

Effect of exclusive breastfeeding on incidences of illness in infant's first 6 months of life

Abstract

Background: The first six months of life represents a critical period in the survival of an infant. There is high morbidity and mortality risk as the newborn with its inherent immunological deficits adapts to the extra-uterine life. Optimal nutrition plays a major role in the survival of infants during this early period of life hence the significance of appropriate feeding practices.

Aim: This study aims to determine effect of exclusive breastfeeding on incidences of illness in the first six months of life. It also seeks to ascertain maternal and child factors associated with development of these illnesses in infants.

Methods: This is a cross-sectional descriptive and analytical study involving four hundred mother-infant pairs attending the infant welfare clinic of the Nnamdi Azikiwe University Teaching Hospital (NAUTH) enrolled after meeting the study criteria.

Results: Exclusive breastfeeding (EBF) was practiced by 33.5% of surveyed mothers. Mothers educational level ($p=0.003$), socioeconomic class ($p=0.018$), occupational status ($p=0.025$), antenatal care ($p=0.015$), mode of delivery ($p=0.001$), age of child ($p=0.001$) and giving of colostrums following birth ($p=0.002$) significantly predicted the feeding option adopted by mothers. Exclusively breastfed infants were 0.4 times less likely to visit hospital due illnesses (OR 0.41, CI 0.22-0.73). Frequent watery stooling (OR 0.05, CI 0.03-0.94), cough and/or catarrh with difficulty in breathing (OR 0.04, CI 0.01-0.85) and rashes and/or boils (OR 0.06, CI 0.01-0.83) were less likely to occur in exclusively breastfed infants. Increasing infant's age and delivery through caesarian section increased the likelihood of infant illness and hospital visits.

Conclusion: EBF is significantly associated with reduction of morbidity in the first six months of life. Efforts to promote EBF should be sustained.

Keywords: exclusive breastfeeding, infant illness, nnamdi azikiwe university teaching hospital

Volume 1 Issue 4 - 2014

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Received: May 09, 2014 | **Published:** September 04, 2014

Abbreviations: EBF, exclusive breastfeeding; PBF, predominant breastfeeding; CBF, complementary breastfeeding; NAUTH, nnamdi azikiwe university teaching hospital; WHO, world health organization; UNICEF, united nations children's fund; BFHI, baby friendly hospital initiative; IWC, infant welfare clinic; PASW, predictive analytics software; NAUTHEC, nnamdi azikiwe university teaching hospital ethics committee; PROBIT, belarus promotion of breastfeeding intervention trial; AAP, american academy of paediatricians

Introduction

According to a recent UNICEF publication optimal breastfeeding of infants has the greatest potential impact on child survival of all preventive interventions, with the potential to prevent over 800,000 deaths; a staggering 13% of all deaths in children under five in the developing world. According to the same publication an exclusively breastfed child is 14 times less likely to die in the first six months than a non-exclusively breastfed child and breastfeeding drastically reduces deaths from acute respiratory infection and diarrhea which account for majority of under-5 deaths in Africa.¹ For over two decades now, the World Health Organization (WHO) and United Nations Children's Fund (UNICEF) have consistently emphasized and promoted exclusive breastfeeding in the first six months of life as a veritable contributor to child survival.² In an attempt to achieve successful breastfeeding globally by the year 2000, WHO and

UNICEF launched the Baby Friendly Hospital Initiative (BFHI) in 1991. Studies done in both developing³⁻⁵ and developed⁶ countries have validated exclusive breastfeeding as supporting optimal growth and wellbeing of children. In spite of the documented benefit of exclusive breastfeeding some workers have queried its supremacy especially as it concerns the 6 month duration.⁷⁻⁹ Some studies have even reported relative growth faltering in exclusively breastfed infants from 3 months of age compared to non exclusively breast fed infants.^{8,10} It becomes very pertinent to regularly examine the relationship between feeding practices, nutritional and health status of infants to guide and support improved child survival. There are few studies that have looked into the effect of exclusive breastfeeding on the nutritional and health status of infants under six months of age in Anambra state and Nigeria in general. This study therefore tries to bolster the existing knowledge base by examining the effect of exclusive breast feeding on the incidence of illness in infants in the first 6 months of life.

Methodology

Study area

This study was conducted in the Infant Welfare Clinic (IWC) of Nnamdi Azikiwe Teaching Hospital (NAUTH), Nnewi, Anambra state, Nigeria. Nnewi, the host town of NAUTH is located on latitude 6° 01' N and longitude 6° 55' E.¹¹ NAUTH is the only tertiary health institution in Anambra State providing health services to a catchment population of about 25,430,493 as at 2005 with an average annual

growth rate of 3.4%.¹² It draws clientele from the entire state and also the neighbouring communities from the surrounding states of Enugu, Delta, Abia, Ebonyi, Kogi and Imo. The hospital maintains an Infant Welfare Clinic which runs three times in a week and is manned by trained nursing staff. On the average the centre attends to 30-40 mother-infant pair on each clinic day. The clinic provides mainly free routine immunization services. Other services rendered include growth monitoring, and counseling in diverse aspects of child survival strategies.

Study population and selection process

This cross-sectional descriptive and analytical study carried out over a 6 months period involved apparently healthy infants under-six month with their mother and/or care-giver who attended the Infant Welfare Clinic of NAUTH Nnewi from August to December 2012. The mother-infant pairs who fulfilled the study inclusion and exclusion criteria were consecutively enrolled.

The inclusion criteria includes:

- i. Infants below six months of age who after history and physical examination were found not to have any acute or chronic systemic illness.

- ii. Infants below six months of age whose mothers have given informed written consent.

Exclusion criteria:

- i. All infants who were HIV exposed, infected or whose HIV status were not known.
- ii. All infants delivered prematurely.
- ii. Low birth weight infants.
- iii. Infant below six months of age who on history and physical examination were found to have organic or congenital abnormalities.
- iv. Infant below six months of age who had suffered from acute illness within the preceding two weeks.
- v. Infant whose mothers have died and
- vi. Mothers-infants pair of multiple deliveries i.e. twins, triplets etc.
- vii. Infants below six months of age whose mothers refused given consent.

Table 1 WHO definition of selected feeding practices

Infant-feeding (IF) Option	What this Type of IF Requires Infants to Receive	What this Type of IF Allows Infants to Receive	What this Type of IF Does not Allow Infants to Receive
Exclusive breastfeeding (EBF)	Breast milk (including milk expressed or from a wet nurse)	ORS, Drops or syrups (vitamins, minerals, medicines)	Anything else
Predominant breastfeeding (PBF)	Breast milk (including milk expressed or from a wet nurse) as the predominant source of nourishment	Liquids (water and water based drinks, fruit juices, ORS), ritual fluids and drops or syrups (vitamins, minerals, medicines)	Anything else (in particular, non-human milk, food-based fluids)
Breastfeeding with complementary foods (CBF)	Breast milk (including milk expressed or from a wet nurse) and solid or semi solid foods	Anything else : any food or liquid including non-human milk and formula	No restrictions applicable
Breastfeeding (BF)	Breast milk (including milk expressed or from a wet nurse)	Anything else: any food or liquid including non-human milk and formula	No restrictions applicable

Table 2 Characteristics of study participants and infant feeding practices

Variables	Infant Feeding Practice				P-value
	Nn (%)	Exclusive EBF	Predominant PBF	Complementary CBF	
Age of Mother (Years)	N=398	N=134	N=110	N=154	
25 or less	106(27)	34(25)	27(25)	45(29)	0.116
26-30	159(40)	59(44)	51(46)	49(32)	
31-35	98(25)	32(24)	26(24)	40(26)	
36 or older	35(9)	9(7)	6(6)	20(13)	
Mother Educational Level	N=400	N=134	N=110	N=151	
Primary	89(22)	18(13)	26(24)	45(30)	0.003
Secondary	170(43)	55(41)	51(46)	63(42)	
Tertiary	137(34)	61(46)	33(30)	43(28)	
Socioeconomic Class	N=400	N=134	N=111	N=155	
Lower	185(46)	48(36)	57(51)	80(52)	0.018
Middle	150(38)	55(41)	42(38)	53(34)	
Upper	65(16)	31(23)	12(11)	22(14)	
Occupation of Mother	N=400	N=134	N=111	N=155	
None	97(24)	37(28)	26(23)	34(22)	0.025
Unskilled	140(35)	31(23)	41(37)	68(44)	
Semi-skilled	86(22)	34(25)	25(23)	27(17)	
Skilled	77(19)	32(24)	19(17)	26(17)	
Maternity Leave	N=288	N=93	N=77	N=118	
No	154(54)	44(47)	36(47)	74(63)	0.032
Yes	134(46)	49(53)	41(53)	44(37)	
Mother Parity	N=400	N=134	N=111	N=155	

Table Continued...

Variables	Infant Feeding Practice				P-value
	Nn (%)	Exclusive EBF	Predominant PBF	Complementary CBF	
1	128(32)	34(25)	36(33)	58(37)	0.200
2-3	152(38)	57(43)	38(34)	57(37)	
4 or more	120(30)	43(36)	37(33)	40(26)	
Antenatal Care	N=400	N=134	N=111	N=155	0.015
No	32(8)	6(4)	6(5)	20(13)	
Yes	368(92)	128(96)	105(95)	135(87)	
Mode of Delivery	N=400	N=134	N=111	N=155	0.001
Vaginal (Spontaneous or assisted)	320(80)	122(91)	92(83)	106(68)	
Caesarean section (CS)	80(20)	12(9)	19(17)	48(32)	
Age of Child (Months)	N=400	N=134	N=111	N=155	0.001
Less than 1	53(13)	22(16)	27(24)	4(3)	
1-2	25(6)	84(63)	70(63)	71(46)	
3-4	76(19)	23(17)	13(12)	40(26)	
5-6	46(12)	5(4)	1(1)	40(26)	
Sex of Child	N=400	N=134	N=111	N=155	0.838
Male	211(53)	68(51)	59(53)	84(54)	
Female	189(47)	66(49)	52(47)	71(46)	
Birth Rank	N=400	N=134	N=111	N=155	0.437
First	126(32)	34(25)	36(32)	56(36)	
Second	80(20)	31(23)	18(16)	31(20)	
Third	73(18)	26(19)	20(18)	27(17)	
Fourth	62(16)	26(19)	19(16)	18(12)	
Fifth and higher	59(15)	17(13)	19(17)	23(15)	
Colostrums Given	N=400	N=134	N=111	N=155	0.002
No	46(12)	127(95)	100(90)	127(82)	
Yes	354(88)	7(5)	11(10)	28(18)	
Ever Visited Hospital for Ill Health	N=400	N=134	N=111	N=155	0.000
No	210(53)	92(69)	52(47)	66(43)	
Yes	190(47)	42(31)	49(53)	89(57)	
Reason for Hospital Visits	N= 190	N=42	N=59	N=89	0.049 0.006 0.025 0.529 0.038 0.065 0.000
Frequent watery stool	24(6)	5(21)	6(25)	13(54)	
Cough and catarrh with breathing difficulty	12(3)	1(8)	2(17)	9(75)	
Cough and catarrh without breathing difficulty	85(21)	24(28)	32(38)	29(34)	
Ear discharge	1(0.3)	1(100)	0(0)	0(0)	
Rashes and/or boil (generalized or localized)	15(4)	2(13)	4(27)	9(61)	
Fever without any other focal features	35(9)	8(23)	12(34)	15(43)	
Multiple features and/or visits (>1)	18(5)	1(6)	3(17)	14(78)	

Table 3 Logistic regression analysis of maternal, child and outcome variables

Variables	Exclusively Breastfed		Odd Ratio OR (95% Confidence Interval)			
	No†	Yes	Crude OR	p	Adjusted OR	p
Ever Visited Hospital for ill Health						
No	118(56)	92(44.0)				
Yes	148(77.9)	42(22.1)	0.36(0.23-0.56)	0.001	0.40(0.22-0.73)	0.030
Reason for Hospital Visits						
Frequent watery stool	19(79.2)	5(20.8)	0.34(0.12-0.94)	0.037	0.05(0.03-0.94)	0.045
Cough &/or catarrh with breathing difficulty	11(91.7)	1(8.30)	0.12(0.02-0.92)	0.041	0.03(0.01-0.85)	0.043
Cough &catarrh without breathing difficulty	61(71.8)	24(28.2)	0.51(0.29-0.87)	0.014	0.49(0.24-1.05)	0.065
Ear discharge	0(0.00)	1(100.0)	0.00(0.00-0.00)	-	0.00(0.00-0.00)	-
Rashes and/or boil (generalized or localized)	13(86.7)	2(13.3)	0.20(0.04-0.89)	0.036	0.06(0.01-0.83)	0.026
Fever without any other focal features	27(77.1)	8(22.9)	0.38(0.17-0.88)	0.023	0.32(0.10-1.10)	0.070
Multiple features and/or visit (>1)	17(94.4)	1(5.60)	0.06(0.01-0.56)	0.013	0.48(0.20-1.90)	0.630
	No	Yes	Crude OR	p	Adjusted OR	p
Mode of Delivery						
Vaginal (Spontaneous or assisted)	68(85.0)	12(15.0)	0.41(0.19-0.87)	0.020	0.53(0.24-0.74)	0.003
Caesarean section (CS)	198(62)	122(38)	1	-	1	-
Age of Child						
Less than 1	31(58.5)	22(41.5)	1	-	1	-
1-2	141(63)	84(37.0)	2.44(1.05-5.69)	0.038	2.28(1.02-5.08)	0.043
3-4	53(69.7)	23(30.3)	4.28(1.63-11.24)	0.003	3.56(1.43-8.87)	0.006
5-6	41(89.1)	5(10.9)	6.99(2.24-21.8)	0.001	5.72(1.98-16.5)	0.001

†Reference category for regression analysis for upper half of table

Data collection

Data collection was done by the principal investigators and two research assistants (interns who are qualified medical doctors). They were trained in the art of interviewing the mothers, desired clinical examination and the protocol of referral if need be. The correct answers to the questions designated "interviewer to determine" were communicated to them just before commencement of data collection daily. The researchers often randomly went back to interview the mothers for quality control checks. These were all geared towards ensuring that study criteria were well applied. The data collection tool was a pre-tested interviewer administered questionnaire which was completed by questioning the mother and taking measurements of the infants' weight, length and head circumference.¹³ Information was collected regarding parental place of origin and domicile, maternal age, marital status, educational attainment and occupation. The parental socio-economic class was derived based on Oyediji's classification.¹⁴ Data were also collected about mother's antenatal clinic attendance, place of delivery and mode of delivery. Baby's first feed, everything baby took in the previous 24hours, reason for stopping breastfeeding etc were documented. There was also a section that sought to find out whether the mothers ever heard of exclusive breastfeeding, mothers' knowledge of the meaning, willingness to practice it if given the opportunity and reason for rejecting exclusive breastfeeding (where applicable). Prior to enrolment of any mother-infant pair, specific enquiries on the elements of the exclusion criteria were made including the HIV status. The HIV status of mothers who delivered in NAUTH and gave consent to participate in the study was additionally confirmed from the hospital's record. For mothers who gave birth outside the hospital, their confidentially volunteered status was admissible. Mothers who did not have antenatal HIV or who could not recall their status were excluded from the study. Mothers with ongoing respiratory illness were also excluded alongside their babies. For the infants, information on the sex, birth rank, time of first intake of feed and/or water, reasons for delay beyond 30minutes, first feed given to baby, colostrums intake, etc were sought from the mothers.

Predictor variables

Mothers were asked to recall what they had given to their infants in the past 24 hours. Based on WHO definition^{15,16} of infant feeding methods (Table 1), infants were categorized into those exclusively breastfed, predominantly breastfed and complementary breast feeding. The number of mothers and the feeding option adopted for their infants aged 0-6 months was further categorized into

- i. Yes, for mothers who practised exclusive breastfeeding and
- ii. No, for mother that did not practice exclusive breastfeeding.

Maternal age in years was categorized as 25 or less, 26-30, 31-35, and ≥ 36 ; educational level of mothers categorised as no education, primary education, secondary and tertiary education; occupation of mothers during previous 6 months after birth was categorised as none, unskilled, semi-skilled and skilled; socioeconomic class of mother was categorised as; lower, middle, rich (using oyediji's classification scale¹³ validated for developing countries). Parity of mother was categorised as 1, 2-3, and ≥ 4 ; maternal leave entitlement for working mothers was categorised as no and yes; use of antenatal care was categorised as no or yes; mode of delivery was classified into vaginal and caesarean section. Infant's age was categorised as <1 months, 1-2 months, 3-4 months and 5-6 months; gender of the infant grouped as male or female; and birth rank grouped into first, second, third, fourth and fifth or higher.

Outcome variable

The primary outcome variable was the proportion of infants who had been taken to the hospital for ill health in the last 6months. Due to the cross-sectional design of this survey only illness that were serious enough to warrant a hospital visit were considered. Visits to natural healers, patent drug stores, chemists, churches or self medications were excluded. Secondary outcome were the incidences of features infants which warranted hospital visit by mothers. Mothers were asked to recall features the infant developed that prompted the hospital visit. These clinical features were classified as follows- Frequent watery stool, cough and/or catarrh with breathing difficulty, cough and/or catarrh without difficulty in breathing, ear discharge, rashes and/or boils (localized or generalized), fever alone without any other focal features and multiple features (>1 clinical features).

Data analysis

The Predictive Analytics Software (PASW) Statistic 19.0 statistical package was used for data analysis. The Pearson chi-square (χ^2) test was used to study the differences between the independent variables and EBF practice. Binary logistic regression was also used for analysis of maternal and child variables, in order to examine their individual effects on EBF practice. Multivariate analysis was performed to determine factors which predicted ill health in infants after adjusting for confounders. All predictors were entered in the same block and the largest category in each predictor variable used as the reference category. For all statistical tests performed, it was ensured that the assumptions for carrying out these specific tests were met. Results are presented using percentages, Odds Ratios and 95% CIs where appropriate. Statistical significance was set at P-value <0.05.

Ethical consideration

Ethical clearance was obtained from the Nnamdi Azikiwe University Teaching Hospital Ethics committee (NAUTHEC) with reference number NAUTH/CS/66/VOL3/50. Informed consent (written) was obtained from every mother in her own right and on behalf of her child before recruitment. Participation in the study was entirely voluntary and no financial inducement whatsoever was involved. Voluntary withdrawal at any stage of interaction was guaranteed for all subjects without any adverse effect for the mother or the baby. All information was handled with strict confidentiality.

Results

From a primary sample of 1865 mother-infant dyads seen during the period of study, 534(28.6%) were found eligible having met the set study criteria. One hundred and thirty- four (134) declined participating in the study. This gives a recruitment fraction of 0.75 (400/534) and a final sample size of 400 mother-infant dyads (Figure 1). Exclusive breastfeeding (EBF) was practiced by 134(33.5%), predominant breastfeeding (PBF) by 111(27.8%), and complementary breastfeeding (CBF) by 155(38.8%) of the surveyed mothers. Table 2 shows the socio-demographic characteristics of study participants and factors that are associated with feeding option adopted by the surveyed mothers. Maternal variables like mothers educational level ($p=0.003$), socioeconomic class ($p=0.018$), mothers occupational status ($p=0.025$), antenatal care ($p=0.015$) and mode of delivery ($p=0.001$) were strongly associated with feeding practices adopted by mothers. Similarly, age of child ($p=0.001$) and giving of colostrums following birth ($p=0.002$) also significantly predicted the feeding option adopted by mothers. Of the 400 mothers interviewed, 190(47%) have visited the hospital at least once on account of their infants ill-health. Eighteen (9.5%) of these have visited the hospital more than once not including follow up visits.

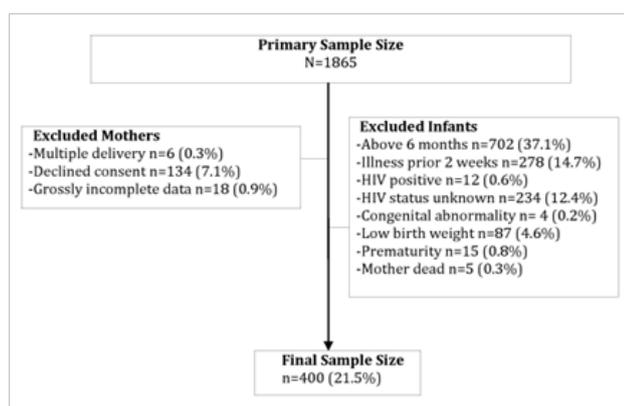


Figure 1 Process of study subject selection.

Mothers who exclusively breastfed their infants significantly visited hospital less (31%) compared to those who predominantly breastfed their infants (53%), and those who practiced complementary breastfeeding (57%) ($p=0.030$). Apart from ear discharge ($p=0.529$) and fever without any other focal features ($p=0.065$), other clinical features which prompted mothers to take their infants to the hospital was significantly less in exclusively breastfed infants compared to infants that were predominantly and complementarily breastfed (Table 2). Adjusted regression analysis shows that mothers of infants that were exclusively breastfed were 0.4 times less likely to visit hospital for infants ill health (OR 0.41, CI 0.22-0.73) compared to those not exclusively breastfed. Further analysis also showed that exclusively breastfed infants were less likely to develop features that warranted mothers visit the hospital on account of their infants illness. These include frequent watery stooling (OR 0.05, CI 0.03-0.94), cough and/or catarrh with difficulty in breathing (OR 0.04, CI 0.01-0.85) and rashes and/or boils whether localized or generalized (OR 0.06, CI 0.01-0.83).

Fever without any other focal features (OR 0.32, CI 0.10-1.10), multiple symptoms (OR 0.48, CI 0.20-1.93) and cough and/or catarrh without difficulty in breathing (OR 0.49, CI 0.24-1.05) lost significance after adjusting for confounders on multivariate regression analysis. Only mode of delivery and age of infant were retained on multivariate analysis as important socio-demographic predictors of mothers visiting the hospital due to their infant's illness. Infants delivered vaginally were 0.5 times less likely to be taken to the hospital due illness compared to those delivered through caesarian section (OR 0.53, CI 0.24-0.74). Likewise, infants who are aged 1-2 months (OR 2.28, CI 1.02-5.08), 3-4 months (OR 3.56, CI 1.437-8.87) and those aged 5-6 months (OR 5.72, CI 1.98-16.5) were about twice, four and six times respectively more likely to be taken to the hospital due to ill health compared to newborns below one month of age (Table 3).

Discussion

This study demonstrated the relative advantage of exclusive breastfeeding in protecting under-6 month infants from certain illnesses when compared with the other feeding practices. Most notably, it further highlighted the protective relationship existing between exclusive breastfeeding and incidence of diarrhoeal disease and pneumonia. It showed that non- exclusively breastfed babies were more than twice times more likely to suffer from serious respiratory illness and diarrhoeal disease. This is corroborated by a study done in Peru which showed that exclusively breastfed children are less likely to experience diarrhoea and respiratory illnesses in the first six months of life.¹⁷ Similarly an observational analysis in the Belarus promotion

of breast feeding intervention trial (PROBIT) cohort also showed a significantly reduced risk of gastroenteritis (adjusted odds ratio 0.61; 95% confidence interval 0.41 to 0.93).¹⁸

With acute respiratory infection and diarrhoeal diseases being the dominant cause of under-5 mortality, it is inferable that promotion of exclusive breastfeeding holds a pivotal key to attainment of MDG 4. This relatively greater immunity amongst exclusively breastfed infants is most likely from the array of undiluted anti-infective factors which is replete in breast milk. Babies who receive other things beyond breast milk are likely to have these anti-infective factors watered down in addition to the pathogenic inoculum which are almost always present in these other feeds. It therefore underscores the long documented health-protecting benefit of exclusive breastfeeding over other feeding practices. It is an extended revalidation of the recommendation of exclusive breastfeeding in the first six months of life by both World Health Organization (WHO), American Academy of Pediatricians (AAP) and even Nigeria's national guideline on Infant and Young Child Feeding.

This study also showed normal vaginal delivery was predictive of relatively reduced rate of hospitalization when compared with Caesarean section. This is hardly surprising because mothers with normal vaginal birth are more likely to offer their babies colostrums and breastfeed exclusively both of which have been documented to exert illness-sparing effect on babies. Also, the indication for Caesarean delivery might have negatively impacted on the babies' health status all increasing the risk of illness and consequent hospitalization. Neonatal period was also found to be associated with less hospitalization which showed incremental trend with advancing age. Younger babies are more likely to be breastfed exclusively as also observed in this study, less exposed to other environmental pathogens and also have greater titers of maternal antibodies all of which combine to keep them relatively free of infectious illnesses.

Exclusive breastfeeding has extensive health benefits to babies and it is therefore crucial to their survival and well-being. It affords babies greater protection from childhood killer illnesses like pneumonia and diarrhea disease besides other numerous benefits to the parents which indirectly posses positive health impact. It is therefore vitally expedient that exclusive breastfeeding receives all the attention from clinicians, mothers and other stakeholders to ensure its protection, promotion and sustenance.

Limitation

Breast Feeding practice information could only be acquired from the questions concerning a 24 hour recall of infant feeding practice. Several misclassifications might have occurred since a mother who has been exclusively breastfeeding her infant but for some reasons gave her infant water or some other liquids the previous day is considered not exclusively BF and vice-versa. Secondly some symptoms like poor suck and/or refusal to feed and vomiting were excluded from the analysis. This is because almost all mothers mentioned poor feeding in addition to other features as part of the symptoms which informed their visit to the hospital and also some mothers confused regurgitation for vomiting in their newborns during interview.

Conflicts of Interest

DICO and SO conceived and designed the study. SO, UE and IN were responsible for supervision of data collection and quality control. DICO analyzed the data, wrote the result and the first draft of the manuscript. SO, DICO and UE contributed to discussion, editing, and approved the text. JE and CE reviewed the final manuscript and supervised the work. All authors read and approved the final manuscript.

Acknowledgements

We thank the Hospital Management from the ethical clearance to carry out this study. Special thanks to the mothers and their infants for consent to participate in this study. We are also very grateful to Dr(s) Okoli Innocent and Nwosu for their contribution in proforma filling and data collection.

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