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(RESEARCH ARTICLE)

Feeding practices of children born to HIV-AIDS positive mothers in the democratic Republic of Congo

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Abstract

The objective was to identify feeding practices among children born to HIV/AIDS-positive mothers in the Democratic Republic of Congo.

Methods: The study was conducted in the provinces of Haut-Katanga and North Kivu in Haut-Katanga, the study included seven ZS in the city of Lubumbashi and the ZS Sakania, Kipushi the ZS Nyiragongo, Rutshuru, Binza, Goma, Karisimbi and Kirotshe were included in the study. A total of 1234 HIV+ mother couples with children at least six weeks old and with early HIV diagnosis results available were included in the study. For each mother-child couple, data were sought from three complementary sites: ANC, Maternity Hospital and the Provincial AIDS Reference Laboratory.

Results: The majority of mothers were between 20 and 34 years of age (83.9 percent); three-quarters of these mothers were married (73.4 percent); half had a high school education (56.5 percent), and their main occupation was housework (41.9 percent); (96.8%) of women had given birth in a health facility; (79.8%) had done so in the health facility where they had attended ANC; only 11.3% of these women had experienced complications during delivery; two thirds of the women surveyed had children aged between 7 and 24 months (66.2%). The options cited were exclusive breastfeeding (61.3%) and artificial breastfeeding (46.0%). It was noted that 82.3% of women had put their baby to the breast within one hour of delivery. 90.3% reported exclusive breastfeeding, and it was noted that women chose to breastfeed because they were unable to buy artificial milk (42.9%). No mothers reported using a wet-nurse or the milk bank to feed their babies. However, the fact that mothers' child feeding options are not mutually exclusive suggests that although choice was made over one option, many mothers combine several to feed their children.

Conclusion: It is important to consider, in the current context, the threat that mixed feeding poses to efforts to prevent MTCT Emphasizing follow-up home visits that not only retain women in the program, but also ensure that they and their infants are adhering to treatment, can continue to maintain undetectable viral load and reduce the risk of MTCT associated with mixed feeding. These visits should be carried out by trained, motivated and accountable staff to ensure that they have maximum impact in reducing the rate of MTCT.

Keywords: Practice; Feeding; Newborn; Mother; HIV-positive

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1. Introduction

The HIV/AIDS epidemic has become a major problem in many countries. A very sad aspect of the epidemic is the number of infected newborns. This is one cause of the increasing number of deaths among young children. Indeed, 180 million children under the age of 5 suffer from stunted growth and 55 million suffer from wasting (including 20 million in its severe form). The most affected regions are southern central Asia and sub-Saharan Africa, the DRC being 9th. Thus, approximately 90% of stunted children live in 36 countries, 20 of which are in sub-Saharan Africa. The highest prevalence is therefore found in Africa, but when it comes to the number of stunted children, the sad record goes to Central and South Asia (1).

In 2010, UNICEF drew up a map of the factors affecting the survival, growth, and development of children around the world, among which is a low rate of exclusive breastfeeding in the first 4 months. She also believes, with the WHO, that if all infants and young children were exclusively breastfed for the first six months of their life and then received nutritious foods in addition to breastfeeding until the age two years, 1.5 million children's lives could be saved each year (2). Although significant progress has been made, UNICEF considers that in most countries breastfeeding rates are still too low. Indeed in 2002, less than 50% of children in the world are exclusively breastfed during the first 4 months (3). UNAIDS, WHO and UNICEF, in 2009, estimate that nearly 1,200 children under the age of 5 contract HIV infection every day and that nearly 2 million are living with HIV, 91% of them in Africa (4). Sub-Saharan Africa still bears most of the burden of the global epidemic with 2/3 of all children living with HIV worldwide and almost 3/4 of all infant deaths due to AIDS (5).

In many developing countries, HIV infection affects almost as many women as men. HIV infection affects 25–45 percent of infants born to HIV-positive mothers (6). HIV/AIDS affects 3.2 million children. 90% live in Africa and an estimated 52,000 children are infected in Côte d'Ivoire (7). It has been shown that a mother with HIV can transmit it to her child through her milk (8). This virus has been isolated from human breast milk. It was thought that this fragile virus could be destroyed by stomach acid and enzymes present in the baby's intestine, and that the latter's digestive system could act as a barrier to the virus. This is undoubtedly partly true (9).

Many children breastfed by mothers infected with this virus are not contaminated by breast milk. It is difficult, however, to determine when a child can be infected: before delivery, during delivery or when it is breastfed by its mother. This uncertainty is partly due to the fact that infected children, like those who are not, passively produce antibodies against HIV in response to their mother's infection, but the presence of antibodies (10) in standard HIV tests cannot be interpreted as a sign of active infection. A pregnant woman with vitamin A deficiency is more likely to transmit her HIV infection to her fetus. Transmission through breast milk is probably a relatively rare occurrence. Apparent differences in transmission rates among groups of women in several countries appear to be related to, among other factors, vitamin A intake (11).

A meeting of WHO and UNICEF experts issued very clear recommendations in this area, despite the fact that there is evidence of transmission of HIV through breast milk: in regions where infectious diseases and malnutrition are the main causes of death of children and the high infant mortality rate, breastfeeding should be advised to pregnant women including those with HIV, the reason for this is that the risks of viral infection through breast milk in infants remain lower than the risk of death from malnutrition(6). Many children in Africa, Asia and Latin America live in settings where gastrointestinal infections are common, poor sanitation and suspect water supplies, in these conditions the many benefits of breast-feeding far outweigh the risk of a child suckling from an HIV-infected mother. The use of bottles instead of breasts to reduce the risk of HIV/AIDS contamination should only be advised by the public authorities in regions where the common causes of infant morbidity and mortality are not infectious diseases (12).

Children infected with HIV/AIDS face many complications, one of which is malnutrition. Constituting a major public health problem in countries with limited resources, malnutrition is strongly involved in the development of HIV infection in children, an infection which in turn has deleterious effects on their nutritional status. However, the place of nutrition in the management of pediatric HIV remains insufficiently studied and integrated, particularly in West Africa. Indeed, few studies have documented the extent of malnutrition among these children in this region. The effects of antiretroviral therapy on growth and metabolism need to be further explored, and specific nutritional support for HIV-infected children is still limited. Breastfeeding significantly improves child survival. It protects against illnesses such as diarrhea, pneumonia and other infections that can be fatal (12).

Breastfeeding also offers other advantages such as better intellectual potential for breastfed children compared to those fed formula. Thus, the discovery that breastfeeding is also a route of transmission of the AIDS virus between an HIV-positive mother and her baby has posed a public health dilemma, particularly in countries where HIV affects

considerable proportions of the population and where breastfeeding is the cultural norm. However, the finding that exclusive breastfeeding may be as effective as complete cessation of breastfeeding in reducing vertical (mother-to-child) transmission of the disease gives hope that vertical transmission can be reduced under conditions where complete cessation of breastfeeding is difficult to accomplish during the first six months of a child's life (3).

In poor countries, HIV-positive women have difficult choices to make about feeding their infants. On the one hand, they risk contaminating the infant through breastfeeding. But if they stop breastfeeding, they don't have access to safe water, they don't have enough fuel to sterilize bottles and prepare replacement foods, or they can't buy enough special formula to ensure the nutrition of their babies. This dilemma is heartbreaking, and no mother can solve it alone (2).

2. Material and methods

2.1. Study environment and selection of subjects

The study was carried out in the provinces of Haut-Katanga and Nord-Kivu. In each of these provinces, all health facilities (FOSA) having integrated PMTCT, whatever the option, were included in the study. In Haut-Katanga, the study included seven HZs in the city of Lubumbashi and the Sakania, Kipushi and Kasenga HZs. In North Kivu, the ZS Nyiragongo, Rutshuru, Binza, Goma, Karisimbi and Kirotshe were included in the study. In each FOSA, the records of mother-child couples treated in the PMTCT program between November 2013 and January 2016 were considered as study units. In each FOSA, the mothers of the children included in the study were invited to participate in an interview on the practice of breastfeeding and infant nutrition, as well as on community interventions aimed at eMTCT.

2.2. Collection and analysis

All data collection tools were pre-tested before use and trained interviewers oversaw their collection. The data of each woman was handled confidentially. The authorization of the ethics committee of the University of Lubumbashi was obtained before the start of the study. For the interviews of the women, the free and written consent to participate in the study was obtained. Stata 10 and Mendeley software

The variables were: the package of care received, the ART regimen, adherence to ART represented by the frequency of ANC, the mode of delivery as well as the presence or absence of complications during delivery. In children, these were birth weight, maturity, taking ARVs, type of breastfeeding, feeding practices

3. Results

3.1. Sociodemographic characteristics of mothers

A total of 116 mothers were interviewed at the study sites about their breastfeeding and infant nutrition practices. Table 1 presents the distribution of these mothers according to the sites and HZs.

More than two-thirds of women interviewed were from Haut-Katanga (Table 1). In this province, it is in Lubumbashi, in the ZS of Lubumbashi that the majority were. No woman had been selected in the ZS Tshamilemba, Mumbunda, Kamalondo and Katuba. In North Kivu, it is in ZS Karisimbi and Nyiragongo that many mothers were recruited.

Many mothers interviewed were between 20 and 34 years old (83.9%), those under 20 and at least 35 years old represented 4.8% and 11.3% respectively. Three quarters of these mothers were married (73.4%), only 26.6% were not. Concerning the level of study, half had a secondary level (56.5%), 17.7% had a primary level, while 21.0% and 4.8% were respectively without level of study and had a university education level. The main occupation of these mothers was cleaning (41.9%) followed by sales (20.2%), the liberal trade (15.3%), the field (12.1%) and the public service (8.9%). Only 0.8% of mothers were involved in activities in private business or studies (pupils or students).

Almost all the mothers interviewed (96.8%) had given birth in a health facility, only 3.2% had done so at home. Among the women who had given birth in the FOSAs, 79.8% had given birth in the FOSA where they had followed the CPN while 16.9% had given birth in a FOSA other than that of the CPN. Only 11.3% of these women had experienced complications during childbirth and the same proportion (of all women) had delivered by caesarean section. On the day of the survey, two-thirds of the women surveyed had children aged 7 to 24 months (66.2%) 11.3% had children aged six months or less and 22.6% had those aged at least 25 months.

Health zones	Workforce	Percentage
Haut-Katanga	71	61.2
Kampemba	4	3.2
Kasenga	1	0.8
Kenya	1	0.8
Kipushi	7	5.7
Kisanga	1	0.8
Lubumbashi	53	46.0
North-Kivu	45	38.8
Binza	6	4.8
Goma	7	5.7
Karisimbi	16	12.9
Kirotshe	2	1.6
Nyiragongo	14	11.3
Rutshuru	8	6.5

Table 1 Number of breastfeeding mothers interviewed by data collection site

3.2. Information received by mothers, in FOSAs, on infant feeding options

During the ANC and in the maternity ward, the mothers had learned about several breastfeeding or infant feeding options. In order of frequency, the options cited were exclusive breastfeeding (61.3%) and formula feeding (46.0%). Mixed breastfeeding was mentioned by 16.9% of women. Similarly, 19.4% of mothers had mentioned other forms of breastfeeding and infant feeding such as cow's milk-based feeding, heated breast milk, the milk bank or the use of a wet nurse. Figure 5 presents the breastfeeding options mentioned by the mothers according to whether they had learned about them in.

3.3. Feeding the newborn within 24 hours after birth

In Figure 2, 41.1% of women had put their babies to the breast within 30 minutes after delivery, and 41.1% did so within an hour. Thus, in total, it was noted that 82.3% of women had put their baby to the breast within one hour of delivery. Only 17.7% of women had put the children to the breast beyond 24 hours after delivery (Figure 2). In addition to breast milk, 6.5% had given another drink to newborns. Among these drinks, the most given were artificial milk (50.0%), boiled water (37.5%) and honey (12.5%).

3.4. Feeding options chosen by HIV-positive mothers

During this survey, 90.3% had declared practicing exclusive breastfeeding, 26.6% artificial breastfeeding, 25.8% feeding with fresh cow's milk, while 6.5% had said to heat their milk before giving it to the baby. None of the mothers reported using a wet nurse or the milk bank to feed their baby. However, the fact that mothers' infant feeding options are not mutually exclusive suggests that although the choice was made on one option, many mothers combine several to feed their infants. Figure 3 presents mothers' reports of the feeding options chosen for their children.

The reasons for choosing the mode of feeding varied from one mother to another (Figure 4). It was noted in general, for breastfeeding, that women had chosen it because they were unable to buy artificial milk (42.9%). They also considered that breast milk was the best food for their baby (35.7%) that this mode of breastfeeding is the one recommended; 0.9% had other reasons for breastfeeding. Regarding artificial breastfeeding, some women had chosen it to avoid transmitting the infection to their children (64.7%); 17.7% had chosen it either because they had received the gift of milk or because they had no milk flow; 2.9% of women had given this milk because of a breast disease. Infant feeding practices in the 12 hours before the interview among children at least six months old, the exclusive breastfeeding rate was 36% at three months and 22.6% at six months. Moreover, while the majority of mothers declared having made the choice between exclusive breast-feeding and strict artificial feeding, it was noted that during the 12 hours which preceded the data

collection, the mothers' practices on feeding their infants were not consistent with these statements. Although breast milk was the staple food for all children, overall, the majority of mothers practiced mixed breastfeeding. Among children six months or younger, it was noted that although all children were breastfed, 70% had consumed the water in the 12 hours preceding the survey and 20% respectively had also consumed the milk. Artificial, tea and juice (Figure 5). The proportion of children six months or younger who were exclusively breastfed in the 12 hours preceding the survey was 28.6%, the rest were mixed breastfed. For children aged 7 to 12 months, none were exclusively breastfed. In addition to milk, the drinks given concomitantly to the children were water, tea, juice, yogurt, honey, and Lolly. Among those aged 13 to 24 months, it was noted that in addition to breast milk, water, tea, artificial milk, and Lolly were the drinks commonly given. Nearly 10% of children had respectively received liquid foods such as soup, yogurt, honey, Lolly and Maheu. Among children aged 25 months or older, water, tea and artificial milk were the most consumed drinks, followed by yogurt, Lolly and Maheu. Only 7.1% of children were still breastfed.

Not only were the children mixed feeding, but their diets were generally also mixed. In addition to the beverages mentioned in figure 5 A&B, we note that more than 21.4% of children under six months already ate, respectively, imported soy porridge, bread and 14.3%, porridge from maize or cassava flour base. For children aged 7 to 12 months, 15.4% already consumed maize-based foufou, 10.3% porridge made from maize or cassava flour, 7.7% bread, 5.1% fish and rice and 2.6% soy porridge, imported or meat. From 13 months, although these children continued to be breastfed, it was noted that 44.2% consumed fourfou and 23.3% rice. The other foods most consumed in this age group were imported porridge, bread, soy porridge or porridge made from corn or cassava flour, meat, or cereals. At 25 months, breastfeeding was almost non-existent, fourfou (64.3%) with fish (46.4%), bread (50.0%), imported porridge (39.3%) and rice (25%) had become the main meals for the children of the mothers surveyed.



Figure 1 Knowledge of infant feeding options based on information received at health facilities



Figure 2 Feeding practice of the newborn in the hours after delivery: (A) time to breastfeed, (B) other drinks given to the baby





Figure 3 Infant feeding options chosen by HIV-positive mothers to feed their infants

Figure 4 Reasons for choice of main feeding methods: (A) breastfeeding, (B) formula feeding



1 Sweetened drink made from water, sugar and colouring

Figure 5 Beverages (A) and foods (B) other than breast milk and formula given to children 12 hours before the survey

4. Discussion

Regarding child nutrition practices among infected mothers, only 22.6% of infected women had practiced exclusive breastfeeding up to six months. For the most part, they practice weaning too early, around three months of age. While many of these mothers reported practicing exclusive breastfeeding, in practice it was mixed breastfeeding that they used for their infants. Although breast milk was the staple food for most children, it was usually combined with other foods such as tea, yogurt, foufou, fish and even sugary drinks.

Regarding the feeding of infants exposed to HIV, breast milk is recognized as the food of choice for the growth of newborns. In low-income countries, exclusive breastfeeding is recommended for up to six months, before supplementing breast milk with other foods such as formula, water, or porridge.

Exclusively breastfeeding infants is associated with a large reduction in mortality from diarrheal diseases, but also from other infectious diseases. However, in the context of HIV infection, breastfeeding is associated with an MTCT rate of 15%, and this risk may be higher when the mother uses mixed breastfeeding than if she exclusively breastfeeds. Is explained using inappropriate and unsuitable foods for the age of the child, which are often responsible for the irritation of the intestinal mucosa, thus increasing the risk of MTCT through breast milk? Given that milk substitutes are not accessible to all HIV-affected households, and because food hygiene practices are still poor, the National Program for the Fight against AIDS (PNLS) recommends, for the infant feeding, to use exclusive breastfeeding up to six months. After this period, mothers should add other foods suitable for the nutritional needs of infants. This was not the case in our study sites. Failure to comply with this recommendation is first and foremost a question of precariousness in which the majority of women treated within the framework of PMTCT activities find themselves. Economically, they are poor and cannot afford the cost of milk substitutes after the planned six months and, culturally, they have no arguments to justify the strict cessation of breast-feeding after these six months. This cessation is thus perceived as a stigmatizing factor given the increasingly growing information in the population concerning the conduct of breastfeeding among PLWHA.

This study, carried out on the one hand from medical files, on the other hand, by interview, has certain biases. We have already discussed, above, the selection bias linked to the management of information among women and children in care. Regarding breastfeeding practices, the data reported in this study may also have been influenced by this same bias. Indeed, given that the interviews were carried out in the FOSAs, only consenting women able to pay for transport to the FOSAs were recruited in this study, but this does not in any way affect the quality of the data.

PMTCT guidelines in the Democratic Republic of Congo endorse WHO guidelines on infant feeding. Thus, the question of breastfeeding in the African context remains a dilemma for many non-breastfeeding women, at the risk of being rejected or stigmatized, as Oladokun shows in her study (13). This ultimately results in inappropriate infant feeding practices as evidenced by the rate of mixed feeding practiced by HIV-positive mothers in this study. Many women, after having chosen formula feeding, practice mixed feeding, which is the riskiest method in vertical transmission. In Ngwej's study, he found that HIV infection was significantly associated with mixed feeding (p<0.05) (14). This finding is like those reported in several studies, there is evidence that mixed feeding, compared to exclusive breastfeeding and strict artificial feeding, is associated with an increased risk of HIV transmission (15, 16, 17).

During mixed feeding, the beneficial immune factors of breast milk are likely to be outweighed by damage to the child's intestinal lining by contaminants or allergens in mixed foods. In contrast, exclusive breastfeeding promotes the maintenance of the integrity of the child's gastrointestinal barrier (considered the primary mode of infection). Recently, it has been established that immunological factors in breast milk are likely to reduce viral activity in human milk (18).

Transmission through breast milk can occur at any time during breastfeeding and the cumulative probability of acquiring infection increases with the duration of breastfeeding (19). In the Mbori study, the only randomized intervention trial published to date, the morbidity and mortality of breastfed and formula-fed infants born to HIV-1-infected women were assessed in four prenatal treatment centers in Nairobi, Kenya. Mothers were randomly assigned to formula feeding (n=186) or breastfeeding (n=185) and infants (n=371) were followed at 12 and 24 months. Formula was not provided free of charge to mothers in this study. The number of children alive and free of HIV infection at 2 years was significantly lower in the breastfeeding group than in the formula group. The cumulative proportion of HIV infection at 2-year follow-up was 21% in the formula group and 37% in the breastfeeding can be a safe alternative to breastfeeding for infants born to HIV-1 infected mothers in resource-limited settings (20).

A study conducted in Harare, Zimbabwe, assessed the effect of exclusive breastfeeding on infant mortality in 14,110 mother/newborn pairs. Of these, 4,495 mothers were HIV-infected and 2,060 of their pregnancies had breast or formula within 48 hours of birth. Feeding choice by the mother was mostly self-determined (43%), while social workers (22%) and grandmother (16%) were cited as the main sources of advice. These findings suggest the need for more effective social worker training and advocacy campaigns targeting women in resource-limited settings on the importance of exclusive maternal nutrition during the 4– first 6 months (21).

A study conducted in South Africa evaluated the influence of feeding mode on infant morbidity at clinic visits at 1 and 6 weeks, at 3 months and then every 3 months thereafter for 15 months. The results showed that the prognosis was worse for HIV-infected infants who had never been breastfed than for those who had. Nine or 60% of infants who had never been breastfed presented at least 3 morbid episodes compared to 15 (32%) of breastfed children. During the first 2 months of life, non-breastfed infants (regardless of HIV status) had nearly twice as many episodes of illness as breastfed infants. These findings underscore the risk of formula feeding in resource-limited settings. This study was conducted before the implementation of the South African comprehensive antiretroviral program (22).

Further research is needed to better understand the reasons why mothers choose not to exclusively breastfeed, particularly in communities with limited resources. In addition to the above factors, working mothers may not have adequate maternity leave, lack facilities to care for their child in the workplace, and work long hours, all of which make very difficult to initiate and maintain exclusive breastfeeding. In many countries, there is no legislation protecting breastfeeding in the workplace (23).

Infant leaky gut has also been associated with a higher risk of MTCT. A study in which 272 infants of HIV-infected women in South Africa were examined at ages 1, 6 and 14 weeks and tested with the two sugars lactulose/mannitol showed that Infants infected with HIV during the first 14 weeks had significantly higher intestinal permeability at 6 and 14 weeks and one resulted in a live birth. They were followed for up to 2 years. The overall rate of postnatal MTCT was 12.1 and 68.2% of these transmissions occurred after 6 months, highlighting the increased risk associated with prolonged breastfeeding beyond the first semester (23). Early mixed feeding has been associated with a higher risk of MTCT at 6, 12, and 18 months (16). In 2000, Coutsoudis et al. (24) drew widespread attention to the potential risk of

mixed feeding compared to exclusive breastfeeding. The results of a study conducted in Durban, South Africa, which assessed the infant feeding practices of 549 HIV-infected women indicated that, at around 3 months of age, exclusively breastfed infants had been less often infected with HIV (14.3%) than those receiving a mixed diet (24.1%) (25). A metaanalysis of data on late postnatal transmission of HIV-1 in 4085 breastfed infants in 9 trials indicated that the time of infection was known in 539 of these infants, and that, among these, 225 (42%) had contracted the virus in the late postnatal period (breastfeeding beyond 6 months). The cumulative probability of late postnatal transmission at 18 months was 9.3%. The prevalence of mixed feeding (instead of exclusive breastfeeding) appears to be high in countries where resources are limited (26). Some studies have indicated that many women institute mixed feeding due to insufficient milk. The decision to perform a mixed diet, however, is probably made based on several factors. A longitudinal study in a rural community with limited resources and high HIV prevalence (Mtubatuba, Kwazulu Natal, South Africa) followed 119 infants for 16 weeks. In addition, a cross-sectional survey was conducted among the mothers of 445 infants. The results showed that a small proportion (10%) of infants were exclusively breastfed for 6 weeks. The most common perceived reason for mixed feeding was insufficient breast milk. Another concerning observation in the longitudinal aspect of the study was that 46% of infants were given fluids other than milk slightly higher urinary excretion of neopterin (an indicator of immune system activation) at all-time points. Measurement compared to uninfected infants. Diet had no effect on the excretion of neopterin, an index of immunostimulation. These results suggest that infant HIV infection induces changes in intestinal permeability and, possibly, immune system activation before the onset of clinical manifestations (27). If mothers are to be encouraged to stop breastfeeding at 6 months, policymakers and social workers need to ensure that they receive adequate information and counseling on complementary or weaning foods.

5. Conclusion

In this work, we have shown that breastfeeding and child feeding practices carry a high risk of transmission. Very few mothers practice exclusive breastfeeding. Most practice mixed breastfeeding and solid foods are introduced into children's diets a little earlier. Social constraints, in particular: stigmatization, socio-cultural burdens and the poverty of infected mothers, reinforce the attitude of mothers to adopt mixed breastfeeding although they know the disadvantages in terms of risk of MTCT. While continuing to promote breastfeeding up to 6 months, it is important to consider, in the current context, the threat that mixed breastfeeding poses to PMTCT efforts. Emphasizing home-based follow-up visits to not only retain women in the program, but also to ensure that they and their babies are adhering to treatment, can continue to keep the viral load undetectable and reduce the risk of MTCT associated with mixed breastfeeding. These visits should be carried out by trained, motivated and accountable staff, to hope that they have their maximum impact on reducing the rate of MTCT.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors of this article declare that there is no conflict of interest Statement of ethical approval

The Ethics Committee of the University of Kinshasa for approval before carrying out the investigations under number: ESP/CE/091/2015.Statement of informed consent

Informed consent was obtained from all individual participants included in the study".

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