

Seroconversion of children following natural measles infection and vaccination

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Abstract

Objective: To evaluate the relationship between measles virus (MV) antibodies (abs) in sera and breast milk of nursing mothers, their contributions in seroconversion of children (0 - 9 months) post vaccination, prevalence of prevaccination measles abs in sera of children brought for measles vaccination and seroconversion rate in vaccinees from nursing and lactating mothers. Also to determine the potency of vaccines available in Nigeria in relation to seroconversion.

Methods: One hundred and twenty pre- and post-vaccination sera and breast milk samples were collected from each nursing mother while corresponding number of finger prick pre- and post-vaccination sera samples were collected from children on filter papers. These were tested for mv abs using serological techniques.

Results: Eighty (20.0%) mothers had measles haemagglutination inhibition (HI) abs in sera and 88(27.2%) had mv HI abs in breast milk. Eight (2.0%) children who had prevaccination mv abs in sera came from mv ab negative mothers. Forty- four (37.0%) came back for post vaccination sera, sero-converted while 76(63.3%) gave low sero-conversion rate of 37.0%. Results showed that mv abs in sera or breast milk of mothers did not interfere with mv vaccination in children. The low sero-conversion rate obtained was due to low vaccine potency with titres ranging between (log10-10 - log10-2.5)TCID₅₀/per dose, besides non-specific antiviral substances exhibited virus neutralizing activity.

Conclusion: Poor sero-conversion due to loss of passive immunity arose from undernourishment while low ab titres came with natural infection. This suggested mv vaccination did not immunize following natural mv infection or any other previous immune status (JPMA 58:501;2008).

Introduction

National Programme on Immunization (NPI) formerly Expanded Programme on Immunization (EPI) was launched in 1979.¹ Programme boasts routine vaccination coverage and long-term sustainability for immunizable childhood diseases by emphasizing National, State and Local Immunization Days (NIDs, SIDs and LIDs respectively). Coverage for vaccination of childhood preventable diseases improved.²

Measles is characterized by coryza, cough, conjunctivitis and specific exanthema and maculopapular eruption.³ Transmission is by inhalation of secretions from patients' respiratory tract during prodromal phase and rash.⁴ In tropics, 50% of children

are infected before 6 years age and 80% - 90% at less than 20 years.⁵ Vaccination efficacy is high when appropriately utilized and vaccine is protective unlike in the past.⁶ Nigerian Government (FGN) and World Health Organisation (WHO) reported that single dose vaccine given at nine months age produced immunity in 80 - 85 % children.⁷

Immune development is either congenital or after vaccination or wild type of an infection. Poor immune response is due to poor storage of vaccines or improper vaccination.⁸ Adu et al.⁹ and Onoja et al.² attributed low sero-conversion to potency of vaccines. The role of mother-to-child in sero-conversion is not documented. Children may derive abs from breast milk when sucking and in-utero.¹ Wild mv infection causes mobilization of

immune defenses seen as a rash. Sub-clinical infections in non-immunized or after active immunization have virus replication in the body. Abs titres are present throughout life in some cases after infection in the absence of re-exposure.¹⁰ Therefore, public health problems are not due to lack of adequate coverage or laboratory back up system. It is mainly due to lack of serologic response to initial vaccination.

Measles before 6 months age is rare due to protection by maternal abs although cases have been observed in the first year of life.^{1,9} Disease occurrence in previously immunized subjects has been reported.^{11,12} Measles can be fatal for 1-5 years old children.¹³ This study was designed to see the prevalence of prevaccination mv abs in children, its relationship in sera and breast milk of nursing mothers and contribution to seroconversion. We also assayed sero-conversion rate by determining vaccine potency.

Subjects and Methods

Institute of Child Health (ICH), University College Hospital (UCH), Ibadan and two comprehensive health centers (CHCs) in Ekpoma, Edo State Nigeria serve as children vaccination centers. Children $\leq$ 5yrs enjoy EPI which provides free vaccines.

The study subjects, included children less than 9 months and nursing and lactating mothers, from different socioeconomic status ranging from peasant farmers to highly placed government officials. Consent was sought and gotten from mothers before vaccination and collection of blood samples. Nursing and lactating mothers were interviewed to obtain past history of measles and assess children's health. Well children were considered healthy-looking brought to health centres for immunization only and confirmed healthy by physical examination were vaccinated and studied. Nutritional status was assessed by weight for age, compared with WHO parameters for median weight for age and sex.¹⁴ Those below 2SD of median weight for age and sex were classed under nourished.

A total of 396 blood specimens were collected from nursing mothers and children as pre-vaccinated samples. One hundred and twenty nursing mothers returned with children for post vaccination blood tests; therefore, 120-paired sera were available for seroconversion studies. Breast milk of 396 mothers was used for analysis.

Pre-vaccination blood samples were collected from children by finger puncture on ROPACCO^(R) (Rochester, USA) rectangular filter paper described by Nekano et al.¹⁵ The punctured and bleeding thumb was

placed on each circle mark (12mm in diameter) on filter paper having information: name, age, sex, date, vaccine collected, vaccination date, vaccine batch number, expiry date and location. Blood filled filter papers were dried at ambient temperature, stored in plastic bags at 20°C before serum extraction.

Three ml breast milk was collected from each nursing/lactating mother into clean nunc tube/bijou bottle. They were advised to bring their children back, after 7 - 8 weeks for post vaccination blood evaluation. Questionnaires containing subject's biodata were collected.

Several batches of lyophilized Ruvax vaccines (13178, L5690 and M5652) were used and maintained in ice packs before being reconstituted according to manufacturer's instruction. Each child received 0.5ml of Schwarz hyper attenuated live measles vaccine intramuscularly after reconstitution.

Determined by titration using Vero cells, 0.5ml of 1.5×10^5 cells / ml was seeded into tubes containing 1% minimum essential medium eagle (MEME), a maintenance medium, incubated at 37°C until cells became confluent. Reconstituted vaccine aliquots were collected after vaccination and ten serial fold dilutions made using 1% maintenance medium. Growth medium was poured off and 0.2ml of each vaccine dilution (virus) was inoculated into cell culture tubes. Controls for both cells and virus were uninoculated and inoculated respectively with undiluted virus (neat). Adsorption of virus was made by addition of maintenance medium. Virus titer was read and calculated on 7th day using Kärber method.¹⁶

AGM-Rbc was collected into alservers solution and washed by centrifugation at 3000 g / 10mins in phosphate buffer saline (PBS). About 10mm diameters were punched out from soaked ROPACCO filter papers into Khan Tubes containing 0.5ml PBS/Bovine Saline Albumen (BSA) PH 7.2, and later soaked at 40°C for 24 hours. Thawed frozen breast milk was spun at 2000g for 10 min and to each 0.05ml of middle layer collected was added 0.05ml of PBS/BSA PH 7.2 and stored at -20°C. Spurned (10min, 2000 g) sera and breast milk were treated (0.025 and 0.02) ml respectively using 50% AGM-RBC to remove unspecific agglutinins giving false negative results.

About 0.05ml undiluted measles antigens were added to the first well containing 0.025ml of 2 folds diluted PBS/BSA and 0.05ml 1% AGM-RBC while last well served as a control cell. Virus HA titer was expressed as reciprocal of highest dilution of antigen causing complete agglutination of AGM-RBC. For HI,

0.025ml 0.05ml of 1:10 dilution sera and breast milk was each mixed with 0.025ml PBS/BSA (PH 7.2) 4HA unit's measles HI antigens. This gave a range of two-fold serial dilutions of 1:10 to 1:256, except sera and breast milk of controls. Identifying factors correlating with sero-conversion was carried out by Chi-square (X²) distribution method using Microsoft excel.

Results

Table 1 shows immunological status of nursing, lactating mothers and children. Eight (2.0%) prevaccinated children and 80 (20.2%) nursing mothers

Table 1: The immunological status of nursing and lactating mothers and their corresponding children

Nursing Mothers		Result of HI test	
Samples Test	No.of samples tested	No+ve(%)	No-ve(%)
<u>Nursing/lactate. Mothers:</u>			
Serum	396	80(20.2)	316(79.8)
Breast milk*	324	88(27.2)	236(72.8)
Serum+ve/breastmilk+ve	396	16(4.04)	38.(95.96)
Serum+ve/breast milk-ve	396	48(13.0)	348(87.0)
Serum-ve/breast milk+ve	396	72(18.2)	324(81.8)
Serum-ve/breast milk-ve	396	192(48.5)	204(51.5)
<u>Children:</u>			
Prevaccination serum	396	8(2.0)	388(98.0)
Post vaccination	120	44(36.7)	

Note * 324 nursing mothers did not have mv HI abs in the serum while 72 samples have mv HI abs in both serum and breast milk and (This may not have been sufficient for the study).
Therefore sum 324 and 72 = 396

were positive for mv HI abs, whereas 388 (98.0%) children and 316 (79.8%) mothers were negative. Ab titre ranged between 10 and 40. Eighty-eight (27.2%) were positive for mv HI abs in breast milk while 236 (72.8%) were negative. Sixteen nursing mothers had abs in serum and breast milk, 324 (81.8%) had no mv HI abs in sera but 72 (22.2%) had it in breast milk.

Table 2 shows immune status of nursing / lactating mothers and sero conversion of children in

relationship to HI titers from pre- and post vaccinated sera and breast milk. Eight pre-vaccination HI positive children belonged to negative mothers. From 388 pre-vaccination HI negative children, 80 were from HI serum positive mothers and 308 from serum negative mothers. Whereas 16 were from mothers with mv HI abs in breast milk and serum, and 204 from mothers without abs in serum and breast milk. No child had prevaccination abs from 88 breast milk positive nursing mothers while 8 children had prevaccination abs in sera from 236 breast milk negative mothers. Of the 120 children who were tested post vaccination, 44 had (36.7%) sero converted, and 76 (63.3%) did not seroconvert. Titre range at sero-conversion was between 10 and 20. From 44 sero-converted children, 4 had positive mothers while 40 had HI negative mothers. Sixteen and 60 children from positive and negative mothers respectively did not seroconvert. Sixteen and 28 positive children were from breast milk positive and negative mothers respectively. No child belonged to a milk/serum positive mother. Whereas 24 children had breast milk/serum negative mothers. From 76 non-sero-converted children, 16 were from breast milk positive mothers and 60 from breast milk negative mothers. HI titers at 1:10 were 8, 32, 40 and 88 for pre-, post-vaccinated (children), mothers' sera and breast milk respectively. At 1:20 dilution, titers were 12 and 28 for post vaccinated and mothers' sera while at 1:40 dilution, titer was 12 for mothers' sera. Total HI titers for pre-, post vaccinated sera (children), mothers' sera and breast milk were 8, 44, 80 and 88 respectively. The postvaccination status of 32 (72.7%) children was a titer of 10 and in 12(27.3%) that of 20.

Table 3 shows the relationship between sero-conversion and mv ab in sera and breast milk. Test of significant association showed non- significance ($P>0.05$ and $P=0.005$) in sera and breast milk respectively. Four (20.0%) and 40 (40.0%) children seroconverted from positive and negative mothers respectively, whereas 16 (50.0%) and 28 (31.8%)

Table 2: Immune status of nursing/lactating mothers and seroconversion of their children in relation to level of HI titers from pre-, post-vaccinated sera and breast milk.

Serum	Children				Immune Status of Nursing Mothers				
	No. tested			Breast milk		Serum		Breastmilk+ve/ /Serum -ve	Breast milk-ve Serum -ve
		No. +ve	No. -ve	No. +ve	No. -ve	+ve	-ve		
Prevaccination	396	8	388	0	8	0	8	0	0
HI + ve serum	8	8	0	0	8	0	8	0	8
HI -ve serum	88	0	388	88	236	80	308	16	204
Postvaccination	120	44	76	32	88	20	100	12	108
HI+ve serum	44	44	0	16	28	4	40	0	24
HI-ve serum	76	0	76	16	60	16	60	12	64

Table 3: Relationship between seroconversion and measles antibody in the sera and breast milk of mothers.

Seroconversion (Children)	Serum (HI) from mothers			Breast milk (HI) from mothers		
	+ve(%)	-ve(%)	Total	+ve(%)	-ve(%)	Total
Yes	4(20.0)	40(40.0)	44	16(50.0)	28(31.8)	44
No	16(80.0)	60(60.0)	76	16(50.0)	60(68.2)	76
Total	20(100.0)	100(100.0)	120	32(100.0)	88(100.0)	120

X² = 2.85 df = 1 (P>0.05)

X² = 3.39 df = 1 (P>0.005)

Comparisons.

Sero-conversion of children:

Yes, Serum HI +ve/Breast milk HI +ve - P<0.03(significant)
 Serum HI -ve/Breast milk HI -ve - P<0.05(significant)
 No, Serum HI +ve/Breast milk HI +ve - P>0.05(non significant)
 Serum HI -ve/Breast milk HI -ve - P>0.08(non significant)

children seroconverted from breast milk positive and negative mothers respectively. Three vaccine batches were collected and titrated to determine potency before and after vaccination. Five vials were titrated after rehydration during vaccination exercise. These were stored at -20°C and freeze thawed once before re-titration. One vaccine had expired. Non-reconstituted vaccine had a titer close to World Health Organisation (WHO) standard of Log10^{-3.5}/TCID₅₀/per dose. Others were Log10^{-1.0} to Log10^{-2.5}/TCID₅₀/per dose.

Discussion

There is rapid transmission of the measles virus in the prodromal period.¹ Importations occur during resurgence, and susceptibility in Nigerian population during resurgence could be high enough sustaining continuous transmission without accumulation of variants or displacement by imported viruses. Maternally transmitted abs is either abs transferred in utero via placenta or the colostrums (breast milk). Result of HI test from sera and breast milk of mothers and children revealed abs presence in 80 (20.2%) sera and 88 (27.2%) breast milk. Pre vaccination sera from children of negative mothers at vaccination age showed that in 8(2.0%) abs was not maternally derived. This contrasted with Adu and Adeniji¹ who had presence of HI abs in 38 children and 50 lactating nursing mothers from the same environment. This study showed that no child from positive mothers had abs at the pre-vaccination stage. Authors reported 7 children from HI positive mothers. Without disputing maternal abs passed to children, our study showed abs wane before 9 months. These two authors did not indicate the ages of the studied children. This is agreeable with Kimati et al.¹⁷ who opined that maternal abs wanes rapidly, leaving children virtually unprotected before 9 months age.

Contrary opinions from Onoja et al.² and Albrecht et al.¹⁸ revealed maternal abs presence at 12 months. Adu and Adeniji¹ reported lack of complement fixation (CF)

abs in children of positive mothers, suggesting that no abs was transferred to children through breast milk. Ogra et al.¹⁹ observed low transfer of abs in humans through breast milk. However, Onoja et al.² reported a higher abs level in children who were longer breastfed. Our study showed no significant (P>0.05) difference between breast-fed controls and patients. IgG measures late infections while CF abs indicates early infection. We observed no significant difference (P>0.05) between IgG abs level and children's history of contact with measles infection. Therefore, with waning of IgG over time, if CF abs is detected it should justify early infection amongst the breastfed. We conclusively explain the reason for not detecting CF abs, as heat labile substances present in colostrum and breast milk neutralize them. The IgG sero-prevalence was due to previous vaccination and IgG abs from sub acute infection (wild measles virus) was taken into consideration. Hospital visits, incidence of measles in the family and neighbourhood in the last one-month, were all associated with level of IgG antibodies in children.

Lawton and Shortridge²⁰ observed significant quantity of cells, humoral factors and nonspecific antiviral substances in breast milk and colostrums exhibiting neutralization activity. This could explain undetection of CF abs in breast milk. Presence of HI measles abs in mothers without previous history of measles vaccination and children of sero-negative mothers was indication of circulation of wild mv in environment. Since specific abs present in breast milk of mothers depend on previous contact with the microorganism or vaccination antigen, our results confirmed wild spread mv in the local population. This was reflected in the number of positive children with measles HI abs not from positive mothers.

The negative results for measles abs before vaccination showed the presence of mv abs in milk and sera of mothers but did not interfere with vaccination. This indicated no abs transfer to children from breast

milk and sera positive mothers. Amongst seroconverted children, 40 (40.0%) and 28 (31.8%) were from negative sera and breast milk mothers respectively. There was no significant difference in seroconversion rate or antibody interference with mv vaccine ($P>0.05$) in both categories (serum and breast milk). The low conversion rate observed among children that seroconverted, 40 (40.0%) and 28 (31.8%), with ab titer ranging between 10 and 20, indicated low potent vaccines used for immunization. There is need for field evaluation of measles vaccine efficacy to complement, validate and backup laboratory findings, which was suggested²¹ by Walter et al. From 5 vials titrated, one was close to WHO recommended $\text{Log}_{10}^{-3.5}/\text{TCID}$ per dose, others had titers ($\text{Log}_{10}^{-1.0}$ and $\text{Log}_{10}^{-2.5}$)/TCID per dose.

Viral importation continues fuelling sporadic outbreaks and epidemics in areas with good control measures. This strengthens the need to accelerate global measles control activities. Genetic characterization of mv wild type provides means to measure level of virus circulating in areas beginning to implement measles control plans.

Pan American Health Organisation in South and Central America adopted introduction of two-dose schedule. This boosted immunogenic response in those who failed to seroconvert after the first dose. It reduced measles cases in United States in 1993 and during a 6 weeks period at the end of 1993, no indigenous case of measles was reported.²² We recommend breastfeeding for 12 months with the belief that nonspecific factors other than specific abs do not have blocking effect on immunization.

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References

1. Adu FD; Adeniji JA. Measles antibodies in the breast milk of nursing mothers. *Afr. J. Med. Med. Sci.* 1995, 24:385-88.

2. Onoja AL, Adu FD, Tomori O. Evaluation of measles vaccination programme conducted in two separate centers. *Vaccine* 1992, 10: 49-52.
3. Krugman S, Katz SL, Gershen AA, Wilfert CM. Measles (Rubeola) In: *Infectious disease of the children*. St. Louis: CV Mosby 1985, Pp. 152 - 166.
4. Kempe CH, Fulginiti F. The pathogenesis of measles virus infection. *Arch. Gesa mte Virusforsch* 1965, 16:103-128.
5. Benenson, AS . Ed. *Control of Communicable diseases in man*, 11th ed., Washington, American Public Health Ass. 1970.
6. de Quadros C.A, Izurieta H, Venczel L, Carrasco P. Measles Eradication in the Americas, Progress to date. *J Inf. Dis*, 2004; 189: 227-35.
7. FGN/ WHO. Emergency preparedness and response to epidemics. 1996, April: Pp. 29.
8. Anne SY, Joseph HD, Lawrence AR, Biot H. Measles Immunization, success and failures. *JAMA* 1977, 237: 347-351
9. Adu FD, Akinwolere OAO, Tomori O, Uche LN. Low seroconversion rates to measles vaccine among whildren in Nigeria. *Bull. WHO* 1992, 70: 457-460.
10. Black FL, Rosen L. Patterns of measles antibodies in residents of Tahiti and their stability in the absence of re-exposure. *J. Immunol.* 1962, 88:725-31.
11. World Health Organization. Expanded Programme on Immunization. Global advisory Group weekly Epidemiological record 1993a, 65: Pp. 5 - 12.
12. World Health Organization. The immunological response. Measles: immunological basis for immunization module 7 WHO/EPI/GEN. 1993b, 93. 17. Pp. 2-16.
13. Morley P. Overview of the present situation as to measles in the developing World. *Trans. R. Soc. Trop. Med. Hyg.* 1975, 69:22-23.
14. World Health Organization. Measuring change in nutritional status, guideline for assessing the nutritional impact of supplementary feeding programme for vulnerable groups. WHO Geneva 1983, Pp. 63-101.
15. Nekano JH; Miller DL; Foster DS; Brink WE Microtitre determination of measles haemagglutination antibody with filter papers. *J. Clin. Microbiol.* 1983, 17:860-65
16. Kärber G. Beitrag Zur Kollektiven behandlung pharmakologischer reihenversuche. *Arch. Exp. Pathol. Pharmacol.* 1931, 162:480-83
17. Kimati VO, Loretn K, Munub GMR, Kimboi F. The problem of measles virus, response with reference to vaccine stability, age, protein-energy malnutrition and malaria in the tropics, *J. Trop. Paediatr.* 1981, 27:205-09
18. Abrecht P, Ennis FA, Salzmann EJ, Krugman S. Persistence of maternal antibody in infants beyond 12 months: mechanism of measles vaccine failure. *J. Paediatr.* 1977, 91:915-18.
19. Ogra SS, Weintraub D, Ogra PL. Immunologic aspects of human colostrum and milk. *J. Immunol.* 1977, 119:245-48
20. Lawton JWM, Shortridge KF. Protective factors in human breast milk and colostrum. *Lancet* 1977, 1: 253
21. World Health Organisation. EPI measles immunization of ill children. *Weekly Epid. Records* 1987, 62: 315.
22. Centers for Disease Control and Prevention. Absence of reported measles - United States. November MMWR Morb. Mortal. Wkly Rep. 1993, 42: 925-926.