Humoral Immune Response to Mumps (RS-12 strain) 
Vaccine of Children 1-7 Years Old

Short Communication

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Summary
The immunogeneticity of monovalent mumps vaccine, in order to conferring long time immunity against the wild virus, prepared from RS-12 local strain was evaluated. The paired serum samples were collected from a total of 160 children before and after vaccination against mumps with the monovalent vaccine. From these children, 156 ones showed an increased anti hemmaglutinin antibody by HI test. The geometric mean titer (log2) was calculated 3.04 from 96 seronegative cases, 95 of them were produced detectable antibodies. Seroconversion of RS-12 strain is estimated 99%. Booster effect of the vaccine is confirmed by 4-fold increasing in HI titer in 64 seropostitive cases. Increasing of immune case up to 140 (87%) is the other reason for effectiveness of the vaccine. Consequently RS-12 attenuated strain can be a suitable candidate for production of monovalent mumps and polyvalent vaccines, in combination with the other infectious agents, which set up on conventional vaccination program.

Key words: mumps, vaccination, immunization, SN test, HI test

Introduction
More than fifty years for prevention from mumps contagious infection due to numerous complications particularly aseptic meningitisis, encephalitis,

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meningoencephalitis, orchitis and ophoritis, that all groups frightens, productions and using of types of mumps inactivated and attenuated vaccines have been an effective and marked attempt (Belshe 1991). Inactivated vaccines made by formalin, ether and UV, are not used many years because of various defects including unstable protection, necessity of booster injection repeating, lack of producing antibodies against fusion glycoprotein (F) and delay in stimulation of cellular immunity (Belshe 1991, Fields et al 1996, Rooyen & Rhodes 1948, Norrby & Penttinen 1978). Successes in immunization with attenuated strains were achieved by subculture and using of low temperatures for the virus growing (Belshe 1991, Buynak & Hilleman 1966, Sasaki et al 1976).

At present more than ten vaccine strains of mumps are used. The RS-12 and Rubini are two strains that were attenuated on human diploid cells and cause fewer problems in sensitive persons (Plotkin & Orenstein 1999, Sassani et al 1991, Ikic et al 1970). Live mumps vaccines are produced and used as monovalent and polyvalent in combined with every vaccine strains of measles, rubella or both (MMR) (Plotkin & Orenstein 1999). In future common form of using mumps vaccine is MMR and according to WHO time-table best age for first and second dose inoculations are 12-15 months and 4-6 years, respectively (Fields et al 1996, MMWR 1998). Some researches have indicated that the immune stability period is 8-12 years after vaccination (Mirchamsy 1996, Plotkin & Orenstein 1999).

In study on immunization of mumps vaccine strains among six major structural proteins, hemagglutinin-neuraminidase (HN) glycoprotein is a proper antigen evaluates success of vaccine in effective stimulation of immune system in vaccinated persons (Ennis et al 1968, Fields et al 1996). So study on neutralization antibodies, other assure quefitient will be for certificating of vaccine immunization (Hilleman 1967). Antibodies against HN antigen maximize during about 3-4 weeks, until six months fix in most level, then decrease duration two years, at last fix in a level for years. Detection of certain titer is a reason for
protection (Ennis et al 1968, Nelson 1996). In this study the advantages use of RS-12 strain instead of other mumps vaccine strains were discussed.

**Materials and Methods**

**Vaccine.** The monovalent mumps vaccine (RS-12 local strain) including $10^5$CCID$_{50}$/ml of attenuated virus (Razi Ins.) was used.

**Clinical trial process.** Groups under studying with agreeing of their parents were including heath children that come to Treatment and Health Center of Razi Institute. The first bleeding performed in vacuum 1-2ml from anticubital fosa area of 160 children (76 girls and 84 boys) with 1-7 years old. Then one dose of the mumps vaccine was injected in deltoid. The second bleeding was done 5-7 weeks later.

**Laboratory process.** Part one). Serum from each blood sample was collected and stored in $-20^\circ$C. Vero cell line bottles were washed by PBS buffer and inoculated with 18-20ml virus suspension of RS-12 strain and incubated in 33$^\circ$C for 1h, then 200ml DMEM was added and re-incubated in 33$^\circ$C. After six days and during three processes freeze-thawing, viral antigenic suspension was prepared. Titer of antigenic suspension was determined by hemagglutination test (HA) (Albrecht & Klutch 1981). This suspension was distributed in proper and sterile bottles and stored in $-40^\circ$C. The titer of coupled serum samples (related to before and after vaccination) was determined by using HI test.

Part two). Serum neutralization (SN) test (Collee et al 1989, Leland 1996) was performed on remains paired sera that had proper vacuum with using of virus suspension including $10^3$CCID$_{50}$/ml. The titer of neutralization antibodies were determined by carrying out of hemadsorption (HAd) test and appearing of CPE.

**Results and Discussion**

The titers of paired serum samples related to 160 children were determined by HI test. Maximum geometric mean of titer addition (GMat) was seen after
vaccination in age group 1-2 years (Figure 1). Same parameter in sex group was shown 0.01 log difference also it was estimated 2.61 in children with previous vaccination in comparison 3.2 for children without previous history of vaccination. Based on time interval of vaccination till secondary bleeding, samples were grouped and SN titers were calculated and compared (Figure 2). It was distinguished that after vaccination from 96 serum negative samples (>1/2), 95 samples had titer and their GMat was 3.79. Therefore seroconversion of RS-12 strain was calculated equivalent 98.96%.

![Graph showing GMat (log) before and after vaccination](image)

Figure 1. Competition of serum titer (HI) in injection of mumps vaccine RS-12 strain

Before vaccination 35 samples had immune titer (≤1/8) and after vaccination immune cases raised to 140 cases. By using t pair test a significant difference (P<0.05) of geometric mean of serum titer before and after vaccination was observed. The vaccine seroconversion and its success for immunizing children with confidence coefficient 95% were certificate by McNemar test. One week after vaccination, fever was determined as most common clinical symptoms (17 cases), 5 cases of parotitis and ear pain in 8 cases was also recorded. All these cases were removed in duration three days.
Comparison of HI and SN results for 29 coupled samples showed that number of titer raised cases after vaccination (28 cases) and GMat of these cases were equivalent in two tests. Comparison of GMat in two groups of girls and boys presented that difference between them wasn’t significant. Difference of GMat was estimated 0.58 (log₂) in two groups of children with and without history of vaccination. The results of this study indicated that in despite of available information (Belshe 1991) the best time for blood collection is 7–9 weeks after injection. From viewpoint of seroconversion, this vaccine strain was efficient (99%) in comparison with other strains such as Jerry Lynn (92%-98%), Urabe (94%), Hoshino (93%-95%) (Plotkin & Orenstein 1999). In addition RS-12 strain propagates on human diploid cell (MRC-5) whereas the other mentioned vaccine strains propagates on chicken embryo fibroblast (CEF) that can cause sensitivity in persons who sensitive to compounds of chicken embryonic and egg. In comparison with Rubini strain, which propagates on human diploid cell (WI–38) the seroconversion of RS-12 strain is more. Numerous records of vaccination failure with Rubini strain that were related to excess attenuation actually caused its

Figure 2. Comparison of titer addition (HI) based on interval of vaccination till secondary bleeding
useless (Germann et al 1996, Plotkin & Orenstein 1999). On the other hand, in comparison with Makino et al (1990) study booster injection of RS-12 strain mumps vaccine and its ability to raise first titer till 4-fold confirmed the efficacy of the strain. Moreover, lack of nervous complications such as aseptic menigitis and encephalitis 2-3 weeks after injection that were reported on other vaccine strains (Plotkin & Orenstein 1999) was considerable. In conclusion according to the results of this study since RS-12 strain has been isolated, prepared and produced in our country, it can be used in vaccine production procedure instead of other strains.

References


