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Review Article

A Review on Rubella Vaccine: Iran (1975-2019)

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Abstract

The first Attenuated rubella vaccine was developed by Parkman and Meyer in 1966. Ten years later in the 1975s, the rubella vaccine was developed in Razi Vaccine and serum research institute (RVSRI). In 1977, the rubella TAKAHASHI vaccine successfully passed the clinical trial and was initially used voluntarily only in the private sector. Since 1987, the administration of rubella as MMR (Measles/AIK-C; Rubella/TAKAHASHI; Mumps/HOSHINO) strain vaccine has been included in the immunization program in Iran. This review article focused on the development and production of the rubella TAKAHASHI/HDC vaccine in RVSRI. The herd immunity and rubella cases were investigated in the pre- and post-vaccine era. The effectiveness and proper coverage of the rubella vaccine led to the elimination of rubella from Iran in 2019. The current study aimed to assess local rubella vaccine manufacturing and its consequences on rubella elimination from Iran, using various search engines. A complete search was carried out in medical databases, including PubMed, Scopus, Web of Science, Scientific Information Database, IranMedex, Magiran, and Google Scholar. Within 1972-1975, Rubella TAKAHASHI/HDC vaccine was developed by RVSRI and successfully passed clinical trial in 1977. Over the four last decades (1980-2020**)**, more than 40 million infants, young, and adults were vaccinated by million doses of local Rubella, measles-rubella (MR) or measles-mumps-rubella (MMR) vaccine in Iran. In 1972, the pre-vaccine era, the overall sensitivity to rubella infection was 69% in one-year-old Iranian children and 23% in childbearing women. The use of a safe, inexpensive, and effective vaccine increased herd immunity to 95% (85%-99%) in our country. During the last two decades, we have witnessed a 91% decline in the confirmed rubella cases, from 1124 in 2000 to 33 cases in 2018. The current article presented the process of vaccine development, tracked it through more than four decades, and discussed disease status before and after the rubella vaccine era, as well as the history of its elimination from Iran. The effectiveness of the local Razi Rubella vaccine resulted in a significant increase in seroprevalence in Iran. Expanded vaccination against rubella, usually with measles, has led to the elimination of Rubella from Iran as confirmed by World Health Organization in 2019.

Keywords: Iran, Razi, Vaccine, Rubella, TAKAHASHI, Production, Congenital Rubella Syndrome, Elimination

Un examen du vaccin contre la rubéole: Iran (1975-2019)

Résumé: Le premier vaccin atténué contre la rubéole a été mis au point par Parkman et Meyer en 1966. Dix ans plus tard, dans les années 1975, le vaccin contre la rubéole a été développé dans l'Institut de recherche sur les vaccins et les sérums de Razi (RVSRI). En 1977, le vaccin antirubéoleux TAKAHASHI a passé avec succès l'essai clinique et n'a été initialement utilisé volontairement que par le secteur privé. Depuis 1987, l'administration de la rubéole sous forme de Vaccin contre la souche RRO (rougeole/AIK-C; Rubéole/TAKAHASHI; Oreillons/HOSHINO) a été incluse dans le programme de vaccination en Iran. Cet article de synthèse portait sur le développement et la production du vaccin contre la rubéole TAKAHASHI/HDC dans la RVSRI. Les cas d'immunité collective et de rubéole ont été étudiés avant et après la vaccination.

L'efficacité et la couverture adéquate du vaccin contre la rubéole ont conduit à l'élimination de la rubéole d'Iran en 2019. L'étude actuelle visait à évaluer la fabrication locale du vaccin contre la rubéole et ses conséquences sur l'élimination de la rubéole en Iran, à l'aide de divers moteurs de recherche. Une recherche complète a été effectuée dans des bases de données médicales, notamment *PubMed*, *Scopus*, *Web of Science*, *Scientific Information Database*, *IranMedex*, *Magiran* et *Google Scholar*. Entre 1972 et 1975, le vaccin contre la rubéole TAKAHASHI/HDC a été développé par la RVSRI et a passé avec succès un essai clinique en 1977. Au cours des quatre dernières décennies (1980-2020), plus de 40 millions de nourrissons, jeunes et adultes ont été vaccinés par des millions de doses de Vaccin contre la rubéole, la rougeole-rubéole (RR) ou la rougeole-oreillons-rubéole (ROR) en Iran. En 1972, à l'époque pré-vaccinale, la sensibilité globale à l'infection à la rubéole était de 69% chez les enfants iraniens d'un an et de 23% chez les femmes enceintes. L'utilisation d'un vaccin sûr, peu coûteux et efficace a augmenté l'immunité du troupeau à 95% (85% -99%) dans notre pays. Au cours des deux dernières décennies, nous avons assisté à une baisse de 91% des cas confirmés de rubéole, passant de 1124 en 2000 à 33 en 2018. L'article actuel présentait le processus de développement du vaccin, le suivait pendant plus de quatre décennies et discutait de l'état de la maladie avant et après l'ère de la vaccination contre la rubéole, ainsi que l'histoire de son élimination d'Iran. L'efficacité du vaccin local de la rubéole de Razi a entraîné une augmentation significative de la séroprévalence en Iran. La vaccination étendue contre la rubéole, généralement avec la rougeole, a conduit à l'élimination de la rubéole d'Iran, comme l'a confirmé l'Organisation mondiale de la santé (OMS) en 2019.

Mots-clés: Iran, Razi, Vaccin, Rubéole, TAKAHASHI, Production, syndrome de rubéole congénitale, Elimination

1. Context

1.1. Rubella Virus

Although the isolation of the rubella virus in cell culture dates back to 1962, the first report of congenital rubella was published in 1941. In 1815, W.G. Maton was the first who distinguished rubella from other exanthema, measles, and scarlet fever. The disease was named after him as Rutheln (Banatvala and Peckham, 2007; Hobman and Chantler, 2007; Plotkin and Orenstein, 2012). Therefore, in English, it is commonly referred to as German measles. Rubella has been reported contagious to humans and primates from the early nineteenth century. Nonetheless, 20 years later, in 1962, the causative agent of rubella was simultaneously isolated by cell culture techniques (Parkman et al., 1962) in two centers in the United States (Mirchamsy, 1996). In 1856, Veale (1866), a British vascular surgeon, coined the term "Rubella", a Latin name meaning small red. In 1914, Alfred Fabian Hess proposed that rubella is a viral disease. In 1938, Hero and Tosca demonstrated that discharge collecting from a monkey in the acute phase after filtering could transmit this disease (Hilleman et al., 1969). In 1962, the presence of the rubella virus was determined in human amniotic cell culture by virus isolation and its cytopathic effect. In 1962, Boston and Parkman used the technique of interference with the growth of enteroviruses, such as *ECHO11* and *Coxsackie A9*, as well as such other viruses, such as *Sindbis virus* and *Vesicular Stomatitis virus*, in African green monkey kidney (AGMK) cells (Hobman and Chantler, 2007). This phenomenon is the only method demonstrating the growth of the virus in cell culture. In 1963-1965, this event led to the discovery of the virus since the scientists were able to study virology and serology for the diagnosis and pathogenesis of this virus.

Rubella (RV) virus is the only member of the rubivirus genus of the Togaviridae family. The RV single-stranded positive-sense RNA genome is about 10 kb long with a guanine-cytosine (GC) content of 69.5%, the highest value among RNA viruses to date. The 5 'end of this RNA has a cap of 7 methyl guanosine, while at the end of 3 ', there is a poly-A with an average length of 53 nucleotides. The genome consists of two open reading frames (ORF) that do not overlap and are separated by a non-translatable region of 123 nucleotides. The viral genome codes for two non-structural (p90, p150) and three structural proteins (C, E1, and E2) (Banatvala and Peckham, 2007; Hobman and Chantler, 2007).

The rubella virus passes through 300 microns in diameter filters and can be concentrated in (100,000

rpm) interval. If the pure virus is suspended in a solution containing 2% of chicken serum, its titer will be reduced by 0.3-0.4 log/h at 37ºC. The virus is completely destroyed after 1 h at 56ºC under the aforementioned conditions. The virus resistance to heat increases with an elevation in the amount of protein in the maintenance medium. Humans are the only natural hosts and reservoirs for RV.

2. Evidence Acquisition

The aim of this article is to review the production process of local rubella vaccine and its consequences in Iran, using different search engines. Almost complete search of medical databases including PubMed, Scopus and Web of Science, Scientific Database, IranMedex, Magiran and Google Scholar.

3. Results

3.1. Phylogeography of Rubella Virus in the World and Iran

The rubella virus has only one serotype in two clades consisting of at least three genotypes, respectively (Hobman and Chantler, 2007). Genetic differences between clades do not appear to be translated into antigenic differences despite the frequency changes of 3%-6% amino acids in viral proteins (Abernathy et al., 2011). These genotypes are immunologically identical in terms of both natural infection and vaccination. The genome is approximately 10 kb in size; however, genotyping is based on a 739-nucleotide sequence window of the E1 structural protein, which contains antigenic sites and other important functional domains necessary for cellular invasion (Banatvala and Peckham, 2007). Intraspecific genotypic variation is approximately 5% across genotypes of group 1 and 8% for group 2; moreover, the maximum variation between groups 1 and 2 is 8%-10%. Such genotype classifications are an essential component of virological surveillance and are primarily used to determine whether outbreaks are due to endemic circulation or importation events. Out of currently active genotypes, 1E, 1 G, and 2B have achieved the broadest global distributions. In Iran, the isolation of wild virus from progressive rubella pan-encephalitis patients by Shafyi et al. (2005) made it possible to report information about virus genotyping. This locally isolated rubella virus is known as MF and registered in the GenBank with accession number DQ975202 (Shafyi et al., 2005; Bordbar et al., 2010; Rivailler et al., 2017). On the other hand, the lineage of 2B-L2 is genetically diverse, and its distance in the group was obtained at 1.8%. Based on the result of the mantle test, which was performed on 75% of viruses collected from 19 different countries in 2010, geographical clustering (P=1104) in sequence 94, 2B-L2 showed a new introduction of the phylogenetic tree topology for 2B-L2 plotted as 2B-L2a, 2B-L2b, and 2B-L2c. Although 2B-L2b and 2B-L2c are supported with boot values greater than 80%, and all three 2B-L2 subtypes probably have a common ancestor of Indian origin, epidemiological and sequencing data are not sufficient to assess the genetic origin of this lineage. The 2B-L3 and L4 lineages are mainly from East Asia (Banatvala and Peckham, 2007). There is a dearth of molecular data on specific characteristics of rubella virus genotyping in Iran. According to phylogenetic analysis, the Iranian isolated MF rubella virus was classified into genotype 2B (Rvi / Iran / 00 / DQ975202.1) (Shafyi et al., 2005; Bordbar et al., 2010). The Iranian isolate is located at a great distance from other existing isolates and does not fall into any of the subdivisions (2BL0-2BL4); therefore, it is still identified under genotype 2B (Figure 1). A virus similar to this sequence has not been found in the Gene Bank registered viruses for more than four decades. Therefore, it is necessary to conduct further extensive studies with a larger sample size from all parts of the country to confirm whether the dominant virus of Iran belongs to the 2B and its subdivision genotype, or a new genotype has been circulated in our country. A broader and deeper understanding of virus transmission during an outbreak is required to support the elimination of rubella from

Iran. This molecular monitoring is of utmost importance in attributing the circulating virus to imported, native, or rubella vaccine-associated strains. The necessary research on sub-divided genotypes or clades that benefit from standard methods can provide better explanations for the genetic diversity of the RuV sequence since 2B viruses have a wide geographical distribution. Access to this information from different Iranian isolated rubella viruses can provide more information in terms of time and location since this isolated virus is separated from other isolates in the Gene bank**.**

3.2. Rubella and Congenital Rubella Syndrome: an Importance of Public Health Problem

In 1941, Gregg reported that out of 78 cases of infantile cataracts, 68 ones were due to maternal rubella early in pregnancy (Hobman and Chantler, 2007). Consequently, congenital cataract was linked to intrauterine maternal rubella. In later years, the reports from epidemiologists and teratologists in Australia, Sweden, the United States, and the United Kingdom confirmed the role of rubella in congenital cataracts and also found an association between coronary heart disease and deafness in infants. Therefore, the triple characteristic of congenital rubella syndrome (CRS) was determined as cataracts, coronary heart disease, and deafness. These characteristics changed the rubella infection from a mild rash to a major threat in pregnancy (Polk et al., 1982). Between 1964 and 1965, the widespread epidemic in the United States affected thousands of pregnancies and led to the development of CRS with diverse manifestations affecting the brain, lungs, liver, spleen, kidney, and bone marrow. This anomalous pathogenesis was associated with hepatitis, splenomegaly, thrombocytopenia, encephalitis, mental retardation, pneumonia, metaphysical defect, diabetes mellitus, and thyroiditis. Moreover, cochlear atrophy and patent ductus arteriosus are typical in CRS after frequent manifestation in the eye, ear, heart, including glaucoma, central auditory in fereruption, and peripheral pulmonic stenosis (Peetermans and Huygelen, 1967). Since the rubella vaccine was not widely available until 1965, parties were organized where children were naturally vaccinated against rubella in contact with adults at the party. In 1963-1965, the availability of in-vitro cell culture enabled the researchers to cultivate the virus in the cell substrate. Since then, the possibility of virological and serological studies has increased in the field of diagnosis and pathogenicity of the rubella virus. The reports related to CRS incidence among childbearing women increased from 1% in the pre-vaccination era to 10%-20% during 1964-1967. Following that, the possibility of assessing and reporting the risk of rubella infection in early pregnancy also increased (Shafyi et al., 2005).

The high prevalence of congenital malformations following maternal infection during these years (1964 - 1965) included an estimated number of 12.5 million rubella cases. More than 30,000 pregnancies were affected by the epidemic, and about 5,000 pregnant women decided to have a medical abortion, which was certainly underestimated, and there were 6,250 cases of spontaneous loss of the fetus. This epidemic led to 11,000 miscarriages or therapeutic abortions and 20,000 cases of CRS. The outbreak resulted in 2,100 deaths in infancy, 12,000 cases of deafness, 3,580 cases of blindness due to cataracts and/or microphthalmia, and 1,800 births with mental disabilities. The economic costs for each child with CRS are estimated at $ 221,660, and the total cost of the epidemic could be $ 1.5 billion by 1965 (Peetermans and Huygelen, 1967). The last major epidemic that began in Europe in 1962-1965 and spread to the United States within 1964-1965 exerted devastating effects on peoples' lives. This highlights the importance of rubella and CRS as major public health problems. Before the use of the vaccine, rubella epidemics affected about 5% of the population, although only about 10% of these cases were reported to public health officials. In the pre-vaccination period, the analysis showed that rubella affected 60% of cases among children under 10 years of age and 23% of adults over 15 years of age. The introduction of the vaccine and its application did not significantly reduce the incidence in adolescents and adults, and CRS has

continued to be observed in the community for several years after vaccination (Polk et al., 1982). In New York City alone, CRS reportedly affected 1% of all neonates born at that time (Peetermans and Huygelen, 1967).

3.3. Pre Vaccine Era in Eastern Mediterranean Region

Rubella is a contagious viral infection that occurs most often in children and young adults. Infection in the first trimester of pregnancy can lead to miscarriage, fetal death, stillbirth, or congenital anomalies, known as CRS. The highest risk of CRS development was detected in countries where childbearing women were not immune against Rubella. The WHO reported that before the introduction of the vaccine, in every 1,000 live births, up to 4 neonates were born with CRS in epidemic and non-immune communities (WHO, 1991).

The introduction of the Expanded Program on Immunization (EPI) in the early 1980s reduced the average number of rubella cases from 39,000 to 9,400 per year. Since the production of the rubella vaccine, it has been introduced in most countries across the globe. According to statistics provided by WHO (1991), 236,000 neonates with the CRS are born in developing countries each year. In 1996, when CRS was non-epidemic, it was estimated that about 110,000 infected neonates were born in developing countries (Cutts and Vynnycky, 1999). In 2004, a rubella case fatality rate of 3% (70,000 deaths) was reported in the East Mediterranean Region (WHO, 2009).

In 2005, WHO Regional Office for the Eastern Mediterranean (EMRO), Department of Communicable Disease Control, estimated that about 12,000 neonates were born with CRS in this area each year. The CRS incidence has been reported differently in live births in various parts of the world. For example, during the rubella epidemic, out of every 1,000 neonates, 1.7 cases in Israel, 1.7 in Jamaica, 0.7 in Oman, 2.2 in Panama, 1.5 in Singapore, 0.9 in Sri Lanka, and 0.6 in Tobago had CRS. In this region (EMRO), nearly 1, 1000 measles cases and a similar number of rubella cases were reported in 2007 (WHO, 2005).

There was a growing interest in vaccination during 1990-1995 in Eastern Mediterranean Region when rubella outbreaks were reported in the Islamic Republic of Iran, Iraq, Kuwait, Oman, Saudi Arabia, and the United Arab Emirates. In December 1995, six Gulf countries agreed to focus their efforts on the prevention of rubella and CRS through childhood vaccination that increases the average age of infection in women of childbearing age, and subsequently, decreases the incidence of CRS (WHO, 1991). Following this approach, the immunization program started by Mass campaign for childbearing women aged 15-25 and 1-14-year old children, in addition to routine measles-rubella (MR) or measles-mumps-rubella (MMR) vaccination of children. This program increased the number of countries using the rubella vaccine in their national vaccination programs. Nowadays, 11 countries (12% of the regional population) reported national use of the rubella vaccine (Figure 2). In this Region (EMRO), 470 million doses of vaccine were administrated during the last decades, and this vaccine saved 2.5 million lives in the two last decades. The WHO Strategic Action Plan, 2012-2020, aimed to successfully eliminate measles and rubella from at least five WHO regions by the end of 2020 (WHO, 2018).

In EMRO, measles elimination and the prevention of measles virus transmission were targeted till 2010. Following the use of the MR vaccine, 173 countries (out of 194 countries) introduced the rubella vaccine by the end of 2019, and its global coverage was estimated at 71%. Rubella disease has reportedly decreased by 97%, from 670,894 in 102 countries in 2000 to 14,621 cases in 151 countries in 2018. On 28 May 2019, WHO declared that measles and rubella have been eliminated from both Bahrain and Oman, and the Islamic Republic of Iran has eliminated rubella and is about to eradicate measles. According to the 2018 Global Vaccine Action Plan (GVAP) on immunization, rubella control is lagging behind since 26 countries are still performing vaccination, and two regions (Africa

and the Eastern Mediterranean) have not yet set targets for the eradication or control of rubella (WHO, 2018).

3.4. Rubella in Iran at Pre-Vaccination Era, Epidemiology, Pathogenicity, and Complication

There is a dearth of research on the identification and herd immunity to the rubella virus in the pre-vaccination era in Iran. In 1970, the immunity rate for women ≥ 15 years of age in Tehran province was reported as 97% (Naficy and Saidi, 1970). One of the most interesting studies regarding the impact of socioeconomic conditions, natural infection, virus circulation, and its effectiveness in immunity against rubella before the EPI era was conducted in 1972. In the referred study, 1559 blood samples of young women and girls were tested, and 85% of cases had immunity (Saidi, 1972). A direct relationship was observed between serum prevalence and age, from 29% in one-year-old children to 97% in 15-year-old girls. In the mentioned study, 93% and 89% of urban and rural women were seropositive, respectively. In the low-income classes of urban women and the affluent classes, immunity scores were obtained at 99% and 92%, respectively. The results of the stated study concluded that rubella vaccination is not necessary since mild natural rubella infection and virus circulation have led to sufficient rubella antibodies in females of childbearing age. However, it is necessary to monitor and evaluate the level of immunity against this disease in young girls (Saidi, 1972). A decade later in 1985, another study conducted on girls aged 15-20 years reported an immunity level of 90% in the same province (Nategh and Ebrahimpour, 1985). In another study in Tabriz, 18.8% of girls aged 18-15 years were sensitive to rubella (Nategh and Ebrahimpour, 1985). In a study conducted in the south of Iran, Khuzestan province, Dezful, among all age groups, 30% of pregnant women showed sensitivity to rubella (Ghafourian Boroujerdinia and Pakzad, 1996).

In 1996, the result of another investigation conducted on 81 girls aged 18-26 in Zanjan city showed that they all had adequate antibodies against rubella (Amini, 1996). In 1985, a serological study among the adults, pregnant or non-pregnant women residing in the south of Iran reported 90% immunity to rubella (Pakzad and Moattari, 1987). However, 10 years later in this region of the country, pregnant women's immunity to rubella was reported as 74.8 (36) that increases to 90% in 2000 (Ghafourian Boroujerdinia et al., 2003).

In the pre-vaccination era, published serological articles indicated that an increase in age from 1-15 years led to a arise in seropositivity from 29% to 97%. This report pointed to the low incidence of rubella in urban areas of the country and reported that about 90% of children under 9 years of age were seropositive to rubella. These articles suggested that due to the high prevalence of rubella infection in Iran, CRS should be rare in the country (Kabiri and Moattari, 1993). Different studies conducted in various provinces of Iran reported that ≥ 70% (70-97%) of women of reproductive age (15-45 years) were seropositive against rubella infection. Overall immunity rates in pregnant Iranian women before and after the national vaccination campaign were reported to be 88.6 and 90.4%, respectively (Saidi, 1972; Rahimi et al., 1994; Sadigh et al., 1998; Sadighi et al., 2005).

There are three different reports on field trials using imported vaccines before the development of the local rubella vaccine in Razi institute. The first rubella vaccine trial was assayed to compare the effect of subcutaneous and intranasal administration on immunization levels. In this article, two groups of 27 and 25 children received the RA 27/3 Rubella vaccine via subcutaneous and intranasal administration. All the children who underwent SC injection showed adequate antibodies in their blood. In the second group that received nasal drops, out of 25 vaccinees, 21 children were seropositive (Naficy et al., 1967).

Another study compared and tested the immunization level of three different rubella strains of the vaccine (HPV-77DE5, Cendehill-5, and RA27/3). The result of the mentioned research revealed that all three rubella strains yielded similar results (Naficy et al., 1970). The third study assessed the administration of RA 27/3 rubella vaccine alone and in combination with the

measles vaccine. The results showed that the use of two viruses in one vaccine did not contradict each other (Naficy et al., 1967).

3.5. Congenital Rubella Syndrome in Iran

The rubella disease has been recognized as a distinct viral disease and its related complications for more than 100 years. A fetus infected with the rubella virus may be born with CRS. Some CRS-related defects may be diagnosed at birth, while others are reported months or even years later. The manifestations of CRS may be transient (such as purpura), permanent (such as deafness, central nervous system dysfunction, congenital heart disease, or cataracts), or as late symptoms (such as diabetes mellitus). The clinical manifestations of congenital rubella include miscarriage and stillbirth, low birth weight, mental retardation, sensorineural hearing loss, unilateral or bilateral central hearing loss, speech problems, cardiovascular disorders (VSD-PS-PDA), arterial duct residue, pulmonary artery stenosis, and interventricular wall defect. The neonates with CRS shed the rubella virus with tears for a long time. The rubella virus can be present in nasopharyngeal secretions in 80% of neonates with CRS during the first month of life, 62% in 1-4 months, 33% in 5-8 months, 11% in 9-12 months of age, and 3% during the second year of life. Infants with CRS who excrete the rubella virus through tear secretion are infected and need appropriate precautions. According to UNICEF and WHO, out of 1,000 live births, about 0.2 of neonates are born with CRS in non-epidemic years. Although a comprehensive nationwide study has not been conducted on the percentage of newborns with CRS, given the current rate of population growth in Iran, 16,400 children with this syndrome are expected to be born in Iran at present (Farhoud et al., 1996). The endemic infection has been eliminated from Iran, and a high percentage of women of childbearing age are immune to rubella. Nonetheless, there is no published information on congenital rubella cases, as well as the incidence and number of neonates born with CRS in our country. It is of paramount importance to prevent the exposure and transmission of the virus from unimmunized pregnant women to the fetus (Hobman and Chantler, 2007; Plotkin and Orenstein, 2012). Vaccination against rubella until the age of five leads to the shift of disease from children to adults, increasing CRS in adults. To plan for the prevention of CRS, especially in the Middle East, monitoring should be annually conducted in geographic areas in the public and private sectors in all target groups receiving the vaccine. The CRS care should be reviewed and tested on newborns case-by-case (Cutts and Vynnycky, 1999; WHO, 2009), apart from laboratory examination of any suspected case (Mulders et al., 2016). Monitoring of rubella and CRS case care should be performed by examining and performing a laboratory test on each suspected case (Sabin, 1969).

Due to the need for accurate information on the epidemiology of rubella and CRS, the following suggestions are put forward:

1. Rubella antibodies should be evaluated in people vaccinated in 2003 and 2005.
2. The CRS cases should be tracked and examined after the Campaign.
3. A comparison should be made between CRS cases before and after the campaign in terms of relevant symptoms.
4. Antibody levels (IgG, IgM) should be examined in campaign participants and compared with those who have not been vaccinated. An appropriate surveillance system is needed since the absence of epidemiological data on CRS and adequate monitoring could result in shifting of rubella cases to higher ages and increasing the incidence of CRS.

3.6. Rubella Vaccine

The epidemic picture of rubella infection resulted in extensive efforts to produce an effective vaccine for the disease as follow:

3.6.1. Killed Vaccines

The use of inactivated rubella virus has been studied by different scientists (Sever et al., 1963; Buynak et al., 1968; Beck, 1969). Live attenuated vaccines have received great attention due to the following reasons:

inability to replicate high-titer rubella viruses in cell culture, the need to inoculate high titer of antigen to produce adequate antibodies, failure in the production of enough immunogenicity, re-infection with live rubella virus after vaccination by inactive antigen, and the complications following the repeated injections of killed vaccines.

3.6.2. Live Attenuated Vaccine

The following five rubella strains were licensed in the USA during 1969-1970, and safe and effective attenuated vaccines containing some of them were widely available.

a- The first rubella virus was recovered from army recruits in 1962 and named HPV-77 since it was developed after 77-120 serial passages on primary GMK cell culture (Parkman et al., 1962; Hobman and Chantler, 2007; Plotkin and Orenstein, 2012). An attenuated rubella vaccine was developed and tested using this strain within 1965-1967 and has been reported safe, immunogenic, and non-contagious in humans (Meyer et al., 1966).

b- The second live attenuated rubella virus vaccines were prepared by the adaptation of HPV-77 after five passages in duck embryo cell culture and named HPV77. DE5 (Hilleman et al., 1969; Huygelen et al., 1969).

c- Adaptation of DE5 HPV77 on Dog kidney cell cultures produced the third strain after 12 passages and was named DE5 HPV77. DK12 (15).

d- "Cendehill 51 strain" the other attenuated rubella virus vaccine was isolated and grown in primary rabbit kidney cells after 51 passages (Peetermans and Huygelen, 1967).

e- RA/27 virus strain was developed by growth on human embryonic kidney and attenuated by human fibroblasts in 1964 (Plotkin et al., 1969).

The first attenuated rubella vaccine was developed and tested between 1965 and 1967. Two years later, in 1969, the HPV77.DE5 and Cendehill vaccine strains were licensed in the United States (Hilleman et al., 1969; Huygelen et al., 1969). In Japan, similar results were obtained from inoculation of the rubella vaccine. In addition to the aforementioned viruses, the following vaccine strains were available and used only in Japan since 1968. We will mention the rubella virus strains made in Japan following two purposes:

Firstly, the isolated virus in Japan were much milder with fewer abnormalities in the fetus, compared to those responsible for the epidemic in the United States between 1966 and 1969 (Kono et al., 1969). Secondly, one of these Japanese strains was adapted to human diploid cells at the Razi Institute and used for rubella vaccine preparation. In Japan, six rubella vaccine strains have been reported as follows:

i- Matsuura (ME) virus (ME-P) has been isolated from green monkey kidney cells and then adapted in primary chick embryo cell culture and has been assigned two names: EM6 and ME11 (Kono et al., 1969).

ii- MEQ7 and MEQ11 were prepared by the cultivation of ME-P in Japanese quail embryos (Kono et al., 1969).

iii. TAKAHASHI strain used a wild-type rubella virus which was adapted in the testicular and kidney cell-substrate of rabbits after 36 subcultures (Owada et al., 1973).

iv- To-336 which passed seven times in African Green Monkeys kidney cells and then attenuated by primary guinea pig embryo kidney after 20 culturing passages.

v- SK was prepared by three serial cultures in the green monkey kidney cells and then attenuated through 60 serial cultures in rabbit kidney cells.

vi- TCRB-19 and TCRB-12 were two strains with the same name isolated from the throat of patients. These isolations were prepared after two cultures in the African green monkey kidney, 33-53 passages in the primary calf kidney cell, and several cultures in primary rabbit kidney cells (Shishido and Ohtawara, 1976). Immunity in the first rubella vaccine prepared with HPV-77DE strain resulted positive after seven years follow-up in small amounts in children's blood (Meyer et al., 1966). According to detailed studies conducted on six aforementioned Japanese rubella virus

strains, all of these agents are harmless and do not change their genotypes after entering the human body; moreover, significant antibodies were produced in several years (Shishido and Ohtawara, 1976). A single dose of rubella-containing vaccine (RCV) can provide lifelong protection (Abernathy et al., 2011).

3.6.3. Subunit and Virus-Like Particles Vaccine

Advances in molecular biology have led to the development of inactivated subunit vaccines. These vaccines were developed by the genome sequence of the E1-protein of the virus and contain several neutralizing epitopes. Although the immunogenicity of this component is moderate, using the strong adjuvant has induced good responses in animals. In addition to these vaccines, virus-like particles containing three major rubella virus proteins have also been produced with relatively good immunogenic properties. They may be useful in the immunization of adult women.

The first trivalent vaccine "MMR1", containing rubella virus (HPV77.DE5), measles virus (Moraten strain), and mumps virus ((Jeryl Lynn strain), was licensed in 1970. However, since 1972, the (RA27 / 3) strain of the rubella vaccine has been licensed in the United Kingdom. It was replaced by the HPV77.DE5 strain seven years later in 1979 in the United States (Peetermans and Huygelen, 1967; Plotkin et al., 1967; Plotkin and Orenstein, 2012). There are three reasons for this delay. Firstly, the primary culture of animal tissues was preferred at that time; however, after the development of human diploid lung cell strain at the Wistar Institute, the evidence showed a standard human cell that was useful for vaccine production and did not cross-contaminate with other adventitious agents (Sabin, 1969). Secondly, in the next decade, the evidence obtained from the use of primary animal tissue culture in rubella vaccines demonstrated some complications, such as joint reactions caused by the use of duck embryo and canine kidney cells, and has since been withdrawn. Thirdly, the evidence regarding various vaccines suggested that all strains of the rubella vaccine, except for RA27/3 strain, showed re-infection on exposure to wild-type virus. Finally, the good safety record of the RA27/3 strain was significantly greater, as compared to other strains. The majority of these documents encouraged the US Food and Drug Administration to license RA 27/3 (Plotkin and Orenstein, 2012).

The second trivalent MMRT vaccine containing rubella virus (strain RA27 / 3), measles virus (Schwartz strain), and mumps virus (species Urabe AM / 9 or Jeryl Lynn strain), was licensed in the United Kingdom in 1988.

The first WHO requirements for the rubella vaccine were published in 1976 (TRS 610., 1976). An overview of rubella virus vaccine strain, in vitro process of development, the passage number, type of cell-substrate, growth characteristics, and year of derivation in experimental vaccines are summarized in Table 1.

3.7. Development of the Rubella Vaccine in Iran

During the rubella pandemic in the world (1964-65), RVSRI undertook a mission to launch the production of a vaccine to prevent the spread of vertical transmission (mother-to-child) of rubella. There was little publicized evidence regarding rubella epidemics and the prevalence of CRS in Iran. However, depending on the disease situation in the world and the EMRO region, its rate was indirectly measured and estimated at 0.2/1000 live births before rubella vaccination (Sadighi et al., 2005).

Rubella vaccine has been produced in Iran since 1975. The result of the first clinical trial about combined MMR vaccine, Razi institute (AIK, TAKAHASHI, and HOSHINO) was Published in 1977 (Mirchamsy et al., 1976; Shafyi and Mohammadi, 2018). Nevertheless, it was not introduced to the national immunization program until 2003. During this period (1975-2003), the rubella vaccine was only available to physicians and private clinics (Figure 3). The CRS in Bahrain, Kuwait, and Israel led to the implementation of the rubella vaccine in the vaccination strategy in the Eastern Mediterranean Region, combined with Measles (MR) and Mumps as

MMR from 1995. Since 2003, according to studies on the necessity of the inclusion of rubella vaccine in other countries, the following recommended vaccination program has been implemented in Iran: Compulsory MMR vaccination at 12-15 months of age, followed by a second dose of MMR vaccine in 4-6 years of age (Sadighi et al., 2005).

During the four past decades, different information has been published about rubella and the prevalence of CRS in Iran due to the production of high-quality vaccines and the implementation of EPI. In addition, massive progress in monitoring and reporting systems for disease, as well as data on the prevalence of rubella and CRS, revealed that the disease is eliminated from Iran. The present study has now cumulated 45 years of lessons on rubella production and its effect on the incidence of rubella and CRS in Iran using a local vaccine. This review explained scientific and documented issues about how this vaccine was produced and incorporated into MMR vaccination (Mirchamsy et al., 1976; Sadighi et al., 2005; Shafyi and Mohammadi, 2018).

3.7.1. Preparation of Vaccines

3.7.1.1. Source of the Vaccine Strain

TAKAHASHI wild virus Rubella strain was isolated from a throat swab of a rubella patient by Shishido and Ohtawara (1976). This isolate passaged six times in primary GMK cells and had a titer of 10 4. 7 CCID50 Iml in rabbit kidney (RKI3) (Shishido and Ohtawara, 1976). The close scientific relationship between the Kitazato and Razi institute regarding the development of MMR vaccines led to the TAKAHASHI vaccine strain gifted being Iran.

3.7.1.2. Development of Attenuated Rubella TAKAHASHI/HDC Vaccinal Virus

It was decided to change the cell-substrate due to the problems with the preparation of primary testicular and kidney cultures of rabbits, and the MRC-5 cell strain was selected for rubella vaccine production due to its availability. Using our previous experiment on measles and poliomyelitis based on WHO requirement, the received rubella virus passage three times in GMK. The harvested virus successfully passed the sterility and potency test. It was adapted for growth in the MRC-5 cell-substrate by four other passages. Different serial harvests of rubella TAKAHASHI attenuated strain in HDC-MRC-5 produced a total volume of 83 liters containing Log 5.25 CCID50/1ml as the first step (Mirchamsy et al., 1976; Shafyi and Mohammadi, 2018).

Safety testing for new rubella/HDC strain was obtained by the evaluation of a laboratory animal. The consistency of the virus genome is the main concern for virus vaccines previously attenuated through the non-primate passage. To observe non-reversibility to virulence and stability of virus after adaption to HDC, rct40 and T50 marker of viruses were studies. In this regard, this strain was passaged 20 times in MRC-5 cells of a baby hamster for the assessment of changes in its neurovirulence after consequent passages in diploid cells. The results of the test revealed that the variation of neurovirulence during the passages in MRC-5 was not significant (Table 2). The result of rct40 marker test is displayed in Table 3. In rct^{40} marker test, the result did not change after five serial passages in MRC-5c cells similar to the original virus before adaptation to HDC (Table 3). The result of the T50 test is presented in Table 4. As illustrated, this value did not change after 5-20 passages in MRC -5 cells (Table 4).

As this result will be described, no reversion was determined in the attenuated virus. According to stability in this genetic marker, the rubella TAKAHASHI showed a fixed phenotype after adaptation on MRC-5, in comparison with the original virus before adaptation. The most important criteria for the selection of a virus vaccine are reproducibility and consistency during production. The potency of the virus was log 4.7CID50 when received. After adaptation to MRC-5, this titer increased and showed 5.7 TCID50 in potency test on RK-13.

3.7.1.3. Evaluation of Virus Infectivity by Potency Measurement

The cytopathic effect (CPE) induced by the attenuated rubella virus was not so clearly defined in cell cultures. In this regard, two different established rubella vaccine virus RA/27-HDC and TAKAHASHI/HDC were

harvested in various bathes of vaccines. The test was performed by interference with vesicular stomatitis (VSV) as a challenge virus.

The infectivity of the harvested fluid was conducted on RK-13. The potency of the virus was determined by calculating the log of 50% interfering dose (log CCID50/0.1 mL) using the Spearman-Karber method (WHO; TRS 610.1976). An in-house standard was designed as a positive control for the validity and sensitivity of the tissue culture. These standard vials were duplicated and simultaneously included in each assay in parallel to the vaccine samples. The samples are serially diluted, added to RK-13 cells in a culture tube using an automated workstation, and incubated at 33ºC. Each sample was replicated four times in a potency test. The comparative potency assay data of different vaccine batches are summarized in tables 2-4) (WHO; TRS .610.1976).

3.7.1.4. Clinical Trials and Reactions on the New Vaccines

The clinical evaluations of test vaccines were conducted in two, close, and open steps. In this process, antibody responses and transmission of the virus from vaccinees were investigated, in addition to clinical reactions, such as skin rash, fever, and three-week post-vaccination lymphadenopathy. The first injection was administered to immunized children ages 9-11 (≤ 12) months (Table.5). The second dose (re-immunization) with the same vaccine was programed at 18-24(≥18) months of age. The result was indicative of a 2-3-fold increase in HI titer after the second dose in all vaccinees.

The immunogenicity results of the vaccine were confirmed in this step. The second evaluation was performed in a larger (n=130) population of susceptible and seronegative children and young adult females (Table 8).

The antibody responses were followed by the HI-antibody titration. The blood samples were taken from the vaccinees before injection and in the next 6-8 weeks (Tables 5, 6, and 7).

In these trials, clinical reactions between children and adults who had close contact with the vaccinated group were closely monitored. In almost all the vaccinees, the Seroconversion rate was higher than 95% (Table 8). The geometric mean of antibody titers in the serologically converted children ranged from 26.3-27.6 using TAKAHASHI/MRC-5. The incidences of fever, rash, and lymphadenopathy among vaccinees indicated that the vaccine virus did not change regarding antigenicity and immunogenicity after passage in MRC-5 and is attenuated enough for general use in children. Consequently, TAKAHASHI/MRC-5 vaccine was included in the formulation with Measles and Mumps and used for field trials (Mirchamsy et al., 1976; Shafyi and Mohammadi, 2018). About 10 years later in 1986, other field trials were conducted on MMR-containing Rubella TAKAHASHI/HDC combined with Mumps (RS#12/HDC) and Measles (AIK/HDC) (Sadigh et al., 1998) (Figure4).

This trial was performed on adult volunteers aged 17-37 by the assessment of 94 vaccine recipients. The highest and lowest number of subjects were related to the age groups of 20-19 years (n=31) and 15-16 years (n=9). There were between 15 and 16 cases in other groups. Moreover, 41.7% and 30.8% of the participant were in the age group of 26-25 and 21-22 years, respectively. A total of 17 cases had a history of receiving the vaccine, and 11 recipients had reported rubella disease in their history. Based on the serosurvey of the study group, 97.9% of cases had a history of rubella disease or vaccination. They were placed in the immune group who were seropositive before receiving the vaccine (Figure 4A and Figure 4B). After receiving the vaccine, 14.2% of cases in this group showed the effect of a booster dose, and the antibody titer increased. In a similar vein, the increase has doubled in 33.9% of participants, and the antibody titer has remained constant in 41.3% of cases. In the other group with no history of infection or vaccination (non-immune), in 100% of cases, the average geometric titer was increased to 2.5 based on Log 2. As illustrated by the results, the antibody titer increased after injection in both groups in cases with or without the disease or a history of vaccination. As displayed in Figure 4A and Figure 4B, the upward trend of immunity in the age groups of 15-20 has fluctuations; nonetheless, since then, it has had a direct relationship with increasing age. It reached its

maximum of 100% in the oldest age group (≤ 25-26). In general, the percentage of antibody titer after vaccination was lower in immune groups, as compared to that in unimmune ones. In the assessment of the immunogenicity of the vaccine, no specific trend was observed regarding titer increase in different age groups. As mentioned in this study, 97% of immune groups showed high levels of antibodies.

Since 1984, this vaccine is also produced in combination with Measles (MR) and Mumps (MMR) (Figure 3). The EPI was started in 1984 to provide active immunity against vaccine-preventable diseases, including diphtheria, tetanus, pertussis, measles, polio, and tuberculosis in children less than one-year-old. Since 2003, after the national immunization of measles and rubella (MR), the measles-rubella-mumps combined vaccine (MMR) replaced the measles vaccine in the current immunization.

3.8. Durability of Immunity in Rubella Vaccine

Regarding the rubella vaccine, Iran adopted a policy of delayed immunization until 2003, compared to other countries. This policy provided the opportunity for virus circulation, allowing natural infection to immunize the population after transient infection. This phenomenon was also mentioned regarding the measles virus in other articles (Shafyi et al., 1996). As depicted in Figure 5A and 5B, 73% of subjects demonstrated immunity to rubella prior to EPI implementation. In other words, contact with the patient and the natural circulation of the virus as a natural booster has played a major role in increasing antibodies in the pre-vaccine era (Saidi, 1972). The maternal antibody in comparison with umbilical cord blood also confirmed the high seropositivity of the community in 1994.

The seroprevalence of rubella antibodies in 632 cord blood samples of newborns showed that 627 (99.4%) of cases were seropositive for rubella in HI titer. In this study, mothers were 14-31 years old (Figure 6) (Shafyi and Mohammadi, 2018). Based on the epidemiologic situation of rubella in six Persian Gulf countries and the EMRO region, the routine vaccination for rubella was recommended by National Committee on Immunization. The emphasis was placed on children (Cutts et al., 1997). Although National Immunization Committee decided on the use of the same policy in Iran, all the people aged 5-25 years were initially vaccinated. This approach regarding rubella vaccination will undoubtedly serve as a useful tool for the protection of the fetus from CRS.

A rubella TAKAHASHI vaccine follow-up study on 20-35-year-old subjects reported that the antibodies remained stable in 75% and 98% of cases after receiving one dose and two doses of vaccine in childhood, respectively. In the stated study, the results were evaluated using the HI test (Sadigh et al., 1998). A seroprevalence study using Sero-neutralization and hemagglutination-inhibition assay pointed out that more than 95% of TAKAHASHI rubella vaccine recipients were still seropositive after 10 years (Jafari et al., 2012). The evaluation of cellular immunity after receiving the RAZI Rubella TAKAHASHI vaccine and the comparison of data with previous studies on specific lymphocyte proliferation in response to rubella vaccination confirmed good durability in cell-mediated immunity for local vaccines. In this test, people who had received the rubella TAKAHASHI vaccine about 10 years ago displayed activated cellular immunity in a timely and significant manner (Jafari et al., 2012). The examination of cord blood of women who were vaccinated during childhood and tested during pregnancy showed that about 7% and 9% of cases were negative and weak positive, respectively, and the rest had significant protective immunity after 20 years (Figures 7, 8) (Shafyi et al., 1996). Rubella immunity rates in pregnant Iranian women were 88.6% (95% CI: 80.6-93.6) and 91.5% (95% CI: 88.1-93.9) before and after the national vaccine campaign, respectively. The last article based on systematic review and meta-analysis of 15 articles involving 7,601 pregnant Iranian women revealed that the overall pooled rubella immunity rate was 90.1% in 2019 (Azami et al., 2019). Following two vaccine campaigns in 2003 and 2005 with Bivalent measles and rubella, both vaccines were included in EPIsince 2004. The initial results of this policy seemed to be successful based on more than 90% Seropositivity in different age groups (Esteghamati et al., 2007).

Different studies have investigated the persistence of the circulating rubella antibody in vaccinees after

immunization with live attenuated rubella vaccines. Although these articles have pointed to a high level of immunity, there is great variation in the specificity and sensitivity of the method used for rubella prevalence studies in different socio-economic regions of the country. Some studies presented age-stratified data; however, either the age groups were not narrow enough or the sample size was not sufficient for modeling the mean age relative to the total population involved in the infection. Furthermore, the lack of positive and negative controls in the evaluation of samples is another shortcoming that can be observed in the evaluations presented on immunity level against rubella. According to financial support, various serological methods, such as ELISA, HI, and SN have been used in these articles to evaluate the results in different geographical areas. In some studies, positive and negative controls, international or in-house standards, were not included in the experiments. In this regard, the analysis and comparison of results need to be made cautiously. The comparison of results and validation of a high immunity level that existed in different geo-economic regions before EPI could be possible using a standard method with high sensitivity and specificity.

3.9. Impact of Rubella Vaccination Strategy on the Occurrence of Congenital Rubella Syndrome

Before the introduction of the rubella vaccine, the incidence of CRS ranged from 0.1-0.2/1000 and 0.8-4/1000 live births during endemic and rubella epidemics, respectively (WHO, 1991). In the past decades, rubella vaccination has drastically reduced and practically eliminated rubella and CRS in many developed and some developing countries. In 2006, there were less than 3,000 reported cases in the United States. In January 2014, Colombia was confirmed as the first Latin American country which was free of rubella. The last non-imported case of rubella in the USA was reported in 2009. The United States became the first country to have eliminated rubella On April 26, 2015 (Kirby, 2015).

Epidemiological studies of rubella and congenital rubella can be assigned to the following two seasons in Iran: 1) before EPI within 1972-1995 and 1995-2000 and 2) after EPI during 2018-2000. Epidemiological studies in different parts of the world have reported that rubella antibodies were 50% for children aged 6-8 years and 80% for women of childbearing age (Hobman and Chantler, 2007; Jafari et al., 2012). Before the vaccination era in 1970, the overall immunity of the population in Iran was reported to be 85%. Although immunity has been reported to be high in childbearing women, it was significantly low in rural areas (89%), as compared to urban areas (93%). It can be ascribed to population density and socioeconomic status. In 1972, only 3% of children ≥15 years old were reported Seronegative. About 60% of children ≤ 5 years old had seropositive mothers. This rate reached 90% by increasing the age to ≥9. These data show that the incidence of rubella in urban areas of Iran is low, and about 90% of children up to nine years of age are immune against rubella. This immunity appears to be higher than what is commonly found in the United States, Europe, and Asia. Based on the results of studies conducted within 1968-1995, the prevalence of rubella in Iran is very high; therefore, CRS should be rare in this country. These investigations indicated that rubella immunity in women of childbearing age fluctuated between 70% and 95%, and it was higher (99 %), compared to those in high socioeconomic groups (92 %) in Iran. (Saidi, 1972; Kabiri and Moattari, 1993; Rahimi et al., 1994; Modarres and Oskoii, 1996; Pakzad and Ghafourian, 1996). A decade later, in a study on pre-marriage women in Iran from 2000-2012, 98.4% (95% CI: 97.5% −99.2%) of participants were immune against rubella. Moreover, in 2018, the rates of immunity in low and high incidence regions was reported as 99.3% (95% CI: 97.8–99.9%) and 97.5% (95% CI: 96.4–98.5%), respectively (P=0.05) (Zahraei et al., 2019). There is a dearth of information in Iran on rubella suspected cases bases on laboratory findings or CRS monitoring, confirming clinical data. Epidemiological studies before and after measles-rubella mass campaigns during 2003-2005 reported that the immunity rate in women of reproductive age (15-45 years) ranges from 69.9-97% (Esteghamati et al., 2007; Mahmoudi and Vahedi, 2007). Other Systematic meta-analysis studies have been conducted on 7,601 cases of pregnant women aged 18-35 years in all geographical

areas of the country between 1986 and 2018. In this study, the seropositivity rate in pregnant women was reported to be 91 %(86.1-93.1)(Azami et al., 2019). The above data during 1986-2019 pointed to the satisfactory level of immunity to rubella in women of reproductive age in Iran. Epidemiological evidence has demonstrated that there are high coverage and immunization levels against rubella achieved by the circulation of rubella virus in the pre-vaccine era and effective vaccination after vaccine production. There is still a risk of infection in pregnant women, even though only ≤7% of them are non-immune, and there is little prospect of CRS elimination (1.25). The CRS has been on the decline worldwide due to increased rubella vaccine coverage (Metcalf et al., 2012). However,where women of childbearing age do not have protective immunity against the virus, there is still the threat of costly CRS. Rubella immunity rates among pregnant women have been reported as 74%-98%, 53%-95%, and 54%-96% in European, African, and Asian countries, respectively (Azami et al., 2019). An appropriate CRS monitoring is required since Seroepidemiological data are indicative of a decrease in seroimmunity in older ages (>30years). Consequently, it may result in the shift of the age distribution of cases of rubella to older individuals and increased incidence of CRS in Iran.

Table 1: General Review of some of live attenuated rubella vaccine strain, the origin of virus sample, cell-substrate and Growth characteristics, and year of derivation. Number of passages

Country of Origin	Vaccine Strain	Sample /Place (City)	Cell Substrate / No of Passage	Year of Derivation	Reference
USA	HPV77	Army personnel with rubella/USA	Vervet monkey kidney/(77);	(1961)	(PAHO, 1998)
	HPV77. DE5	As above/USA	Vervet monkey kidney/ (77);duck embryo/(5)	(1961)	(Hobman and Chantler, 2007)
	HPV77. DK12	As above/USA	-Vervet monkey kidney/ (77); duck embryo/ (5); Dog kidney cell cultures/ (12).	(1969)	(Esteghamati et al., 2007)
	Cendehill	Urine-postnatal acquired rubella/ Louvain, Belgium.	-primary rabbit kidney/ (51).	(1963)	(Peetermans and Huygelen, 1967; Huygelen et al., 1969; Hobman and Chantler, 2007)
	RA27/3	Kidney rubella-infected fetus	Human embryonic kidney/ (4); WI-38 / (17-25).	(1964)	(Plotkin et al., 1967; Plotkin and Orenstein, 2012)
Japan	DCRB 19	patient Throat swab Tokyo	VMK / (1); bovine kidney/ (53); rabbit kidney/ (3).	(1967)	(Kono et al., 1969; Kabiri and Moattari, 1993)
	KRT/ TAKAHASHI (MAT)	patient Throat swab / Matsue city	VMK/ (4); primary rabbit testicle (36); primary rabbit kidney/ (1).	(1968)-	(Kono et al., 1969; Kabiri and Moattari, 1993)
	MEQ11/ Matsuura	patient Throat washing / Osaka	VMK/(14); Chick amnion/(65); quail embryo fibroblast cells/(11);	(1966)	(Kono et al., 1969; Kabiri and Moattari, 1993)
	TO-336	Pharyngeal secretion postnatal acquired child with /Toyama, Japan	VMK/(7); primary guinea-pig kidney/(20); primary rabbit kidney /(3);	(1967)	(Kono et al., 1969; Kabiri and Moattari, 1993)
	SK/ Matsuba strain	patient Throat washing / Kumamato Japan	VMK/ (1); swine kidney/ (60); rabbit kidney/ (6).	(1969)	(Kono et al., 1969; Kabiri and Moattari, 1993)
China	BRD-2/2BS	postnatal acquired case/ Beijing, China	Human diploid cells / (30).	(1980)	(Kirby, 2015)

Table 2. Neurovirulence of TAKAHASHI/HDC strains for baby hamster after different passages in diploid cells

	Virus Strain	**Titer ($CCID_{50}$/ baby Hamster brain) In different passages on diploid cells**				
		0*	5*	10*	15*	20*
Attenuated Strains	TAKAHASHI/RT	4.45	4.75	5.25	5.55	5.75
	TAKAHASHI/HDC	4.55	4.75	5.55	5.45	5.75
	RA-27 /HDC	4.17	4.65	5.4	5.15	5.75
Wild Strain	M-30	4.45	4.67	5.14	4.37	5.4

*****Number of passages of the virus in MRC-5 cells
TAKAHASHI/HDC strain was passaged 20 times in MRC-5. An inoculum of 0.02ml of these viruses was inoculated intracerebrally into day-old hamster. The brains of three animals in each group were harvested daily. The titer was measured after 20 passages by $CCID_{50}$/dose in five intervals.

Table 3. Determination of the rct40 Marker of Rubella TAKAHASHI after adaptation on HDC

Virus Strain	**passage At ^{O}C**	**No. of passages At the given temperature**	**Log TCD_{50}/ml (mean values)**		**Log I 40***	**Index rct40+**	**rct40 character +**
			35 ^{O}C	**40 ^{O}C**			
TAKAHASHI/RT	33	0	5. 55	2.25	3.30	0.94	-
		5	4.75	1.25	3.50	1.00	-
TAKAHASHI/HDC	33	0	5.75	2.25	3.50	1.00	-
		5	5.55	2.25	3.30	0.94	-
RA-27/ HDC	33	0	4.75	1.25	3.50	1.00	-
		5	4.55	1.25	3.30	0.94	-

*Log I 40= index of inhibition at 40ºC, i.e. the difference between Log TCD_{50} at 35°C and 40ºC. + Index rct40= ratio of Log I40 of a given strain passed 5 times in MRC-5 cells to that of its original virus. + Character rct40 was determined on the basis of indices as follows: >0.75=rct40 -, 0.75-0.33= rct40 ±, <0.3= rct40 +.
This result of rct40 marker value showed that after five passages in MRC-5/HDC cells, it was comparable with the original virus before adaptation to HDC. This marker did not change in 5 serial passaged in MRC-5c ells.

Table 4. Determination of the T50 Marker of Rubella TAKAHASHI after

Virus Strain	Passage At ^{O}C	No. of Passages At the Given Temperature	Log TCD_{50}/ml (mean values)		Log I T50*	Index T50*	T50*character
			50^{0}/15 min	No treatment			
TAKAHASHI/HDC	33	0	4.37	5.75	1.38	1.0	-
		5	1.62	4.75	3.25	1.3	-
		10	3.25	6.50	3.25	0.9	-
		15	3.12	6.50	3/38	0.75	-
		20	3.3	6.50	3/20	0.80	-
RA-27/ HDC	33	0	4.37	5.37	1.0	1.14	-
		5	4.62	5.87	1.25	1.43	-
		10	4.25	6.5	2.25	0.9	-
		15	3.50	5.50	2.0	0.80	-
		20	3.12	5.00	1.87	0.78	-

*Log T50= index of inhibition at 50ºC/15min i.e. the difference between Log TCD_{50} with treatment at 50°C for 15 minutes and without treatment

Table 5. Close Study: Seroconversion rate in seropositive children and increase titer following two doses of Rubella TAKAHASHI

Virus Strain	Virus content CCID50/dose	Age at first vaccination Month(Number)	Age at Second vaccination Month(Number	Total children inoculated	% Seroconversion After first vaccination	Increase of Titer after the second vaccination
TAKAHASHI	3.87	9-11(8)	18-24(16)	24	100	2-3 folds
RA/27	4.00	9-11(8)	18-24(16)	24	100	2-3 folds

An increase of at least 2-3 folds in HI titers was observed following reimmunization with both vaccines.

Table 6. Seroconversion rate after vaccination against Rubella/TAKAHASHI in Seronegative

Virus Strain	Total children inoculated	Seroconversion after 1st dose		Seroconversion after 2nd dose	
		Number	% Percentage	Number	Percent
TAKAHASHI	120	95	79/1	120	100
RA/27	126	76	60.3	126	100

Table 7. Seroconversion rate in susceptible children after vaccination against rubella TAKAHASHI

Strain	Pre vaccine HI Titer	No of children	Post vaccination		Titer Increase
			Decrease	No change	
TAKAHASHI	1:8*	130	53	0	0	53(≤95%)
	1:16		25	0	1	24(96. %)
	1:32		20	3	0	17(85. %)
	1:64		15	2	0	13(86. %)
	1:128		12	2	0	10(83.33)
	1:256		5	1	3	1(20. %)
RA/27	1:8	130	53	0	0	58(≤95%)
	1:16		25	0	2	18(90%)
	1:32		20	2	4	14(70. %)
	1:64		15	1	2	12(80. %)
	1:128		12	--	1	12(92.30)
	1:256		5	1	3	0(0. %)

*Pre vaccine HI Titer in 76 seronegative cases increased to ≤ 1/8. Post-vaccination Rubella antibody increased to (≤95%) in all cases.

Table 8. Bootstring Effect against Monovalent Rubella TAKAHASHI Vaccine in Children after two Doses

Virus Strain	Total children Inoculated (CCID50/dose)	Pre Seronegative		Post Seropositive	
		No	%	No	%
TAKAHASHI	130 (3.87 - CID50/Dose)	95	73.0*	130	100
RA/27	130 (4.0 - CID50/Dose)	87	66.93	130	100

*In %73 Pre Seronegative children, antibody increased to %100 by a Booster dose

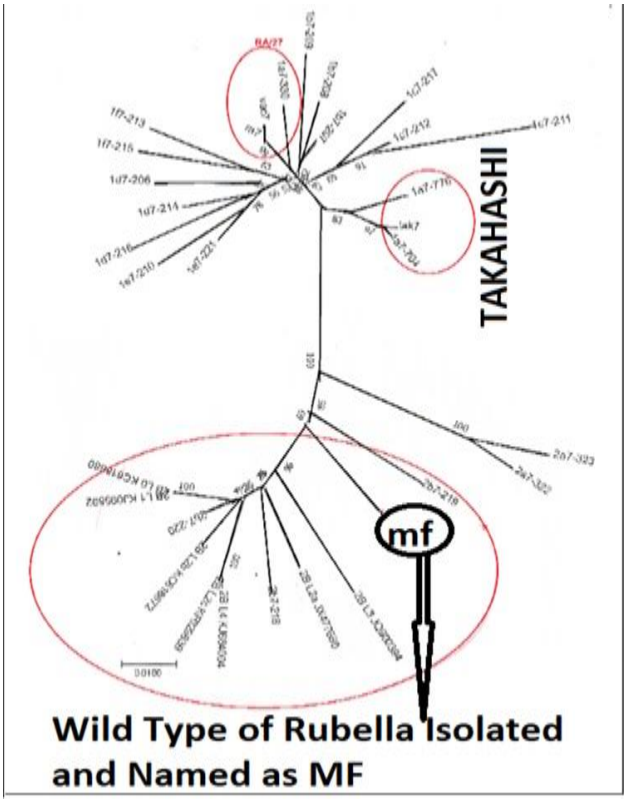
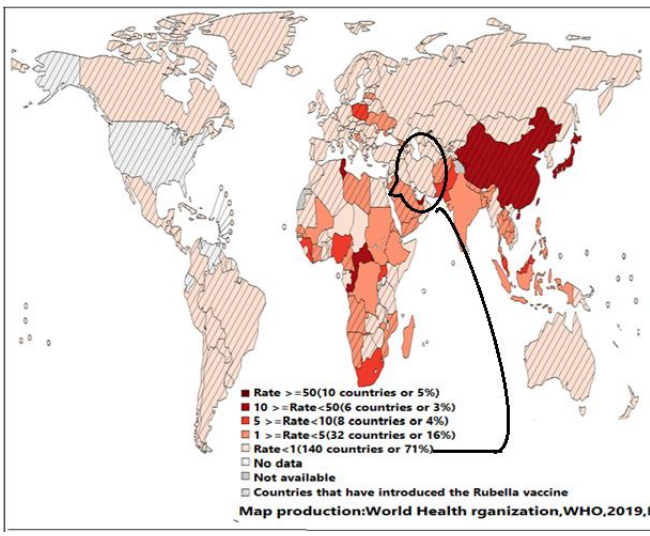


Figure 1. Phylogenetic analysis of Iranian isolated local rubella virus sequence (Rvi / Iran / 00 / DQ975202.1) that was classified into 2B genotype
Although this isolate is located far away from other isolates (2BL0-2BL4), it does not fall into any of the subdivisions and is still identified under genotype 2B, compared to the isolates in the gene bank.



Top 10 countries (last 12 M 2018-2019)			
label	RCV in RI	Cases	% of Total
Pakistan	No	1056	66
Sudan	No	148	9
Tunisia	2004	121	8
United Arab Emirates	1985	102	6
Afghanistan	2018	43	3
Saudi Arabia	1991	38	2
Others	-	25	2
Libya	1995	24	2
Iran (Islamic Republic of)	2004	14	1
Yemen	2015	12	1
Morocco	2014	7	0

Figure 2. Rubella cases-EMRO: In the last decade, 470 million doses of vaccine were administrated and in the last two decades, 2.5 million lives were saved using the vaccine (WHO/2019.https://www.who.int/immunization/global vaccine action plan/GVAP review lessons learned/ (accessed November 21, 2019).

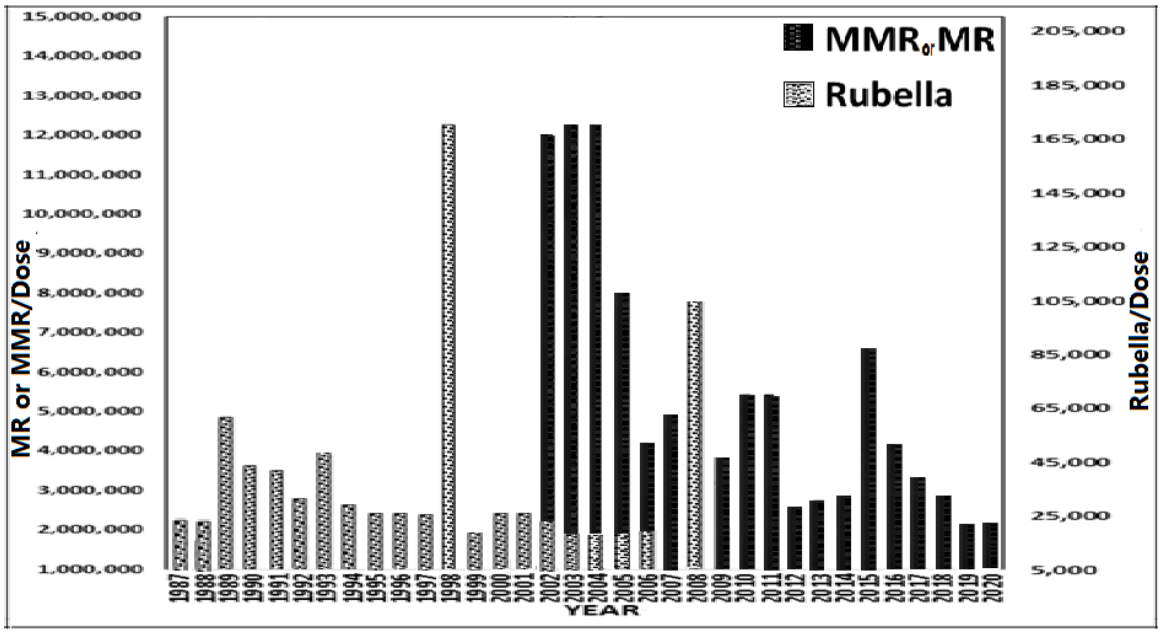


Figure 3. Local Rubella Vaccine Supply between 1987 and 2020. During this period, more than million doses of vaccine were produced as monovalent or in combination with MEASLES (MR) OR MUMPS (MMR) used for immunization.

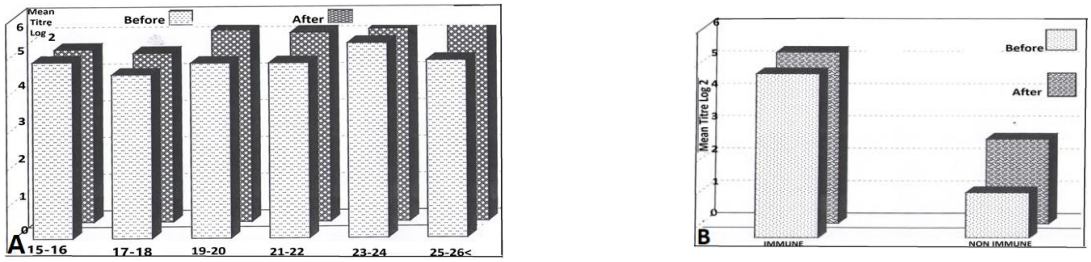


Figure 4. Evaluation of the immunogenicity of Rubella TAKAHASHI in combination with MMR vaccine (AIK-C, S#12,) strains in adult volunteers.
A. After receiving the vaccine, among the adult volunteers aged 17-37, 14.2% of cases showed the effect of a booster dose, and the antibody titer increased. In a similar vein, in 33.9% of cases, the increase has doubled, and the antibody titer has remained constant in 41.3% of subjects. **B.** In the with no history of infection or vaccination (Nonimmune), in 100% of cases, the average geometric titer was increased to 2.5 based on Log 2. As depicted in Figure B, 97.9% of cases reported a history of rubella disease or vaccination who were in the immune group and were seropositive before receiving the vaccine. In this study, the results were evaluated by the HI test.

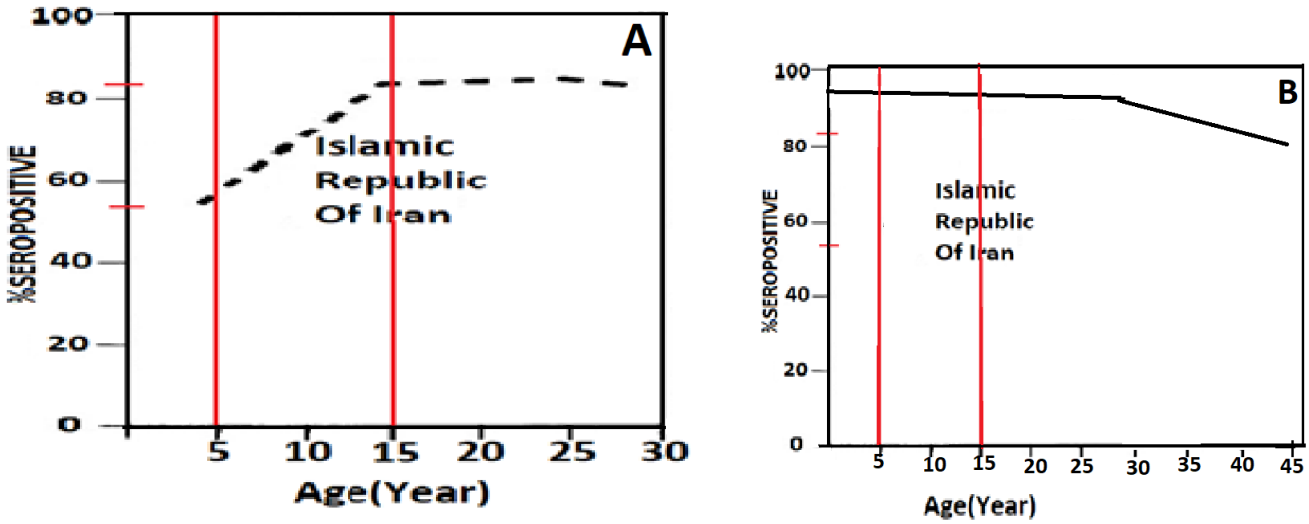


Figure 5. Rubella susceptibility among women of childbearing age in Iran: **(A)** Before vaccination era; **(B)** after vaccination era: Prior to Expanded Program on Immunization (EPI), the overall immunity was 29% at 1 year of age in Iran. It reached >95% after EPI by vaccination. According to epidemiological studies in the age of> 30, the seropositivity rate reached 75%.

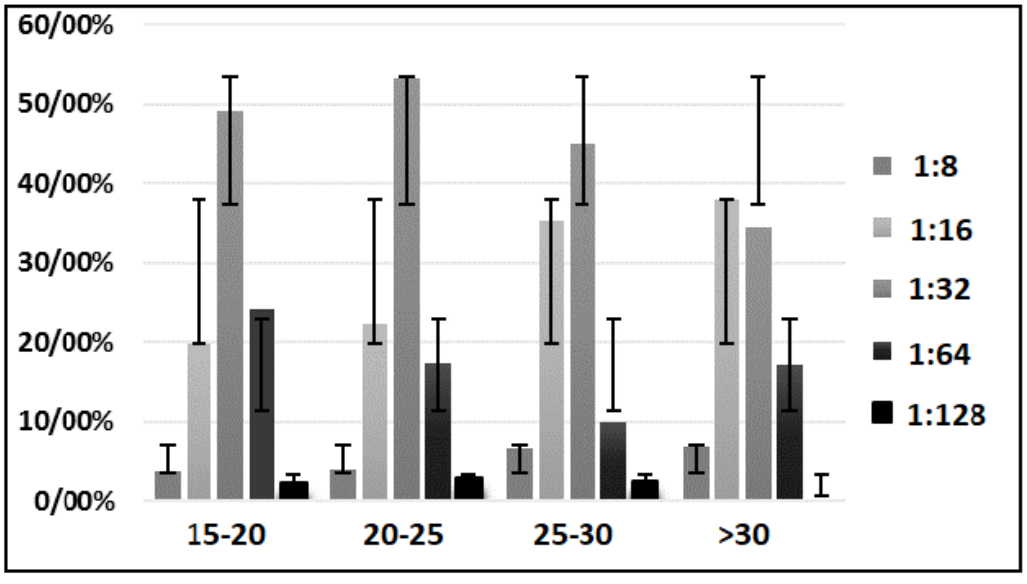


Figure 6. Distribution of maternal antibody in childbearing women according to age before Expanded Program on Immunization

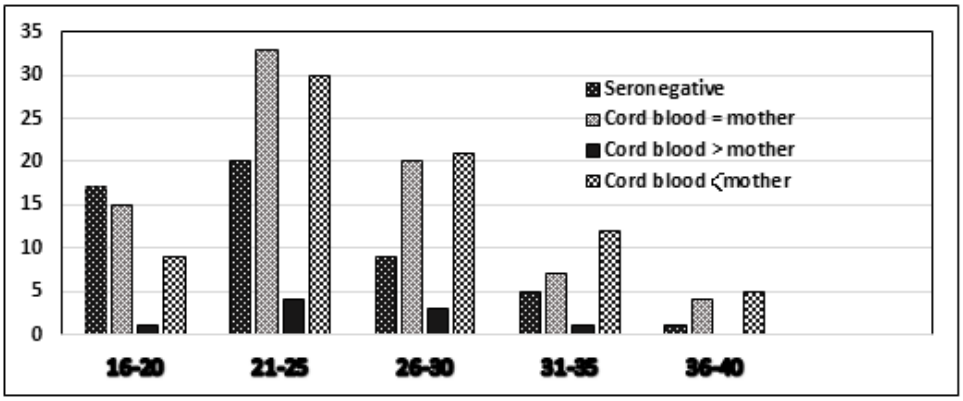


Figure 7. Comparison of Rubella maternal antibody among women of childbearing age in comparison with their infant's cord blood

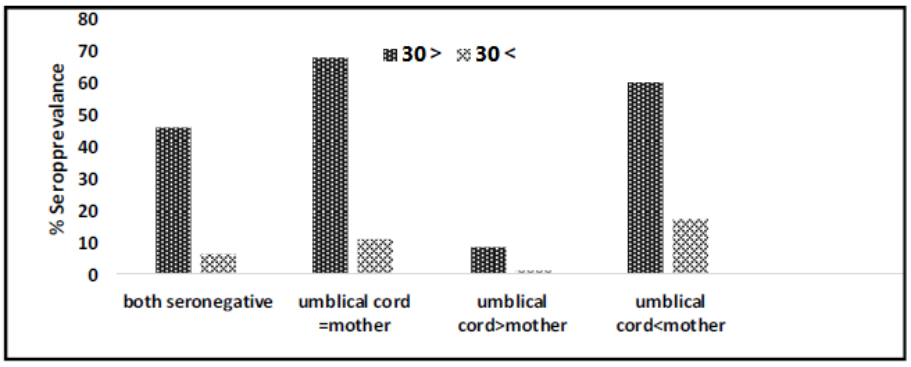


Figure 8. Comparison of Rubella Ab in two groups of mothers (under 30 and above 30) with respect to their infant's cord blood

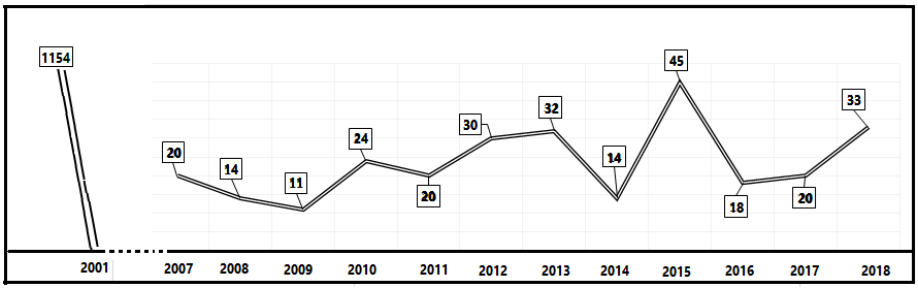


Figure 9. Confirm Rubella Case 2001-2018**:** Incidence rate of Confirmed Rubella cases from 2001-2018 in Iran. (https://www.who.int).

4. Conclusions

All new or unknown viral pathogen causes epidemics of infectious disease in society; nonetheless, these diseases can be preventable in the case of pathogen recognition and the development of effective vaccines. Rubella is a leading cause of vaccine-preventable birth defects. Rubella virus infection usually causes a mild febrile rash illness in children and adults, infection during pregnancy, especially during the first trimester. Nonetheless, it can result in miscarriage, fetal death, stillbirth, or a constellation of birth defects known as CRS. The development of the rubella vaccine has been a progressive change introduced by RVSRI in IRAN. Progress toward rubella vaccination has accelerated since 1972-1977 after the production and successful trial of the local vaccine that was merely available in the private sector. When the relative immunity level of the community was not low, this vaccine has increased the immunity of the population against the disease from 1980. In 1989, a routine second dose of MMR-containing rubella vaccine was introduced for preschool-aged children as the commencement of the elimination effort. In 1993, the rubella immunosurveillance of Iranian females was an indication of the emergence of the rubella outbreak in Shiraz, Iran (Kabiri and Moattari, 1993). The increased number of CRS was also reported in some of the East Mediterranean Region countries at the same time.

Due to the increased cased of CRS in this region, it was decided to include the rubella vaccine in the national immunization program. A local vaccine was used and helped to assess rubella vaccine introduction among all at-risk population that increased to 44,263,660 million (1.4%) during four decades. In the pre-vaccine era, the overall immunity was 29% among one-year-old neonates, and approximately 23% of women of childbearing age were sensitive to rubella infection (Saidi, 1972). Within four decades (1975-2005), since the production of the MR (measles and rubella) vaccine in Iran, the epidemiology of both diseases underwent some dramatic changes.

The measles and rubella vaccines were locally produced and used for immunization in the private sector since 1967 and 1977, respectively. In 1984, EPI began in Iran. More than > 95% coverage of two doses of MMR has been constant since 1993 (Zahraei et al., 2019). The access to vaccines led to two mass campaigns in 2003 and 2005. The introduction of RCV into national immunization schedules and two campaigns in 2003 and 2005 covered approximately 50% (33,579,082 million of 68 million) of people in the age group of 5-25 years who received the rubella vaccine combined with measles (MR). The ease of access to the vaccine resulted in a three-fold increase in coverage (38%-99%). After the EPI initiation in Iran, the proportion of immune people increased to 91%. The overall pooled rubella immunity rate increased to 90.1% among pregnant women in 2019 (Azami et al., 2019). The inclusion of the local MMR vaccine in the EPI in order to reduce measles mortality and eliminate this disease from Iran has significantly reduced the incidence of rubella to <1 case per 1,000,000 since 2010. However, sporadic transmission continues, especially in areas with immigrant and nomadic populations.

Not only an epidemic of rubella occurrence but also a high incidence of CRS had never been experienced in this country based on a review of the published article. Access to a local vaccine facilitated widespread rubella vaccination .This vaccine has been noticed among children and young adults in various parts of Iran during the four past decades from 1980 till now (Esteghamati et al., 2007). The WHO (2018) has reported a 91% decrease in the confirmed cases from 2000-2018 in Iran. These results are displayed in Figure 9.

There are a few investigations about CRS surveillance not only before but also after the introduction of the rubella vaccine. Although various studies have shown no evidence of CRS from vaccine strains, it is reasonable to assume that there is no need for surveillance of CRS after vaccination. In addition, after the rubella vaccination in Iran, the

epidemiological pattern of disease changed and maybe impossible to assess.

The protective nature and durability of rubella TAKAHASHI immunity after vaccination is the most important factor for the assessment of the value of this vaccine. Different descriptive cross-sectional studies were conducted on women under 25 and those who aged over 25 years before and after receiving the Rubella TAKAHASHI vaccine in Iran. These Serosurveillance data assumed that immunity to rubella significantly increased more than 95% with aging, and a significant association was found between age and antibody titer. Along with the timely production of the rubella vaccine, the achievement of EPI and a high coverage level in vaccination has also been applied. Therefore, an increased level of immunity in women of childbearing age can decrease the risk of rubella and CRS in children. Highly affordable local vaccines have played a critical role in constant efforts and led to dramatic changes in the epidemiological patterns of rubella and related complications. The results of most studies published in the post-vaccination period pointed out an increase in Seropositivity and a decrease in rubella cases, signifying the effect of vaccine use, its coverage, and viral vaccine circulation (Figures 4, 5, 6, and 7).

Out of 196 countries of WHO, rubella elimination has been verified in 81(42%) countries (2018). In Iran as one of these countries, the number of confirmed rubella cases declined from 1124 (53%) in 2000 to 33 (91%) in 2018 (Figure 9). Meanwhile, the population increased from 65.5 in 2000 to 82 million in 2018 (Figure 4B). National progress toward the control and elimination of rubella and CRS from 1972-2018 (the most recent data) resulted in an increase in herd immunity from 72% in 1972 to ≥95% in 2019 (Figure 4).

On 28 May 2019, WHO declared that rubella has been eliminated from the Islamic Republic of Iran, and Measles is about to be eradicated. The vaccination coverage and surveillance for rubella and CRS should be continued to make further progress and maintain an eliminated country. According to WHO, many children worldwide die from vaccine-preventable diseases each year. In 2015, the number of deaths in children aged <5 years in the world was 25% which can be easily prevented with vaccines. According to Iran's Ministry of Health report, the death rate in children under five years of age in 1978 was 154 /1000 live births (%15/4) which decreased to 16/1000 (%1/6) live births in 2016.

Since 1829, the production of the local vaccines has been made possible by the great efforts of scientific members, appropriate scientific background, suitable context, and capability of the RVSRI, as well as the financial support. The Iranian children were vaccinated in the 1940s and 1950s by the local Razi vaccine, while there was no EPI to organize appropriate vaccination services. The death rate in children under five years of age in 1978 was about 154/1000 live births which decreased to 20/1000 in 2010. These results indicate a 70% reduction in pediatric mortality by vaccine-preventable diseases.

The immunity situation of the population in our country against vaccine-preventable diseases and elimination of rubella, measles, poliomyelitis, despite the high-risk borders, is one of the most important economic effects brought about by native vaccine production in Razi Institute.

Undoubtedly, this huge success would not have been possible without the commitment and support of the country's top policymakers. This strategic production will continue on the basis of the existing technology, along with updating and transferring advanced technologies. Only by this way can we ensure that the disease is eliminated with the timely implementation of public health measures. Failure to support the technology of vaccine production is neither practical nor desirable for public health. Diseases can be eliminated from the country with continued production of the above strategic products despite the numerous existing problems.

Authors' Contribution

Study concept and design: A. M. and A. Sh.
Acquisition of data: A. M. and A. Sh.
Analysis and interpretation of data: A. M. and A. Sh.
Drafting of the manuscript: A. M. and A. Sh.
Critical revision of the manuscript for important intellectual content: A. M. and A. Sh.
Statistical analysis: A. M. and A. Sh.
Administrative, technical, and material support: A. M. and A. Sh.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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