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Antibody Response to Measles Vaccination in Turkish Children

Summary: In recent years, there has been a remarkable increase in measles cases among preschool and secondary school children in Turkey, as in many other countries. The seroconversion and coverage rates of measles vaccine should therefore be evaluated in order to obtain data that could be used to determine the vaccination policy for Turkey. Measles immunity status was studied by an enzyme-linked immunosorbent (ELISA) test determining the anti-measles IgG antibody levels. Measles specific IgG antibodies were found to be positive in 77.88% of the entire study group of 800 children aged 11 months to 12 years, while 21.25% had negative sera. Seven (0.87%) subjects had borderline results. The results of this study indicate the need to administer a second dose of measles vaccine, preferably at 18 months of age concomitant with other vaccines. This vaccination policy, together with an increase in the extent of immunization coverage, may help to achieve the World Health Organization's (WHO) target of the complete eradication of measles.

Introduction

Measles is a viral disease which may result in acute respiratory tract infection in addition to fever and rash. Although it's usually a self-limited illness, measles continues to be a major cause of childhood mortality in developing countries due to its complications during the acute phase and thereafter [1, 2]. It is estimated that 1.6 million children die annually from measles and its complications [3]. With the introduction of measles vaccine in the 1960's, worldwide incidence of measles infection has decreased dramatically [4, 5]. In the first few years of its administration, the vaccine was thought to confer lifelong immunity. However, the increase in measles cases among previously vaccinated school children, especially junior and senior high school students, in the 1980's has led to new studies about post-vaccination seroconversion rates [4–7].

In Turkey, measles vaccination began in 1968 with Schwarz strain given as a unique dose at 12–15 months of age only during epidemics. Routine administration of measles vaccine at the age of 9 months, as recommended by WHO for developing countries, began in 1987. However, preceding this policy, an accelerated Expanded Programme on Immunization (EPI) covering children 6–60 months of age had been held in 1985 in order to eliminate five contagious diseases, including measles. This programme resulted in a substantial decrease in the measles prevalence rates of the subsequent few years. Measles prevalence rates in Turkey were 132/100,000 in 1970, 4/100,000 in 1987, 41.6/100,000 in 1992 and 56.7/100,000 in 1993. The age distribution of cases in 1992 was 8.35% aged 0–11 months, 23.79% aged 1–4 years and 59.55% aged 5–14 years. The ratio of the latter age group was 60.77% in 1993. Measles immunization coverage rates were 34% in 1986 and 65% in 1992. Immunization coverage rates should be increased to 95%. Besides, it is necessary to assess the seroconversion rates against measles vaccine in previously vaccinated pre-school and school children in order to establish the measles vaccination policy. According to these data, the need for a second dose of vaccine, and if necessary, the optimal age of administration need to be discussed.

Materials and Methods

Study population: A total of 800 children referred to outpatient clinics of the Department of Pediatrics, Ankara University Faculty of Medicine between August 1993 and December 1993 were included in the study for assessment of measles vaccine serology in different age groups. These children were divided into three groups in order to evaluate the early vaccine response and secondary vaccine failure: 200 infants aged 11–24 months (Group 1), 300 children aged 4–6 years (Group 2) and 300 children aged 10–12 years (Group 3). Group 1 and Group 2 had been administered measles vaccine at 9 months of age, and Group 3 had been vaccinated at the age of 2–4 years during the accelerated EPI in 1985. All of these children had been vaccinated with Schwarz strain. To be included in our study, the follow-up requirements had to be fulfilled: 1) for Groups 1 and 2, history of vaccine administration at 9 months of age had to be verified by personal records of vaccination or by parental information; 2) for Group 3, history of vaccine administration at 2–4 years of age in 1985 had to be verified from the records of regional primary health care centers; and 3) for all groups, no history of verified or suspected measles infection after vaccination, any chronic illness, or an immunodeficient state. Parents were informed of the study protocol and their consent was obtained.

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Table 1: Serological data of the study population.

Groups	Seropositive No. (%)	Seronegative No. (%)	Equivocal No. (%)
Group 1	143 (71.5)	56 (28)	1 (0.5)
Group 2	207 (69)	89 (29.67)	4 (1.33)
Group 3	273 (91)	25 (8.33)	2 (0.67)
Total	623 (77.88)	170 (21.25)	7 (0.87)

Table 2: Seropositivity rates of age subgroups.

Age subgroups		Seropositive No. (%)
Group 1		
	11–15 months	64 (73.56)
	16–20 months	50 (75.76)
	21–24 months	29 (61.60)
Group 2		
	4 years	73 (76.29)
	5 years	70 (66.67)
	6 years	64 (65.98)
Group 3		
	10 years	115 (91.27)
	11 years	69 (84.15)
	12 years	89 (94.62)

Serology: Separated serum samples were stored at −20°C until tested for IgG antibodies to measles virus. The antibody levels were measured by means of an enzyme-linked immunosorbent (ELISA) IgG antibody test, using a commercial kit (Incstar Rubeola IgG Clin-ELISA Cat. No.: 4600, Lot # Ch-B 402033, USA). To sum up briefly: microtiter wells as a solid phase were coated with measles virus antigens. Control and test sera at a dilution of 1/51 were added to the wells. Corresponding specific antibodies that were present in the specimens were bound to the antigen at the solid phase. After a washing step to remove unbound material, anti-human IgG conjugated to peroxidase was added. After a second washing step to remove unbound conjugate, the enzyme-linked complexes were detected by incubation with a substrate (p-nitrophenylphosphate) for development of a blue colour that changed into yellow after stopping the enzymatic reaction with 3N NaOH (sodium hydroxide). The optical density, which is directly proportional to the amount of anti-measles IgG antibodies in the specimen, was measured by an ELISA microtiter plate reader (Awareness, Stat-fax-2100 microtiters ELISA reader). Linear regression graphics were drawn for each box, using absorbance values of calibrator-I, calibrator-II and calibrator-III, and standard ELISA values for each calibrator (10 for calibrator-I, 205 for calibrator-II and 520 for calibrator-III). ELISA values corresponding to absorbance of each test serum were found by this regression graphic. An ELISA value equal to or greater than 110 was interpreted as indicative of the presence of specific antibodies to measles and immunity to future infection. Values between 101–109 were considered equivocal, while an ELISA value of 100 or less denoted no detectable antibody to measles and indicated absence of immunoprotection.

Statistical analysis was done by Chi-square test and Z test for comparison of values for groups and for percent values within each group, respectively.

Results

Eight hundred subjects were included in the study. Of these, 439 (54.87%) were male and 361 (45.12%) were female. The serological data of the entire study population is presented in Table 1. Comparison of the three groups for seropositivity and seronegativity with Chi-square test revealed a statistically significant difference (χ^2: 49.748, $p<0.01$). Z test was performed for group pairs in order to determine the group and/or groups causing the statistical significance. There were statistically significant differences between Groups 1 and 3, and Groups 2 and 3 ($p<0.01$), while the difference was not statistically significant between Groups 1 and 2 ($p>0.05$). When the seropositivity and seronegativity rates were compared according to sex with Z test, there were no significant differences ($p>0.05$). Each group was divided into age subgroups and seropositivity rates were evaluated for them (Table 2). When these age subgroups were compared with each other within each group, only the difference between the 11-year-old and 12-year-old age groups was found to be statistically significant ($p<0.05$).

Discussion

Although measles vaccine confers a high level of protection, and coverage rates of 80–95% have been accomplished in developed countries, there has been a substantial increase in measles cases in these countries in recent years [5, 6, 8]. It is suggested that an anti-measles seroconversion of 70–80% is sufficient to protect against outbreaks in communities where vaccination coverage rates are above 80%, especially among children under 2 years of age [9]. However, the immunity level should be at least 95% in crowded areas such as schools and campuses in order to prevent virus transmission and outbreaks of disease [10, 11]. The seroconversion rate of 77.88% that was determined in a total of 800 children in our study, together with a coverage rate of approximately 65% in Turkey, seems insufficient to protect against sporadic or epidemic measles infection.

Administration of measles vaccine at 9 months of age confers a seroconversion of about 80–90% and a substantial protection from the disease [4, 8]. The seroconversion rate of 71.5% which was found in our study for the 11–24-month age group, is somewhat lower than these values. There were similar rates (approximately 70%) also for the 11–15, 16–20 and 21–24-month age groups. These insufficient rates may be the result of interruption of vaccine cold chain, administration errors, or the maternal antibody level, which is the most important factor determining early vaccine response and vaccine failure at the age of administration. Studies have shown that causes other than maternal antibodies have a minor role in early vaccine failure [6]. Although disappearance of maternal anti-measles antibodies takes place at variable ages in different communities, there is an inverse relationship between disappearance age and socio-economic status [12–14]. It is not exact-

ly known for our country when these antibodies are eliminated. *Ceyhan* et al. [15] have found that maternal anti-measles antibodies were present in 20% of infants at 30–31 weeks of age. In the view of these data, the low seroconversion rate (71.5%) that we found in our study could be explained, at least in part, by the persistence of maternal anti-measles antibodies at 9 months of age in Turkey.

In our study, we obtained a lower, though statistically insignificant ($p>0.05$), seroconversion rate in Group 2 when compared to Group 1 (69% vs. 71.5%). This result, together with the vaccine coverage rate of 65% in our country, shows that the 11-month-old to 6-year-old age group possesses a high risk for measles infection and points to the need to administer a second dose of vaccine at approximately 2 years of age.

In developed countries, recent measles outbreaks have been noted mainly in the 10 to 12-year age group [4, 5, 8]. The seropositivity rate of 91%, which we found in this age group (Group 3), is significantly higher than the values of Groups 1 and 2 ($p<0.01$). This high seropositivity rate found for Group 3 can be explained by three factors: 1) this group of children had been vaccinated during the accelerated EPI held in 1985 when they were 2–4 years old, 2) they could have developed measles infection, even though this was not specified by their families, and this native immunity could have been enhanced by the vaccine administered during that program, 3) a subclinical measles infection following vaccination could have resulted in an increase in vaccine-induced antibody levels.

Results of our study show that measles continues to be an important health problem in our country. It has been established that a primary vaccine failure of 30% after vaccination at 9 months of age persists through 11 months to 6 years of age. This susceptible group of 30% of the community, together with those not included in the coverage rate of 65% in our country, are potentially at risk for measles infection.

In conclusion, vaccine coverage rates should be increased and a second dose of vaccine should be administered, preferably at 18 months of age, as it is feasible to administer it with other vaccines. It seems reasonable; at least till the results of this second dose policy are obtained, to vaccinate 4–6-year-old children as well at the beginning of primary school together with the other vaccines, because this age group has low immunization rates similar to those of children at 11–24 months of age. In addition, serologic studies should be performed periodically, and vaccination programs appropriate for our country should be determined according to these data.

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Zusammenfassung: Antikörperantwort auf Masernimpfung bei türkischen Kindern. Masernfälle haben in den letzten Jahren bei Vorschul- und Schulkindern in der Türkei erheblich zugenommen. Diese Beobachtung wurde auch in anderen Ländern gemacht. Es ist daher nötig, Untersuchungen zur Serokonversion und Durchimpfung mit Masernimpfstoff durchzuführen, um Daten zu haben, die als Grundlage für die Impfstrategien in der Türkei verwendet werden können. Der Masern-Immunstatus wurde mittels enzymgebundenem Immunsorbent-Assay (ELISA) bestimmt. Die Messung der anti-Masern IgG-Antikörperspiegel ergab einen positiven Befund bei 77,88% der gesamten Gruppe von 800 Kindern im Alter von 11 Monaten bis 12 Jahren. 21,25% der Seren wurden als negativ beurteilt. Sieben Kinder (0,87%) hatten grenzwertige Ergebnisse. Die Daten der Studie sprechen dafür, vorzugsweise im Alter von 18 Monaten eine zweite Dosis Masernimpfstoff zu applizieren, wenn auch andere Impfungen erfolgen. Mit dieser Impfstrategie und einer besseren Durchimpfung könnte das Ziel der Weltgesundheitsorganisation (WHO), Masern völlig auszurotten, leichter erreicht werden.

References

1. **Gershon, A. A.:** Measles virus. In: *Mandell, G. L., Bennett, J. E., Dolin, R.* (eds.): Principles and practice of infectious disease. Churchill Livingstone, New York 1995, pp. 1519–1526.
2. **Chui, L. W. L., Marusyk, R. G., Pabst, H. F.:** Measles virus specific antibody in infants in a highly vaccinated society. J. Med. Virol. 33 (1991) 199–204.
3. **Aaby, P., Clements, C. J.:** Measles immunization research: a review. Bull. OMS 67 (1989) 443–448.
4. **Markowitz, L. E., Orenstein, W. A.:** Measles vaccines. Pediatr. Clin. North. Am. 37 (1990) 603–625.
5. **Adcock, L. M., Bissey, J. D., Feigin, R. D.:** A new look at measles. Infect. Dis. Clin. North Am. 6 (1992) 133–148.
6. **Markowitz, L. E., Preblud, S. R., Fine, P. E. M., Orenstein, W. A.:** Duration of live measles vaccine-induced immunity. Pediatr. Infect. Dis. J. 9 (1990) 101–110.
7. **Christenson, B., Böttiger, M.:** Methods for screening the naturally acquired and vaccine-induced immunity to the measles virus. Biologicals 18 (1990) 207–211.
8. **Cutts, F. T.:** Measles. World Health Organization, Geneva 1993.
9. **Chen, R. T., Goldbaum, G. M., Wassilak, S. G. F., Markowitz, L. E., Orenstein, W. A.:** An explosive point-source measles outbreak in a highly vaccinated population. Am. J. Epidemiol. 129 (1989) 173–182.
10. **Schlenker, T. L., Bain, C., Baughman, A. L., Hadler, S. C.:** Measles herd immunity. JAMA 267 (1992) 823–826.
11. **Lerman, Y., Riskin-Mashiach, S., Cohen, D., Slepon, R., Shohat, T., Harari, H., Wiener, M., Danon, Y. L.:** Immunity to measles in young adults in Israel. Infection 21 (1993) 154–157.
12. **Albrecht, P., Ennis, F. A., Saltzman, E. J., Krugman, S.:** Persistence of maternal antibody in infants beyond 12 months: mechanism of measles vaccine failure. J. Pediatr. 91 (1977) 715–718.
13. **Shasby, D. M., Shope, T. C., Downs, H.:** Epidemic measles in a highly vaccinated population. N. Engl. J. Med. 296 (1977) 585–589.
14. **Yeager, A. S., Davis, J. H., Ross, L. A., Harvey, B.:** Measles immunization: successes and failures. JAMA 237 (1987) 347–351.
15. **Ceyhan, M., Kanra, G., Vargel, S., Isikcelik, Y.:** The evaluation of vaccination against measles at nine months of age. Turkish J. Pediatr. 343 (1992) 127–133.