Congenital rubella infection in neonatal cord blood samples of newborns in hospitals affiliated to Tehran University of Medical Sciences

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Introduction:
Rubella is a disease caused by the rubella virus and it is usually mild and self-limiting. Infection of a developing fetus is serious and important because the child may be born with congenital rubella syndrome. Its symptoms include mental retardation, heart defects, cataract, etc.
In 2003, mass vaccination against measles and rubella in individuals 5-25 years old was done. One of the main objectives of this study was to survey congenital rubella infection status with the presence of IgM antibodies against rubella virus in cord blood samples and also the immunity assessment of maternal IgG antibodies against rubella virus in the above samples.

Methods:
The cross-sectional study was to determine the transfer of congenital rubella in 358 cord blood samples collected in hospitals affiliated to the Tehran University of Medical
Sciences that was done in 2008-2009

The collected samples were analyzed by two ELISA methods for detection of IgG and IgM antibodies, RT-Nested PCR tests was applied on samples of IgG–negative and IgM-positive and also some of randomly IgG-positive samples for identifying the presence of the virus genome.

In this study two groups of mothers were tested, one consisted above 29 years of age (at the time of vaccination) with the frequency of 73.4% and the other one below 29 years of age with the frequency of 26.6%.

Results:
Of the 358 samples, 91.1% IgG and 2.8% were found to be positive. None of the 31 samples were positive according to the presence of the virus genome via the method of RT-Nested PCR.

Conclusion:
According to high immunity of mothers, the probability of congenital rubella transmission was low, but because of low immunity of mothers of >29 years of age, it is much better to upgrade the age of vaccination to 28 years old.

Keyword: CRS,Cord blood,Elisa, PCR

Introduction:
Rubella is a viral disease which is coused by a member of Rubivirus genus of the Togavirus family. Rubella virus etiologic agent is gezantomy mild disease with low fever, lymphadenopathy and short term of morbiliform rash. Rubella virus is the cause of congenital rubella syndrome and congenital abnormalities when infection occurs during pregnancy for the fetus.The most common complications of congenital rubella syndrome are as follows:
1-Transient conclusions include low birth weight, thrombocytopenia, and hepatosplenomegaly, jaundice, pneumonia, and bone lesions, menengoencephalitis (stable or complications).
2-Permanent conclusions including cataract, retinopathy, deafness, glaucoma, and microcephaly.
3-Developmental conclusions which includ diabetes mellitus, mental backwardness, and disorder and sympathy.
Multiple complications due to congenital rubella syndrome will impose heavy economical, emotional or social burden on families and societies. The importance of the matter will be more significant when percentage of disabled babies born whose complications are heavy and irreparable. But this problem can be resolved by the exact evaluation of women's immunity in childbearing age, therefore, the risk of exposure to congenital rubella syndrome can be assessed and non-immune (equivocal) women will be protected from CRS via vaccine-injection.
Diagnosis of CRS cannot be based on symptoms in a newborn baby. This operation requires virus isolation or serological testing in an infant. In countries where vaccination is not done or in the early stages, consideration of CRS is very important. Diagnosis of rubella after birth and congenital infection rubella specific IgM by studying via EIA are carried out with commercial kits. Considering that these antibodies do not have the ability to cross the placenta, and fetus is capable of producing these antibodies therefore these antibodies indicate presence of fetal intrauterine infection. IgM is usually recognizable in congenital infection for one year. In countries where vaccination is carried out for long term, positive-IgM rate is decreased and risk of false positive is increased.

Methods of study
The descriptive / cross-sectional study was done on 358 collected cord blood samples of newborn babies in hospitals affiliated to Iran University of Medical Sciences in 2004-2005. The necessary information for the specifications of mothers and neonates was filled out by the questionnaire.
The cord blood samples were collected in sterilized tube and immediately after blood coagulation, their serum was isolated by centrifugation. All samples were kept in the fridge at -20 C^0 until used.

ELISA test: ELISA kit (VIR-ELISA) made in Germany was used for qualitative and quantitative analysis of rubella virus specific IgM and IgG in serum and plasma samples.
Extraction of RNA: RNA extraction kit using the High Pure Viral Nucleic Acid kit (Roche in Germany) was used in accordance with manufacturer instructions.

Synthesis of cDNA: cDNA synthesis was performed using RT mix from Construction Company Bioneer. In this method, 5 micro liters of RNA extracted was added to the tube and then heated for an hour at 42 ° and followed by heat at 70^0 for 5 min to inactivate RT.
Nested PCR test for diagnosis of rubella:
The test consisted of two phases PCR (PCR1, PCR2). Using two primers, R2 and R7 to R11 and R8C for PCR1 and for PCR2. Primers R2 and R7 outer primers and 185 bp region of the genome were used Rubella E1 part too .(Table1)

Table1: Primers used in Nested PCR Reaction

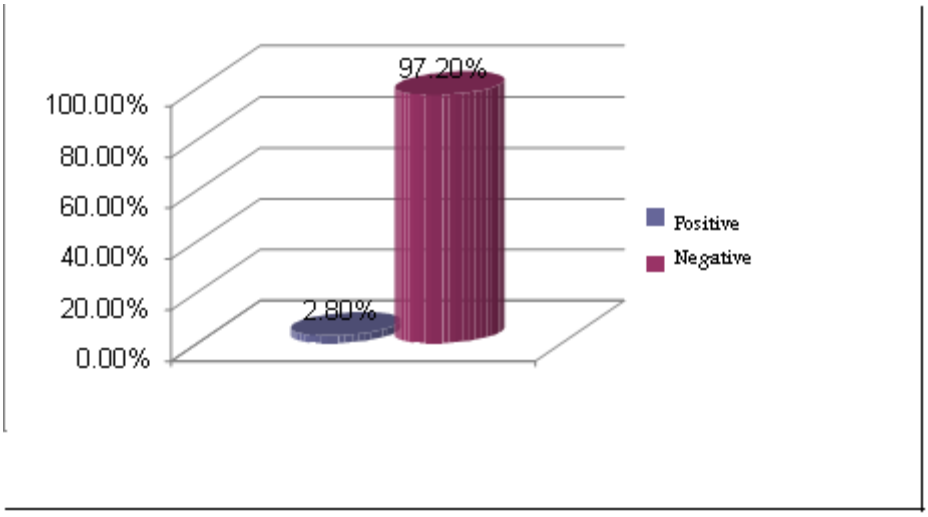
Primers used in PCR1		
Name	***Forward***	***Reverse***
R2/R7	CAA CAC GCC GCA CGG ACA AC	CCA CAA GCC GCG AGC AGT CA
	Primers used in PCR2	
R11/R8C	CTC GAG GTC CAG GTC CYG CC	GAA TGG CGT TGG CAA ACC GG

Reaction PCR2 and PCR1: First cycle 3 minutes at 95 °c, followed by 40 cycles of 95 ° 30 seconds, 60 degrees 30 seconds and 72 degrees 60 seconds, reaction was performed finally at 72 degrees 5 minutes. PCR2 similar program was applied the first step with the difference that the number of cycles from 40 cycles to 25 cycles decreased. PCR products were analyzed using 1.5% agarose electrophoresis and Ethidium bromide was observed under the final test results using SPSS-16 software and test and Pearson Chi-square analysis were compared.

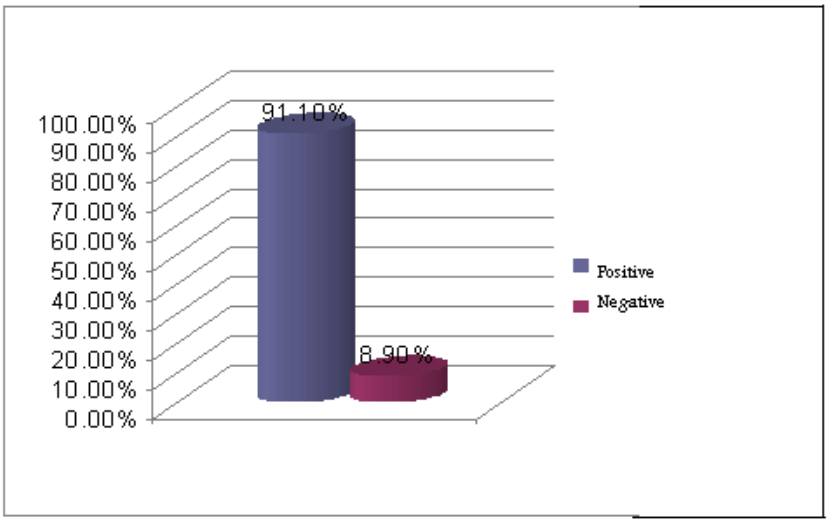
Results:

Presence of specific IgM antibodies of rubella in cord blood

A total of 358 case studies, 348 were found to lack IgM antibodies against rubella virus and only 2.8% were positive (Graph no.1).



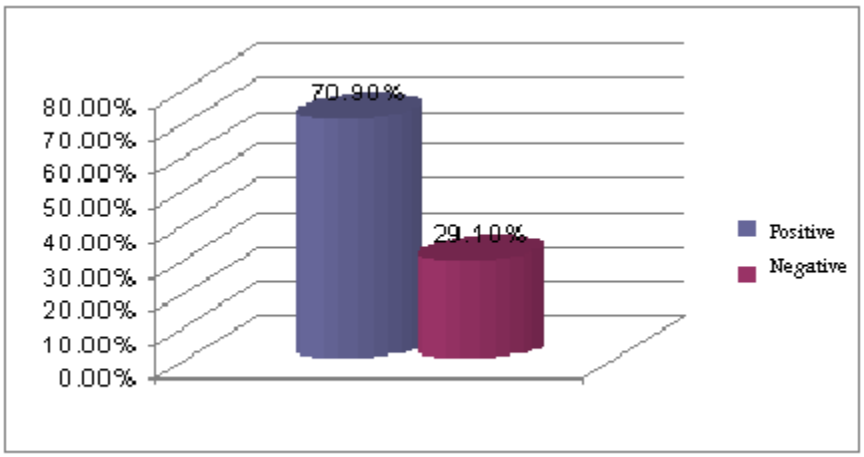
Graph no.1: frequency percentage of the results of IgM antibodies presence against rubella virus.



Graph Number2: frequency percentage of the results of IgG antibodies against rubella virus.

Comparison the results of rubella specific IgG and mother's with differed ages at delivery time

In this study, mothers are divided into two age groups:
The first one in a mass vaccination at the age of vaccination were delivery have less than 29 years old in 2003 and the other older than 29 years who did not receive vaccine at that time. Of 358 mothers, 254 (70.9%) were less than 29 years old and 104 (29.1%) more than 29 years old. By doing the statistical chi-square test of, there was a significant connection between mother age and being a negative rubella specific IgG(Chart 3)..



Graph Number2: frequency percentage of the results of maternal age at delivery

From the number of 358 newborn babies, 190 (53.1%) were Male and 168 (46.9%) were Female. There was no significant relation for the gender.

Analysis of RT-Nested PCR

The samples that were negative in regard to IgG (16 samples) and also positive IgM samples (5 samples) and some of randomly positive IgG samples (10 samples which is statistically important) and were analyzed for the presence of virus genome via RT-Nested PCR.
Also the sample of attenuated rubella vaccine samples was considered as a positive control.
From the 31 samples, none of them were positive according to the presence of genome(Figure 1).

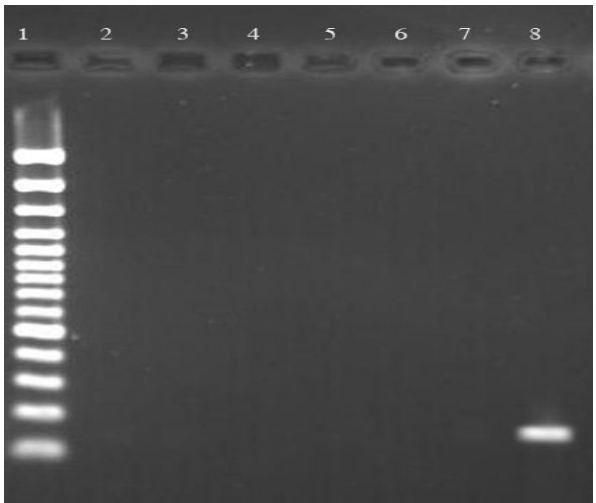


Figure 1: RT-Nested PCR results

1: Ladder 100 bp, 2-6: Test,7: Neagtive control, 8: Positive control

Discussion:

Rubella epidemic cycle is one of 6- to 9-year intervals. In adults, the highest rate of exposure to this disease has been reported for women with congenital rubella.
In fact, 80% of pregnant women in the United States are safe against rubella and it is estimated that about 12500000 rubella cases occur over there.
Congenital rubella has occurred in 30000 pregnancy cases with 10000 fetal deaths or abortion treatment and 20,000 CRS cases. This rate put 2 billion dollars financial costs on US government shoulders. According to the report of WHO, at least 236000 CRS has been seen in developing countries in non epidemic years and this range will be increased in least ten folds in epidemic ones. But there are limited reports of CRS in these countries. In addition, lacks of vaccination planning and epidemiological information are factors which can result in increasing exposure to rubella and CRS.
In 2005, 32 million doses of vaccine was applied to people of 5-25 years old against measles and rubella in Iran. Diagnosis of rubella infection based on clinical observation can determine less then 50% of cases. In addition presence of IgG antibodies against rubella virus is not always indicative of epidemiological status of rubella infection. One of the objectives of this study was to survey congenital rubella infection in collected cord blood samples via ELISA method for identifying IgM antibodies against rubella virus.

The results of IgM ELISA tests were positive. The results of a study done in Pasteur Institute with ELISA method in 1992-1997 identified presence of specific IgM antibodies to rubella in 2.5% of pregnant women. Also, in this study about 94% of pregnant women had IgG antibodies via HI method. The results of this study are congruent with our ELISA test results. Another study was done in Bandar Abbas province in women of fertility ages in 2003 which 1% of the subjects had IgG Ab. Another purpose of this study was to survey the immunity-protective status of pregnant mothers and identifying specific IgG antibodies of rubella in cord blood samples of newborn babies which would show their immunity status. From 358 studies cases, 326 cases (91.1%) and 32 ones (8.9%) are respectively positive and negative. These results are confirmed

the results obtained in other studies that the range of immune protective of mothers has been reported to be 50-96.4%.
Of 32 individuals with IgG-negative, 22 cases in a group were more than 29 years old (68.75%) and another 10 cases (31.25%) in the group have less than 29 years old. The levels of immunity in these two groups are 79% and 96% respectively. These differences were statistically significant.
According to the results of this study it can be predicted that the immunity status of mothers who are not vaccinated is low therefore for prevention of CRS in this group of age it must be recommended to become vaccinated. At last it can be said that immunity status and respond to vaccination in society under survey is in an acceptable level.

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