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Maternal Breastfeeding Methods, Breastmilk-Derived Antibodies and Cells Concentrations and Their Impact on Infant Morbidity: Results from a Prospective Cohort in Southern Benin

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ABSTRACT

Breastfeeding is associated with long-term well-being including low risks for infectious diseases and non-infectious diseases such as asthma, cancer, autoimmune diseases and obesity during childhood. However, very few studies assessed the real-impact of different methods of breastfeeding on infant morbidity in low-income countries such as Benin. Our study aimed to evaluate the dynamic change of immune components and the impact on infant morbidity among Beninese children population.

Seventy-six children and their mothers were enrolled in colostrum, transitional and mature milk groups at the District Hospital of Comè in Bénin. Breast milk samples were collected one-time from mothers to assess total IgA, IgG and IgM and leukocytes by using spectrophotometry and level of microscopy, respectively. Mean or proportion comparisons were appreciated using a Mann-Whitney or Fisher's exact tests, when appropriate.

Forty (54.63%), six (7.89%) and thirty (39.47%) mother-infant pairs were enrolled in colostrum, transitional and mature milk groups respectively. The total number of leucocytes and antibodies were different in colostrum, transitional and mature milk.

The prevalence of breastfeeding was 90.79% (n=69) in the population with 69.74% (n=53) of exclusive breastfed. Forty-five infants (84.91%) among exclusively breastfed infants were healthy whereas 4 (25%) among mixed breastfed infants and 4 (57.14%) among formula group were healthy (p=0.001). The total leucocytes count and IgA, IgG and IgM concentration decreases from colostrum through transition milk to mature milk.

Our data showed a prevalence of exclusive breastfeeding which is associated to a positive clinical outcome on infant's health. We have also confirmed decrease of antibodies and leucocytes during the maturation of breast milk.

Keywords:

Human breast milk, Breastfeeding, Infant health, Immunity, Antibodies

Introduction

Human Breast Milk (HBM) is considered as the optimal food to provide the developmental nutrients needs for infants under 2 years as well as shape their immune system [1,2]. In addition, to provide nutrients (proteins, lipids, and carbohydrates), HBM provides immune and non-immune components, bioactive molecules (cytokines/chemokines, lipids, hormones, enzymes and antibodies) and microbiota favoring the breastfed infant protection against numerous diseases [1,3,4]. Moreover, the infant's immune system matures by interacting with the environment including encountering pathogens, establishing adequate tolerance to the environment and the developing microbiota. [2,5–7]. The roles of the different breast milk components are far from being completely understood. These cells include immune cells (B and T lymphocytes, regulatory cells, monocytes/macrophages, neutrophils, natural killer cells and innate immune cells), stem cells, progenitor cells, lactocytes, and myoepithelial cells. Various antibodies are also found in HBM [1,8,9]. In the past decades, several studies on humans have shown that influencing factors such as geography, infant sex, or the number of pregnancies impact the heterogeneity and composition of HBM. Indeed, Holmlund et al. have shown that the maternal country of birth influences the pro-and anti-inflammatory contents of HBM, resulting in susceptibility or not to immune-mediated diseases such as allergy or necrotizing enterocolitis [10]. Despite this fact, too many children are malnourished and a high number of newborns and infants continue to die due to infectious diseases because of a lack of knowledge about the composition of HBM and how these components shape the infant's immune system. It has been

reported that through a process called microchimerism, maternal cells are transferred to infant intestinal mucosal tissue, and from there, enter their circulatory system to populate other infant immune tissues [11]. In Benin, very few studies on breast milk and its benefits are available. Our study could modify the national policies and put breast milk back at the heart of the diet of Beninese infants by extending maternal leave for this purpose or facilitating the breastfeeding in public and private workers environment.

Herein, our study aimed to evaluate the dynamic change of immune components in human colostrum, transitional and mature milk and the impact of breastfeeding on infant morbidity.

Material and Methods

Participants and data collection

From July to October 2018, we enrolled in a prospective study 40, 6 and 30 mother-infant pairs in colostrum, transitional and mature milk groups at the District Hospital of Comè in Bénin. Inclusion criteria were as follows: women whose infants were cared in pediatrics, women who gave birth in the maternity ward or who were transferred to the neonatology ward with their infants. Twins were excluded from the study. None of these women had any acute infection and complications during pregnancy and breastfeeding. This study was approved by the Institutional Review Board of the District Hospital of Comè, Bénin. Written consent was obtained from the mothers prior to the one-time sampling and information collection.

Questionnaires were used to collect the mother-infant pairs' demographic and clinical information. Mothers' information included age, type of milk, and breastfeeding. Infant's information included gender and age.

Sample collection

Colostrum was collected within 5 days after delivery, transitional milk 15 days after delivery and mature milk 35 days postpartum. After cleaning the nipples with cotton soaked in alcohol, the breast was pressed and the milk collected in a sterile tube. All samples were collected from one side of breast, and transferred on ice to laboratory where samples were immediately processed. Sera from infant's peripheral blood samples (7 ml) were collected one-time at inclusion and were stored at -20ºC.

Breast milk leucocytes count

The white cell count of the different types of milk was done using the microscope (Olympus CX23, Olympus-life science, France) after 1/20 dilution with Lazarus liquid (Dutscher, France).

Antibodies measurement

The total concentration of immunoglobulins A, G and M was determined using semi auto chemistry analyzer spectrophotometer (Rayto RT-9200, Shenzhen, China) after 1/10 dilution of milk and or colostrum and sera.

Statistical analysis

Differences in participants' characteristics in colostrum group, transitional group and mature milk group were compared using Fisher exact and Mann–Whitney test, when appropriate. Differences of the means of leucocytes and antibodies at different stages of lactation were evaluated by Kruskal-Wallis test. Statistical analyses were performed using Prism 5 software (San Diego, CA). All hypotheses were two-sided, and p-values<0.05 were considered statistically significant.

Results

Characteristics of the mothers and their infants

A total of 40 (54.63%) colostrum, 6 (7.89%) transitional and 30 (39.47%) mature were collected in this study. As showed in Table 1, the mean age of mothers was 28.18 ± 5.55 years whereas their infants aged from 1 day to 2 years after birth. Table 1 outlines the characteristics of mothers-infants in each group. Fifty nine children (77.63%) were aged under 6 months whereas 17 (22.36%) were aged between 7 and 24 months. The prevalence of breastfeeding was 90.79 % (n=69) in the population with 69.74% (n=53) of exclusive breastfed, 21.05% (n=16) of mixed breastfeed and 9.21% (n=7) of formula fed.

Table 1: The characteristics of the infants and their mothers.

Factors	Colostrum (n=40)	Transitional milk (n=6)	Mature milk (n=30)	P value
Maternal characteristics				
Age (Years)	26.95 (± 5.05)	28.31 (± 5.72)	29.80 (± 5.92)	0.06
Mode of breastfeeding n (%)				
Exclusive	30 (%)	4(%)	19 (%)	-
Mixed	9 (%)	1 (%)	6 (%)	-
Formula	1 (%)	1 (%)	5 (%)	-
Infantile characteristics				
Age (Months)				
0 to 6	40	6	13	-
7 to 24	0	0	17	-
Gender				
Male	23	6	12	-
Female	17	0	18	-
Exclusive breastfed under 6 months	30 (75%)	4 (66.66%)	7 (23.33%)	-

The influence of human breast milk type on leukocyte count and antibody levels

Figure 1 shows that depending on the phase and stage of lactation, a dynamic change in the proportion of white blood cells in colostrum (5.07 109/L), in transitional milk (2.64 109/L), and in mature milk (5.34 108/L) (P<0.0001).

Figure 1: Variation of leucocytes number depending of the stage of lactation.

As showed in Figure 2A, IgG level is higher in colostrum (1344.13 ± 122.57 mg/L) compared to transitional milk (1121.33 ± 114.21 mg/L) (P=0.0003) and mature milk (800.33 ± 191.84 mg/L) (P< 0.0001). In the same manner, IgA level in colostrum (4626.09 ± 438.83 mg/L) is higher than transitional milk (4114.84 ± 376.49 mg/L) (P=0.0036) and mature milk (2224.38 ± 680.19 mg/l) (P<0.0001) (Figure 2B). IgM was also higher in colostrum (1914.40 ± 440.89) than in transitional milk (1054.76 ± 426.18 mg/l) (P<0.0001) and in mature milk (509.54 ± 243.88 mg/l) (P<0.0001) (Figure 2C). HBM antibody concentrations decrease during the time and become stable when the milk is mature. Indeed, as shown in Figure 2D, IgA is most concentrated antibodies in human breast milk whether in colostrum or in transition milk or even in mature milk (Figure 2D). The influence of maternal age at delivery on immunoglobulin concentrations was also studied. No significant relationship could be observed for IgA and IgG concentration according to maternal age. However, a trend towards higher IgM concentration seems to occur after 20 years of age (Table 2).

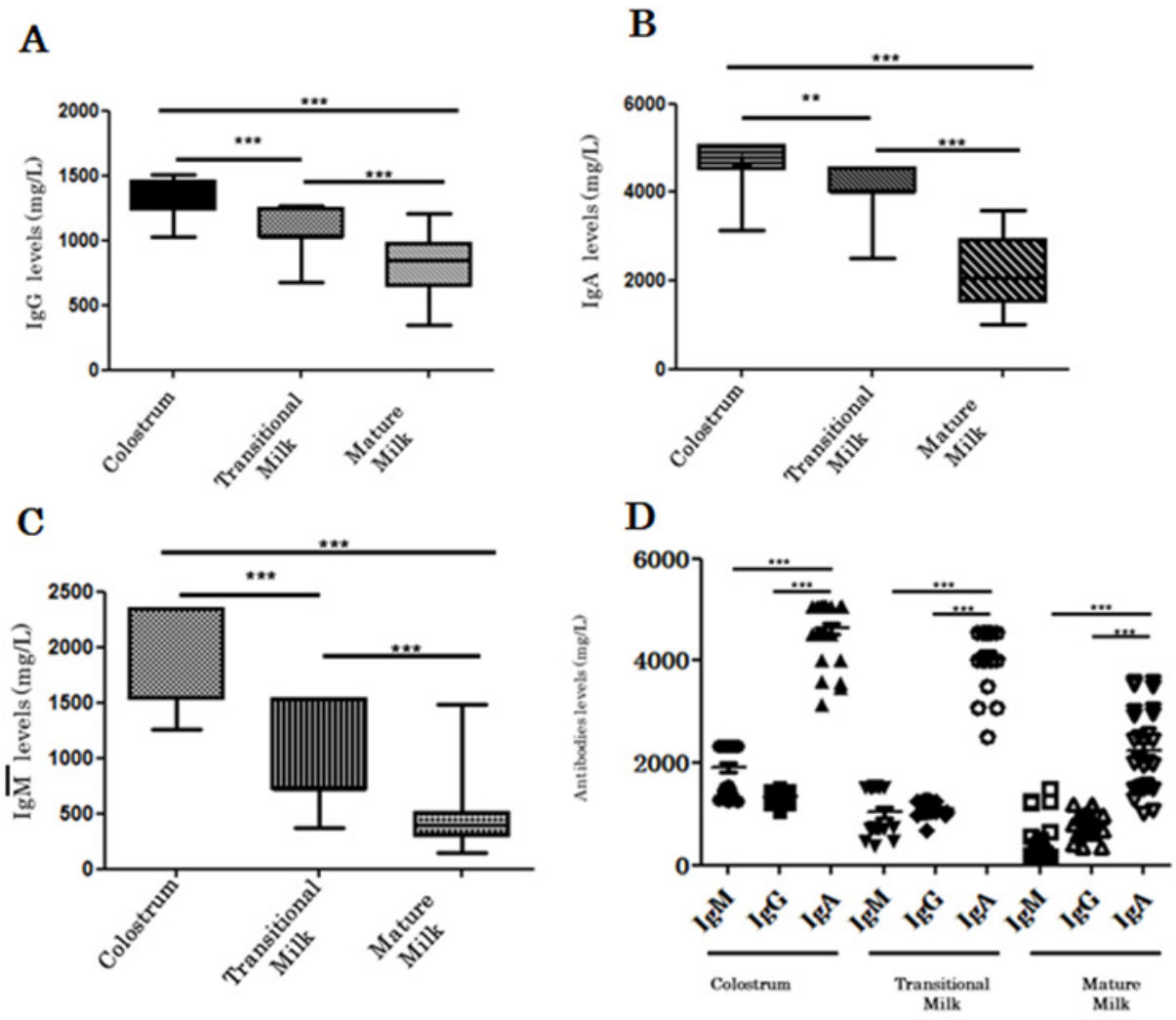

Figure 2: Change in IgA, IgG and IgM levels during human breast milk maturation.
IgG (**A**), IgA (**B**) and IgM (**C**) levels are higher in colostrum and decrease while human breast milk becomes mature. IgA is most concentrated antibodies in human breast milk whether in colostrum or in transition milk or even in mature milk (**D**).

Table 2: Correlation between antibodies levels and mother's age at delivery.

Age (years)	[10–20]	[20–30]	[30–40]	P value
Parameters				
IgA mg/L	3068.09 ± 837.50	3809.29 ± 1080.97	3561.23 ± 975.46	0.22
IgG mg/L	963.24 ± 165.18	1129.89 ± 231.91	1071.88 ± 236.03	0.148
IgM mg/L	719.11± 464.78	1271.83 ± 647.09	1170.69 ± 618.88	0.08

The effect of type of breastfeeding on childhood illness during the first 6 months after birth

In the exclusive breastfed group, 84.91% (n=45) of infants were healthy whereas 25% (n=4) and 57.14% (n=4) where healthy in the mixed breastfed and in the formula groups respectively (Table 3). In the exclusive breastfed group, 15.09% (n=8) infants suffered from different pathologies such as malaria, cold and cough, jaundice, bacterial infection or diarrhea (Table 5). In the mixed breastfed group, 75% of infants suffered from cold, jaundice, bacterial infection, diarrhea, abscess or respiratory distress (Table 5). Three formula fed infant suffered from respiratory distress or fever. Table 4 showed that the majority of sick children were aged less than 6 months (Table 4).

Table 3: Exclusive breastfeeding allows healthy children.

Type of breastfeeding	Healthy children	Sick children	Total	P value
Exclusive breastfed % (n)	84.91 (45)	15.09 (8)	100 (53)	0.001
Mixed breastfed % (n)	25 (4)	75 (12)	100 (16)	
Formula % (n)	57.14 (4)	42.86 (3)	100 (7)	

Table 4: Exclusive breastfeeding correlate with good health during the first 24 months after birth.

Type of breastfeeding	0 to 6 months	7 to 24 months
Exclusive breastfed	(n=41)	(n=12)
Healthy children	33	12
Sick children	8	0
Mixed breastfed	(n=14)	(n=2)
Healthy children	4	0
Sick children	10	2
Formula	(n=4)	(n=3)
Healthy children	2	2
Sick children	2	1

Table 5: Different diseases observed depending of the type of breastfeeding.

Diseases	Exclusive breastfed (n)	Mixed breastfed (n)	Formula (n)
Abscess	0	1	0
Bacterial infection	1	1	0
Cold	1	1	0
Diarrhea	2	3	0
Fever	0	0	1
Jaundice	3	4	0
Malaria	1	0	0
Respiratory infection	0	2	2

Furthermore, in breast milk from mother who exclusively breastfed, the concentrations of IgA (3.903 ± 1.151 g/l), IgG (1.147 ± 2.639 g/l) and IgM (1.308 ± 0.747 g/l) were higher compared to milk from mothers who have given mixed breastfeeding (IgA=3.33 ± 1.16 g/l, IgG=1.15 ± 0.26 g/l and IgM=1.04 ± 0.63g/l) or those who have given formula to their infant (IgA=2.627 ± 1.228 g/l, IgG=0.892 ± 0.294 g/l and IgM=0.401 ± 0.047 g/l) (p=0.013, p=0.018, p=0.017 respectively) (Table 6).

Peripheral blood IgA, IgG and IgM levels in infants according to the type of breastfeeding

Our observations showed that between 0 to 6 months, the mean of IgG levels were 3982.06 mg/L in healthy infants while it was slightly higher in infants who suffered from bacterial infection (4191.13 mg/L), jaundice (4197.41 mg/L), respiratory infection (5030.49 mg/L), digestive infection leading to diarrhea (4819.91 mg/L) or fever (4212.55 mg/L) (Figure 3). Peripheral blood IgM levels were relatively lower in children who had been

Table 6: Exclusive breastfeeding favor higher levels of antibodies in human breast milk.

Type of breastfeeding	Exclusive breastfeeding	Mixed breastfeeding	Formula	P value
IgA mg/L	3903.34 ± 1151.93	3329.07 ± 1165.75	2627 ± 1228.2	0.013
IgG mg/L	1147.82 ± 263.97	1019.81 ± 293.11	892.71 ± 294.77	0.018
IgM mg/L	1308.90 ± 747.85	1036.42 ± 634.00	401.76 ± 46.89	0.017

ill compared to healthy children (Figure 3). Similarly, IgA levels were relatively lower in the peripheral blood of children who had been sick, except in children who had diarrhea where the IgA concentration was slightly higher (Figure 3).

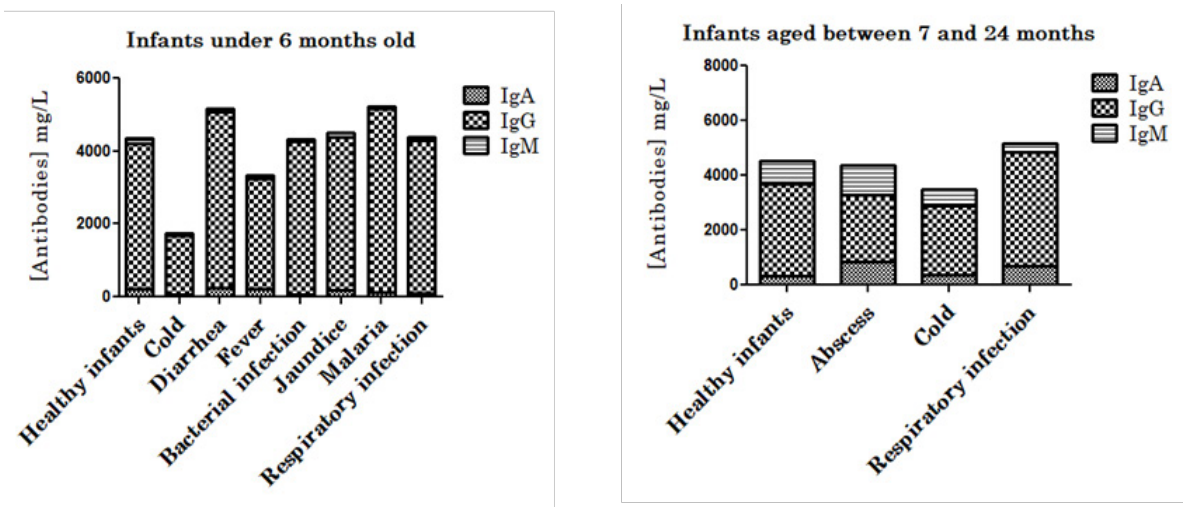

Figure 3: Peripheral blood IgA, IgG and IgM levels in healthy vs sick infants depending of age. Antibodies levels in infants under 6 months (**A**) and infants aged between 7 and 24 months (**B**).

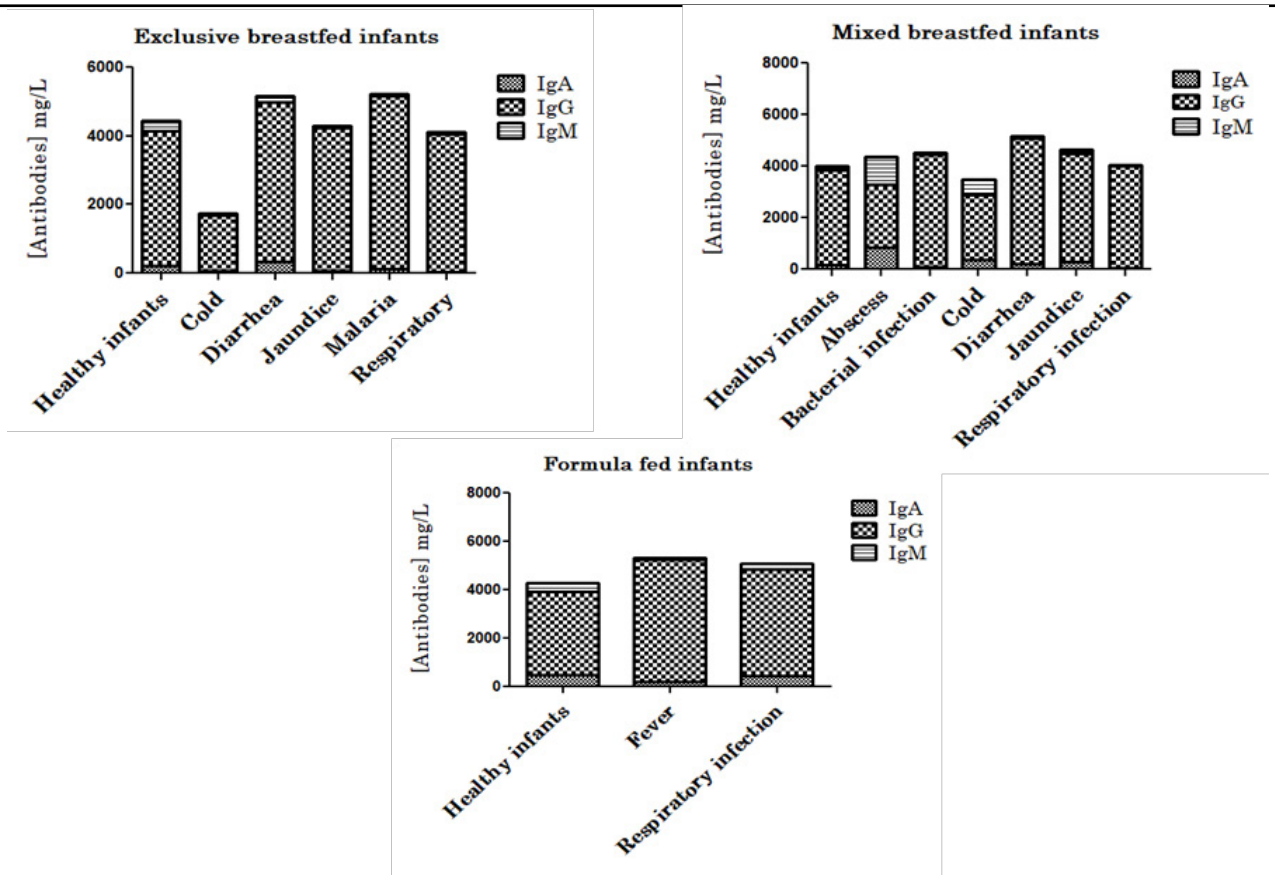

Figure 4: Peripheral blood IgA, IgG and IgM levels in healthy vs sick infants depending of the type of breastfeeding. Antibodies levels in exclusive breastfed (**A**), mixed breastfed (**B**) and formula fed (**C**) infants.

Among infants aged 7 to 24 months, mean IgG levels were 3376.83 mg/L in healthy infants and 4136.97 mg/L in those with respiratory infection. Moreover, it is lower in children who have had a cold (2501.79 mg/L) or an abscess (2425.02 mg/L) (Figure 3). The IgA levels in these children who had been sick were also slightly higher, whereas the IgM level was only elevated in a child who had had an abscess (Figure 3).

Our observations also showed that in healthy infants fed exclusively with breast milk, the mean level of serum IgG was 3934.95 mg/L, while in infants who suffered from diarrhea or jaundice or a bacterial infection, the level of total IgG was higher (Figure 4A). On the other hand, this level is lower in children who have had a cold (Figure 4A).

In healthy infants who received mixed breastfeeding, the mean level of total serum IgG was 3670.42 mg/L while in infants who had diarrhea, or jaundice or a bacterial or respiratory infection, the level of total IgG was higher (Figure 4B). However, it is lower in children who have had an abscess or a cold (Figure 4B).

In healthy infants who were formula fed, the mean peripheral IgG level was 3461.96 mg/L, while in infants who suffered from respiratory infection or who had a fever, the mean IgG levels were respectively 4427.09 mg/L and 5030.49 mg/L (Figure 4C).

Peripheral IgA and IgM levels are low and vary little depending on breastfeeding mode (Figure 4).

Discussion

Numerous immunological and cellular components are present in Human Breast Milk (HBM) and significantly affect the development of the newborn, its immune development and health [1]. Although the presence of these components has been well demonstrated, less is known about their specificity against microbes and childhood diseases [1,12].

Many factors, including race, ethnicity, environment, diets, culture, age etc might influence the composition of human breast milk [1]. In this study, we confirmed in a part of Beninese population the composition of colostrum, transitional and mature milk and the importance of exclusive breastfeeding in prevention against communicable and non-communicable disease. It was clearly demonstrated in this study that the total cell count and immunoglobulin concentration decreases from colostrum through transition milk to mature milk, as in previous observation [1,13-15]. Our data shown that HBM antibody concentrations decrease during the time and become stable when the milk is mature (Figure 2D) [15,16].

This observation confirms the importance of colostrum in the immune protection transmitted by the mother to the newborn [17,18]. Our major finding is the role of exclusive breastfeeding in protection against childhood disease which confirm the previous observations [19-21].

The influence of maternal age at delivery on immunoglobulin concentrations was also studied. No significant relationship could be observed for IgA and IgG concentration according to maternal age. However, a trend towards higher IgM concentration seems to occur after 20 years of age [22,23].

Although breast milk provides different immunoglobulin isotypes, IgA is more prominent and in the neonatal gut neutralizes bacterial and viral pathogens by binding to them. This neutralization of the microorganisms limits then their ability to interact and interfere with epithelial cells [1,24-27]. These characteristics easily explain the high concentrations of IgA found in human breast milk according to different types of lactating, especially in colostrum [1,22].

In addition, 90.79 % of the children in our study were breastfed. Children under-6 months were the most likely to be breastfed [28,29]. Among the breastfed children, 69.74 % had been exclusively breastfed. Among exclusive breastfed infants 77.36 % (n=41) were aged less than 6 months [1,28]. This confirms the natural tendency of mothers to breastfeed their newborns without necessarily understanding the immunological benefits and easily support the recommendation of the World Health Organization [2].

Depending on the type of breastfeeding, the concentration of immunoglobulins in breast milk varies considerably. Indeed, the concentration of IgA (2627 ± 1228.2 mg/L), IgG (892.71 ± 294.77 mg/L) and IgM (401.76 ± 46.89 mg/L) decreased in the milk of mothers who did not breastfeed their child compared to those who exclusively breastfed their child (IgA=3903.34 ± 1151.93 mg/L, IgG=1147.82 ± 263.97 mg/L and IgM=1308.90 ± 747.85 mg/L) or those who have given mixed breastfeeding (IgA=3329.07 ± 1165.75 mg/L, IgG=1019.81 ± 293.11mg/L and IgM=1036.42 ± 634.00 mg/L). Considering the amount of antibodies in maternal milk, the passive transfer of antibodies is very useful and depends on the previous and current exposure of the mothers to microorganisms [1,18,30].

Furthermore, our observations showed that children who were formula breastfed (42.85%) or mixed (75%) were more likely to be sick. Nowadays, it is clear that human breast milk and especially colostrum are protective against childhood infectious diseases and their recommendation must be strengthened [1,2,18,22].

Our study still has some limitations. Firstly, the number of the participants was too small and might skew the statistical analysis. More other, it would be better for this study to collect human colostrum, transition milk and mature milk from the same mother, which could better reflect the change in breast milk. Finally, more efforts are still needed to explore the specificity of breast milk-derived cells and antibodies and their effects on childhood disease.

Peripheral blood IgM levels were relatively lower in children who had been ill but overall elevated IgG levels suggesting that these children have an established memory response or have benefited from transfer of IgG to peripheral blood after breastfeeding. Moreover, the levels of IgG in sick children are high and suggest an increase of IgG levels in the mammary glands of mother's breastfeeding their child. Indeed, during breastfeeding, the infant's mouth-derived pathogen may be retro-transferred via the nipple to breast and results in a mammary immune response [18,31,32]. These immune response leads to specific antibodies production in milk and fed to the infected infant [33-35]. Cleary, maternal, and infant infections stimulate a rapid leukocyte response in breast milk [18]. IgA levels were relatively lower in the peripheral blood of children who had been ill except in children who had diarrhea whose IgA concentration was slightly higher. This suggests a probable passage of IgA from the digestive mucous membranes

into the blood, especially since it has been demonstrated that a sick child induces more antibody production by the breastfeeding mother [33-35].

Conclusion

The majority of infants cared at the district hospital of Comè in Bénin had been exclusively breastfed. The latter were much less likely to suffer from childhood diseases than those who had received mixed breastfeeding or formula. It is clear that the antibodies and cells transferred by the mothers during breastfeeding contributed significantly to this natural protection. It is also essential to assess the interference between maternally induced antibodies and cells following natural exposure to microorganism and vaccine antigens.

Author Contributions

Gatien AG Lokossou and Pierre Bessan Adjévi conceived and designed the study. Material preparation, data collection and analysis were performed by Pierre Bessan Adjévi, Julien Azonnakpo and Gatien AG Lokossou. Gatien AG Lokossou conceived and wrote the manuscript. All authors commented, revised and edited the manuscript.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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