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Serosurveillance of Measles Antibodies in Vaccinated Children of Different Age Groups in Khartoum State, Sudan

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ABSTRACT

Background: Francis Home, demonstrated in 1757 that measles was caused by an infectious agent present in the blood of patients. David Edmonston (1954) had isolate measles virus in Boston, USA, from an 11 year old boy and adapted and propagated on chick embryo tissue culture.

Objective: This study was conducted to assess the efficacy of currently used MV vaccine in Khartoum state.

Materials and methods: The measles virus (MV) antibody titers were screened for 73 serum samples using virus neutralization test. Twenty of seventy three tested using hemagglutination inhibition test. Serum samples were taken from different age groups, 20 samples from Bahri and 17 from Khartoum and 36 from Omdurman. Fifteen before vaccination, 19, 11 after the first and the second dose of vaccine respectively and 28 at age of school entry.

Results: In this study 43.8% (32/73) Samples had neutralizing index (NI) to measles virus above the protective titer (1.5), 42.5% (31/73) had an NI less than the protective titer and 13.7% (10/73) had no detectable neutralizing antibodies.

Conclusion: A significant difference is observed in the titer of measles antibodies between the unvaccinated group and groups of children after second dose of vaccine and at age of school entry. And no significance within children after first dose of vaccine $p<0.005$. Using hemagglutination inhibition assay, 18 (18/20) of the tested sera had a protective antibodies level ≥ 4 and 2 samples (2/20) had no protective antibodies level ≤ 4. Fourteen serum samples were screened using both HI and VNT, 14/14 and 5/14 were protected, respectively. The study intended to provide a rational basis for improvement of measles vaccination strategies and strengthen measles research in Khartoum. Therefore, booster doses for measles immunization are required to prevent virus circulation.

Keywords: MV, HI, NT, Vaccine, Antibodies

BACKGROUND

Measles is caused by the measles virus, a single-stranded, negative-sense, enveloped RNA virus. Humans are the natural hosts of the virus; no other animal reservoirs are known to exist. Monkeys in the wild apparently are free of measles, contracting infection only after contact with humans [1], causes a highly contagious viral infection, with typical clinical symptoms including maculopapular rash, fever, cough, coryza and conjunctivitis. Measles continues to be a major cause of childhood morbidity and mortality worldwide. Measles, which is an acute systemic infection of, in most cases, young children ,is responsible for 10% of deaths from all causes among children less than 5 years old [2].

In the early 1960s, the advent of a live attenuated measles virus (MV) vaccine reduced the incidence of measles in many parts of the world, including developed and developing countries. In some developing countries, fatality rates for measles can still be as high as 15%, causing about 770, 000 deaths among infants and children and this is probably due to lack of vaccination of many individuals in the population [3].

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Although the introduction of live attenuated MV vaccines had largely reduce the endemic circulation of wild-type MV in the industrialized world, vaccination has been less successful in large areas of Africa and Asia [4]. Elimination of MV requires increasing the vaccination coverage levels, In this context, it is very important that reliable and sensitive laboratory methods are used to accurately determine the antibody level and protection achieved after vaccination and the level of antibodies that persists in those who were previously vaccinated [5].

It is likely that many factors contribute to the presence of susceptible individuals among mass vaccinated populations. These include failure to seroconvert and decline of immunity with time after vaccination [6]. Other important factors that might affect the immune response, the age at the time of vaccination, the number of doses, and the strain included in the vaccine [7]. Measles vaccination is not effective in very young infants, mainly due to interference with MV-specific maternal antibodies and immaturity of the immune system. In most industrialized countries with low measles incidence the first measles vaccination is, therefore, carried out between the ages of 12 and 15 months [8]. The protective immunity induced by vaccination may not be life long without being boosted by an exposure, mostly sub clinically, to a naturally circulating virus [9].

One year after the vaccination, 16% of the children who received the vaccination were no longer protected [10]. More than 70% of children who received the first vaccination before 10 months of age were shown to have NT antibodies at 15 months or older and that 98% of children who received the second dose at this timing had measurable NT antibodies 8 months after revaccination [11].

MATERIALS AND METHODS

Study area

This study was conducted in three localities in Khartoum state during the period from February to August 2014.

Study population

Children between the ages of 7 months and 7 years were included in this study. Details of the vaccination history were obtained from there parental, concerning, date of vaccination and the type of vaccine given.

Inclusion and exclusion criteria

The children were selected based on the following criteria: age; number of vaccine doses and no past history of severe illnesses.

Sample size and sampling method

One hundred blood samples were collected based on the compliance of criteria, seventy three of them were subjected to Neutralization test and 20/100 samples subjected to hemagglutinition inhibition assay

Study design

This is an investigatory study and it embraced children at different age group (7-9 months) – (11-15mounths) – (19-22 months) and (6-7years). Three localities were chosen for this study (Bahri, Khartoum and Omdurman) and this was done to compare the immune status of children in different parts after different number of vaccinations.

Ethical considerations

The study was approved by Ministry of Health and the hospital from which samples were collected and verbal consent was obtained from subjects enrolled in the study.

Blood samples collection, transport, processing and storage

3 ml of blood were collected in plain vacuum tubes through venipuncture. The blood was kept for 2 h at 25°C then centrifuged at 3000 rpm for 10 min. Serum was carefully removed with a Pasteur pipette and transferred to sterile labeled cryovials, then stored at -20°C until used.

Source of vaccine virus

The vaccine strain was obtained from the Public Health Centralized Immunization Clinic. It was manufactured by the Serum Institute of India Ltd. (Pune, India).

Virus propagation

The measles virus stock was propagated in a vero cell line in two T.C 75 cm^2 flasks, which were inoculated with 0.4 ml of reconstituted measles vaccine virus the other was left as a control, with daily observation until 90% CPE was reached on the fifth day post-inoculation. The tissue culture flask was then frozen and thawed three times. The measles virus culture was then centrifuged at 3000 rpm for 20 min using a cold centrifuge to obtain suspension of the viral particles which were then dispensed in aliquots of 1 ml into cryovials, and stored at -20°C. The polyethylene glycol (PEG) method of virus concentration was carried out overnight at +4°C and then 5 µl of tween 80 detergents was added on ice and vigorously shaken. 2 ml of ether were added and spun for 20 min at 3000 rpm in a cold centrifuge. Purified MV HA Ag was collected from the interphase and tested for hemagglutination activity as recommended by Rota et al. [12].

MV neutralization assay

Serum was heat-inactivated at 56°C for 30 min. Tenfold serial dilution of the virus from10^{-1} to 10^{-5} (1 ml from the virus stock in 9 ml of EME medium), was prepared and 50 µl of each dilution were distributed in 12 wells of 96 well micro titer plate except those of the two last row (G-H) which was considered as positive control (vero cells/medium), each row contained certain dilution (10^{-1} in the first row A, $10^{-1.5}$, in row B, 10^{-2} in C and so on). Then 50 µl of 1:4 dilution of test serum (0.5 ml of serum in 1.5 ml

of GEME medium) was added to each virus dilution in duplicate the last two column (11-12) represent negative control (virus dilutions/vero cells) then incubated at 37°C for 1 h. Then 100 µl of vero cell line suspension were added and incubated at 37°C for 6 days with daily observation.

Neutralizing index

The neutralization index (NI) was calculated according to Sperman and Karber method.

VNT antibody Index ≤1.5 was considered protective.

Hemagglutination assay (HA)

25 µl of PBS were added into 2 row of micro titer plate and then 25 µl of virus suspension were added and mixed after that 25 µl from this well were transferred to the next well and mixed, this step repeated to make twofold serial dilution along the row. 25 µl of 1% monkey red blood cells were added to each well then incubated at 37°C for 1 h. The reciprocal of the highest dilution that produce positive HA was considered as the virus titer that would give one HAU.

Monkeys' washed red blood cells (RBCs)

2.5 ml of monkeys' blood were collected by venipuncture using 5 ml sterile disposable syringe containing 2.5 ml Alsevier solution. The mixture was then dispensed in a tube and centrifuged at 1000 rpm for 5 min then the supernatant was discarded. An equal volume to Alsevier solution, of normal saline was added to the pelleted RBCs and the suspension was centrifuged. This washing process was repeated three times and 1% suspension of washed Monkey RBCs was prepared and preserved at 4°C.

Hemagglutination inhibition test

The test was carried out using the MV HA Ag to obtain I HA unit, from which the 4 HA units (standard working concentration) was obtained. Then the HI was performed. HI antibody titers were measured by a standard micro titer method using African green monkey red blood cells [13].

The HI test may be complicated by the presence of non-specific inhibitors of viral hemagglutination and naturally occurring agglutinins of the erythrocytes. Therefore, the sera were treated before use. Serum was heat-inactivated at 56°C for 30 min. Non-specific inhibitors of hemagglutination and non-specific agglutinins were removed from test sera by the addition of 0.1 ml of each serum to 0.4 ml borate saline and 0.5 ml of a 25% suspension of acid washed kaolin. The mixture was spun at 1200 rpm for 10 min and 25 µl of monkey erythrocytes was added to remove non-specific agglutinins, then spun at 1200 rpm for 10 min and supernate collected in new labeled cryoviale [14]. 25 µl of PBS were added to each of 96 U-shaped wells of micro titer plate, then 25 µl treated sera were added to well-1 and 25 µl from this well were transferred to well-2 and twofold serial dilution was carried out along the row, this steps were done to each serum sample, then 25 µl of 4HAU of antigen was added to each well of the serially diluted sera except for the monkey erythrocytes control wells. The plate was then allowed to stand for 1 h at 37°C. After that 25 µl of 1% monkey red blood cells (baboon red blood cells), were added to each well and incubated for 1 h at 37°C [15].

STATISTICAL ANALYSIS

Statistical analysis was carried out with SPSS version 20.0 to test for equality of the means using the Chi square and T test.

RESULTS

Description of the study population

Overall 58 (79.5%) were vaccinated, 15 (20.5%) were unvaccinated. The age distributions of the infants recruited into the study were as follow; the majority, 59.0% (28/73) were aged 6-7 years, 21.0% (19/73) were aged 10-15 months. The others 15/73 (14.0%) were aged 7-9 months and 6.0% (11/73) were aged 19-22 months **(Table 1)**.

Table 1. Distribution of the immune status of children at different age groups.

Immune status * Age Cross-tabulation							
			Age				Total
			Unvaccinated (7-9 months)	After dose 1 (11-15 months)	After dose 2 (19-22 months)	At School entry (6-7 years)	
Immune status	Protected	Count	5	7	7	13	32
		% within Immune status	15.6%	21.9%	21.9%	40.6%	100.0%
		% within Age	33.3%	36.8%	63.6%	46.4%	43.8%
		% of Total	6.8%	9.6%	9.6%	17.8%	43.8%
	Unprotected (<1.5)	Count	10	12	4	15	41
		% within Immune status	24.4%	29.3%	9.8%	36.6%	100.0%
		% within Age	66.7%	63.2%	36.4%	53.6%	56.2%
		% of Total	13.7%	16.4%	5.5%	20.5%	56.2%
Total		Count	15	19	11	28	73
		% Immune status	20.5%	26.0%	15.1%	38.4%	100.0%
		% within Age	100.0%	100.0%	100.0%	100.0%	100.0%
		% of Total	20.5%	26.0%	15.1%	38.4%	100.0%

P value=0.411

Cytopathic effect of the measles virus in tissue culture

After 5 days, maximum development of the CPE from measles virus vaccine in vero cells was seen using an inverted microscope. Uninfected vero cells are shown in **Figure 1A**, are spindle cells. Infected cells **(Figure 1B)** are shining, round and stellate cells, followed by formation of syncytia, (multinucleated cells) as well as which showed vacuolization of cells, a CPE of special peculiarity **(Figure 2)**.

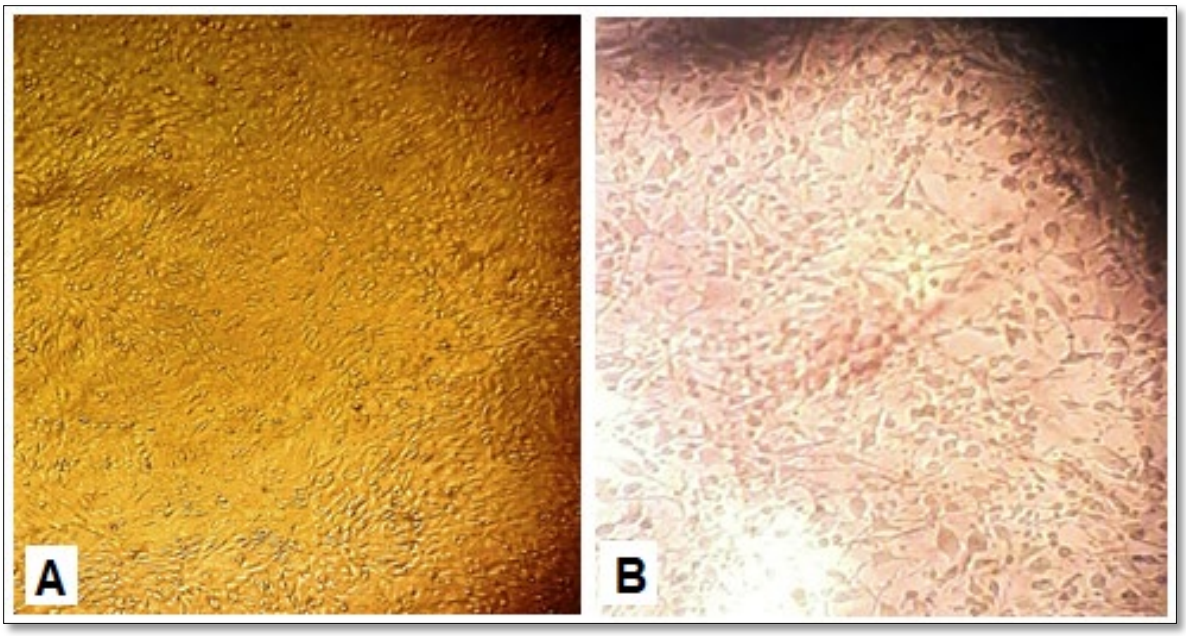

Figure 1. Vero cell line using 10X objective (A) Uninfected cells (negative control**)**, B (cells infected with measles virus, cells are becoming glistering, stellate and round, characteristic of early CPE.

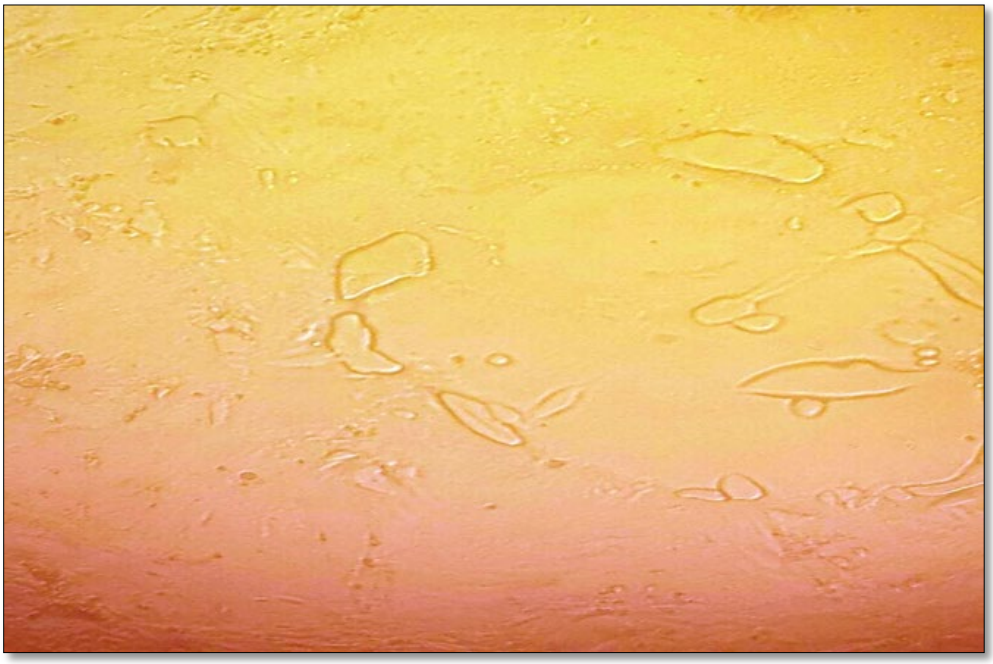

Figure 2. Infected vero cells with measles virus using 40X objective. (Advanced CPE, retraction of monolayer, formation of large number of syncytia, followed by cell necrosis and vacuolation).

Measurement of antibodies against measles using neutralization test

In this study 32 (43.8%) children of the population had a protective neutralizing antibody titer to measles virus (≥ 1.5) while 41 (56.2%) had detectable neutralizing antibody less than the protective titer. Before vaccination 5 (33.3%) of children were protected 10 (66.7%) unprotected, after the first dose 7 (36.8%) were protected and 12 (63.2%) were unprotected, after the second dose 7 (63.6%) were protected, 4 (36.4%) unprotected and at the School entry age 13 (46.4%) were protected, 15 (53.6%) unprotected **(Table 1)**. There was no significant difference in the level of protection between the children in Bahri, Khartoum and Omdurman ($p>0.05$) as shown in **Table 2**.

Table 2. Distribution of immune status of the children at selected areas in Khartoum state.

Immune Status * Area Cross tabulation						
			Area			Total
			Bahri	Khartoum	Omdurman	
Immune status	Protected	Count	8	9	15	32
		% within Immune status	25.0%	28.1%	46.9%	100.0%
		% within Area	40.0%	52.9%	41.7%	43.8%
		% of Total	11.0%	12.3%	20.5%	43.8%
	Unprotected	Count	12	8	21	41
		% within Immune status	29.3%	19.5%	51.2%	100.0%
		% within Area	60.0%	47.1%	58.3%	56.2%
		% of Total	16.4%	11.0%	28.8%	56.2%
Total		Count	20	17	36	73
		% Immune status	27.4%	23.3%	49.3%	100.0%
		% within Area	100.0%	100.0%	100.0%	100.0%
		% of Total	27.4%	23.3%	49.3%	100.0%

P value=0.684

There was no significant difference between titers of children who were not vaccinated and those who took the first dose of vaccine p value=0.641. But there was a significant difference between children of unvaccinated group and the other groups after the second dose and at age of school entry, P=0.006, P=0.002, respectively **(Table 3)**.

Table 3. Comparison between the neutralizing antibodies titer among unvaccinated children and children at different age groups.

Group Statistics						
	Groups	No.	Mean	Std. Deviation	Std. Error Mean	P value
Reading	Control	15	0.9667	0.64688	0.16702	P=0.641
	After First dose (11-15 months)	20	1.0875	0.82028	0.18342	
	After Second dose (19-22 months)	11	1.9318	0.78335	0.23619	P=0.006
	At age of School entry (6-7 years)	27	1.6786	0.82174	0.15530	P=0.002

Measurement of antibodies against measles using hemagglutination inhibition assay (HIA)

The agglutination of the RBCs by the concentrated virus appeared till the sixth well (1:64) which was considered as one HAU (64). Two wells back from the last well in which agglutination appeared, i.e., to wells at dilution (1:16) was considered as 4HAU (1 ml from concentrated virus in 15 ml PBS), as shown in **Figures 3 and 4**.

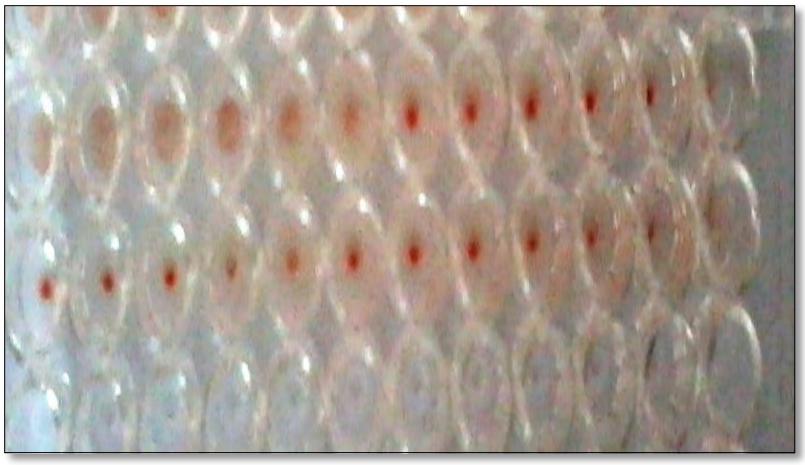

Figure 3. HA titration for measles virus.

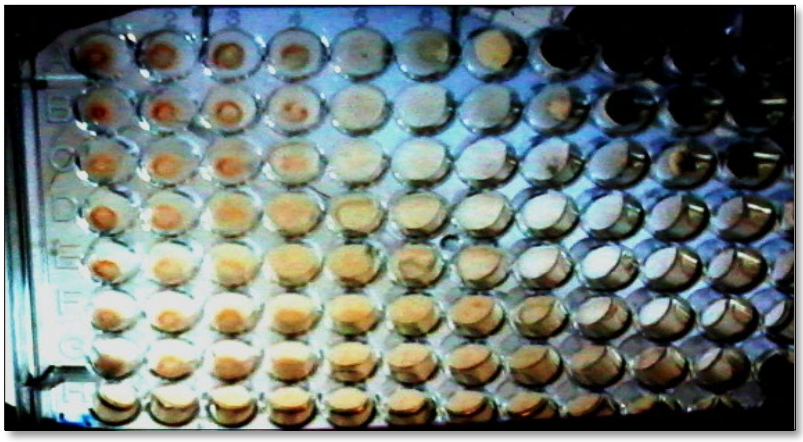

Figure 4. HI titer for measles antibodies.

Complete inhibition of agglutination at 1:4 dilution of serum was considered as the protection level (Titer=4) [19].

Out of the 20 children 18 (90%) had protective antibodies level and 2 (10%) not protected.

The titer of neutralizing antibodies rose slightly with advance in due to increased number of vaccinations **(Table 4 and Figure 5)**.

Table 4. HI titer of measles virus antibodies of children at different age groups.

HI Titer * Age						
HI Titer / Age group	**Unprotected**	**Protected**				
	1:2	**1:4**	**1:8**	**1:16**	**1:32**	**1:64**
Unvaccinated (7-9 months	2	2	2	1	-	-
After the first dose (11-15 months)	-	-	2	4	-	-
After the second dose (19-22 months)	-	-	-	-	1	6
Total	2 (10%)	18 (90%)				

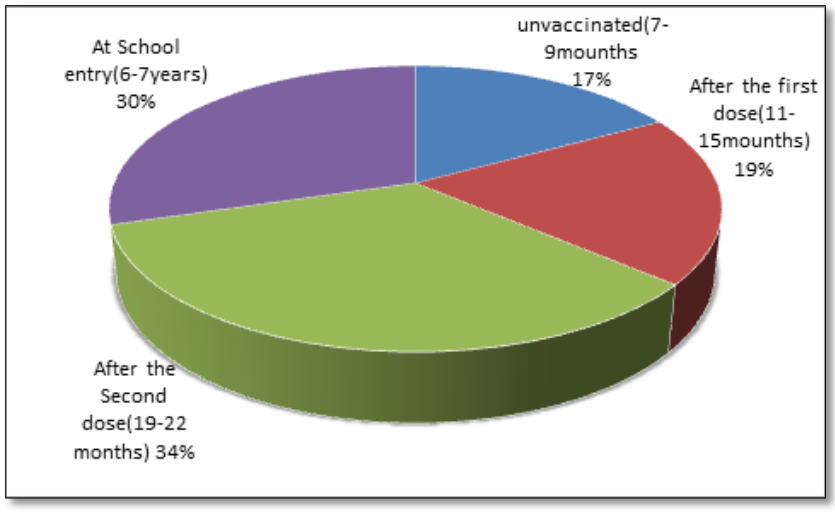

Figure 5. Mean of antibodies titer among different age groups.

DISCUSSION AND CONCLUSION

Antibodies to the hemagglutinin (H) protein are the primary antibodies measured by neutralization of virus infectivity in tissue culture [15], just like hemagglutination inhibition (HI) test which is used to measure the antibodies to the H-protein. Neutralizing antibodies play a very important role in preventing re-infection; therefore, the neutralization test is used to evaluate vaccine response and assess susceptibility to measles. This study examined the level of measles hemagglutinating antibody in children in three selected hospitals in Khartoum state. Using Neutralization test and Hemagglutination inhibition (HI) test, which are classical techniques that have been employed in prevalence studies and have good correlation with ELISA They reflect the level of the population's antibodies [16]. The Hemagglutination inhibition (HI) test is the most widely acceptable test in most developing countries. This is because it is very sensitive and specific and easily performed in any laboratory [17].

The result of the neutralization test showed that (43.8%) had detectable neutralizing antibodies to measles and (56.2%) had no detectable neutralizing antibodies, i.e., are seronegative to the neutralization test, this could be due to vaccine failure due to a break in the cold chain or malnutrition. Past studies have shown that a break in the vaccine cold chain resulted in the inability to develop measles protective antibodies [18].

Of particular interest in this test is the relatively large number of vaccinated children in all locations who were found not to be protected. This could be as a result of vaccine failure; it could also be due to wrong information from parents about the true vaccination status of their children.

Highest titer was found in the age group at entry of school, this rising in titer may be due to post vaccination infection which acted as booster dose. The 27 (46.6%) unvaccinated children might be protected as a result of recovery from natural infection or maternal antibodies in agreement with Adu et al. [19] in that measles antibodies are found in children earlier than 9 months may be maternally derived but not always. It is also possible that the antibodies were acquired post exposure to circulating wild measles virus.

In this study it is clear that the level or percentage of protection increase slightly after the first dose and more after the second, this agrees with Stetler et al. [11]. who reported that more than 70% of children who received the first vaccination before 10 months of age were shown to have neutralizing antibodies which lasted at 15 months or older.

The Hemagglutination inhibition (HI) test showed that out of the 20 children 18 (90%) had protective antibodies level (<4) and 2 (10%) not protected (>4). This agreed with Onoja and Adeniji [20] in that out of the 175 children vaccinated in Ibadan, 60 (34.3%) had an antibody level not sufficient to protect against measles infection and 115 (65.7%) were protected.

From unvaccinated group 71.4% (5/7) showed a protective antibodies titer ≥ 4 this result disagree with Omer et al. [21] in that children in rural Khartoum, had detectable MV-antibodies at 5 months, which dropped sharply to nil at 8-9 months.

The antibody titer rises according to the age of the children; this is in agreement with the study of Onoja and Adeniji [20] who found that antibody level increases with the age. There was no significant differences in antibodies titer of unvaccinated children and after the first dose of vaccine p=0.641, which may be due interference of maternal antibodies with the vaccine, this agrees with Ibrahim et al. [8] in that because the interference of maternal antibodies the first measles vaccination is carried out between the ages of 12 and 15 months in most industrialized countries. But a significant difference appeared after the second dose

p=0.002 and at age of school entry p=0.006 .This means a booster dose is needed in order to attain protection against measles infection. This agrees with Masae et al. [8], in that protective immunity induced by vaccination may not be life long without being boosted either via a second dose of vaccine or natural infection.

From the comparison between the two test used, HI was found to be more sensitive in evaluating antibodies titer and that may be as a result of treatment of the virus stock using PEG/Tween 80 treatment, which increased the potency of virus H Ag. This is in agreement with Rota et al. [12] in that the polyethylene glycol (PEG) method of virus concentration increased the potency of virus hemagglutinin antigen. Also this agrees with Motayo et al. [17], in that hemagglutination inhibition (HI) test is the most widely acceptable test in most developing countries .This is because it is very sensitive and specific and easily performed in any laboratory.

Finally these results allow us to conclude that the titer of measles antibodies at different age groups of children in Khartoum State, increased with age and was related to the number of vaccinations, out of 14 serum samples screened using HI and NT100% (14/14), 35.7% (5/14) were protected, respectively. This is evidence that HI could be a standard test in laboratory for measles diagnosis. Due to the importance of the disease and the lack of definitive treatment to it, procedures for evaluating the efficacy of vaccine must be maintained and further studies are required.

REFERENCES

1. Kalter SS, Herberling RL, Barry JD (1991) Detection and titration of measles virus antibody by hemagglutination inhibition and by dot immunobinding. J Clin Microbiol 29: 202-204.
2. Olivé JM, Aylward RB, Melgaard B (1997) Disease eradication as a public health strategy: Is measles next? World Health Stat Q 50: 185-187.
3. Youwang Y, Ping W, Feng C (2001) Serological and epidemiological effects and influence factors of primary immunizations with current live attenuated measles vaccine (Hu 191) among infants aged 6-15 months. Vaccine 19: 1998.
4. Clements CJ, Cutts FT (1995) The epidemiology of measles: Thirty years of vaccination. Curr Top Microbiol Immunol 191: 13-33.
5. Argüelles MH, Orellana ML, Castello AA, Villegas GA, Masini M, et al. (2006) Measles virus-specific antibody levels in individuals in Argentina who received a one-dose vaccine. J Clin Microbiol 44: 2733-2738.
6. Mossong J, O'Callaghan CJ, Ratnam S (2000) Modeling antibody response to measles vaccine and subsequent waning of immunity in a low exposure population. Vaccine 19: 523-529.
7. Kumar ML, Johnson CE, Chui LW, Whitwell JK, Staehle B, et al. (1998) Immune response to measles vaccine in 6 month old infants of measles seronegative mothers. Vaccine 16: 2047-2051.
8. Ibrahim SA, Abdallah A, Saleh EA, Osterhaus AD, De Swart RL (2006) Measles virus-specific antibody levels in Sudanese infants: A prospective study using filter-paper blood samples. Epidemiol Infect 134: 79-85.
9. Itoh M, Okuno Y, Hotta H (2002) Comparative analysis of titers of antibody against measles virus in sera of vaccinated and naturally infected Japanese individuals of different age groups. J Clin Microbiol 40: 1733-1738.
10. Mitchell LA, Tingle AJ, Décarie D, Lajeunesse C (1998) Serologic responses to measles, mumps and rubella (MMR) vaccine in healthy infants: Failure to respond to measles and mumps components may influence decisions on timing of the second dose of MMR. Can J Public Health 89: 325-328.
11. Stetler HC, Orenstein WA, Bernier RH, Herrmann KL, Sirotkin B, et al. (1986) Impact of revaccinating children who initially received measles vaccine before 10 months of age. Pediatrics 77: 471-476.
12. Rota JS, Hummel KB, Rota PA, Bellini WJ (1992) Genetic variability of the glycoprotein genes of current wild-type measles isolates. Virology 188: 135-142.
13. Orvell C (1976) Identification of paramyxovirus-specific hemolysis inhibiting antibodies separate from hemagglutinating-inhibiting and neuraminidase-inhibiting antibodies. Acta Pathol Microbiol Scand B 84: 441-450.
14. Gershon AA, Krugman S, Lennette EH, Schmidt NJ (1979) Diagnostic procedures for viral, rickettsial and chlamydial infections. Am Public Health Assoc, Washington DC, pp: 665-693.
15. Norrby E, Gollmar Y (1975) Identification of measles virus-specific hemolysis-inhibition antibodies. Infect Immun 11: 231-239.
16. Cox MJ, Azevedo RS, Massad E, Fooks AR, Nokes DJ (1998) Measles antibody levels in a vaccinated population in Brazil. Trans R Soc Trop Med Hyg 92: 227-230.
17. Motayo BO, Okonko IO, Faneye A, Adu FD (2011) Preparation of high potent measles hemagglutinating antigen from wild type measles virus in Ibadan, Nigeria. N Y Sci J 4.
18. Adu FD, Ikusika A, Omotade O (1997) Measles outbreak in Ibadan: clinical, serological and virological

identification of affected children in selected hospitals. J Infect 3: 241-245.

19. Adu FD, Odoemele FC, Bamgboye E (1999) Effect of measles antibodies in the breast milk and sera of mother on seroconversion to measles vaccine. Afr J Biomed Res 2: 7-11.
20. Onoja AB, Adeniji AJ (2013) Kinetics of measles antibody by hemagglutination inhibition assay in children in south-west and north-central Nigeria. Int J Infect Dis 17: e552-e555.
21. Omer MI, el dawla IG, Nicolas A, Roumiantzeff R, Lapeyssonie L (1986) Humoral antibody responses to biannual multiantigen vaccination: Report of a field trial on children in Sudan. Ann Trop Pediatr 6: 243-248.