European Journal of Epidemiology **18**: 1085–1089, 2003.

INFECTIOUS DISEASES

Seroprevalence of neutralizing antibodies to measles virus in a vaccinated population in Iran, 1998

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Accepted in revised form 16 May 2003

Abstract. Measles is an acute highly infectious viral disease. Although live attenuated vaccine is used throughout the world, outbreaks of disease still occur in many countries including Iran. In this cross-sectional study, by implementing a viral neutralization test and cell culture techniques, the seroprevalence of neutralizing anti-measles antibodies was assessed. Three hundred and fifty-four blood samples were collected and random-cluster classified from healthy subjects 6 months to 16 years old, residing in the town of Khodabandeh and its rural areas. Of the total subjects, 174 (49.2%) were girls and 180 (50.8%) were boys. From 354 subjects studied, 310 (87.6%) had neutralizing anti-measles antibody titer of 1:8 or higher and were considered to be immune and 44 (12.4%) had lower antibody titers. At the time of specimen collection, information with regards to age, sex, history of vaccination and place of residence were collected. χ^2 statistical test demonstrated a significant association between immune status and grouped age at the time of first vaccination ($p < 0.009$). The proportion test indicated significant differences in rate of seropositivity in paired age groups (3–8 vs. 9–11 and 9–11 vs. 12–64 months) ($p < 0.02$). The use of reliable techniques for assessing success of vaccination programs and performing seroepidemiological studies in order to organize national programs of control and eradication of measles are necessary.

Key words: Immunization, Measles, Microneutralization assay, Seroprevalence

Introduction

Seroepidemiologic studies reporting prevalence of antibodies to specific viral infections are increasingly being used in infectious surveillance, such as measuring the impact of vaccination on population levels of immunity, identifying groups at risk of infection during outbreaks, and development of better vaccination policies [1–5]. If administered properly, live attenuated measles vaccine can induce life long immunity in greater than 85% of those vaccinated with one dose and about 90% with two doses [6]. Mass vaccination campaigns and Expanded Program of Immunization (EPI) have increased vaccine coverage in the world with a substantial impact on reduction of measles morbidity and mortality, but permanent elimination has proven to be difficult considering travel to and from endemic areas. Such a goal requires globally organized strategies. Understanding measles outbreaks that occur after the initiation of measles elimination efforts will be critical in refining the strategies for measles elimination [6, 7].

Measles vaccination was begun in Iran in 1967. In 1970, an efficient measles vaccine (AIK) was produced by Razi institute in Iran and vaccination programs were deployed. Until 1980, measles vaccination was given to 9 months old children and from 1980 the age of vaccination was reduced to 6 months. The current vaccination program was deployed from 1988; this new policy mandated the use of two doses of measles vaccines, one at 9 months and one at 15 months of age. The reported cases of measles was reduced from 346/100,000 in 1970 to 34/100,000 in 1976 and to 10/100,000 in 1991 [8].

In 1998, 2885 suspected cases of measles and four cases of death due to the disease were reported with the disease rate of 4.7 in 100,000 and mortality rate of 0.14%. Forty-two percent of confirmed cases of measles were detected in vaccinated individuals and in 58% of cases vaccination history was not clear or could not be found. In the same year, in the province of Zanjan, suspected and confirmed cases of measles were reported to be 13 and 8.2 in 100,000, respectively [7].

This cross-sectional study was designed to assess prevalence of neutralizing measles antibodies following reports of confirmed cases of measles in the town of Khodabandeh and its rural areas in Zanjan as an example of one of the districts in Iran in which higher incidence of measles was reported during recent years [7].

Materials and methods

Study design

Previous studies have indicated measles vaccine coverage of over 95% in Iran [7]. Therefore, prevalence of measles antibodies in the study group was expected to be 95%. Based on 1996 census in Iran, with $\alpha = 0.05$ and desired precision equal to 0.025, statistical analysis indicated that 300 sera were required. Considering estimated population growth by 1998, 71,000 children between ages 6 months and 16 years old would be living in the area of study. Therefore, a total of 354 serum samples were collected random cluster classified, 84 from children residing in the town of Khodabandeh (23.7%) and 270 from those living in 19 villages in the area (76.3%). In order to choose subjects for participation in this study, health records kept in health clinics were chosen randomly and subjects who were within appropriate age groups were invited to participate in the study. Parents' agreement was acquired prior to blood collection. Number of sera collected from each sex was almost equal. At the time of specimen collection, information regarding date of birth, sex, health status, number of family members, dates of first and second vaccinations as well as age, profession and level of education of parents were recorded.

Cells and virus

HeLa cells were used for propagation and titration of virus and in microneutralization assays. The source of virus was an Edmonston B (lyophilized vaccine, Razi vaccine and serum production Institute, Karaj, Iran), which was adapted to HeLa cells by repeated passages. Infectious virus titer was determined by preparing serial dilutions of virus in cell culture tubes and measuring TCID50.

Microneutralization test

Sera was heat-inactivated in 56 °C waterbath for 30 min. Two fold serial dilutions (1:2 to 1:256) of specimens along with positive and negative control sera were prepared in cell culture media (DMEM, Sigma). Fifty microliter of 100 tissue culture infectious dose 50 (TCID50) virus was added to 50 µl of each serum dilution and controls in microtiter plates. The plates were put on a shaker to mix their contents before being transferred to 36 °C incubator. After 1 h of incubation, 50 µl of serum–virus mixture was transferred to appropriate wells of another microplate containing monolayers of HeLa cells. The plates were incubated at 36 °C for 1 h in order for free virus to adsorb to monolayers. After this, 100 µl of DMEM containing antibiotics and 2% FBS was added to each well. For each plate, cellular controls (normal cells and medium without virus and serum) and viral controls (50 µl of prepared virus in 50 µl DMEM without serum) were also included. The microtiter plates were then put in 36 °C incubator and checked daily till day 7 for the presence of CPE. The highest dilution of test serum that could prevent CPE of measles virus was recorded as measles antibody titer [9, 10]. Protective neutralizing antibody titer was considered to be >1:8 in protected children and lower titers were considered unprotective in susceptible individuals [1, 11, 12].

Statistical analysis

The results of microneutralization test and its relationship with each of the variables recorded for all subjects was processed by computer using SPSS software and χ^2 test.

Results

Characteristics of enrolled subjects

From total number of subjects studied, 174 (49.2%) were girls and 180 (50.8%) were boys. All were in good health, ranging in age from 6 months to 16 years old. Vaccination history was obtained from vaccination cards. Percent distribution of age at first and second vaccinations is shown in Figures 1 and 2. Most subjects received the first vaccine between 9 and 11 months (60%) but 53 (15%) received it after 12 months and the records for 18.1% were not documented. 102 (29%) had not completed their second dose of vaccine until 16 months and 30 (8.5%) had not received their second dose of vaccine at all. Distribution of number of doses of vaccine given is presented in Table 1. Of note is the percentage of subjects for which no documentation was found.

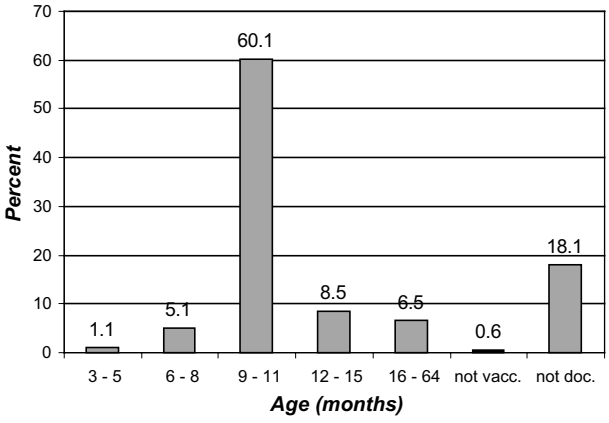

Figure 1. Percent relative frequency distribution of grouped age at first vaccination of measles.

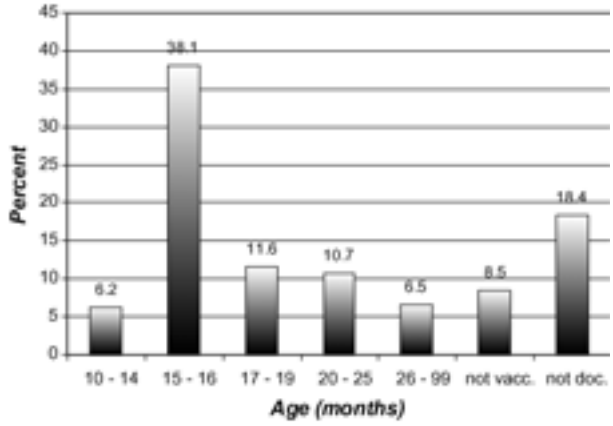

Figure 2. Percent relative frequency distribution of grouped age at second vaccination of measles.

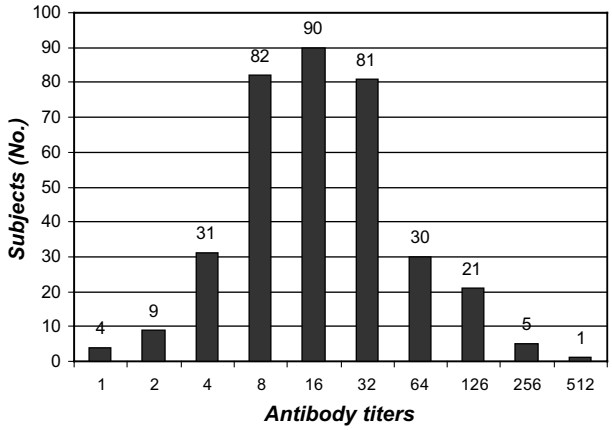

Figure 3. Frequency distribution of neutralizing measles antibody titers.

Table 1. Frequency distribution of number of doses of measles vaccine received

Vaccination dose	No. of subjects	Percent
One dose	27	7.6
Two doses	262	74.0
No vaccination	2	0.6
No documentation	63	17.8
Total	354	100.0

Measles virus neutralizing antibodies

In this study, subjects with titers of 1:8 and higher were considered to be immune. In most studies in which neutralization or plaque-reduction assays have been utilized, titer of 1:8 or higher of antibody has been considered as protective neutralizing titer against measles [1, 11, 12]. Sixty-three subjects had no vaccine records; but of those with vaccination records, 99% had a documented measles vaccine. In addition, the Ministry of Health had carried out a mass vaccination program in 1996, but no records were kept on the vaccination cards.

The results of microneutralization assay indicated that from total of 354 subjects studied, 310 (87.6%) had titers of 1:8 or higher and in 44 (12.4%) measles antibody titer was lower (Figure 3).

The relationship of immune status with other variables studied

χ^2 statistical analysis was applied. Subjects for whom no vaccination record was available were not included in this analysis. Figure 4 shows frequency distribution of various age groups with relation to immune status.

Antibody titers to measles reduced after age 10. Age groups 2–4, 4–6 and 6–8 had higher neutralizing antibody titer relative to other age groups. In some,

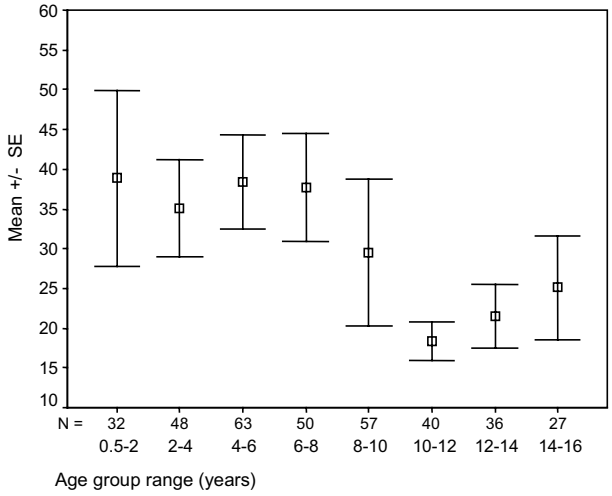

Figure 4. This figure demonstrates frequency distribution of various age groups studied and mean ± SE of reciprocal measles neutralizing Ab titers.

the reason may be the shorter duration between sample collection and vaccination, while in others the reason may be better coverage of vaccination in the recent years. In addition, as is demonstrated in Figure 4, the presence of relatively high titer of antibody shows the effects of mass vaccination in 1996 or possible reinfection or exposure to wild virus, since subclinical measles infection has been reported in seropositive vaccinated individuals [13].

Children with the first measles vaccination under 9 months were significantly less likely to be seropositive compared to those 9–12 months (72.7 vs. 90.1%) ($p < 0.009$). Surprisingly, older children, 12–64 months were less likely to be seropositive (77.6%). The proportion test indicated significant differences in rate of seropositivity in paired age groups (3–8 vs. 9–11 and 9–11 vs. 12–64) ($p < 0.02$) (Table 2). However, χ^2 statistical test did not show any significant relationship between immune status and sex of subjects, the place of specimen collection (urban or

Table 2. Frequency distribution of age at first vaccination related to immune status

Age at first measles vaccination (months)	Immunity			
	Negative		Positive	
	Number	Percent	Number	Percent
3–8	6	27.3	16	72.7
9–11	21	9.9	192	90.1
12–64	11	22.4	38	77.6
Total	38	13.4	246	86.6

rural areas), age at the second vaccination, number of doses of vaccine received, number of family members, level of education, or jobs of parents. No significant relationship between measles antibody titer and these demographic variables could be demonstrated.

Discussion

In most countries in the world, measles vaccination of children has had considerable impact in control of measles disease. But various countries have reported measles epidemics despite high vaccine coverage. Example of these are measles epidemics of 1988–1990 in the United States of America, Canada, Hungary, Taiwan [13, 14] as well as epidemic of the year 1990 in Iran [15].

Previous investigations have demonstrated a strong correlation between the presence and titer of measles neutralizing antibodies and protective herd immunity [6, 13]. In this work, 12.4% of individuals were detected as sensitive to measles. Effective factors in establishment of protective immune responses to an infectious agent include mode of contact, i.e, acquisition by vaccination or natural disease, host factors and persistence of developed immunity. Other factors which are considered to be important in the efficiency of a vaccination program include vaccine coverage, number of vaccination, the age at each vaccination, titer of vaccine, chronic underlying disease in vacinees and vaccine efficacy. One of the critical factors in establishment of immunity against a given vaccine is the age at vaccination. In this research, χ^2 statistical test indicated a significant relationship between immune status and grouped age at the first vaccination ($p < 0.009$). 22 (7.6%) of individuals received their first dose of vaccine prior to 9 months of age (Figure 1). Other studies have indicated that vaccination at younger age may be less protective [1, 16, 17]. Primary vaccine failure has been defined by lack of seroconversion, most common in younger infants. In a recent study, Gans et al. reported diminished capacity of the infant immune system to generate humoral responses to measles vaccine in 6-month-old cohorts in comparison to 9 and 12 months old cohorts [16]. This lower level of responsiveness may be due to the immaturity of some aspects of immune system in vaccinees. In addition, passive antibodies to measles virus may have a particular capacity to interfere with antigen-specific B cell responses. Interestingly, several studies have demonstrated that most children failing to seroconvert at 6 months, seroconverted with a second dose at 12 or 15 months old [16, 17]. However, with respect to cell-mediated immunity, infants evaluated at 6, 9, or 12 months had no age-related differences in T cell proliferation [15]. By use of related assays, Bautista-Lopez et al. [18] also detected cellular immunity to measles in infants 6 or 12 months. Distinguishing between antiviral effects of humoral and cellular responses is difficult because most individuals studied have both. These observations indicate the need to further investigate the functional maturation of humoral as well as cellular components of adaptive immunity in human infants. The optimal age for measles vaccination in any part of the world has to be based on weighing the benefit of vaccination and the risk of disease, complications of disease, and vaccine failures.

Delay in vaccination in endemic areas in which measles outbreaks are reported prior to age 1 is dangerous. In this age group, due to immaturity of the immune system and additional immunosuppression which is normally associated with measles, more intense disease develops which is not always without side effects. Over 50% of people who develop SSPE have contracted natural measles before age 1. Delay or lack of completion of vaccination or any vaccine failure in endemic regions can lead to circulation of wild virus and formation of disease in susceptible individuals [19, 20].

Despite official health reports of over 95% vaccination coverage in Iran and the mass vaccination of 1996 in the area studied, still 12.4% of subjects had $<$1:8 neutralizing measles antibody titer based on the results of the microneutralization test. Vaccination failure due to non-observance of preservation guidelines, use of unsuitable solvents for lypholized vaccines, wrong inoculation techniques or inadequate inoculation, low virus efficiency or vaccination without considering recommended intervals between dosages can be among factors which may be responsible for this lack of responsiveness [15]. In addition, it is important to employ sensitive tests to measure immune responses induced against a particular vaccine in subjects studied [21].

In conclusion, assessment of immunity to measles virus in various regions of Iran particularly in rural areas and among different age groups is essential. Such studies can help in evaluating the vaccination programs as well as the potency and efficacy of vaccines. More importantly, future directions for better elimination of measles would be decided according to the results of such studies.

Acknowledgements

The authors are grateful to Dr Abbas Sedaghat, director of health network in the town of Khodabandeh, Behrooz Kalantari, supervisor of center for disease control and Ebrahim Moosavi, director of central laboratory of Khodabandeh and all children and their parents who participated in this study.

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