In the name of God

S.U.M.S.

Department of Internal Medicine

Shiraz E-Medical Journal

Vol. 7, No. 2, April 2006

Effect of Measles Virus Antibodies from Breast Milk of Nursing Mothers on Seroconversion of Children After Measles Vaccination.

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Abstract:

The effects of measles virus (MV) antibodies (abs) in the sera and breast milk of nursing and lactating mothers on sericonversion to measles vaccination was studied. Three hundred and ninty six samples were collected each from sera and breast milk of nursing mothers and corresponding number of finger prick prevaccination sera samples on filter paper from their children were tested for MV abs. Eighty (20.2%) mothers had measles haemagglutination inhibition (HI) abs in their sera and 88(27.2%) had MV HI abs in their breast milk. Eight (2.0%) of the children who had prevaccination MV abs in their sera were all from MV ab negative mothers. Forty-four (37.0%) who came back for post vaccination sera seroconverted while 76 (63.3%) gave a low seroconversion rate of 37.0%. Results showed that MV abs in either sera or breast milk of mothers did not interfer with MV vaccination in their children. The low seroconversion rate obtained was due to low vaccine potency with titers ranging between (log10-1.0 – log10-2.5) TCID/per dose. This was beside other non-specific antiviral substances exhibited virus neutralizing activity.

Key Words: Measles, Antibodies, Seroconversion, Vaccination.

Introduction:

Measles is one of the most common childhood fevers which is highly contagious and characterised by coryza, conjunctivitis and specific exanthem followed by generalized maculo-papular eruption [1]. Transmission of the infectious virus is probably in secretions shed from the respiratory tract during the prodromal phase and early stages of rash when cough and coryza are intense [2]. The disease is severe in the tropics where about 50% of children are infected before they are 6 years old and 80% - 90% of people under 20 years have suffered from the condition [3].

Development of immunity to this disease is either congenital, vaccination or infection by wild type. Some constraining factors leading to poor immune response from vaccination include poor condition of vaccine storage and improper handling of vaccines during vaccination [4]. Other workers like Adu et al. [5] and Onoja et al. [6] have attributed low seroconversion rate to measles vaccination to low potency of the vaccines.

The role of maternal antibodies (Ab) in the seroconversion to measles vaccination is documented but the role of mother-child factor as it affects measles vaccination has never been determined. Several studies showed in utero transmission as the only source by which mothers transmit abs to their children while Adu and Adeniji [7] showed children derive maternal abs from breast milk when sucking. Placental transfer of immunoglobulin abs is a function of complexity and class of the Ab. Infection with wild measles virus causes extensive mobilization of the immune defenses. Subclinical infections in non-immunity such as maternal abs or active immunity may have virus replication in the body cause mild or no clinical symptoms. After a regular infection, Black and Rosen [8] showed that titers of abs be demonstrated throughout life in essentially all cases even in the absence of re-exposure.

A considerable number of cases of measles occur during the first year of life although overt measles before the age of 6 months is rare because of the supposed protection provided by the maternal abs. Measles takes a serious course and may lead to death in children between 1-5 years and factors not yet identified converge to give this effect [9]. This study investigated relationship between presence of measles virus abs in the sera and breast milk of nursing mothers and their effects on the seroconversion of their children post-vaccination. It evaluated the prevalence of prevaccination measles abs in the sera of children brought for measles vaccination and established the rate of seroconversion to measles vaccination in children of nursing mothers. It determined the potency of the vaccine in relation to seroconversion.

Materials and Methods:

Study centre: The work was done at the Institute of Child Health, University College Hospital (UCH), Ibadan, Nigeria. The centre serves as vaccination centre for children within Ibadan and environs. Ibadan is the

capital of Oyo State occupying the latitudes 40 2' E and 70 10'E and longitudes 70 3' N and 40 15' N. These children enjoy the expanded programs on immunization (EPI) in Nigeria providing free vaccines for the immunization of children under 5 years old.

Subjects: The subjects were mainly children less than 9 months old and their corresponding nursing mothers. These mothers belong to the different levels of socioeconomic status ranging from peasant farmers to highly placed government officials. They were attending the Institute of Child Health, UCH, Ibadan for vaccination against measles and other childhood diseases. Before vaccination and collection of blood samples from children and mothers, due consent from the subjects was sought and gotten after explaining the purpose and concept for the study.

Collection of Blood Samples using filter paper, breast milk and distribution of questionnaires: A total of 396 blood samples were collected from each nursing mother and the corresponding children as pre-vaccinated samples. Only 120 nursing mothers came back with their children for the collection of post vaccination sera and therefore only 120 paired sera were available for seroconversion studies while 394 breast milk collected were used for analysis.

Prevaccination blood samples were collected from children by finger puncture on a ROPACCO(R) (Rochester, USA) rectangular filter paper measuring 7cm by 10cm as described by Nekano et al. [10]. The punctured and bleeding thumb was placed on each of the circle marks (12mm in diameter) on the filter paper. The blood soaked through the filter paper until each circle was completely filled. Also printed on the filter were information like name, age, sex, date vaccine was collected, vaccination date, vaccine batch number, expiration and location. The filter papers were dried at ambient temperature and stored in plastic bags at – 20°c until ready for serum extraction.

About 3ml of breast milk samples were collected from each of the nursing mothers into clean nunc tubes/bijou bottles and they were advised to bring back their children, 7 – 8 weeks after measles vaccination for collection of post vaccination and evaluation of blood samples. Questonnaires containing biodata on both mothers and children were collected after fill out and analyzed accordingly.

Vaccine and Vaccination Schedule: Several batches of lyophilized Ruvax vaccines (13178, L5690 and M5652) were used for the measles immunization. These were obtained from United Nations International Children's Emergency Fund (UNICEF), through the state EPI Unit and were stored in the freezer until required for immunization. The vaccines were maintained in ice packs and reconstituted each time per vial according to manufacturer's instruction. Each vaccine received 0.5ml of reconstituted vaccine intramuscularly.

Vaccine Potency: The potency of the vaccines was determined by titration in vivo cells. Tubes were seeded with 0.5ml of 1.5 x 105 cells/ml and incubated at 37°C until the cells were confluent. Serial ten fold dilution of reconstituted vaccine aliquots which were collected at the end of the vaccination was made in maintenance medium (1% Minimum Essential Medium Eagle – MEME) from 10 to 10. The growth medium was poured off from the confluent tubes and 0.2ml of each vaccine dilution (virus) was inoculated into cell culture tubes in triplicates using separate pipettes. Controls for both cells and virus were uninoculated and inoculated with undiluted virus (neat) respectively. About 1ml of maintenance medium was added to each tube after allowing an hour for virus adsorption. Tubes were incubated at 37oC in stationery sloppy position. Virus titer was read and calculated on the 7th day using Kärber method [11].

Treatment of African Green Monkey Red Blood Cells (AGM-Rbc), (extracted) serum and breast milk: AGM-RBC was collected into abervers solution and washed three times by centrifugation at 3000 g / 10mins in phosphate buffer saline (PBS). A 10% suspension from the packed cells was used. The frozen 12mm and blood soaked ROPACCO filter paper were allowed to attain room temperature – 37°C. The 12mm soaked diameter was punched out (10mm) into a Khan tube and 0.5ml PBS/Bovine Saline Albumen (BSA) PH 7.2 was added. This was allowed to soak for 24 hrs at 4oC. The dark brown eluate composed of immunoglobulin (Ig) and degraded hemoglobin giving a situation of 1:10. The frozen breast milk was allowed to thaw and then spun at 2000g for 10 mins. The uppermost and bottom layers were discarded using pipette while the middle layer was collected and stored at –20°C. About 0.5ml of PBS/BSA PH 7.2 was added to 0.05ml of each breast milk. Both sera and breast milk were treated with 0.025ml and 0.02ml respectively of 50% AGM-RBC and spun for 10mins at 2000g to remove unspecific agglutinins which could give false negative results.

Haemagglutination test (HA): About 0.025ml of PBS/BSA was diluted two fold using microdiluters into microtiter well plate. About 0.05ml of undiluted measles antigen was added to the first well while the last well served as cell control. About 0.05ml of 1% AGM-RBC was added to all the wells after settlement. The virus HA titer was expressed as the reciprocal of the highest dilution of the antigen causing complete agglutination of the AGM-RBC.

Haemagglutination Inhibition Test (HI): About 0.025ml of 0.05ml of 1:10 dilution of sera and breast milk was mixed each with 0.025ml of PBS/BSA (PH 7.2) of the 4HA unit of the measles HI antigen. This gave a range of serial two-fold dilution of 1:10 to 1:256, except the sera and breast milk of the controls. The plates were shaken and mixture incubated at 37oC for one hour. About 0.05ml of 1% washed AGM-

RBC was added and kept at 37oC until the cells in the control wells had settled at the bottom before results were read. The serum HI or the breast milk HI titer was expressed as the highest dilution of the serum showing complete inhibition of haemagglutination.

Data analysis: Identification of factors that may correlate with the seroconversion was carried out by the Chi-square (X^2) distribution method using Microsoft Excel.

Results:

Table 1 shows the result of haemagglutination inhibition test on sera of nursing mothers/children and breast milk. Eight (2.0%) prevaccinated sera samples from children were positive for measles haemagglutination inhibition (HI) abs while 388 (98.0%) were negative. Eighty (20.2%) sera samples from the nursing mothers were positive for measles HI abs while 316 (79.8%) were negative. The Ab titer ranged between 10 and 40. Only 88(27.2%) were positive for measles HI abs in breast milk while 236 (72.8%) were negative.

Table 2 shows the immunological status of the nursing mothers involved and 16 of these nursing mothers who had serum-contained-abs also had abs contained in their breast milk. About 324 (81.8%) of nursing mothers had no measles HI abs in their sera but 72 (22.2%) of them had measles HI abs in their breast milk.

Table 1. Results of haemagglutination inhibition test (HI) on sera (nursing mothers and children) and breast milk.

	No. of Samples	Results of HI screening Number of Positives. (%)
Prevaccination serum	396	8(2.0)
Postvaccination serum (Nursing mothers)	120	44(36.7)
Serum	396	80(20.2)
Breast milk*	324	88(27.2)

* 72 samples may not have been sufficient for the study.

Table 2. The immunological status of nursing mothers involved in the study

Nursing Mothers		Result of HI test	
Samples	No. tested	+ve(%)	-ve(%)
Serum	396	80(20.2)	316(79.8)
Breast milk	324	88(27.2)	236(72.8)
Serum +ve/Breast milk +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)

Table 3 shows the relationship of immune status of nursing mothers and seroconversion of their children. All the 8 prevaccination HI positive sera of the children were from negative mothers. Out of the 388 prevaccination HI negative children, 80 children were from HI serum positive mothers while 308 children came from serum negative mothers. Out of this 388 prevaccination HI negative children, 16 children were from mothers who had measles HI abs in both their breast milk and serum while 204 children from same group were from mothers without abs in their serum and breast milk. No child had prevaccination abs from the 88 breast milk positive nursing mothers while 8 children had prvaccination measles Abs in their sera from the 236 breast milk negative mothers. About 120 children came back for post vaccination sera and 44 (36.7%) seroconverted after vaccination. About 76 (63.3%) did not seroconvert while titer range was observed to be between 10 and 20. Out of 44 seroconverted children, 4 of the HI positive children were from serum positive mothers while 40 were from the HI negative mothers. Sixteen and 60 children from serum positive and negative mothers respectively did not seroconvert. The 16 children who were measles HI serum positive, were from HI breast milk positive mothers while 28 HI positive children were from HI breast milk negative mothers. None of these children came from HI milk/serum positive mothers while 24 children were from breast milk/serum negative mothers. Out of 76 children that did not seroconvert, 16 children were from HI breast milk positive mothers while 60 were from HI breast milk negative mothers.

Table 3. Relationship of Immune Status of nursing mothers and seroconversion of their children.

Serum	No. tested	Children		Immunity Status of Nursing Mothers					
				Breast milk		Serum		Breast milk +ve/Serum +ve	Breast milk -ve/Serum -ve
		+ve	-ve	+ve	-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 4 shows the relationship between level of HI titers of the pre-and post vaccinated children and their corresponding nursing mothers' sera/breast milk. The HI titers at sero conversion ranged between 10 and 20. Thirty-two (72.7%) of the children at postvaccination had HI titer of 10 and 12(27.3%) had HI titer of 20. Seventy-six (63.3%) did not seroconvert.

Table 4. Relationship between level of HI titers of the prevaccinated, postvaccinated and mothers sera/breast milk.

Samples	HI Titers				
	1:10	1:20	1:40	1:80	Total
Prevaccinated serum	8	-	-	-	8
Postvaccinated serum	32	12	-	-	44
Mothers Sera	40	28	12	-	80
Mothers breast milk	88	-	-	-	88

Table 5 shows the relationship between seroconversion and measles Ab in the sera and breastmilk of mothers. A test of significant association between seroconversion and measles Ab in sera and breast milk of mothers showed no significant association (P>0.05 and P.0.005) respectively. Four (20.0%) and 40 (40.0%) of the children seroconverted from the serum HI positive and negative mothers respectively. Only 16 (50.0%) and 28 (31.8%) of the children seroconverted from the beast milk HI positive and negative mothers respectively.

Table 5. Relationship between seroconversion and measles antibody in the Sera and breast milk of mothers: Test of significant association.

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

X^2 = 2.85 df = 1 (P>0.05) X^2= 3.39 df = 1 (P>0.005)

Table 6 shows the result of potency test on the vaccines used during the immunization exercise. Three vaccine batches were collected and titrated to determine their potency before and after vaccination. Five vials from these batches were titrated after rehydration during the vaccination exercise. These were stored at –20^{o}C and freeze thawed once before re-titration. One vaccine was found to have expired. Only the non-reconstituted vaccine had titer close to World Health Organization (WHO) Standard of Log10-3.5/TCID/per dose. Others were between Log10-1.0 to Log10-2.5/TCID/per dose.

Table 6. Results of Potency test of vaccines used for immunization.

Serial No.	Vaccine Batch or Lot. No.	Titer (TCID50) (Kärber method [11])
1	131780103	Log10-1.0 (or 1.0)
2	M5652	Log10-1.5(or 1.5)
3	L5690	Log10-2.5 (or 2.5)
4	M5652	Log10-1.5 (or 1.5)
5	L5690	Log10-3.0 (or 3.0)

Discussion

Measles is one of the most communicable diseases affecting children in the tropics and resurgence of disease provides good example of the rapid transmissibility of the virus [7]. Numerous importations occur during resurgence, apparently meaning that these viruses did not circulate widely enough to be detected by molecular surveillance. Perhaps the number of measles susceptible persons in Nigerian population during resurgence was high enough to sustain continuous transmission without accumulation of variants or displacement by other imported viruses. The sources of maternally transmitted abs in children are the Ab transferred in utero via the placenta and the colostrum (breast milk). The probability of this transmission is a function of complexity and class of the abs.

In our study, the result of the HI test from the 396 sera and breast milk samples from nursing mothers and their children revealed Ab presence in 80 (20.2%) sera and 88 (27.2%) in breast milk. Prevaccination sera from children of negative mothers showed 8(2.0%) at vaccination age indicated that, the source of the Ab was not maternally derived. This was contrary with the report of Adu and Adeniji [7] who showed the presence of HI abs in 38 children and 50 corresponding lactating nursing mothers from same environment. In our study none of the children from positive mothers had abs at prevaccination while the authors reported of 7 of the children were from the HI positive mothers. While not disputing that maternal abs passed from positive mothers to their children, our proof showed such abs would have waned before the 9 months age of measles vaccination in Nigeria. These two authors did not indicate the ages of the children studied however, our observation lent credence to waned Ab activity which did not play any role in interfering with measles vaccination. This observation corroborated the findings of Kimati et al. [12] who opined that maternal abs wane rapidly, leaving children virtually unprotected before the age of 9 months.

Contrary opinions from Onoja et al. [6] and Albrecht et al. [13] have revealed maternal Ab presence at 12 months. Adu and Adeniji [7] reported lack of complement fixation (CF) abs in children of corresponding positive mothers, suggesting very little or no Ab was passed to children through breast milk. Ogra et al. [14] observed very low transfer of abs in humans through breast milk. However, Onoja et al. [6] observed higher Ab level in children who had longer breastfed. Our study showed nonsignificant

difference between controls and patients who were breast fed.

Abs especially IgG, indicate late infection while CFAb indicates early infection. Therefore, with the waning of IgG with time, it was expected CF Ab be detected to justify early infection amongst the breastfed. We did not conclusively explain the reason for the undetection of CF abs but the possible substances outside abs present in colostrum and breast milk could have contributed to the neutralization of the measles virus CF Abs.

This suggestion could be supported by the views of Lawton and Shortridge [15] who observed significant amount of cells, humoral factors and nonspecific antiviral substances in breast milk and colostrum exhibiting virus neutralization activity. The presence of HI measles Ab in these mothers without previous history of measles vaccination and also in children of seronegative mothers was an indication of circulation of wild measles virus in the environment. Also, since specific abs presence in breast milk of mothers depends on previous contact with the microorganism or vaccination antigen, our result confirmed widespread measles virus in the local population. This is also reflected in the number of positive children for measles HI abs not from positive mothers.

The negative results for measles Ab before vaccination showed that the presence of measles virus Ab in milk and sera of the mothers did not interfere with measles vaccination. This is because there was no evidence from our work indicated Ab transfer to children from breast milk and sera positive mothers. Amongst the children that seroconverted, 40 (40.0%) and 28 (31.8%) were from negative sera and breast milk mothers respectively. There was nonsignificant difference in seroconversion rate or antibody interference with measles 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 ranged between 10 - 20 reflected low potent vaccines were used for the immunization. Out of the 5 vials of vaccines titrated, only one was close to the WHO recommended dose of Log10-3.5/TCID/per dose while other vials had titers between (Log10-1.0 and Log10-2.5)/TCID/dose. Our study showed that mothers and child relationship had little or no role to play in response to measles vaccination given the 9 months age of vaccination practiced in Nigeria.

Measles virus introduced by importation will continue to fuel sporadic outbreaks and epidemics even in areas with relatively good control measures. Therefore, there would be no full control anywhere except globally. This observation strengthens the need to accelerate global measles control activities. Genetic characterization of wild type measles virus provides a valuable means to measure the level of virus circulation in areas just beginning to implement measles control plans.

In view of this, more aggressive childhood vaccination programs, introduction of a two dose schedule and successful mass vaccination campaigns be conducted by Nigerian Government as were the measures adopted by the Pan American Health Organization in South and Central America. These reduced the number of reported measles cases in the United States in 1993 and during a 6 – week period at the end of 1993, no indigenous cases of measles were reported [16].

However, our results encourage mothers to continue breastfeeding their children for at least 12 months believing that nonspecific factors other than specific abs might not have blocking effect on immunization.

Acknowledgements:

The authors are grateful to Mr. E. J. Ekpeme from the Institute of Child Health (ICH), University College Hospital Ibadan, for his assistance in the collection of the samples, Messrs LN Uche, MA Ibeh, AE Bamgboye and A Antiabong are gratefully remembered for various assistance and encouragement to Mrs. F. Odoemele.

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