soon after translation in infected cells.

RV capsid formation occurs in association with membrane for which the E_2 signal sequences is required. E_1 & E_2 forms heterodimeric complexes .They are targeted to the golgi apparatus. A trans- dominant golgi retention signal is identified within C terminal region of E_2. Thus all structural protein are transported to golgi complexes. The 40S genomic RNA is encapsidated by the C protein. A 29 nucleotide RNA sequence is essential for binding to capsid protein .Virus is released from cells by budding, probably at both plasma membrane and internal membrane. The nucleocapsid core buds from modified cellular membrane & acquires host cell lipids & viral protein E_1 & E_2 to form viral envelope.

Epidemilogical Determinants

AGENT FACTORS[37]

A. AGENT – rubella is caused by RNA virus of togaviridae family which can be recovered from the nasopharynx, throat, blood, CSF, urine.
B. SOURCE OF INFECTION- clinical or subclinical cases of rubella .There is no known carrier state for postnatally acquired rubella. Infants born with congenital rubella may shed virus for many months. The vaccine virus is not communicable.
C. PERIOD OF COMMUNICABILITY- rubella is less communicable because of absence of coughing in rubella. Infectivity period extend from a week

before syptoms to about a week after rash appears . Infectivity is maximum when the rash is erupting.

HOST FACTORS

A. AGE- Prior to introduction of universal rubella immunization peak infection occurred in 5-9 year old age group. After vaccine implementation , the disease shifted from children to young adults.[38] A concerning statistic is the continued susceptibility to rubella infection of women of childbearing age. Serologic surveys have shown that 10-20% of such women in the united states are susceptible.

   The risk of developing congenital rubella infection depends on the month of pregnancy in which the maternal infections occurred. [20]

B. IMMUNITY- After an attack of rubella, lifelong protection against disease develops in most persons. Antibody titres to rubella virus develop, but the sigificance of the decline of antibody titre with time remain unclear.CMI to rubella virus associated with CD4+ &CD8+ T lymphocytes has also been detected by in vitro assays month to years after an attack of rubella. Persistence of specific antibody for as long as 14 years after immunization is also demonstrated.[39]

Despite this reinfection with rubella virus can occur though most are asymptomatic & detectable only by serological means. Rubella reinfection occuring months or years after reciept of vaccine has also been reported. Most of these reinfection were not characterized by clinical illness but were identified only by rise in antibody titer. Reinfection are more common among vaccinees than among persons

who have experienced natural rubella & they are most common among person with HAI antibody of 1: 64 or less.[39]

Reinfection in pregnancy is hazardous only if viremia occurs, & this has rarely been documented. Following maternal reinfection during the first 16 week in pregnancy, the risk of fetal infection has been estimated to be in order of 8%, although fetal damage is rare. Although it is possible that in such cases, transmission of virus to the fetus may be due to a specific defect in the maternal immune response, rubella reinfection is not associated with a lack of neutralizing antibodies or persistent impairement of rubella –specific lymphoproliferative responses.

Reinfection in pregnancy will be eliminated if high rates of rubella vaccination are achieved & maintained.[10]

ENVIRONMENTAL FACTORS

Disease occurs in seasonal pattern that is in temperate zones during late winter & spring, with epidemic every 4-9 years.[37]

Clinical features

ACUTE RUBELLA

The infection caused by RV in early childhood or adult life is usually mild,upto 50% of infection are subclinical.[40] Clinically apparent rubella is characterized by any combination of symptoms that include maculopapular rash, lymphadenopathy, low-grade fever, conjunctivitis, sore throat, and arthralgia.[41] Rash is first seen on the face and spreads to the trunk and limbs. Lesions appear as distinct pink maculopapules that later coalesce and then fade rapidly over several days. [42]

Signs and symptoms are non-specific, rubella may be mistaken for other rash infections such as measles, parvovirus, adenoviruses, enteroviruses.[43] In children, a prodrome is rare and rash is usually the first manifestation. In older children and adults, there is often a 1 to 5 day prodrome with low-grade fever, malaise, lymphadenopathy and upper respiratory symptoms preceding the rash. The rash starts on the face, becomes generalized within 24 hours, and lasts approximately three days.[44]

RV appears to be spread principally by aerosols. The mucosa of the upper respiratory tract and the nasopharyngeal lymphoid tissue serve as portals of virus entry and are the initial sites of virus replication. Spread of virus through lymphatics or a transient viremia then seeds regional lymph nodes.[38] Enlarged post auricular & suboccipital lymph nodes, which precedes the rash, are characteristic & last for 5-8 days.[45]

Complications

JOINT SYMPTOMS

Natural rubella can be complicated by acute arthralgia or arthritis, especially when it occurs in adolescent and adult women. Incidence rates for arthralgia and arthritis exceed 60% in some outbreaks and may be considered a manifestation of rubella rather than a complication of the infection.[38] Manifestations of rubella arthropathies vary from joint pain alone to joint swelling, effusions, and loss of joint motion with local heat and erythema. Joint symptoms usually begin within 1 week of the appearance of the rash and may involve any joint, with the fingers and knees being

most commonly affected. Symptoms usually resolve within several weeks but may persist for years, are sometimes episodic, and, rarely, can be disabling.[38,46]

THROMBOCYTOPENIA[38]

Transient asymptomatic depression of thrombocyte counts is common with rubella, but symptomatic thrombocytopenic purpura follows rubella in only 1 of 1,500 cases. The latter condition is usually self-limiting and may occur in the absence of rash as a plausible cause of some cases of idiopathic thrombocytopenic purpura. Very rarely, epidemic rubella has been associated with hemolytic anemia. There are sporadic reports of cardiac arrhythmia with acute childhood rubella and of thyroiditis and hepatic dysfunction with rubella in adults.

ENCEPHALITIS

The most serious complication of postnatal rubella is postinfectious encephalopathy or encephalomyelitis. Estimated to occur in 1 of 6,000 cases of natural rubella, the symptoms of postinfectious rubella encephalopathy appear abruptly 1 to 6 days after the onset of rash in an otherwise typical case of rubella. The most frequently encountered symptoms include headache, vomiting, stiff neck, lethargy, and generalized convulsions. Rubella postinfectious encephalopathy usually requires only supportive treatment, and the course of the disease is generally concluded within a few days.[38] Case fatality rate has been found upto 30% .[47]

CONGENITAL RUBELLA SYNDROME

Although postnatal rubella is rarely associated with severe complications, infection of a developing fetus after transplacental transmission of virus from the mother has dire consequences for fetal development. Maternal infection shortly before conception does not appear to lead to intrauterine infection.[38] After conception, however,

infection occurs in 90% of cases during the first 8 weeks of gestation, falling to a low of 25% to 35% during the second trimester and rising again near term.[48]

PATHOGENESIS

Effects on Placenta[38]

RV infection of the placenta in early gestation produces scattered foci of necrotic syncytiotrophoblast and cytotrophoblast cells and damages the vascular endothelium. Infection at later stages induces chronic multifocal mononuclear cell infiltrates in the placental membranes, cord, and decidua, along with vasculitis. These culminate in placental hypoplasia and macroscopic placentitis. After placental infection is established, there can be subsequent dissemination of virus to the fetus. This is not invariant, however, and virus is more often recovered from placental products than from fetal products of conception. After entry, virus is capable of spreading widely throughout the developing fetus, and almost any organ may be infected. A chronic and generally nonlytic infection is then established in the fetus.

Mechanisms of Teratogenesis[38]

The pathogenic mechanisms underlying RV teratogenesis is unknown but it is multifactorial.

Direct effects of virus replication on focal clones of cells and their progeny during critical stages of the ontogeny of specific fetal organs give rise to the wide range of abnormalities that together comprise CRS.

Infection of epithelial cells *in vitro* is associated with a marked depolymerization of actin filaments, and disruption of these cytoskeletal structures could disturb mitosis. Human embryonic cells persistently infected *in vitro* with RV display an altered responsiveness to the growth-promoting properties of epidermal growth factor as well

as a decreased capacity for collagen synthesis. Thus, it is probable that noncytopathic RV infection of selected embryonic cell types *in utero* upsets the normal delicate balance of cellular growth and differentiation and has profound effects on organogenesis. Organs of congenital rubella infants contain reduced numbers of cells.

Direct cytolytic effects of RV also contribute to the observed damage. For example, the cataracts of congenital rubella contain highly characteristic pyknotic cells in the lens nucleus, and noninflammatory necrosis of the pigmented cells of the retinal epithelium produces focal areas of decreased and increased pigmentation of the retina. Focal cellular necrosis has also been noted in the myocardium, skeletal muscle, and inner ear. Necrosis of vascular endothelium is also prominent early in the *in utero* infection. In the CNS, focal areas of noninflammatory parenchymal and perivascular necrosis are common.

In addition to these direct effects of RV replication in host tissue, there is considerable evidence that perinatal and also postnatal damage is, at least in part, immune mediated. Pathologic signs in infants who die at some interval after birth usually include mononuclear inflammatory infiltrates in one or more organs, particularly the lungs and brain. Circulating immune complexes containing RV antigens may involved in the immunopathogens of late-onset disease.

The persistence of RV in fetal tissue throughout gestation, and in infants with CRS for prolonged periods after birth, raises the question of how the virus avoids immune elimination. Antibody may in fact promote persistence, as it has been shown to do *in vitro*. Moreover, fetal IgM is not synthesized until about 20 weeks of gestation, and cell-mediated immune responses are not detectable until 27 weeks, leaving the foetus highly vulnerable.

Clinical Consequences[38]

The effects of RV invasion of fetal tissue are varied. Very early infection may result in resorption of the embryo. Whether placental infection alone can lead to spontaneous abortion or abnormalities of fetal development is conjectural.

Premature delivery and stillbirths are other potential outcomes of the fetal infection, but in most cases, the infected fetus survives, and the pregnancy continues to term. Increasingly, in many countries, clinically recognized maternal rubella now terminates in therapeutic abortion, particularly when infection occurs during the first 8 weeks of gestation when the occurrence of birth defects is extremely high (67% to 85% in different studies) . Fetal damage, therefore, is not universal even when there is serologic or virologic evidence of fetal infection. The current rubella vaccine RA27/3 has extremely low teratogenicity, and inadvertent vaccination in early pregnancy is no longer considered an indication for therapeutic abortion. Viral transmission to the fetus resulting in persistent infection has, however, been described.

The classic triad presentation of congenital rubella syndrome consists of the following[49]

- Sensorineural hearing loss is the most common manifestation of congenital rubella syndrome. It occurs in approximately 58% of patients. Studies have demonstrated that approximately 40% of patients with CRS may present with deafness as the only abnormality without other manifestations. Hearing impairment may be bilateral or unilateral and may not be apparent until the second year of life.
- Occular abnormalities including cataract, infantile glaucoma, and pigmentary retinopathy occur in approximately 43% of children with CRS. Both eyes are

affected in 80% of patients, and the most frequent findings are cataract and rubella retinopathy. Rubella retinopathy consists of a salt-and-pepper pigmentary change or a mottled, blotchy, irregular pigmentation, usually with the greatest density in the macula. The retinopathy is benign and nonprogressive and does not interfere with vision (in contrast to the cataract) unless choroid neovascularization develops in the macula.

- Congenital heart disease including patent ductus arteriosus (PDA) and pulmonary artery stenosis is present in 50% of infants infected in the first 2 month's gestation. Cardiac defects and deafness occur in all infants infected during the first 10 weeks of pregnancy and deafness alone is noted in one third of those infected at 13-16 weeks of gestation.

Other findings in CRS include the following[49]

- Intrauterine growth retardation, prematurity, stillbirth, and abortion.
- CNS abnormalities, including mental retardation, behavioral disorders, encephalographic abnormalities, hypotonia, meningoencephalitis, and microcephaly.
- Hepatosplenomegaly.
- Jaundice.
- Hepatitis.
- Skin manifestations, including blueberry muffin spots that represent dermal erythropoiesis and dermatoglyphic abnormalities.
- Bone lesions, such as radiographic lucencies.

- Endocrine disorders, including late manifestations in congenital rubella syndrome usually occurring in the second or third decade of life (eg, thyroid abnormalities, diabetes mellitus).
- Hematologic disorders, such as anemia and thrombocytopenic purpura.

TABLE 1: Clinico-pathologic Abnormalities in Congenital Rubella

Abnormality	Common/ Uncommon	Early /Delayed	Comment
General			
Intrauterine growth retardation	Common	Early	...
Prematurity	Uncommon	Early	...
Stillbirth	Uncommon	Early	...
Abortion	Uncommon	Early	...
Cardiovascular system			
Patent ductus arteriosus	Common	Early	May occur with pulmonary artery stenosis
Pulmonary artery stenosis	Common	Early	Caused by intimal proliferation
Coarctation of the aorta	Uncommon	Early	...
Myocarditis	Uncommon	Early	...
Ventricular septal defect	Uncommon	Early	...
Atrial septal defect	Uncommon	Early	...
Eye			
Cataract	Common	Early	Unilateral or bilateral
Retinopathy	Common	Early	Salt-and-pepper appearance; visual acuity unaffected; frequently unilateral
Cloudy cornea	Uncommon	Early	Spontaneous resolution
Glaucoma	Uncommon	Early/Delayed	May be bilateral
Microphthalmia	Common	Early	Common in patients with unilateral cataract
Subretinal neovascularization	Uncommon	Delayed	Retinopathy with macular scarring and loss of vision

Ear			
Hearing loss	Common	Early/Delayed	Usually bilateral; mostly sensorineural; may be central in origin; rare when maternal rubella occurs >4 months' gestation; sometimes progressive
CNS			
Meningoencephalitis	Uncommon	Early	Transient
Microcephaly	Uncommon	Early	May be associated with normal intelligence
Intracranial calcifications	Uncommon	Early	...
Encephalographic abnormalities	Common	Early	Usually disappear by age 1 y
Mental retardation	Common	Delayed	...
Behavioral disorders	Common	Delayed	Frequently related to deafness
Autism	Uncommon	Delayed	...
Chronic progressive panencephalitis	Uncommon	Delayed	Manifest in second decade of life
Hypotonia	Uncommon	Early	Transitory defect
Speech defects	Common	Delayed	Uncommon in absence of hearing loss
Skin			
Blueberry muffin spots	Uncommon	Early	Represents dermal erythropoiesis
Chronic rubelliform rash	Uncommon	Early	Usually generalized; lasts several weeks
Dermatoglyphic abnormalities	Common	Early	...
Lungs			
Interstitial pneumonia	Uncommon	Delayed	Generalized; probably immunologically mediated
Liver			
Hepatosplenomegaly	Common	Early	Transient
Jaundice	Uncommon	Early	Usually appears in the first day of life
Hepatitis	Uncommon	Early	May not be associated with jaundice

Blood			
Thrombocytopenia	Common	Early	Transient; no response to steroid therapy
Anemia	Uncommon	Early	Transient
Hemolytic anemia	Uncommon	Early	Transient
Altered blood group expression	Uncommon	Early	...
Immune system			
Hypogammaglobulinemia	Uncommon	Delayed	Transient
Lymphadenopathy	Uncommon	Early	Transient
Thymic hypoplasia	Uncommon	Early	Fatal
Bone			
Radiographic lucencies	Common	Early	Transient; most common in distal femur and proximal tibia
Large anterior fontanel	Uncommon	Early	...
Micrognathia	Uncommon	Early	...
Endocrine glands			
Diabetes mellitus	Common	Delayed	Usually becomes apparent in second or third decade of life
Thyroid disease	Uncommon	Delayed	Hypothyroidism, hyperthyroidism, and thyroiditis
Growth hormone deficiency	Uncommon	Delayed	...
Genitourinary system			
Cryptorchidism	Uncommon	Early	...
Polycystic kidney	Uncommon	Early	

Laboratory diagnosis

LABORATORY ASSESSMENT OF PRIMARY RUBELLA INFECTION IN PREGNANCY[50]

Assessment of primary rubella infection in pregnant women relies primarily on the detection of specific maternal IgM antibodies in combination with either seroconversion or a >4-fold rise in rubella specific IgG antibody titer in paired serum samples (acute/convalescent) . Today, due to the high sensitivity of the ELISA-IgM assays low levels of rubella specific IgM are detected more frequently, leading to an increase in the number of therapeutic abortions and reducing the number of CRS cases. However, frequently the low level of IgM detected is not indicative of a recent primary infection for several reasons:

a. IgM reactivity after vaccination or primary rubella infection may sometimes persist for up to several years.
b. heterotypic IgM antibody reactivity may occur in patients recently infected with Epstein Barr virus (EBV), cytomegalovirus (CMV), human parvovirus B19 and other pathogens, leading to false positive rubella IgM results.
c. false positive rubella specific IgM response may occur in patients with autoimmune diseases such as systemic lupus erythematosus (SLE) or juvenile rheumatoid arthritis, etc., due to the presence of rheumatoid factor (RF).
d. low level of specific rubella IgM may occur in pregnancy due to polyclonal B-cells activation trigerred by other viral infections.

False negative results may also occur in samples taken too early during the course of primary infection. Thus, the presence or absence of rubella specific IgM in an asymptomatic patient should be interpreted in accordance with other clinical and epidemiological information available and prenatal diagnosis may be required. A novel assay developed recently to support maternal diagnosis is the IgG avidity assay which can differentiate between antibodies with high or low avidity (or affinity) to the antigen. It is used when the mother has both IgM and IgG in the first serum collected. Following postnatal primary infection with rubella virus, the specific IgG avidity is initially low and matures slowly over weeks and months. Rubella specific IgG avidity measurement proved to be a useful tool for the differentiation between recent primary rubella (clinical and especially subclinical infection), reinfection, remote rubella infection or persistent IgM reactivity. This distinction is critical for the clinical management of the case, since infection prompts a therapeutic abortion, reinfection requires fetal assessment, while remote infection or non-specific IgM reactivity carry no risk to the fetus.

PRE- AND POSTNATAL LABORATORY ASSESSMENT OF CONGENITAL RUBELLA INFECTION[50]

Maternal primary infection prompts testing for fetal infection. The preferred laboratory method for prenatal diagnosis is determination of IgM antibodies in fetal blood obtained by cordocentesis. Other options include virus detection in chorionic villi (CV) samples or amniotic fluid (AF) specimens. The laboratory methods used for virus detection are virus isolation in tissue culture or amplification of viral nucleic acids by RT/PCR. However, using those methods for detection of rubella virus in AF and CV might be unreliable & virus may be present in the placenta but not in the fetus, or it can be present in the fetus but not in the placenta, leading to false negative

results. Thus, laboratory diagnosis of fetal infection should combine a serological assay (detection of rubella specific IgM) with a molecular method (viral RNA detection) in order to enhance the reliability of the diagnosis. A recent study showed 83–95% sensitivity and 100% specifity for detection of RV in AF by RT/PCR

Postnatal diagnosis of congenital rubella infection is based on one or more of the following:

a) Isolation of rubella virus from the infant's respiratory secretions.
b) Demonstration of rubella specific IgM (or IgA) antibodies in cord blood or in neonatal serum, which remain detectable for 6–12 months of age.
c) Persistence of anti-rubella IgG antibodies in the infant's serum beyond 3–6 months of age.

LABORATORY ASSAYS FOR ASSESSMENT OF RUBELLA INFECTION AND IMMUNITY

1. Rubella neutralization test (NT)

Virus neutralization is defined as the loss of infectivity due to reaction of a virus with specific antibody. Neutralization can be used to identify virus isolates or to measure the immune response to the virus. This test has proven to be highly sensitive, specific and reliable technique, but it can be performed only in virology laboratories which comprise only a small fraction of the laboratories performing rubella serology. Rubella virus produces characteristic damage (cytopathic effect, CPE) in the RK-13 cell line that was found most sensitive and suitable for use in rubella neutralization test. Other cells such as Vero and SIRC lines can be used if conditions are carefully controlled . Principally, 2-fold dilutions of each test serum are mixed and incubated with 100 infectious units of rubella virus under

appropriate conditions. Then cell monolayers are inoculated with each mixture and followed for CPE. Control sera possessing known high and low neutralizing antibody levels and titrations of the virus are included in each test run.[50] The neutralization titer is taken as the reciprocal of the highest serum dilution showing complete inhibition of CPE.[51]

2. Hemagglutination inhibition test (HI)

HI test is based on the ability of rubella virus to agglutinate red blood cells . HI test is labor intensive, and is performed mainly by reference laboratories. HI is the "gold standard" test against which almost all other rubella screening and diagnostic tests are measured. During the test, the agglutination is inhibited by binding of specific antibodies to the viral agglutinin. Titers are expressed as the highest dilution inhibiting hemagglutination under standardized testing conditions. The HI antibodies increase rapidly after RV infection since the test detects both, IgG and IgM class-specific antibodies. A titer of l:8 is commonly considered negative (cut off level: 1:16) and a titer of ≥1:32 indicates an earlier RV infection or successful vaccination and immunity. Seroconversion is interpreted as primary rubella infection, and a 4-fold increase in titer between two serum samples (paired sera) in the same test series, is interpreted as a recent primary rubella infection or reinfection.[50]

Detection of rubella specific IgM class antibodies by HI test which requires tedious methods for purification of IgM or removal of IgG , are no longer in use due to the development of a variety of rapid, easy to perform and sensitive methods, of which ELISA is the most vastly used .[52]

3. Rubella specific ELISA IgG [50]

The ELISA technique was established for detection of an increasing range of antibodies to viral antigens. In 1976, Voller et al. developed an indirect assay for the detection of antiviral antibodies. The technique has been successfully applied for the detection of rubella specific antibodies. Almost all commercially available ELISA kits for the detection of rubella specific IgG are of the indirect type, employing rubella antigen attached to a solid phase (microtiter polystyrene plates or plastic beads). The source of the antigen (peptide, recombinant or whole virus antigen) affects the sensitivity and specificity of the assay. After washing and removal of unbound antigen, diluted test serum is added and incubated with the immobilized antigen. The rubella specific antibodies present in the serum bind to the antigen. Then, unbound antibodies are removed by washing and an enzyme conjugated anti-human IgG is added and further incubation is carried out. The quantity of the conjugate that binds to each well is proportional to the concentration of the rubella specific antibodies present in the patient's serum. The plates are then washed and substrate is added resulting in colour development. The enzymatic reaction is stopped after a short incubation period, and optical density (OD) is measured by an ELISA-reader instrument.

In most commercial ELISA IgG assays the results are automatically calculated and expressed quantitatively in international units (IU). When performed manually, the procedure takes approximately 3 hours but automation has reduced it to about 30 min.

The correlation between the ELISA and HI or NT titers is not always high. This may be explained by the fact that the three methods detect antibodies

directed to different antigenic determinants. Certain individuals fail to develop antibodies directed to protective epitopes such as the neutralizing domains of E1 and E2 due to a defect in their rubella specific immune responses but they do develop antibodies directed to antigenic sub-regions of rubella virus proteins. ELISA assays utilizing whole virus as antigen may fail to distinguish between these different antibody specificities. Thus, seroconversion determined by ELISA based on a whole virus antigen does not necessarily correlate with protection against infection.

4. Rubella specific ELISA IgM

Commercially available ELISA kits for the detection of IgM are mainly of two types:

a) Indirect ELISA: The principle of the assay was described above for rubella IgG except for using enzyme labeled antihuman IgM as a conjugate. In this assay, false negative results may occur due to a competition in the assay between specific IgG antibodies with high affinity (interfering IgG) while the specific IgM have lower affinity for the antigen. In the new generation ELISA assays this is avoided by the addition of an absorbent reagent for the removal of IgG from the test serum. False positive results may occur if rheumatoid factor (RF: IgM anti-IgG antibodies) is present along with specific IgG in the test serum. Absorption or removal of RF and/or IgG is necessary prior to the assay to avoid such reactions.[53]

b) IgM capture ELISA*:* In these assays anti-human IgM antibody is attached to the solid phase for capture of serum IgM. Rubella virus antigen conjugated to enzyme-labeled anti-rubella virus antibody is added for detection. This type of assay eliminates the need for sample pretreatment prior to the assay.

As for the rubella virus antigens, most assays are based on whole virus extracts, but recent developments led to production of recombinant and synthetic rubella virus proteins.[54]

5. Rubella specific IgG-avidity assay[50]

This assay is based on the ELISA IgG technique and applies the elution principle in which protein denaturant, mostly urea (but also diethylamine, ammonium thiocyanate, guanidine hydrochloride, etc.) is added after binding of the patient's serum. The denaturant disrupts hydrophobic bonds between antibody and antigen, and thus, low avidity IgG antibodies produced during the early stage of infection are removed. This results in a significant reduction in the IgG absorbance level. The avidity index (AI) is calculated according to the following formula AI = 100 × absorbance of avidity ELISA absorbance of standard ELISA The AI is a useful measure only when the IgG concentration in the patient's serum is not below 25 IU . Low avidity (usually below 50%) is associated with recent primary rubella infection while reinfection is typically associated with high avidity as a result of the stimulation of memory B cells (immunological memory). In infants with CRS the low avidity IgG continues to be produced for much longer than in cases of postnatal primary rubella, where it lasts 4–6 week after exposure. This may be used for retrospective assessment of initially undiagnosed CRS cases.

6. Rubella virus isolation in tissue culture [50]

Virus isolation is useful in confirming the diagnosis of CRS and rubella virus strain characterization required for epidemiological purposes. Rubella virus can be isolated using a variety of clinical specimens such as: respiratory secretions (nasopharyngeal swabs), urine, heparinized blood, CSF, cataract material, lens

fluid, amniotic fluid, synovial fluid and products of conception (fetal tissues: placenta, liver, skin, etc.) obtained following spontaneous or therapeutic abortion. In order to avoid virus inactivation, specimens should be inoculated into cell culture immediately or stored at 4◦C for not more than 2 days, or kept frozen (−70◦C) for longer periods. Rubella virus can be grown in a variety of primary cells and cell lines, but RK-13 and Vero cell lines are the most sensitive and suitable for routine use. In these cell systems rubella virus produces characteristic CPE. Since the CPE is not always clear upon primary isolation, at least two successive subpassages are required. When CPE is evident the identity of the virus isolates should be confirmed using immunological or other methods.

7. Rubella RT-PCR assay

Reverse transcription followed by PCR amplification (RTPCR) is a rapid, sensitive and specific technique for detection of rubella virus RNA in clinical samples using primers from the envelope glycoprotein E1 open reading frame. Coding sequences for a major group of antigenic determinants are located between nucleotides 731 and 854 of the E1 gene of RV strain M33. This region is highly conserved in various wild type strains and is likely to be present in most clinical samples from rubella infected patients. Specific oligonucleotide primers located in this region were designed for amplification by RT-PCR.[55] Following rubella genomic RNA extraction from clinical specimens and RT-PCR amplification, the product is visualized by gel electrophoresis. Positive samples show a specific band of the expected size compared to size markers. [55,56]

A nested RT-PCR assay, in which the RT-PCR product from the first amplification reaction is re-amplified by internal primers, was developed and shown to provide a higher level of sensitivity for the detection of rubella virus RNA. However, the risk of contamination is markedly increased. The detection limit of the RT-PCR assay is approximately two RNA copies. Clinical specimens for rubella virus genome detection include: products of conception, CV, lens aspirate/biopsy, AF, fetal blood, pharyngeal swabs and spinal fluid (CSF) or brain biopsy when the central nervous system (CNS) is involved .An additional advantage of RT-PCR is that it does not require infectious virus. RV is extremely thermo-labile and frequently is inactivated during sample transporation to the laboratory.[50]

Finally, it should be noted that clinical samples may contain PCR inhibitors (such as heparin and hemoglobin), and the extraction procedure itself may cause enzyme inhibition. This underscores the need and importance for strict internal quality control during each step of the RT-PCR procedure and participation in external quality assessment programs is of a high value.[50]

Treatment[15]

No specific therapy is avilable for rubella virus infection. Symptom – based treatment for various manifestation, such as fever, arthralgia, is appropriate. Immunoglobulin does not prevent its infection after exposure & therefore is not recommended as routine postexposure prophylaxis.Although immunoglobulin may modify or suppress symptoms, it can create an unwarranted sense of security, infant with congenital rubella have been to born to women who received immunoglobulin shortly after exposure. Administration of immunoglobulin should be considered only

if a pregnant woman who has been exposed to rubella will not consider termination of pregnancy under any circumstances. In such cases, intramuscular administration of 20 ml of immunoglobulin within 72 hour of rubella exposure may reduce – but does not eliminate the risk of rubella.

RUBELLA VACCINE

Rubella vaccine is on the WHO list of essential medicines, a list of most important needed in basic health system.[57]

Three rubella vaccines were licensed in the United States in 1969: HPV-77: DE-5 (duck embryo), HPV-77:DK-12 (dog kidney), and GMK-3:RK53 Cendevax (rabbit kidney) strains. HPV-77: DK-12 was later removed from the market because there was a higher rate of joint complaints following vaccination with this strain. In 1979, the RA 27/3 (human diploid fibroblast) strain (Meruvax-II, Merck) was licensed and all other strains were discontinued.[58]

Characteristics

The RA 27/3 rubella vaccine is a live attenuated virus. It was first isolated in 1965 at the Wistar Institute from a rubella-infected aborted fetus. The virus was attenuated by 25–30 passages in tissue culture, using human diploid fibroblasts. It does not contain duck, chicken or egg protein.

Vaccine virus is not communicable except in the setting of breastfeeding, even though virus may be cultured from the nasopharynx of vaccinees.

The rubella vaccine is usually given as a combined measles–mumps–rubella (MMR) but can be administered as a single vaccine or in combination with the measles vaccine, or with the measles, mumps and varicella vaccine (MMRV).[58]

Combination vaccines decrease the number of injections children receive, have the potential to improve vaccination coverage for several diseases and increase the level of compliance.[59]

Immunogenicity and Vaccine Efficacy [58]

RA 27/3 rubella vaccine is safe and more immunogenic than rubella vaccines used previously. In clinical trials, 95% or more of vaccinees aged 12 months and older developed serologic evidence of rubella immunity after a single dose. More than 90% of vaccinated persons have protection against both clinical rubella and viremia for at least 15 years.

Several reports indicate that viremic reinfection following exposure may occur in vaccinated persons who have low levels of detectable antibody. Rarely, clinical reinfection and fetal infection have been reported among women with vaccine-induced immunity.

Vaccination Schedule [19]

An RCV is normally administered as a subcutaneous injection (but may also be given intramuscularly), usually at age 12–15 months, but it can also be administered to children aged 9–11 months and to older children, adolescents and adults. In most countries, rubella vaccine is given as MR or MMR, and the age of administration follows the schedule for measles – that is, the first dose is usually given at 9 months or 12–15 months and a second dose at 15–18 months or 4–6 years.

The high response rate to a single dose of rubella vaccine (≥95%) and the long-term persistence of protection in vaccines do not support a routine requirement for a second dose of rubella vaccine. However, based on the indications for a second

dose of measles-containing and mumps-containing vaccines, a second dose of MR or of MMR is now offered in most countries.

During outbreaks of measles, RCVs may be administered to infants as young as 6 months. Because of the possibility of lower seroconversion, the dose administered at 6 months should not be counted as a valid dose, and the child should be vaccinated with subsequent dose(s) of RCVs according to the usual national immunization schedule.

Rubella Immunity [58]

Persons generally can be considered immune to rubella if they have documentation of vaccination with at least one dose of MMR (or MMRV) or other live rubella-containing vaccine administered on or after their first birthday, have serologic evidence of rubella immunity. Persons who have an "equivocal" serologic test result should be considered rubella-susceptible. Although only one dose of rubella-containing vaccine is required as acceptable evidence of immunity to rubella, children should receive two doses of MMR vaccine according to the routine childhood vaccination schedule. Clinical diagnosis of rubella is unreliable and should not be considered in assessing immune status.

Serologic screening need not be done before vaccinating for measles and rubella unless the medical facility considers it cost-effective. Serologic testing for immunity to measles and rubella is not necessary for persons documented to be appropriately vaccinated or who have other acceptable evidence of immunity.

Neither rubella vaccine nor immune globulin is effective for postexposure prophylaxis of rubella. Vaccination after exposure is not harmful and may possibly avert later disease.

Contraindications and Precautions to Vaccination[58]

Persons who have experienced a severe allergic reaction (anaphylaxis) to a vaccine compnent or following a prior dose of rubella vaccine should generally not be vaccinated with MMR.

Women known to be pregnant or attempting to become pregnant should not receive rubella vaccine. Although there is no evidence that rubella vaccine virus causes fetal damage, pregnancy should be avoided for 4 weeks (28 days) after rubella or MMR vaccination.

Persons with immunodeficiency or immunosuppression, resulting from leukemia, lymphoma, generalized malignancy, immune deficiency disease, or immunosuppressive therapy should not be vaccinated. However, treatment with low-dose (less than 2 mg/kg/day), alternate-day, topical, or aerosolized steroid preparations is not a contraindication to rubella vaccination. Persons whose immunosuppressive therapy with steroids has been discontinued for 1 month (3 months for chemotherapy) may be vaccinated. Rubella vaccine should be considered for persons with asymptomatic or mildly symptomatic HIV infection. Persons with moderate or severe acute illness should not be vaccinated until the illness has improved. Minor illness (e.g., otitis media, mild upper respiratory infections), concurrent antibiotic therapy, and exposure or recovery from other illnesses are not contraindications to rubella vaccination.

Receipt of antibody-containing blood products (e.g., immune globulin, whole blood or packed red blood cells, intravenous immune globulin) may interfere with seroconversion to rubella vaccine. Vaccine should be given 2 weeks before, or deferred for at least 3 months following administration of an antibody-containing blood product. If rubella vaccine is given as combined MMR, a longer delay may be necessary before vaccination.

Previous administration of human anti-Rho(D) immune globulin (RhoGam) does not generally interfere with an immune response to rubella vaccine and is not a contraindication to postpartum vaccination. However, women who have received anti-Rho immune globulin should be serologically tested 6–8 weeks after vaccination to ensure that seroconversion has occurred.

A personal or family (i.e., sibling or parent) history of seizures of any etiology is a precaution for MMRV vaccination. Studies suggest that children who have a personal or family history of febrile seizures or family history of epilepsy are at increased risk for febrile seizures compared with children without such histories. Children with a personal or family history of seizures of any etiology generally should be vaccinated with MMR vaccine and varicella vaccine because the risks for using MMRV vaccine in this group of children generally outweigh the benefits.

Although vaccine virus may be isolated from the pharynx, vaccinees do not transmit rubella to others, except occasionally in the case of the vaccinated breastfeeding woman. In this situation, the infant may be infected, presumably through breast milk, and may develop a mild rash illness, but serious effects have not been reported. Infants infected through breastfeeding have been shown to respond normally to rubella vaccination at 12–15 months of age. Breastfeeding is not a

contraindication to rubella vaccination and does not alter rubella vaccination recommendations.

Adverse Reactions Following Vaccination

Rubella vaccine is very safe. Most adverse reactions reported following MMR vaccination (such as fever and rash) are attributable to the measles component..[58]

The common complaints following vaccination are[49] :-

Fever of 39.4°C (103°F) or higher may develop in 5-15% of vaccine recipients from 5-12 days after immunization.

Rash develops in 5% of patients 7-10 days after vaccination.

Mild lymphadenopathy is common.

Joint pain is observed in 0.5% of young children.

Arthralgia is experienced in 25% of females who are past puberty.

Transient arthritis occurs in 10% of females who are past puberty.

Joint complaints occur approximately 7-21 days following MMR vaccination.

Rare cases of transient peripheral neuritic symptoms, such as paresthesia and pain in the arms and legs, have been reported.

Transient and benign thrombocytopenia within 2 months of immunization has been reported in 1 per 25,000-40,000 immunized children.

CNS manifestations have also been reported, but no causal relationship with rubella vaccine has been demonstrated

Rubella Vaccination of Women of Childbearing Age [58]

ACIP recommends that vaccine providers ask a woman if she is pregnant or likely to become pregnant in the next 4 weeks. Those who are pregnant or intend to become pregnant should not be vaccinated. All other women should be vaccinated after being informed of the theoretical risks of vaccination during pregnancy and the importance of not becoming pregnant during the 4 weeks following vaccination. ACIP does not recommend routine pregnancy screening of women before rubella vaccination. If a pregnant woman is inadvertently vaccinated or if she becomes pregnant within 4 weeks after vaccination, she should be counseled about the concern for the fetus, but MMR vaccination during pregnancy should not ordinarily be a reason to consider termination of the pregnancy.

When rubella vaccine was licensed, concern existed about women being inadvertently vaccinated while they were pregnant or shortly before conception. This concern came from the known teratogenicity of the wild-virus strain. To determine whether CRS would occur in infants of such mothers. CDC maintained a registry from 1971 to 1989 of women vaccinated during pregnancy. This was called the Vaccine in Pregnancy (VIP) Registry.

Although subclinical fetal infection has been detected serologically in approximately 1%–2% of infants born to susceptible vaccinees, regardless of the vaccine strain, the data collected by CDC in the VIP Registry showed no evidence of CRS occurring in offspring of the 321 susceptible women who received rubella vaccine and who continued pregnancy to term. The observed risk of vaccine-induced malformation was 0%, with a maximum theoretical risk of 1.6%, based on 95% confidence limits (1.2% for all types of rubella vaccine). Since the risk of the vaccine to the fetus appears to be extremely low, if it exists at all, routine termination of

pregnancy is not recommended. Individual counseling for these women is recommended. As of April 30, 1989, CDC discontinued the VIP registry.

The ACIP continues to state that because of the small theoretical risk to the fetus of a vaccinated woman, pregnant women should not be vaccinated.

Vaccine Storage and Handling [58]

When stored at +4°C, most RCVs have a shelf-life of 2–3 years. For monovalent rubella, MR and MMR formulations, the vaccine should be stored at +2°C to +8°C. Diluents for RCVs are not as sensitive to storage temperatures as the vaccines with which they are used. Diluents are normally stored at ambient temperature, unless they are packed with the vaccine. In this case they should be kept in the cold chain between +2°C and +8°C. Diluent vials must never be frozen. Diluent may be stored at refrigerator temperature or at room temperature.

After reconstitution, MMR vaccines must be stored at refrigerator temperature and protected from light. Reconstituted vaccine should be used immediately. If reconstituted vaccine is not used within 8 hours, it must be discarded. MMRV must be administered within 30 minutes of reconstitution.

WHO RESPONSE[60]

WHO recommends that all countries that have not yet introduced rubella vaccine should consider to do so using existing well-established measles immunization programmes.

In April 2012, the Measles Initiative – now known as the Measles & Rubella Initiative – launched a new Global Measles and Rubella Strategic Plan which covers the period 2012-2020. The Plan includes new global goals for 2015 and 2020.

By the end of 2015

- Reduce global measles deaths by at least 95% compared with 2000 levels.
- Achieve regional measles and rubella/congenital rubella syndrome (CRS) elimination goals.

By the end of 2020

- Achieve measles and rubella elimination in at least five WHO regions.

The strategy focuses on the implementation of five core components:

1. Achieve and maintain high vaccination coverage with two doses of measles- and rubella-containing vaccines;
2. Monitor the disease using effective surveillance, and evaluate programmatic efforts to ensure progress and the positive impact of vaccination activities;
3. Develop and maintain outbreak preparedness, rapid response to outbreaks and the effective treatment of cases;
4. Communicate and engage to build public confidence and demand for immunization;
5. Perform the research and development needed to support cost-effective action and improve vaccination and diagnostic tools.

Implementation of the Strategic Plan can protect and improve the lives of children and their mothers throughout the world, rapidly and sustainably. The Plan provides clear strategies for country immunization managers, working with domestic

and international partners, to achieve the 2015 and 2020 measles and rubella control and elimination goals. It builds on years of experience in implementing immunization programmes and incorporates lessons from accelerated measles control and polio eradication initiatives.

As one of the founding members of the Measles & Rubella Initiative, WHO provides technical support to governments and communities to improve routine immunization programmes and hold targeted vaccination campaigns

NEED FOR MMR VACCINATION IN INDIA

Choices of vaccines in National Immunization Schedule warrants careful decision and periodic reviews. In 1978, India adopted the Expanded Programme on Immunization (EPI) promoted by World Health Organization (WHO). In 1985, EPI was renamed as Universal Immunization Program (UIP). [61]

Measles-Mumps-Rubella (MMR) vaccine in a two dose schedule has successfully eliminated measles, mumps and rubella from many developed countries.[62] MMR vaccine simultaneously provides protection for measles, mumps and rubella. Nearly 45% females in the reproductive age group in India are susceptible to infection during pregnancy. Congenital Rubella Syndrome (CRS) is likely to result in congenital malformations of various organs.[27]

Indian Academy of Pediatrics (IAP) recommends MMR vaccine to all parents who can afford it as two dose schedule, one at 15-18 months and second at school entry (4-6 yr of age).[63] A study conducted by ICMR found that even after MMR

administration, number of children protected against measles was alarmingly low. Observed protection against mumps and rubella was adequate but durability was questionable.[64] Recently, it has been emphasized that protective immune response to each of the component vaccine remains unchanged in combination vaccine.[65]

Delivery strategies for measles vaccine provide an opportunity for synergy and a platform for advancing rubella and CRS elimination.[66] Many countries in South East Asia Region adopted a resolution to eliminate measles and control rubella by 2020 & have introduced RCV (Rubella containing vaccine) in their national immunization program. Funding is identified as a key challenge for achieving measles and rubella elimination targets. SAGE working group in 2013 found that the vaccine requirement of combined vaccine will increase directly in proportion to decrease in measles only vaccine. Moreover, there is no anticipated shortage in the supply of combined vaccine, and can be completely obviated by planned phase-out of measles only vaccine and gradual introduction of combined vaccine.[67]

There is no evidence to support the routine use of monovalent measles, mumps and rubella vaccines over the combined vaccine, a strategy which would put children at increased risk of incomplete immunization .[68] In high-income and middle-income countries, caring for CRS cases is costly, and rubella vaccination has been found to be cost-effective. However, no such studies have been conducted in low-income countries in Africa and Asia.[69] Economic analysis of the same conducted in United States found the 2-dose MMR vaccination program cost-saving from both direct cost and societal perspectives. The net savings (net present value) from direct cost and societal perspectives was of nearly $3.5billion and $7.6 billion, respectively.

Given the fact that cyclical outbreak of mumps is imminent following no vaccination against this communicable disease and existing burden of rubella, measures to include MMR vaccine in immunization schedule must be considered. There is a need to effectively counter diseases knowing that mortality due to measles is greater cause of concern but threat of complications and morbidity from mumps and rubella might assume significant proportions in coming times. Incorporation of RCV into national childhood immunization schedules is both cost-beneficial and cost-effective . In introducing rubella containing vaccines (RCVs), MR and MMR vaccines can easily replace single-antigen measles vaccines in routine childhood immunization schedules. The substantial morbidity and cost resulting from infants born with CRS and the ease of introduction of RCVs into the routine vaccination program clearly indicate that rubella vaccine should be introduced in the National Immunization Programme in India to ensure high vaccination coverage.[70]

MATERIALS AND METHODS

SOURCE OF DATA

Women of reproductive age group attending Obstetrics and Gynaecology OPD of Shri B.M Patil Medical College Hospital & Research Centre, Bijapur.

SAMPLE SIZE: 120

SAMPLING ANALYSIS

Diagrammatic presentation.

Mean ±SD.

Percentages.

And association between seroprevalence of Rubella and socio-demographic factors will be found by using x^2- test

Statistical analysis was done by software-SPSS17 Version.

INCLUSION CRITERIA:

Women of reproductive age group.

EXCLUSION CRITERIA:

1. Previous known history of Rubella vaccine.
2. Previous history of known Rubella infection.

METHOD OF COLLECTION OF DATA

STUDY DESIGN: This is a Cross sectional study from December 2012 to August 2014.

- A total of 120 women satisfying the inclusion criteria and excluding criteria were included in our study.
- After taking written informed consent & fulfilling inclusion criteria women were included in this study.
- Detailed information including general information like age, residence, level of education, occupation, pregnancy , parity were taken.
- Data regarding education level, occupation & income were taken into consideration & was classified according to socioeconomic status.

After history & examination about 2-3 ml of blood sample was collected by venipuncture with all aseptic precaution in a sterile, dry plain test tube from each women and was sent to laboratory immediately.

Serum was seperated from whole blood and stored at 4°C until analyzed.

INVESTIGATION

- IgG & IgM antibodies against Rubella were detected using Rubella IgG & IgM ELISA kit (DELTA BIOLOGICALS).

➢ Following procedure was followed for rubella IgM antibodies test

Serum was brought back to room temperature whenever deep freeze

↓

Diluted serum (100 µl) was taken in well of the strips

↓

Incubate for 45 minutes at 37* c

↓

Wash with wash buffer 4 times

↓

100 µl of immune complex was added to each well

↓

Incubate for 45 minute at 37* c

↓

Wash with wash buffer 4 times

↓

100 µl of substrate was added to each well

↓

Incubate for 15 minutes at 37* c

↓

100 µl of stop solution added to each well

↓

Reading done by ELISA analyser at 450 nm

- Following procedure was followed for rubella IgG antibodies test

Serum was brought back to room temperature whenever deep freeze

↓

Diluted serum (100 µl) was taken in well of the strips

↓

Incubate for 45 minutes at 37* c

↓

Wash with wash buffer 4 times

↓

100 µl of conjugate was added to each well

↓

Incubate for 45 minute at 37* c

↓

Wash with wash buffer 4 times

↓

100 µl of substrate was added to each well

↓

Incubate for 15 minutes at room temperature

↓

100 µl of stop solution added to each well

↓

Reading done by ELISA analyser at 450 nm

RESULTS

In present study 120 women of child bearing age (16 -45 years) were included. Considering all age groups overall rubella IgG seropositivity was found in 38 (31.66%) women in our study.

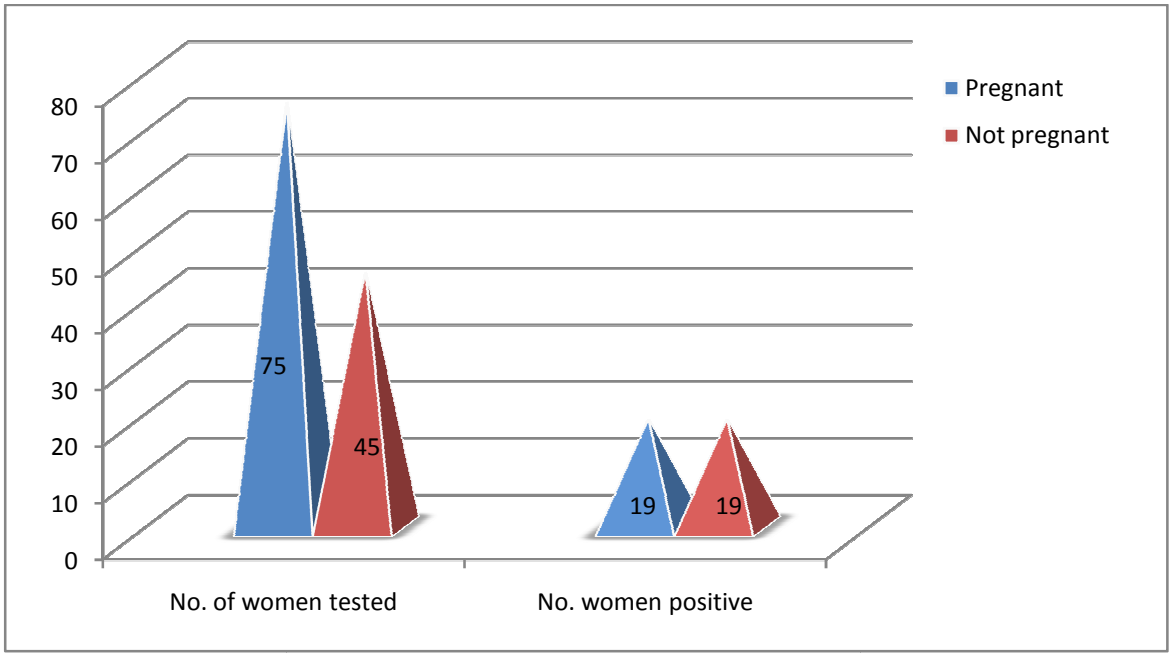
Rubella IgM seropositivity was found only in one case (0.83%) in our study.

Among 120 women tested, 75(62.5%) were pregnant & 45(37.5%) were non pregnant women

TABLE 2: Rubella IgG seropositivity & pregnancy

Pregnant or non pregnant	No. of women tested (%)	No. women positive (%)
Pregnant	75(62.5%)	19(25.3)
Not pregnant	45(37.5%)	19(42.2)
Total	120	38(31.7)

GRAPH 1: Distribution of rubella IgG seropositivity in relation to pregnancy



In our study 75 women were pregnant , among them 19(25.3%) are seropositive for rubella IgG antibodies . Out of 45 non pregnant women 19(42.2%) were positive for rubella IgG

Rubella IgG seropositivity according to different age group

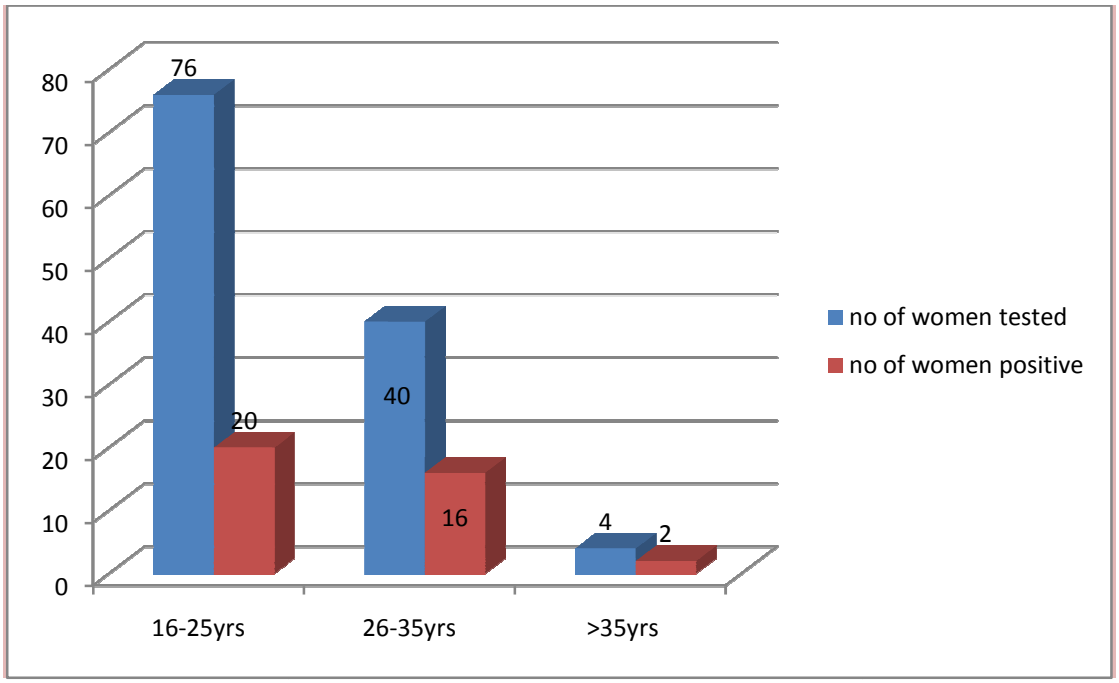
According to age women were divided into 3 groups –

- 16 years to 25 years
- 26 years to 35 years
- More than 35 years

TABLE 3: Rubella IgG seropositivity in different age group

Age group	No.of women's tested (%)	No. women positive (%)	p-value
16-25[a]	76(63.33)	20(26.31)	Between a & b 0.27
26-35[b]	40(33.33)	16(40)	
>35	04(3.33)	02(50)	
Total	120	38	

GRAPH 2: Distribution of rubella IgG seropositivity according to age



16-25 year age group includes maximum (76) women in our study, among them 20(26.31%) women were seropositive for IgG antibodies. Among 40 women of 26-35 years age group, 16 (40 %) were positive . Out of 4 women of >35 years age group 2 (50%)were seropositive for rubella IgG antibodies.

There was an increasing trend in seropositivity from 26.31% in 16-25 years of age group to the maximum incidence of 40 % in the age group 26-35 years .

However, in our study there was insignificant difference of rubella IgG antibodies among 16 -25 & 26-35 years age groups p=0.27(>.05)

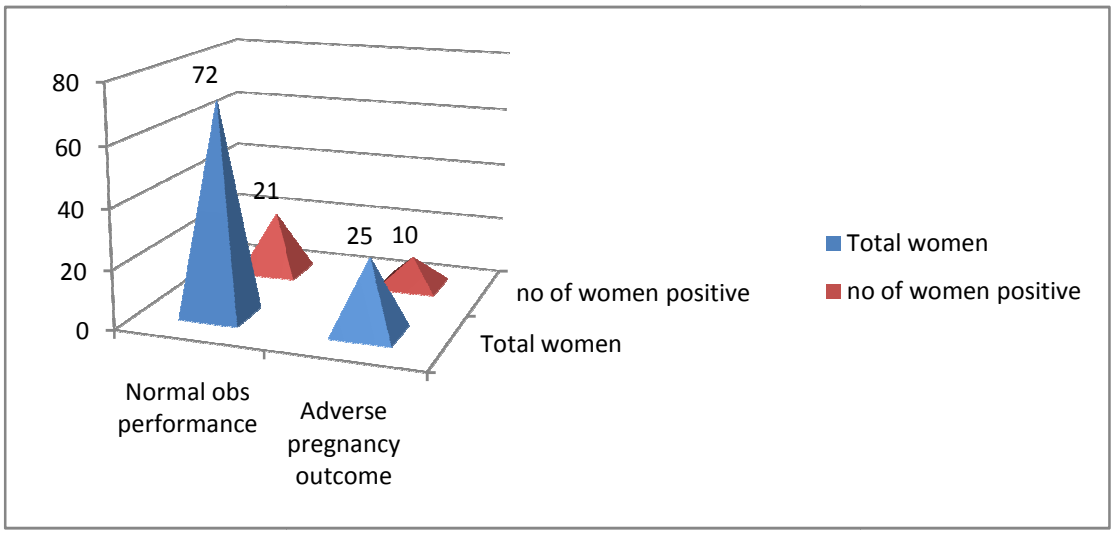
Seropositivity according to previous obstetric performance

Out of 120 women, 25(20.83%) women gave history of previous adverse pregnancy outcome while 72 (60%) gave history of normal obstetric performance.

TABLE 4 : Rubella IgG seropositivity according to previous obstetric performance

Previous obstetric performance	No. of women tested (%)	No. women positive (%)	p-value
Normal obstetric performance	72(60%)	21(29.1)	0.06
Adverse pregnancy outcome	25(20.83%)	10(40.0)	
Total	97	31(31.9)	

GRAPH 3: Distribution of rubella IgG seropositivity according to previous obstetric performance



Out of 25 women with history of previous adverse pregnancy outcome, 10 (40%) were seropositive for rubella IgG antibodies. Among 72 women with normal obstetric performance before, 21 (29.1 %) were seropositive for rubella IgG antibodies.

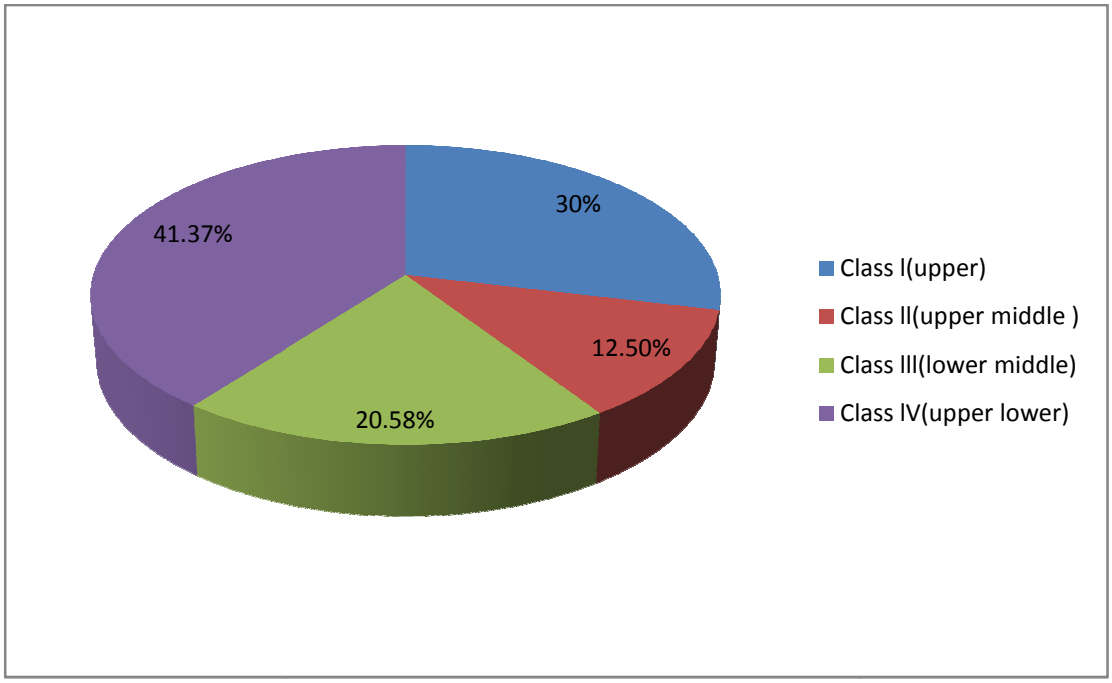
In our study there is insignificant differences between these two group with p = .06 (p >.05)

Socioeconomic status and rubella IgG seropositivity

TABLE 5: Rubella IgG seropositivity & Kuppuswami scale

Socioeconomic classes according to modified kuppuswamy scale	No. of women tested for rubella IgG antibodies (%)	No. of women positive for rubella IgG antibodies (%)
Class l(upper)	20(16.66)	6(30%)
Class ll(upper middle)	8(6.66)	1(12.5%)
Class lll(lower middle)	34(28.33)	7(20.58%)
Class lV(upper lower)	58(48.33)	24(41.37%)
Class V (lower)	0	0

GRAPH 4 : Distribution of rubella IgG seropositivity in relation to socio economic status(according to modified kuppuswamy scale)



Out of positives majority belong to class l V of modified kuppuswamy 's scale that is 41.37% suggesting higher prevalence of rubella antibodies among lower socioeconomic status

All women tested were divided into 3 groups according to socio economic status for comparision -:

Upper (class 1)

Middle (class ll & lll)

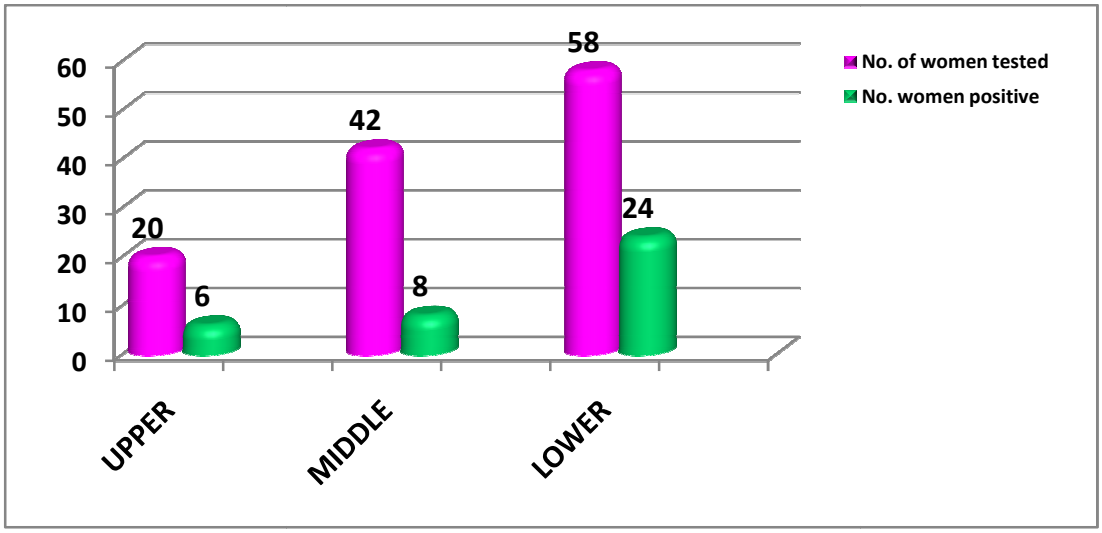
Lower (class lV)

Out of total 120 women 58 (48.33%) belong to lower socioeconomic status, 42 (35%) belong to middle socioeconomic class & 20 (16.66%) belong to upper socioeconomic class.

Table no 6: Rubella IgG seropositivity & socioeconomic status :

Socio economic status	**No. of women tested (%)**	**No. women positive (%)**	**p-value**
UPPER[a]	20(16.66)	06(30)	Between a & b 0.043*
MIDDLE	42(35)	08(19.04)	
LOWER[b]	58(48.33)	24(41.37)	
TOTAL	120	38	

GRAPH 5: Distribution of rubella IgG seropositivity according to socio economic status



In our study 58 women were of low socioeconomic status ,among them seropositivity for rubella IgG antibodies was found in 24 (41.37%). Out of 42 women of middle socioeconomic status 08(19.04%) showed positive results. Out of 20 women of upper socioeconomic status, 6(30%) were seropositive for rubella IgG antibodies.

In our study there was significant difference of rubella IgG antibodies among women of lower & upper socioeconomic class with p = .043(<.05)

Distribution of seropositive patients according to geographical area

With respect to geographical area women of child bearing age was divided into two groups :

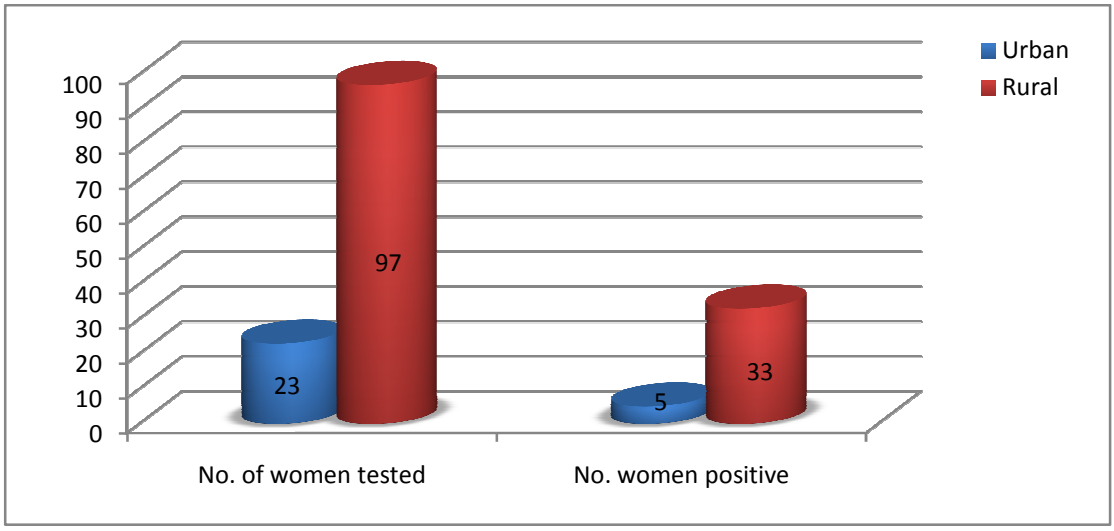
- Urban
- Rural

Out of 120 women 23(19.16%) were from urban area & 97 (80.83 %) were from rural area

TABLE 7: Rubella IgG seropositivity & geographical area

Geographical Area	No. of women tested (%)	No. women positive(%)	p-value
Urban	23(19.16)	05(21.7)	0.01*
Rural	97(80.83)	33(34.0)	
Total	120	38	

GRAPH 6 : Distribution of rubella IgG seropositivity according to geographical area



Out of 23 women of urban area 5 (21.7 %) were seropositive for rubella IgG antibodies compared to 33 (34 %) women out of 97 women of rural area.

There is higher seropositivity for rubella antibodies in rural women then in women of urban area.

Difference between these two categories is statistically significant as P = . 01(<.05).

None of the women included in this study gave history of immunization against Rubella.

DISCUSSION

Rubella is a mild exanthematous disease of worldwide distribution.It is not notifiable in many countries and its clinical diagnosis is frequently inaccurate, serosurveys are used to assess the epidemiologic pattern of rubella in a community. Importance of rubella infection for public health relates to its teratogenic effect on foetus of infected mother In our study 120 women in reproductive age group were tested for rubella IgG & IgM antibodies, among these 38 (31.66%) were positive for rubella IgG antibodies & one (0.83%) for rubella IgM antibodies

TABLE 8: Overall rubella IgG seropositivity

Author	**% of women positive for rubella IgG**
Al – rubaii B[1] et al	77.6%
Singla[26] et al	71.3%
Yadav[27] et al	55%
Raza S [71] et al	90.05%
Nessa A[72] et al	71.99%
Hasan ARSH[74] et al	89.1%
Ouhaiya[73] et al	68.6
Present study	31.66%

There is considerable variation in the prevalence of rubella antibodies among women of childbearing age. European women have relatively higher prevalence of rubella immunity (93.2%) as compared to women of African (86.7%) and Asian origin (78.4%)(R).In India the reported figures vary from 53% to 94.1%.[26] Our finding of 31.66% does not falls within this range and is much lower than those in other studies of Singla[26] et al(71.3%), Yadav[27] et al (55%), Raza S[71] et al(90.05%) conducted in various part of India & Nessa A[72] et al (71.99%),Ouhaiya[73] et al (68.6%)Al-rubaii[1] et al (77.6%),Hasan ARSH [74]et al(89.1%) from outside India.

The reason for this difference in immunity is difficult to explain. However, factors such as net birth rate, population density, opportunities for entry of virus, level of herd immunity at the time of virus introduction and socioeconomic factors of a given community may be responsible for this variation.[26]

In our study rubella IgM antibodies seropositivity is only found in one case (0.83%).Studies of Jubaida N[3] et al, Yasodhara P[75] et al, Chopra S[76] et al had reported 0 .75% , 6.5% & 17.5% seropositivity of rubella IgM antibodies respectively in pregnant women.

Of note, in our participants we do not know the source of anti-rubella IgG whether from natural infection or from previous vaccination during the childhood, because in our country, premarital or prenatal vaccination is not routinely done.

TABLE 9: Seropositivity in non-pregnant Women

Author	% of women positive for rubella IgG
Chandy[25] et al	87.5%
Singla [26] et al	76.9%
Yadav[25] et al	56.2%
Present study	42.2%

In our study 45(37.5%) were pregnant women among which 19(42.2%) were seropositive for rubella IgG antibodies.Studies conducted by Chandy[25] et al, Singla[26] et al , Yadav[27] et al had shown 76.9% ,87.5% ,56.2 % seropositivity respectively in non pregnant women

TABLE 10: Seropositivity in pregnant Women

Author	% of women positive for rubella IgG antibodies
Singla[26] et al	67.2%
Padmaja[28] et al	63.7%
Khare[33] et al	54%
Present study	25.3%

In present study 75(62.5%) women were pregnant out of which 19 (25.3%) showed positive results for rubella IgG antibodies. Singla[26] et al, Padmaja[28] et al, Khare[33] et al had reported 67.2%, 63.7%, 54% seropositivity respectively in their studies

In our study seroprevalence of rubella IgG antibodies in non pregnant women is 42.2% which is more than found in pregnant women 25.3% .Similarly in study conducted by Singla[26] et al it was 76.9% in non pregnant women which was more than found in pregnant women 67.2%. While the study conducted by Al-rubaii[1] in Iraq has reported higher prevalence in pregnant women (78.33%) than in non pregnant women (75.71%).

The reason for this difference is not clear, hence we are in need for further studies stressing on non-pregnant women, then to follow them in pregnancy.

TABLE 11: Seropositivity in different Age-groups

Age groups	Present study (%)	Singla[26] et al (%)	Nessa A[72] et al (%)
16-25	26.31.	69.2	80
26-35	40	77.2	82.3
>35	50	59.3	89.34

In our study in 16-25 year age group prevalence of rubella IgG antibodies was found to be 24.3% which gradually increased in the age group of 26-35year (42.85%) & is still more in 35 year (50%). Similar increasing trend in seropositivity as age increases is found in the study conducted by other authors[26,72] as shown in table no (11). We have not considered third age group for comparision as only four women were included from that group in our study.

However study conducted by Vijaylaxmi P[77] et al & Gupta E[4] et al had reported decreasing seroprevalence of rubella IgG antibodies as age increases.

In study of Vijaylaxmi P[77] et al from three eye hospitals in Tamil Nadu , 1000 female health personnel were tested for IgG rubella antibodies The seropositivity with respect to different age-groups was 18-19y: 87%, 20-24y: 85%, 25-29y: 83.6%, and 30-40y: 76.1%.

In a study conducted by Gupta E[4] et al from a tertiary care hospital in Delhi , The age-wise prevalence of anti-rubella IgG was: 15-19y: 95.2%; 20-24y: 89.5%; 25-30y: 87%, and > 31y: 77.5%.

The gradual increase in seroprevalence of rubella infection with age in our study indicates a continous exposure of population to rubella virus infection.

TABLE 12 : Seropositivity in relation to Previous Obstetric Performance

Previous Obstetric Performance	**Present study (%)**	**Singla [26] et al (%)**	**Jubaida[3] et al (%)**
Adverse pregnancy outcome	**40**	73.2%	86.84%
Normal pregnancy outcome	**29.1**	69.5%	80.65%

In our study the seropositivity of rubella IgG antibodies was more (40%) in women with history of previous adverse pregnancy as compared to women with normal obstetric performance before (29.1%) ,statistically the difference between these two group is insignificant which is similar to the study conducted by Singla[26] et al from Amritsar ,India & Jubaida[3] et al from Bangladesh as shown in table no.(12)
In the study conducted by Gandhoke[29] et al in Delhi over 15 years , 5022 samples from pregnant women were evaluated; the seroprevalence of rubella infection was higher in women with bad obstetric history (87%) compared to those with normal pregnancy outcome (83%).

Higher incidence of seropositivity observed in women presenting with adverse pregnancy outcomes in our study suggests that rubella could be a cause of repeated pregnancy wastage in these women.[26]

TABLE 13: Seropositivity in different socio economic status

Socio economic status	Present study	Singla[26] et al	Jubaida[3] et al	Turgut H[78] et al
Upper	30%	55.9%	72.5%	87.5%
Middle	19.09%	67.3%	89.02%	-
Lower	41.37%	71.8%	91.67%	80%

Considering socioeconomic status in our study, rubella seropositivity rates were found to be higher in women of lower socioeconomic class (41.37%) than in women of upper class (30.%). Similar trend were reported by Jubaida[3] et al, Singla[26] et al, as sown in table no.(13)

In contrast to these studies, study conducted by Turgut H[78] et al, Turkey had reported higher seroprevalence of rubella antibodies among women of higher socioeconomic status(87.5%) than in women of lower socioeconomic status (80%)
One of the probable reason for higher seroprevalence in lower socioeconomic status women in present study may be that in lower class population crowded living conditions might increase the chances of exposure to rubella infection.

TABLE 14 Seropositivity in relation to geographical Area

Geographical Area	Present study	Singla[26] et al	Mwambe B[79] et al	Bamgboye AE[80] et al	Hasan ARSH[74] et al	Ouyahia A[73] et al
Urban	21.7%	76.6%	90.6%	62%	90.4%	56.6%
Rural	34%	58.1%	94.5%	79.7%	84 .2%	43.4%

In our study the seropositivity of rubella IgG antibodies was more in those residing in rural areas (34%) as compared to those of urban areas (21.7%) . Statistically the difference was significant ($p<0.05$). Similarly in study conducted by Bamgboye AE[80] et al in Nigeria & Mwambe B[79] et al seroprevalence of rubella antibodies is higher in women of rural area in comparision to urban area though the differece in there study is insignificant between these two groups. However study conducted by Singla[26] et al, & other authors[73,74,79] have reported higher seroprevalence of rubella antibodies in urban women as shown in table no(14)

The possible explaination in our study of higher prevalence of rubella in rural women could be relatively poor hygienic enviornment in rural area, which might expose them more to rubella virus infection. Thereby developing more natural immunity in rural area compared to urban area.

None of the women included in this study gave history of immunization against Rubella. Similar observations have been made in study conducted by Chakravarti[81] et al & Singla[26] et al from New Delhi. This indicates that the need for immunization to control Rubella has not been duly recognized in India.

Currently, MMR vaccine is not a part of National Immunization Schedule in India. States with immunization coverage more than 80% administer second dose in routine immunization by MMR or measles vaccine. MMR was introduced in state immunization program of Delhi in 1999 as a single dose administered between 15-18 months of age (MMR-I). States of Punjab and Kerala, and Union territory of Chandigarh with high routine immunization coverage are possible candidates to incorporate MMR vaccine in their schedule besides Goa, Puducherry, Sikkim and Delhi which currently have this vaccine in their state immunization schedules. States with immunization coverage less than the above were advised catch up campaigns with measles vaccine.[70]

To our best knowledge, this is the first study in North Karnataka area to provide rubella sero-prevalence data among women of child bearing age. Our study clearly indicate that significant number of women are susceptible to rubella infection in this area which in turn can increase the incidence of CRS in children.

The incidence of CRS has been decreasing worldwide due to increasing coverage of rubella vaccination, but it remains a threatening and costly disease in regions where pregnant women are not immunized and do not have protective levels of IgG against rubella virus.

According to WHO policies, the primary goal of rubella vaccination is to prevent congenital rubella infection and CRS. One of the two approaches to the use of rubella-containing vaccines focuses exclusively on reducing CRS by immunizing adolescent girls or women of childbearing age, or both groups.

Since there is no treatment for an active infection during pregnancy, screening and immunization of women at risk is the mainstay of preventing CRS.

SUMMARY

- The study was conducted in Shri B. M Patil Medical College Hospital & Research Centre, Bijapur, north Karnataka, India.
- Out of 120 women of child bearing age 38 (31.66%) were seropositive for rubella IgG antibody & one (0.83%) was positive for IgM antibody.
- In our study 75 women were pregnant, among them 19 (25.3%) were seropositive for rubella IgG antibodies . Out of 45 non pregnant women 19(42.2%) showed positive results.
- 16 to 25 year age group include 76 women, among them 20(26.31%) were seropositive for rubella IgG antibody. Out of 40 women of age group of 26 -35 years 16(40 %) were seroposive. In age group of more than 35 years 4 women were tested out of which 2 (50%) were positive for rubella IgG antibodies.
- Out of 25 women with history of previous adverse pregnancy outcome, 10 (40%) were seropositive for rubella IgG antibodies. Among 72 women with normal obstetric performance before, 21 (29.1 %) were seropositive for rubella IgG antibodies.
- In present study 97 women belong to rural area among which 33 (34 %) were seropositive for rubella IgG antbodies and out of 23 women of urban area 5(21.7 %) were seropositive for rubella IgG antbodies.
- In our study 58 women were of low socioeconomic status, among them seropositivity for rubella IgG antibodies was found in 24 (41.37%) women. Out of 42 women of middle socioeconomic status 08 (19.04%) showed positive results. Out of 20 women of upper socioeconomic status, 6(30%) were seropositive for rubella IgG antibodies.

CONCLUSION

- It is evident from present study that seropositivity of rubella IgG antibodies in women of child bearing age is very low in our area which suggested that in this region substantial numbers of women reach childbearing age without acquiring natural immunity to Rubella .
- There is considerable variation in the prevalence of rubella antibodies among women of child-bearing age, depending on the socioeconomic strata and selection of study group. The prevalence of rubella immunity varies in different geographical area. Hence serosurveillance of women of childbearing age should be continued in different area of country & there is need to formulate an effective rubella immunization programme to prevent repeated pregnancy wastage and birth of infants with congenital rubella syndrome.
- In most of the study conducted in India, women were referred for rubella screening either due to BOH or possible infection during pregnancy. Therefore, seronegativity in these study is likely to be underreported than general population.
- There was insignificant effect of age and history of previous abortion on the seropositivity rate of IgG specific anti- rubella antibody.
- None of the girls included in this study gave history of immunization against rubella. This undoubtedly shows that, in India, the need for immunization to control rubella infection and congenital rubella syndrome has not yet been recognized.

In conclusion, because of the high rate of anti-rubella IgG seronegativity in our region, we do recommend following

1. Routine anti rubella IgG screening or rubella catch-up vaccination for all women of childbearing age who missed vaccine in childhood.
2. Adding second dose of rubella vaccine to those who have taken vaccine in childhood is must since the concentration of antibodies may drop below the recommended levels.
3. Encourage the health education for the public about the hazard of rubella, the importance of vaccination for prevention of this disease and other information regarding rubella.
4. For non immune women, vaccination at premarital visits, post abortion, post partum, or during any contact with the health care system with warning to avoid pregnancy for 4 week following vaccination will be very useful.

It should be noted that this is a preliminary regional level study & further nationwide surveys with large population sizes will be needed to determine the need for national immunization against rubella or screening of rubella infection among women of child bearing age.

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ANNEXURE-I

PROFORMA

Name	**:**	**IP No**	**:**
Age	**:**	**Case No**	**:**
Address	**:**	**Occupation**	**:**
DOA	**:**	**DOD**	**:**
Time of admission	**:**		
Chief complaints	**:**		

History of present pregnancy :

ANTENATAL HISTORY

Booked/unbooked :

IMMUNISED/UNIMMUNISED :

1st Trimester :

2nd Trimester :

3rd Trimester :

OBSTETRICS HISTORY :

Married Life :

Obstetric Score :

Details of previous pregnancies :

Menstrual History :

PaMC :

LMP :

EDD :

POG :

Past History :

Family History :

Personal History :

GENERAL PHYSICAL EXAMINATION

Build and Nourishment :

Height :

Weight :

Temp :

RR :

Breast :

Thyroid :

Spine :

Pallor / Icterus / Cyanosis / Clubbing / Edema / Lmphadenopathy.

SYSTEMIC EXAMINATION

CVS :

RS :

CNS :

PER ABDOMEN :

AG:

SFH:

EFW:

PER SPECULUM EXAMINATION :

PER VAGINAL EXAMINATION :

INVESTIGATIONS

Hb % :

Blood Grouping and Rh Typing :

Urine Routine :

RBS :

HBs Ag :

RVD :

USG

ANNEXURE-II

CONSENT FORM

TITLE: Seroprevalence of Rubella Antibodies in women of reproductive age.

Dr. Shilpi Gupta
P.G. in Microbiology

I have been informed about the research topic. I am ready to take part in the study and give the samples.

Signature

ನನಗೆ ಈ ಸಂಶೋಧನೆಯ ಬಗ್ಗೆ ಸಂಪೂರ್ಣ ಮಾಹಿತಿ ನೀಡಲಾಗಿದೆ. ನಾನು ಸ್ವಇಚ್ಛೆಯಿಂದ ಸ್ಯಾಂಪಲ್ ಕೊಡಲು ಸಿದ್ದವಾಗಿದ್ದೇನೆ.

ಸಹಿ

मुझे इस रिसर्च के बारे में समझाया गया है। मै अपने मन से स्यांपल देने के लिए तैयार हूं।

सही

ANNEXURE-III

ETHICAL CLEARANCE CERTIFICATE

Institutional Ethical Committee
BIJAPUR-586 103.
OUTWARD
No. 446/12
Date. 18/10/12
B.L.D.E.U's Shri B.M. Patil Medical College.

B.L.D.E. UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 18-10-2012 *at* 3-30pm *to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.*

Title "Seroprevalence of rubella antibodies in Women of reproductive age"

—x—x—

Name of P.G. student Dr Shilpi Gupta
Microbiology

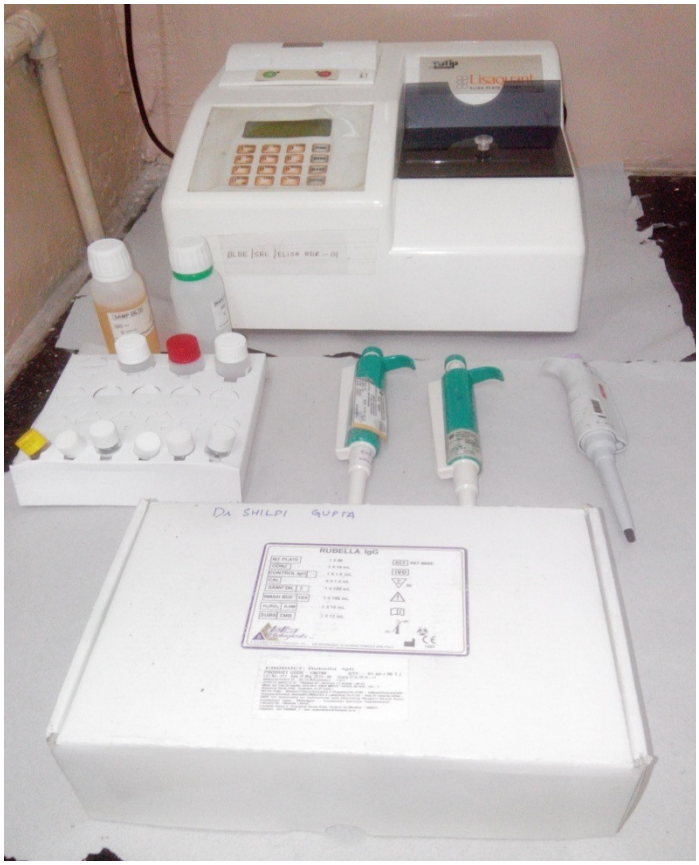
Name of Guide/Co-investigator Dr P.R. Shalapur
Prof. of Microbiology

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

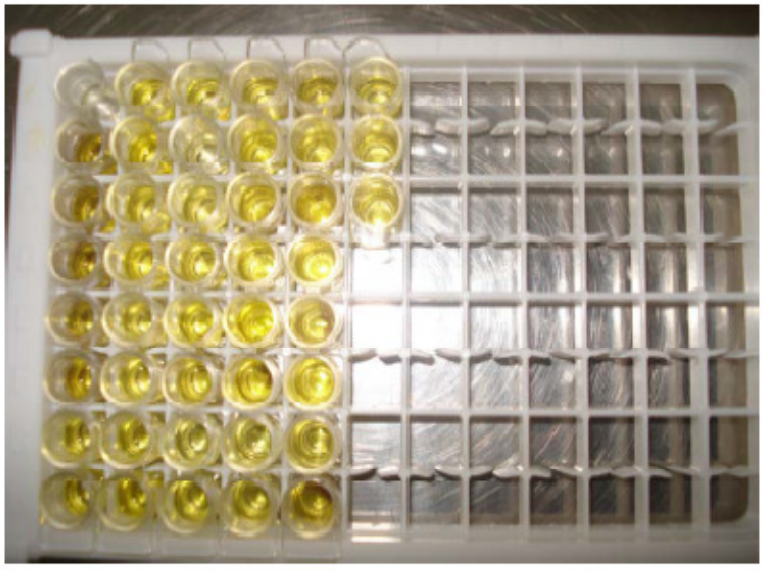
Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

ANNEXURE-IV

PHOTOGRAPHS



ELISA READER AND RUBELLA IgG KIT



ELISA TEST

MASTER CHART

Sl. No	NAME	AGE	Ip/Op No.	PREGNANT/NON PREGNANT	ADVERSE PREGNANCY OUT COME HISTORY	URBAN/ RURAL	SOCIOECONOMIC STATUS	VACCINATION TAKEN	IgG	IgM
1	Savita	30	30510	Non Pregnant	Present	Rural	Lower	No	Positive	Negative
2	Sujatha	22	293007	Non Pregnant	Present	Rural	Lower	No	Negative	Negative
3	Snehlatha	26	26704	Non Pregnant	Present	Rural	Lower	No	Positive	Negative
4	Vijaylaxmi	35	3663	Non Pregnant	Not present	Rural	Lower	No	Positive	Negative
5	sujatha	22	2708	Non Pregnant	-	Urban	Upper	No	Negative	Negative
6	Anitha	25	1648	Non Pregnant	-	Urban	Upper	No	Negative	Negative
7	Basamma	26	33888	Non Pregnant	Not present	Rural	Middle	No	Positive	Negative
8	Geetha	20	5299	Pregnant	Not present	Urban	Middle	No	Negative	Negative
9	Khatunbee	20	2671	Pregnant	Not present	Rural	Middle	No	Negative	Negative
10	Shobhawwa	28	1560	Pregnant	Not present	Rural	Middle	No	Positive	Negative
11	Ambika	20	2423	Pregnant	Present	Rural	Lower	No	Negative	Negative
12	Mebeerunbee	25	2422	Pregnant	Not present	Rural	Lower	No	Negative	Negative
13	Sujatha	22	293007	Non Pregnant	Present	Rural	Lower	No	Positive	Negative
14	Sonali	21	76048	Non Pregnant	-	Urban	Upper	No	Negative	Negative
15	Geetha	28	2794	Pregnant	Not present	Rural	Lower	No	Positive	Negative
16	Sarika	23	2529	Pregnant	Not present	Rural	Middle	No	Negative	Negative
17	Savitha	23	2491	Pregnant	Not present	Rural	Lower	No	Negative	Negative
18	Manjuda	20	2820	Pregnant	Not present	Rural	Middle	No	Negative	Negative
19	Geetha	25	2823	Pregnant	Not present	Rural	Middle	No	Negative	Negative

Sl. No	NAME	AGE	Ip/Op No.	PREGNANT/NON PREGNANT	ADVERSE PREGNANCY OUT COME HISTORY	URBAN/ RURAL	SOCIOECONOMIC STATUS	VACCINATION TAKEN	IgG	IgM
20	Suneetha	23	2607	Pregnant	Not present	Rural	Lower	No	Positive	Negative
21	Anjum	22	3139	Pregnant	Not present	Rural	Middle	No	Negative	Negative
22	Sumithra	28	28424	Non Pregnant	Present	Rural	Lower	No	Negative	Negative
23	Mahadevi	30	38436	Non Pregnant	Not present	Rural	Lower	No	Negative	Negative
24	Kamlabai	25	2933	Pregnant	Not present	Rural	Lower	No	Negative	Negative
25	Renuka	19	3069	Pregnant	Not present	Rural	Lower	No	Negative	Negative
26	Laxmi	24	35499	Pregnant	Not present	Rural	Lower	No	Negative	Negative
27	Roopa	30	3146	Pregnant	Not present	Rural	Lower	No	Positive	Negative
28	Vidya	24	3062	Pregnant	Not present	Rural	Lower	No	Negative	Negative
29	Shreedevi	22	3136	Pregnant	Not present	Rural	Middle	No	Positive	Negative
30	Deepa	23	2962	Pregnant	Not present	Rural	Lower	No	Positive	Negative
31	Kalawati	24	3161	Pregnant	Not present	Rural	Middle	No	Negative	Negative
32	Sushma	22	3019	Pregnant	Not present	Rural	Middle	No	Negative	Negative
33	Sumithra	25	3311	Non Pregnant	Present	Rural	Lower	No	Negative	Negative
34	Mahananda	25	6997	Non Pregnant	Present	Rural	Lower	No	Negative	Negative
35	Savitri	36	29693	Non Pregnant	Not present	Rural	Lower	No	Positive	Negative
36	Sumangla	22	58321	Non Pregnant	-	Urban	Upper	No	Positive	Negative
37	Parvathi	20	3431	Non Pregnant	Not present	Rural	Middle	No	Negative	Negative
38	Himani	19	23618	Non Pregnant	-	Rural	Middle	No	Positive	Negative
39	Jyothi	16	4371	Non Pregnant	-	Rural	Middle	No	Positive	Negative
40	Savishi	30	28571	Non Pregnant	Not present	Rural	Lower	No	Negative	Negative

Sl. No	NAME	AGE	Ip/Op No.	PREGNANT/NON PREGNANT	ADVERSE PREGNANCY OUT COME HISTORY	URBAN/ RURAL	SOCIOECONOMIC STATUS	VACCINATION TAKEN	IgG	IgM
41	Nritya	19	28575	Non Pregnant	-	Rural	Middle	No	Negative	Negative
42	Savita	20	24249	Non Pregnant	-	Rural	Middle	No	Negative	Negative
43	Mallika	25	28576	Non Pregnant	Not present	Rural	Lower	No	Positive	Negative
44	Suneetha	23	7465	Pregnant	Not present	Rural	Lower	No	Negative	Negative
45	Shridevi	20	7592	Pregnant	Present	Rural	Lower	No	Negative	Negative
46	Vijaylaxmi	20	7193	Pregnant	Not present	Rural	Lower	No	Negative	Negative
47	Rekha	29	6982	Pregnant	Not present	Rural	Middle	No	Negative	Negative
48	Jaheera	25	7352	Pregnant	Not present	Rural	Middle	No	Negative	Negative
49	Deepa	22	6921	Pregnant	Present	Rural	Lower	No	Negative	Negative
50	Rekha	19	7092	Pregnant	Not present	Rural	Middle	No	Negative	Negative
51	Renuka	26	46802	Non Pregnant	-	Urban	Upper	No	Negative	Negative
52	Bharti	31	62194	Non Pregnant	-	Urban	Upper	No	Negative	Negative
53	Renuka	31	51124	Non Pregnant	Not present	Urban	Upper	No	Positive	Negative
54	Sangeetha	30	67704	Non Pregnant	-	Urban	Upper	No	Negative	Negative
55	Fatima	30	6229	Non Pregnant	-	Urban	Upper	No	Negative	Negative
56	Sunita	25	51122	Non Pregnant	Present	Urban	Middle	No	Negative	Negative
57	Sangeetha	29	53264	Non Pregnant	Present	Urban	Middle	No	Negative	Negative
58	Akshata	32	5477	Non Pregnant	-	Urban	Middle	No	Negative	Negative
59	Borramma	22	10946	Pregnant	Not present	Rural	Lower	No	Positive	Negative
60	Padmavati	20	11009	Pregnant	Not present	Rural	Lower	No	Positive	Negative
61	Bharti	20	11462	Pregnant	Not present	Rural	Lower	No	Positive	Negative

Sl. No	NAME	AGE	Ip/Op No.	PREGNANT/NON PREGNANT	ADVERSE PREGNANCY OUT COME HISTORY	URBAN/ RURAL	SOCIOECONOMIC STATUS	VACCINATION TAKEN	IgG	IgM
62	Pushpa	25	11589	Pregnant	Present	Rural	Middle	No	Negative	Negative
63	Sujatha	24	11533	Pregnant	Not present	Rural	Middle	No	Negative	Negative
64	Savitha	24	11586	Pregnant	Not present	Rural	Middle	No	Positive	Negative
65	Sheetal	32	11570	Pregnant	Not present	Rural	Middle	No	Positive	Negative
66	Damakka	30	11314	Pregnant	Not present	Rural	Lower	No	Negative	Negative
67	Surekha	28	11087	Pregnant	Not present	Rural	Middle	No	Negative	Negative
68	Parvathi	24	11026	Pregnant	Not present	Rural	Lower	No	Negative	Negative
69	Netra	24	10795	Pregnant	Not present	Rural	Middle	No	Negative	Negative
70	Rohini	23	10709	Pregnant	Not present	Rural	Middle	No	Negative	Negative
71	Vijaylaxmi	26	11712	Pregnant	Not present	Rural	Lower	No	Negative	Negative
72	Bhagyashree	26	1391	Non Pregnant	Not present	Urban	Upper	No	Negative	Negative
73	kiran	26	7572	Non Pregnant	-	Urban	Upper	No	Positive	Negative
74	Rudramma	20	14658	Pregnant	Not present	Rural	Middle	No	Negative	Negative
75	Kadambri	22	14995	Pregnant	Not present	Rural	Lower	No	Positive	Negative
76	Laxmi	22	14922	Pregnant	Not present	Rural	Lower	No	Negative	Negative
77	Umashree	25	14524	Pregnant	Not present	Rural	Middle	No	Negative	Negative
78	Sunanda	24	14789	Pregnant	Present	Rural	Middle	No	Negative	Negative
79	Jayshree	26	14877	Pregnant	Present	Rural	Lower	No	Negative	Negative
80	Naseena	19	146880	Pregnant	Not present	Rural	Middle	No	Negative	Negative
81	Bhagyashree	22	14499	Pregnant	Not present	Rural	Middle	No	Negative	Negative
82	Renuka	20	14510	Pregnant	Not present	Rural	Middle	No	Negative	Negative

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83	Vaishali	22	14773	Pregnant	Not present	Rural	Middle	No	Negative	Negative
84	Rudramma	38	14085	Pregnant	Present	Rural	Lower	No	Positive	Negative
85	Lalita	24	15102	Pregnant	Not present	Rural	Lower	No	Negative	Negative
86	Anitha	25	14915	Pregnant	Present	Rural	Lower	No	Negative	Negative
87	Naseena	20	15025	Pregnant	Not present	Rural	Middle	No	Negative	Negative
88	Vidya	26	18145	Non Pregnant	Present	Rural	Lower	No	Positive	Negative
89	Kamlamma	27	14882	Non Pregnant	Not present	Rural	Lower	No	Positive	Negative
90	Jayshree	21	202934	Non Pregnant	Present	Rural	Lower	No	Positive	Positive
91	Sujata	26	10223	Pregnant	Not present	Rural	Lower	No	Positive	Negative
92	Renuka	21	8184	Pregnant	Not present	Rural	Lower	No	Negative	Negative
93	Jakawwa	18	4391	Pregnant	Not present	Rural	Lower	No	Positive	Negative
94	Umashree	20	10941	Pregnant	Not present	Rural	Middle	No	Negative	Negative
95	Shridevi	21	10330	Pregnant	Not present	Rural	Lower	No	Negative	Negative
96	Kasturibai	30	10978	Pregnant	Not present	Rural	Lower	No	Negative	Negative
97	Shilpa	36	12407	Pregnant	Not present	Rural	Middle	No	Negative	Negative
98	Muskan	28	12404	Pregnant	Not present	Rural	Middle	No	Negative	Negative
99	Savita	22	12466	Pregnant	Not present	Rural	Middle	No	Negative	Negative
100	Shridevi	27	12983	Pregnant	Not present	Rural	Lower	No	Negative	Negative
101	Mangla	22	12983	Pregnant	Not present	Rural	Lower	No	Negative	Negative
102	Enabichavan	23	13645	Pregnant	Not present	Rural	Lower	No	Negative	Negative
103	Drakshayan	32	206566	Pregnant	Not present	Rural	Lower	No	Negative	Negative

Sl. No	NAME	AGE	Ip/Op No.	PREGNANT/NON PREGNANT	ADVERSE PREGNANCY OUT COME HISTORY	URBAN/ RURAL	SOCIOECONOMIC STATUS	VACCINATION TAKEN	IgG	IgM
104	Mahadevi	22	10544	Pregnant	Present	Rural	Lower	No	Positive	Negative
105	Basamma	26	17383	Pregnant	Present	Rural	Lower	No	Negative	Negative
106	Shwetha	28	18187	Pregnant	Present	Rural	Lower	No	Positive	Negative
107	Muniba	25	17179	Pregnant	Present	Rural	Lower	No	Positive	Negative
108	Sulochna	37	12132	Pregnant	Present	Rural	Lower	No	Negative	Negative
109	Jaydevi	24	1263	Pregnant	Present	Rural	Lower	No	Positive	Negative
110	jayshree	30	18859	Pregnant	Not present	Rural	Lower	No	Negative	Negative
111	Priyanka	26	3907	Non Pregnant	-	Urban	Upper	No	Positive	Negative
112	Saumya	19	2761	Non Pregnant	Not present	Rural	Middle	No	Positive	Negative
113	Laxmibai	22	3137	Non Pregnant	-	Rural	Upper	No	Positive	Negative
114	Vijaylaxmi	20	3069	Non Pregnant	-	Urban	Upper	No	Negative	Negative
115	Jyothi	18	4371	Non Pregnant	-	Urban	Upper	No	Negative	Negative
116	veena	24	59131	Non Pregnant	-	Urban	Upper	No	Negative	Negative
117	Hema	29	2158	Non Pregnant	-	Urban	Upper	No	Positive	Negative
118	Roopa	29	22064	Non Pregnant	-	Urban	Upper	No	Negative	Negative
119	Renuka	29	441	Non Pregnant	-	Urban	Upper	No	Negative	Negative
120	Jayshree	27	314	Non Pregnant	-	Urban	Upper	No	Negative	Negative