



Trends in  
**Medical Research**

ISSN 1819-3587



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## Seroepidemiology of Measles in Primary School Students in Tehran, Iran

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**Abstract:** This cross sectional study was performed to estimate the prevalence of serological evidence of immunity to Measles in primary school students (age 6-12) that have a history of twice vaccination against measles, on 9 and 15 month old and determining the appropriate age to re-vaccination. Multistage sampling was used to select students to participate in the present study. Immunization status and documentary evidence of immunization was recorded from the Personal Health Record. Measles antibodies were measured using Enzyme-linked Immunosorbent Assay (ELISA). Sufficient blood for antibody testing was obtained from 1665 children, 975 (57%) were girls and 720 (43%) were boys, respectively and mean age was  $9.17 \pm 1.53$  years. On the whole, 1198 subjects (72%) were seropositive and 467 (28%) were seronegative at all. Among girls and boys, 72 and 71%, were seropositive, respectively. These differences were not statistically significant between the two sexes ( $p = 0.404$ ). Antimeasles antibody titer decreased with increasing age from 6 to 10 years old, (76.5% in 6 years old group vs. 71% in 10 years old group) and then rise to 78% in 11-12 years old group. These differences were not statistically significant between age groups ( $p = 0.775$ ). Results indicated that the immunity produced by the Measles vaccine is not enough. Decrease of protective effect of measles vaccine, suggests the necessary of pre-school revaccination.

**Key words:** Measles, immunization, antibody, children

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### Introduction

Measles is one of the contagious diseases which is still identify as one of fatal diseases in childhood. The incidence rate of measles has obviously decreased after extensive vaccination program, but some limited outbreaks occur in older patients, because of antibody falling down (secondary vaccine failure) (Cutt *et al.*, 1991).

The risk of serious complications is highest in young children and adults (Platkin *et al.*, 1994; Black, 1989). Of vaccine-preventable diseases, measles remains one of the most important causes of childhood morbidity and mortality in developing countries. Almost all unvaccinated children contract measles and over 2 million children die of this disease each year (Anonymous, 1995, 1994). Worldwide, it is estimated that 81% of children aged 1 year receive one dose of measles vaccine (CDC, 1998). An estimated 36.5 million cases still occur each year in the world and measles still accounts for 10% of global mortality from all causes among children aged <5 years (CDC, 1997).

In spite of efficient vaccines and widespread vaccination programs, Measles Virus (MV) remains a major human pathogen. Worldwide, it still causes over half a million deaths per year and is a major

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cause of child mortality. Death is often caused by accompanying secondary infections, which are favoured by MV-induced immunosuppression. Vaccine coverage of over 94% is required to interrupt transmission of measles in a population (Anderson and May, 1985). In under 6 years old children, Vaccine coverage of over 93% reduce the transmission to a very low level (Hutchins *et al.*, 2004). Actual coverage is much lower, particularly in developing countries, where cost and lack of infrastructure are a hindrance. However, even in countries with adequate health systems, vaccine coverage is not high enough to prevent spread of the highly contagious virus. In 2002 over 10, 000 cases of measles were reported in Western Europe and many more may have gone unreported, as measles is not a notifiable disease in many countries (Anonymous, 2002).

Revaccination in the vaccinees with low or undetectable neutralizing antibody titres can maintain a significant lymphoproliferative response 6 months after revaccination (Ward *et al.*, 1995). Since the half-life of human IgG is 1-2 months (Lee *et al.*, 2001; Caceres *et al.*, 2000), the long-term virus-specific antibody levels are maintained by antibody-secreting cells (Plasma cells) and memory B cells (Slifka and Ahmed *et al.*, 1996). Thus, vaccines with a lower decay rate may keep their antibody-secreting cells living longer. Therefore, antibody titers in the 1 and 2-dose vaccinees may not differ significantly in an intermediate period ( $\leq 6$  years) post vaccination but the 2-dose vaccinees may have a better cellular immune response and immune memory. This hypothesis may explain why the 2-dose vaccinees have a lower risk of measles infection than the 1-dose vaccinees who have received vaccination after 12 months of age to reduce the risk of primary vaccination failure in the USA and Canada where the boosting from wild virus is rare (Thomas *et al.*, 1999; De Serres *et al.*, 1999).

Measles protective immunity includes humoral and cellular immune responses. Several studies have found that the vaccinees with undetectable or low antibody titers may not necessarily be susceptible to symptomatic measles infection (Lee *et al.*, 2000; Bin *et al.*, 1991; Bartoloni *et al.*, 1997; Whittle *et al.*, 1999). Therefore, it may be necessary to combine the quantification of antibody titers with the detection of immune memory, such as by the lymphocyte proliferation assay (Ward *et al.*, 1995), to develop more reliable indicators for estimating the levels of population immunity. Routine vaccination against measles for infants was introduced in Iran in 1967. Despite the reduction of measles's mortality and morbidity, we are still having local epidemics especially in ages of 15-20 years and this disease is still one of important health sector problems in Iran (Moradi *et al.*, 2001). Measles is a preventable disease and can be eradicated by increasing the vaccine coverage and promoting the motivation for vaccination, in accordance with the worldwide measles strategy (Nakayama *et al.*, 2003). The effectiveness of vaccination programs varies depends on age of subjects in vaccination time, coverage of vaccination programs, quality and specifications of vaccines and planning of immunization programs (Cvjetanovic *et al.*, 1998).

Community-based seroepidemiologic studies are performed to monitor the effectiveness of measles immunization programs and to estimate the decay rate of vaccine-induced measles IgG titers. This study was performed to estimate the prevalence of serological evidence of immunity to Measles in primary school students (age 6-12) that have a history of twice vaccination against measles, on 9 and 15 month old and determining the appropriate age to re-vaccination.

## **Materials and Methods**

The survey was performed on 6-12 years old primary school students from 19 educational sectors of Tehran (2003-2005). The sample size was determined considering an expected prevalence of measles virus antibodies of 70% with a worst acceptable error of 5% and a confidence interval of 95%.

Multi stage sampling was used to select schools and then students from 19 educational sectors of Tehran; Overall, 1779 students were enrolled voluntarily and Informed consent was obtained from parents or legal guardians of students after informing them about the study.

Samples of 2 mL venous blood were taken from each subjects and the serum was separated and frozen at-20°C. Measles specific antibody titer (IgG) was estimated using MEASLES IgG ELISA DSL-05-10-MEG, DSL (Italy). Sensitivity = 96.4% and Specificity = 100%

Using this kit, IgG titers >1.1 IU mL<sup>-1</sup> were considered as positive. Amounts between 0.9 and 1.1 IU mL<sup>-1</sup> were considered as borderline and IgG titers less than 0.9 were considered as negative. Data analysis was carried out with SPSS 11.5 using Chi -square and Fisher tests.

## Results

Out of the 1779 selected students, 114 were excluded from the study due to incomplete measles two dose vaccination, inadequate amount of blood, hemolysis of blood, or absence of a questionnaire. Thus 1665 subjects were enrolled, 945 (57%) of whom were girls and 720 (43%) were boys. mean age was 9.17±1.53 years.

Anti measles antibody titer was negative in 385 subjects, borderline in 82 subjects and positive in 1198 subjects. Borderline cases were also considered as seronegative because of their immunity status are not acceptable. Thus overall seronegative cases were 385+82 = 467 (= 28% of all cases) subjects and seropositive cases were 1198 (72% of all cases) (Table 1).

385 negative cases consist of 210 girls and 175 boys. Eighty two borderline cases consist of 51 girls and 31 boys. Also from 1198 positive cases, 684 cases were girls and 514 cases were boys. From 945 girls, 261 cases (28%) were seronegative; and from 720 boys, 206 cases (29%) were seronegative. There was no statistically significant difference in seronegativity proportion with regard to gender (29%, in males and 27%, in females (p = 0.404).

The percentage of seronegative subjects in 6, 7, 8, 9, 10, 11, 12 years groups were 23.5, 26, 29, 27, 29, 29, 22%, respectively. There were no statistically significant differences in seronegativity proportion with regard to age (p = 0.775) (Table 2).

Table 1: Prevalence of antimeasles virus IgG by sex in Tehran

Serology	Girls		Boys		Total	
	No	%	No	%	No	%
Negative	220	23	175	24	395	24
Borderline	53	6	31	4.5	84	5
Positive	672	71	514	71.5	1186	71
Total	945	100	720	100	1665	100

Table 2: Prevalence of antimeasles vims antibodies by age group in Tehran

Serology age group	Negative		Borderline		Positive		Total	
	No	%	No	%	No	%	No	%
6	6	17.5	2	6	26	76.5	34	100
7	61	23.5	7	3	192	73.5	260	100
8	74	25	13	4	210	71	297	100
9	71	21	19	6	243	73	333	100
10	79	24	17	5	233	71	329	100
11	25	38.5	5	7.5	35	54	65	100

Table 3: Prevalence of antimeasles virus antibodies by geographical group in Tehran

Serology	Northern <sup>I</sup>		Western <sup>II</sup>		Eastern <sup>III</sup>		Southern <sup>IV</sup>	
	No.	%	No.	%	No.	%	No.	%
Negative	102	20	103	26	77	24	103	24
Borderline	27	5	21	5	13	4	21	5
Positive	385	75	274	69	229	72	310	71
Total	514	100	398	100	319	100	434	100

I: Northern part consists of 1,3,4,7 and 8 educational sectors of Tehran, II: Western part consists of 2,5,6,9 and 18 educational sectors of Tehran, III: Eastern part consists of 12, 13, 14 and 15 educational sectors of Tehran, IV: Southern part consists of 10, 11, 16, 17 and 19 educational sectors of Tehran

Table 4: Prevalence of antimeasles virus antibodies by history of vaccination against mbella in Tehran

Serology	Routine vaccination		Measles vaccine+MMR after 15 month		Total	
	No.	%	No.	%	No.	%
Negative	356	27.5	111	33	467	28
Positive	942	72.5	256	67	1198	72
Total	1298	100	367	100	1665	100

The proportion of seronegativity was highest (31%) in the western educational sector of Tehran and in the northern, eastern and southern educational sectors were 28, 28 and 29%, respectively. These differences were not statistically significant ( $p = 0.450$ ) (Table 3).

From 1298 subjects that had a history of 2-dose measles vaccination in 9 and 15 months of age, 356 (27.5%) were seronegative and 942 (72.5%) were seropositive. From 376 subjects that received 2-dose vaccine with 1 dose of measles vaccine at 9 months of age and 1 dose of MMR after 15 months of age, 111 (30%) were seronegative and 256 (70%) were seropositive. There was no statistically significant difference in seronegativity proportion with regard to kind of vaccination ( $p = 0.296$ ) (Table 4).

## Discussion

In the present study 1665 school age children (6-12 years old primary school students) from the 19 educational sectors of Tehran were enrolled, 945 (57%) of whom were girls and 720 (43%) were boys. Anti measles antibody titer was positive in 1198 subjects, borderline in 82 subjects and negative in 385 subjects. Thus there were 467 (28%) of study subjects susceptible to disease. All of subjects have a history of twice vaccination against measles, on 9 and 15 month old. In a study that was performed in Mashhad City in 2002 the measles IgG seroprevalence, reached almost 70%. They collected 172 students from 7 parts of Mashhad and tested for measles specific IgG antibodies by ELISA method. The age of these subjects was 14-19 years and divided in 6 groups. They found that 50 (29%) of the subjects were seronegative (Sarvghad, 2003). In another study that performed in less than 5 years old children, of them 668 subjects from western Azarbaijan, 507 subjects from southern part of Tehran and 887 subjects from Boushehr, showed that immunity ratio in western Aazarbaijan was 80%, in southern part of Tehran was 79% and in Boushehr was 78% (Saidi *et al.*, 1984). The first reason of these differences is difference of age between these studies. The second reason is that answer to vaccine and antibody decay phenomenon are depend on several factors that maybe different in some areas. Secondary vaccine failure in this study was 13-20% that increase with age and in other studies has reported between 2 and 20% (Saidi *et al.*, 1984; Mathias, 1989; Zhonghua, 1987; Xiang and Chen, 1983; Krugman, 1983) and alter in different populations (Christenson and Bottiger, 1994; Markowitz, 1990; Olsha, 1994).

In our study from 945 girls that enrolled to study, 684 cases (72%) were seropositive and 261 cases (28%) were seronegative and from 720 boys that enrolled to study, 514 cases (71%) were seropositive and 206 cases (29%) were seronegative (Table 1). There was no statistically significant difference in seronegativity proportion with regard to gender 206 of 720, 29%, in males and 261 of 945, 28%, in females ( $p = 0.404$ ). Present result is comparable with similar studies. In study that was performed in 1988, in 3 level of primary school, secondary School and high school, girls were more susceptible to measles; but there was not a statistical significant difference between two sexes (Mokhtari Azad *et al.*, 1993).

Males were significantly more seronegative than females ( $p < 0.0001$ ) (Sarvghad, 2003).

In a study that was performed to evaluate the immune status to measles among 375 school children in south of Tehran, seronegativity was 20%. Similar to our study they didn't detect a significant difference and also between two gender groups, but they detect a significant difference between age groups and lower age groups were more susceptible to measles. The reasons for this difference in the children in parts of Iran are unknown and need further study to clarify (Mokhtari Azad *et al.*, 1993).

The percentage of seronegative subjects in 6, 7, 8, 9, 10, 11 and 12 years groups were 23.5, 26, 29, 27, 29, 29 and 22%, respectively. It seems that the age group of 8-10 years old, are the most susceptible group to measles; but there were no statistically significant differences in seronegativity proportion with regard to age ( $p = 0.775$ ) (Table 2).

The most cases of seronegative subjects found in age 14-17. Thus, the correlation of the age and negative results in that study was significant ( $p = 0.0003$ ) (Sarvghad, 2003). In comparison with the present study, we don't detect a significant difference between our age groups probably because that the age of our groups were very close and so did not differ.

As regards vaccination coverage is higher than of 1988, all of our subjects have a history of twice vaccination against measles, but in previous study subjects had a history of one dose vaccination; maybe because of absence of disease in community, the possibility of contact with virus and then increase in antibody titer has reduced and gradually in primary school students, immunity level due to vaccination has decreased (Mokhtari Azad *et al.*, 1993; Nkowane *et al.*, 1987). But in 1988, because of lower vaccination coverage, measles has existed in the community and causes relative increase in antibody titer.

Although in this study, subjects had a history of twice vaccination against measles, on 9 and 15 month old, the maximum positive antibody titer was 76.5% in 6 years old group, which can be due to primary failure and secondary failure.

#### *Primary Failure of Vaccine*

Vaccination program is appropriate if by use of it, after 12 month of age, more than 90 to 98% of susceptible individuals, become immune (Mokhtari Azad *et al.*, 1993), that show us between 2 to 10% of population would be susceptible (AAPC, 1990; Chen *et al.*, 1989; CDC, 1989).

Primary failure of vaccine has different causes include age of subjects in vaccination time, coverage of vaccination programs, vaccines quality, planning of immunization programs and specifications and type of vaccines (Cvjetanovic *et al.*, 1998). vaccination with the high-dose Edmonston-Zagreb or the AIK-C strain at 4-5 months was as good as vaccination with the AIK-C strain at 8-10 months and better than vaccination with the standard dose Schwarz strain at 8-10 months (Tidjani *et al.*, 1989; Morkowitz, 1990).

Method of prescription, maintenance quality of vaccines, method and amount of injection, exposure of light and temperature, vaccine usage in accompany with immunoglobulin and existence of maternal antibodies (Shelton *et al.* 1978; Wesley *et al.*, 1978) and nutritional status of individual (Wesley *et al.*, 1978), existence of an acute disease in the time of vaccination (Halsey *et al.*, 1985; Krober *et al.*, 1991; Ndikuyeze *et al.* 1988), vaccine usage in accompany with immunoglobulin (Krugman, 1971), race, environmental factors and hygiene status (Black *et al.*, 1986; Nieburg and Dibley, 1986) sex (Bromberg *et al.*, 1994) and immunity system status (Dai *et al.*, 1991; Markowitz *et al.*, 1992) are from primary failure factors, that consist some of our susceptible individuals.

#### *Secondary Failure of Vaccine*

Is the losing of vaccine induced immunity. Some of individuals in our study maybe lose their immunity to measles because of secondary vaccine failure.

In this study after the age of 10 years antibody titer rise and in 12 years old group, seropositivity receives to 78% and only 22% of individuals were susceptible to disease. In a similar study that performed at 1988 in southern part of Tehran, the results were similar to this study and the percentage of susceptible subjects in primary school students (9%) was higher than in guidance

School students (4%) and in high school students (4%) ( Mokhtari Azad *et al.*, 1993).

Increasing of antibody titer after the age of 10 years, in these studies is maybe due to contact with measles disease in the community and contact with wild virus (boosting phenomenon). In another study that performed in Ethiopia the seronegative proportion ( $< 100 \text{ IU L}^{-1}$ ) was 20% (95% CI: 16-25) in children 9-59 months old, declining to 9% (7-12) in 5-9 year olds and 5% (4-7) in 10-14 year olds (Enquselassie, 2003). In other studies that have performed in china, secondary vaccine failure after the age of 8 years, receives to 13%. They haven't contact with wild virus (Xiang and Chen, 1983).

Antimeasles antibody titer in four geographical area of Tehran was different. The proportion of seronegativity was highest (31%) in the western educational sector of Tehran and in the northern, eastern and southern educational sectors were 28, 28 and 29, respectively. These differences were not statistically significant ( $p = 0.450$ ) (Table 3). In other studies that have performed in less than 5 years old children, proportion of immunity have been 80% in azarbaijan, 79% in southern part of Tehran and 78% in Boushehr (Saidi *et al.*, 1984). In another study that performed in southern part of Tehran in 1988, from 165 student, 120 had positive titer (73%) that is similar to our result (Mokhtari Azad *et al.*, 1993).

Comparing routine 2-dose measles vaccination in 9 and 15 months of age, with 2-dose vaccine with 1 dose of measles vaccine at 9 months of age and 1 dose of MMR after 15 months of age, showed that from 1298 subjects that had a history of 2-dose measles vaccination in 9 and 15 months of age, 356 (27.5%) were seronegative and 942 (72.5%) were seropositive and from 376 subjects that received 2-dose vaccine with 1 dose of measles vaccine at 9 months of age and 1 dose of MMR after 15 months of age, 111 (30%) were seronegative and 256 (70%) were seropositive. There was no statistically significant difference in seronegativity proportion with regard to kind of vaccination ( $p = 0.296$ ) (Table 4).

According to these results and other studies that previously have performed in Iran, in 6-19 years old groups that have a history of one dose vaccination in 6 months of age, 80% of individuals had positive antibody titer. In comparing with studies that have performed in the United States and England, reported that 6-17 years after vaccination, more than 90% of individuals that had a history of measles vaccination in 1 year old, had positive antibody titer, indicate that vaccination before the age of one year, not only can induce primary vaccine failure, but also reduce vaccine stability. On the

other hand serologic studies indicate that vaccine induced immunity is persistent and has been reported between 8 to 23 years (Nkowane *et al.*, 1987). These studies indicate that if we perform vaccination in appropriate time and reduce primary vaccine failure, effectiveness and persistence of vaccine will be increased.

Recent studies from Croatian population showed that vaccination coverage higher than the reported 90-94% should be attained if one is to expect measles elimination (Borcic *et al.*, 2003). The immunization campaign prevented 90-95% of predicted cases. The campaign was appropriately targeted at children aged 10 years and under (Hellenbrand *et al.*, 2003; Mansoor *et al.*, 1998).

Significant findings of this study are:

- 28% of school age children are susceptible to measles.
- There were not statistical significant difference with regard to age and sex.
- There was not statistical significant difference with regard to geographic parts of Tehran.
- Antimeasles antibody titer reduces with age increasing in primary school students.
- The lowest antimeasles antibody titer was in 8-10 years old group.
- Maximum antimeasles antibody titer was in 6 years old group.
- There is a rising in antimeasles antibody titer, after the age of 10 years.

### **Conclusions**

In comparison of this study with previous 1988 study, susceptible age group decrease from 10-12 years to 8-10 years old, that maybe due to higher vaccination coverage and lesser virus contact in community; that indicate the necessity of revaccination in children under 10 years old. For availability, the best time is the time of school beginning (6 years of age).

### **Acknowledgements**

We would like to greatly thank the Deputy of Health and Physical Education of the Ministry of Education and the Chief Manager and Deputy of the school health system of Tehran as well as the health teachers who guided and explained the procedure of the study to parents and convinced them to give inform consent for enrollment into our study. We would also like to thank laboratory technicians and colleagues in Noor laboratory who helped us in conducting the study.

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