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Original Article

An Investigation on Antirubella Immunoglobulin Titeres and the Level of Protectivity Among 5 Major Highly Populated Cities of Gilan Province

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ABSTRACT

Objective: Rubella is a viral disease with minor morbidity, unless it is contracted by a pregnant woman in early pregnancy that leads to congenital Rubella syndromes. Rubella occurrence during the first trimester leads to fetal death or severe congenital defects. Present study was conducted to survey the protection level against Rubella in the pregnant women of Gilan in IRAN, to be used for the vaccination and prevention programs. **Methods:** This investigation was carried out in six cities of the province, 97 pregnant women aged between 17-38 years old serum samples were taken, Serum IgG and IgM levels were measured by inhibitory competitive ELISA. All cases had a protective level of anti-rubella IgG due to <5 IU/ml (negative), >10 (positive) determination, whereas two women had an equivocal level of IgM referred to <0.9 IU/ml (negative) and >1.1 (positive) calculations. **Results:** According to the obtained results 2.06% of women were suspected to active Rubella infection. Significant correlations were found between seroprevalence of rubella IgG and geographical residency and seasonal fluctuations. **Discussion:** Our findings indicated that population general immunity exists nowadays, but this immunity level might not only be a result of vaccination, we strongly recommend routine antirubella IgG screening for women at the age of childbearing before marriage or conception as well as vaccinating non immune women.

1. INTRODUCTION

Rubella infection is caused by an RNA virus (Chiang Lin et al., 2010) a biological teratogen of the TORCH complex (Agbede et al., 2011). The etiologic agent rubella virus (RV) is an enveloped, positive-sense, ssRNA virus in the genus *Rubivirus* of the family *Togaviridae* (Obijimi et al., 2013). Rubella is an exanthematous illness characterized by nonspecific signs and symptoms including transient erythematous and sometimes pruritic rash, postauricular or suboccipital lymphadenopathy, arthralgia, and low grade fever (Namaei et al., 2008). Within a few days after naturally

acquired rubella infection, RV-IgM usually peaked then decreased sharply (levels divided by 2 every 3 weeks) while RV-IgG was generally detected within 2 to 3 weeks after the onset of infection, in contrast, after vaccination, RV-IgM peaked at a lower level and could always be detected for at least 3 months after vaccination. RV-IgM is detected longer after vaccination than after natural infection even the level of RV-IgG was usually higher after primary infection than after rubella vaccination and, in most cases, remained significantly higher. Additionally, RV-IgG levels are higher after natural infection (Vauloup-Fellous et al., 1982). Therefore, only rubella-specific IgM is used

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to diagnose congenital rubella infection in infants with congenital rubella syndrome (CRS), rubella-specific IgM can be detected in nearly 100% at the age of 0-5 months, about 60% at age 6-12 months, and 40% at the age 12-18 months, IgM is rarely detected after the age of 18 months (Chantler *et al.*,1982). Accurate diagnosis of acute primary rubella infection in pregnancy is imperative and requires serologic testing, since an important number of cases are subclinical. Serology by Enzyme-linked immunosorbent assay (ELISA) to measure rubella-specific IgG and IgM is convenient, sensitive, and accurate. With respect to sensitivity, specificity, reproducibility, and ease of performance, ELISA has distinct advantages over existing techniques to determine antibody titres to different aetiological viral agents including rubella virus (van Loon *et al.*,1982). According to the World Health Organization (WHO), at least 236,000 CRS cases occur in every non-epidemic year in developing countries, and this increase by up to 10 fold during epidemic years. The CRS cases are rarely reported in these countries, and the extent of the problem remains unknown. The precise prevalence of CRS in Iran is not known, but it was measured indirectly and estimated to be 0.2/1000 live births before rubella vaccination. According to the Expanded Immunization Program, in Iran all 12 and 18 month old infants are immunized with MMR vaccine, but in response to the increased numbers of cases in older age groups during 1996–2002, a nationwide MR vaccination campaign was conducted in December 2003, and 33.5 million persons (99%) aged 5 to 25 years were vaccinated (Honarvar *et al.*,2013). Due to effective vaccination programs, rubella and CRS have become rare diseases in many industrialized countries. CRS remains a major cause of developmental anomalies in many developing countries where rubella vaccines have not yet been introduced. It is estimated that more than 100 000 infants are born with CRS each year, mostly in developing countries (Adam *et al.*,2013).

2. MATERIALS AND METHODS

The study was conducted in Gilan province, the North of Iran. The state is comprised of 6 cities and regions: *Rasht*, *Manjil*, *Roudbar*, *Lowshan*, *Tarom* and *Rostamabad*. This cross-sectional study was conducted from *December 2012* to *september 2013*. The study samples were 97 pregnant women aged 17-38 years old who were resident in the province of Gilan. Having obtained their informed consent, a blood sample was taken from each woman to assay the anti-rubella antibodies IgG and IgM. Five mL of blood were collected in plain tubes allowed to clot and centrifugated at room temperature. Then sera were stored at -20°C and furthermore transported to laboratory in dry ice for analyses. Enzyme-linked

immunosorbent assay (ELISA) was used for rubella (IgG and IgM) using commercial diagnostic kits (ACON, USA, GENESIS, England). IgG test results were interpreted as a ratio of the sample optical density (OD) of 450 nm and the sample rate/cut-off value as follows: $< 5\text{U/ml}$ = negative, > 10 = positive. IgM test results were interpreted as similarly as follows: $< 0.9\text{U/ml}$ = negative and > 1.1 = positive. Data entry and analysis were carried out with the SPSS 19 and Microsoft Excel 2007 software. Data was statistically tested using the Chi-square and the T-test. Results were considered to be statistically significant where $p < 0.05$.

3. RESULTS

Ninety-seven women that referred to medical laboratories, participated in this study. Their average age was 25.1 years, 90 women (91.4%) were 31 years old or younger (Fig. 1).

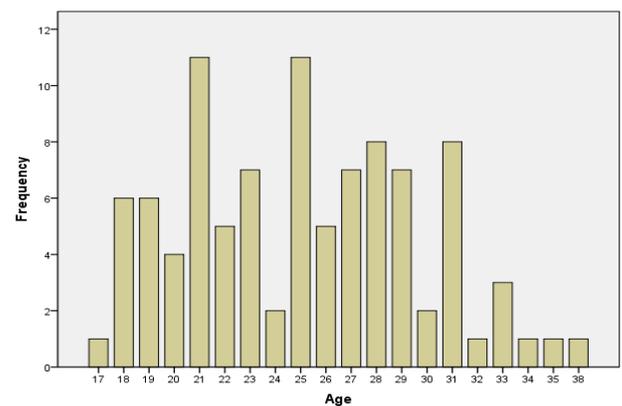


Figure 1. Graph depicting frequency of age in pregnant women surveyed.

All pregnant women had a positive history of MR vaccination, 36 women (37.1%) resided in Rasht city, 29 women (29.9%) in Manjil, 17 (17.5%) women in Roudbar, 7 women (7.1%) in Lowshan, 4 women (4.1%) in Tarom and 4 women (4.1%) in Rostamabad cities (Fig. 2).

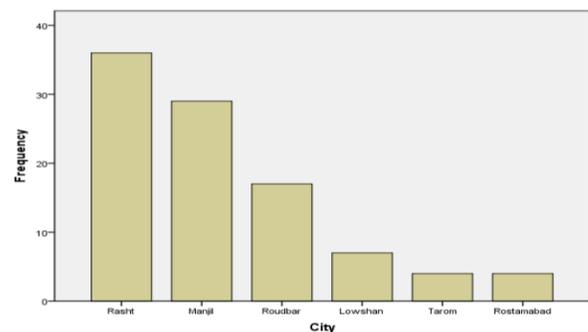


Figure 2. graph depicting frequency of women in Cities surveyed.

At all ,91 women had a history of abortion in previous pregnancies 65% cases had referred in spring ,26 in summer and 6 cases in winter 2012-2013.The mean concentration of anti-rubella IgG and anti-rubella IgM was 34. 88 IU/mL , 1.02 IU/mL respectively (Fig. 3,4).

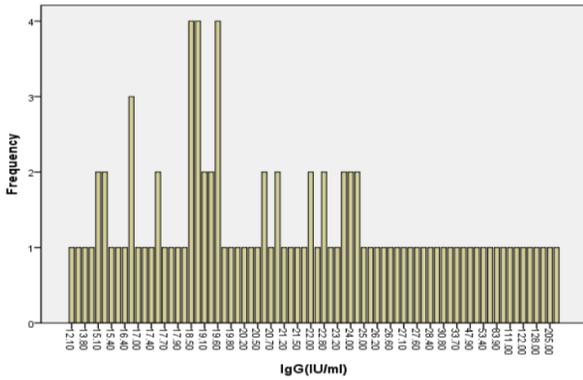


Figure 3 . graph depicting variations of IgG antibody level.

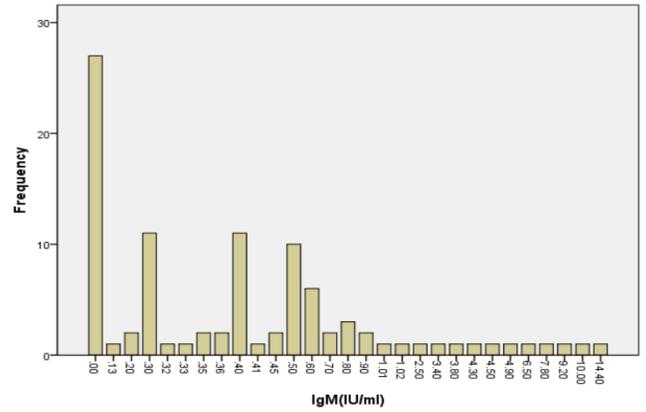


Figure 4. graph depicting variations of IgM antibody level.

All of the cases had a protective level of anti-rubella *IgG* due to vaccinations which conducted in final cut off determination and calculations , Whereas 2 women had an equivocal level of immunoglobulin M . According to the obtained results 2 persons with a borderline titrations, 2.06 % of women were suspected to active *Rubella* infection.(Table 1,2).

Table 1.

comparision IgG Antibody levels in cities surveyed

City	IgG Antibody levels						Total
	50.1-100	150.1-200	15-50	100.1-150	200>	15<	
Rasht	3	1	26	4	1	1	36
Manjil	0	0	28	0	0	1	29
Roudbar	1	0	16	0	0	0	17
Lowshan	1	0	6	0	0	0	7
Tarom	0	0	2	1	0	1	4
Rostamabad	0	0	2	0	1	1	4
Total	5	1	80	5	2	4	97

Table. 2.

comparision IgM Antibody levels in cities surveyed.

City	IgM Antibody levels						Total
	9.1-12	6.1-9	3.1-6	1-3	>12	<1	
Rasht	2	2	4	2	0	26	36
Manjil	0	0	0	1	1	27	29
Roudbar	0	0	0	0	0	17	17
Lowshan	0	0	0	0	0	7	7
Tarom	0	0	1	0	0	3	4
Rostamabad	0	0	0	0	0	4	4
Total	2	2	5	3	1	84	97

There were not significant correlations in seroprevalence of anti-rubella *IgG* and *IgM* in this group . Significant correlations were found between seroprevalence of rubella *IgG* and geographical residency Rasht , Rostamabad , Tarom , Roudbar , Lowshan and Manjil cities and also between seroprevalence of *rubella IgG* and the , spring , summer and Winter seasons.

4-DISCUSSION

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 for Iran as a member of WHO , the prevention and control of measles and rubella is a high priority (Esteghamati *et al.*,2007) .It is believed that based on several studies conducted during 1968 - 2002 in Iran showed that the immunity level against rubella was from 69.9% to 97% among women aged 15 to 45 years (Mahmoodi *et al.* ,2007) that our study showed increased immunity level against rubella and also shows the efficacy of the vaccination program. Earlier findings

had indicated that 61.9% of the target population was immune and 38.1% were susceptible to rubella before vaccination. After the 2003 MR mass vaccination campaign, 98% of the susceptible group acquired immunity and 2% of them did not acquire immunity to rubella (Hamkar *et al.*,2006) . Another survey showed that after the vaccination campaign , rubella immunity reached 91.0% , 99.6%, 99.6% and 97.0% respectively for the 6–10 , 1–15 , 16–20 , and 20–26 year old aged groups . Seropositivity for the rubella virus in the target population was high, especially in women of childbearing age (98.9%) , thereby preventing congenital rubella infections (Pourabbas *et al.*,2008). The antibody response to rubella after MMR vaccination was more than 90% in most studies (Shamsizadeh *et al.*,2012) as our study showed . In other studies performed by Raut *et al* in 2005 in india , Antibody levels in 41 Indian girls were measured 6 years after measles-mumps-rubella (MMR) vaccination. Rates of seropositivity were 88% (measles antibodies) , 95% (mumps antibodies) , and 100% (rubella antibodies) (Raut *et al.*,2007) . even by Halperin *et al* in 2009 in Canada , Before administration of study vaccines , seropositivity rates were 96.4% for measles , 94.3% for mumps , 99.5% for rubella , and 97.9% for varicella .Post-immunization , seropositivity rates were 99.5% for

measles and mumps and 100% for rubella and varicella in the MMR+varicella group (Halperin et al.,2009). the result of studies from Spain and Turkey was between 95-98% that close to our study. By August 2006 ,117 countries had implemented rubella vaccines as part of their routine national vaccination programs (World Health Organization.,2004,2009). Therefore , rubella infection remains endemic in many countries in Asia , and immigrants from these countries pose a new challenge in the global effort to decrease rubella (Chiang Lin et al.,2010). In 2005 , however ,a nationwide epidemic began , 520 rubella cases were reported. In 2007 , the epidemic peaked with 11,345 cases reported .Following the 2008–2009 MR vaccination campaign described , only 14 cases of rubella were reported in 2009 , and by December 2010, only 15 confirmed cases of rubella have been reported (El Sayed et al.,2011).

In Algeria the national prevalence of rubella infection is unknown ,and there is no surveillance system for CRS . Currently , the vaccination against rubella is not integrated into the Algerian National Immunization Program . Data acquired showed that one in three of the women aged between 15 and 49 years is susceptible to rubella infection. There is a need to protect this set of people considering the potential dangers associated with rubella infection (Ouyahia et al.,2013) .in Sudan , Rubella vaccine is not included in the Sudanese national immunization program , and data on the prevalence of rubella are inadequate. Furthermore, there is no routine surveillance for CRS , and data on its incidence are extremely scarce (Adam et al.,2013).In Pakistan , little data is available regarding the prevalence of congenital rubella infection and cost of MMR vaccine is relatively high , Thus majority of women of childbearing age remain unvaccinated and susceptible to rubella infection , in contrast in a study in Pakistan , findings shows that out of 100 Infants , only three (3%) met the definition for confirmed rubella whereas thirty-four patients were positive for Rubella IgG only . The relatively high rate of susceptibility indicated a risk of a rubella outbreak , and the results (Hussein et al.,2006).In our study, we investigated the seroprevalence of rubella antibodies in Gilan province , our findings indicated that general immunity exists nowadays at a rate of 97.7% , but looks that this immunity level is not only the result of vaccination , whereas by the pass of time,immunity level will probably decrease ,we recommend routine antirubella IgG screening for women of childbearing age before marriage or conception or to vaccinate non immune women.

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