Efficacy of flash heat-treated breast-milk in improving health outcomes in HIV-exposed preterm infants: a prospective cohort study

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Lay Abstract

Formula feeding of infants in sub-Saharan Africa increases the risk of mortality due to diarrheal illness and other infections in the first year of life. Furthermore, mixed breastfeeding (breastmilk supplemented with formula or solid food) increases the risk of early HIV transmission when the mother is HIV-infected. Therefore, the WHO currently recommends that when formula feeding is not completely "acceptable, feasible, affordable, and safe," HIV-infected mothers should exclusively breastfeed their infants for the first six months of life. Breastfeeding, however, causes almost a half of all the pediatric HIV infections in sub-Saharan Africa. For that reason, WHO recommends heat treatment of breastmilk prior to giving it to the child, as a way to reduce the risk of passing HIV on to the baby. "Flash" heat treatment of milk in particular has been shown to kill HIV virus present in milk without damaging important vitamins and immunoprotective proteins. So far, there are no studies comparing outcomes in infants of HIV infected mothers fed with heat treated breastmilk compared to untreated milk or formula. We would like to evaluate the safety and effectiveness of heat-treated breastmilk among HIV positive mothers and their infants participating in an ongoing pilot program in Durban, South Africa. Specifically, we will look if heat-treating breast milk will make a difference in HIV transmission rate and general development of the infant compared to formula feeding.

Scientific Abstract

Formula feeding of infants in resource-poor settings increases the risk of mortality due to diarrheal illness and other infections in the first year of life. Furthermore, mixed breastfeeding (breastmilk supplemented with formula or solid food) increases the risk of early HIV transmission when the mother is HIV-infected. Therefore, the WHO currently recommends that when formula feeding is not completely "acceptable, feasible, affordable, and safe," HIV-infected mothers should exclusively breastfeed their infants for the first six months of life. Breastfeeding, however, accounts for up to 40% of pediatric HIV infections in sub-Saharan Africa at an estimated constant rate of 0.74% infections per month. One WHO-recommended method for reducing early HIV transmission from mother to child while retaining the nutritional and protective benefits of breastmilk involves expression and heat treatment of breastmilk prior to administering it to the child. "Flash" heat treatment of milk in particular has been shown to denature HIV virus present in milk without disrupting important vitamins and immunoprotective proteins, but no studies have been done documenting and evaluating clinical outcomes in HIV-exposed infants fed with heat treated breastmilk compared to untreated milk or formula. The purpose of this study will be to evaluate the safety and effectiveness of heat-treated breastmilk among HIV positive mothers and their infants participating in an ongoing pilot program in Durban, South Africa that facilitates heat-treatment of breastmilk for HIV-I positive mothers according to WHO guidelines. Information about the occurrence of infectious disease, HIV transmission rate and general development of the infant will be collected. These outcomes will be compared between three groups of infants: those who received heat-treated breastmilk, formula or non-treated breastmilk. We aim to elucidate whether heat treatment of breastmilk leads to clinically significant improvement in health outcomes and decreased HIV transmission rate in HIV-exposed preterm infants.

A. Study Purpose and Rationale

Up to 40% of pediatric HIV infections in sub-Saharan Africa can be attributed to breastfeeding.[1] The risk of postnatal HIV transmission has been estimated at 8.9 transmissions per 100 child-years of

breastfeeding, at a constant rate of transmission (0.75) over the period of breastfeeding.[2] However, replacement formula feeding has been associated with a 14- to 25-fold increase in diarrhea-related mortality in resource-poor settings,[3] presumably due to water contamination, unsanitary conditions, lack of immunoprotective factors compared to breast milk, decreased adherence due to cost and availability, and sociocultural factors.[4] Meta-analysis has demonstrated that lack of breastfeeding compared with any breastfeeding exposes infants to increased risk of malnutrition, diarrhea, and pneumonia, especially in the first year of life.[5] In resource-poor settings, replacement feeding during the first six months of life has been associated with up to a tenfold increase in mortality due to infectious diseases.[6]

Current WHO guidelines only recommend replacement formula feeding for HIV positive mothers when it is completely "acceptable, feasible, affordable, and safe". If these conditions are not met, mothers are advised to exclusively breastfeed until the child is 6 months old.[5] The increased mortality associated with formula feeding and mixed breastfeeding in the first six months of life compared to exclusive breastfeeding in HIV-positive, under-resourced populations[7] confirms a need for further research identifying safe methods of exclusive breastfeeding. It is absolutely imperative that recommended methods are socially acceptable, culturally appropriate, and minimize the risk of transmission of HIV while maximizing nutritional and immunological benefit.

WHO, UNICEF, and UNAIDS recommend manually expressed, heat-treated breast milk as an alternative to breastfeeding for HIV-positive mothers in developing countries. However, to date the feasibility and efficacy of using heat-treated expressed breast milk (HTEBM) have not been adequately explored in the literature. In terms of specific technique, previous work suggests that direct boiling of milk causes significant nutritional damage, and Holder pasteurization (milk heated to 62.5 degrees Celsius for 30 minutes) inactivates HIV by > 3 logs but requires temperature gauges and timing devices that may not be widely available in resource-limited communities. A newer and simpler technique, flash heating (described in detail below under "Study procedure"), inactivates > 3 logs of cell-free HIV-1 virus as detected by PCR, and eliminates all detectable enzymatic reverse transcriptase activity in samples infected in the lab and naturally.[8] Flash heating does not cause a significant decrease in vitamin content or antimicrobial activity of breastmilk, in fact retaining greater antibacterial activity compared to other heating methods.[9] Data also suggests that a majority of antibodies in breast milk survive flash-heat[10], and that flash-heated milk can be stored safely for up to 8 hours.[11]

Qualitative research in Zimbabwe and unpublished data from Tanzania[12] show that many HIV-positive mothers and health care workers are initially unaware of heat-treatment as a feeding option despite the WHO recommendation, but desire more information about the technique. Education on the treatment's virucidal effect, counseling, and practical demonstration led the majority of participants in both studies to conclude that if nutritious and safe, heated breast milk could be a feasible and sustainable mother-controlled alternative to formula in their communities.[13]

Studies comparing human milk from preterm mothers with that of term mothers clearly demonstrate that the immunologic benefits of breast milk are even greater for low birth weight and very low birth weight preterm infants due to relatively increased concentrations of secretory IgA, lysozyme, lactoferrin, and interferon.[14] There is extensive evidence that even very low birth weight preterm infants fed with breast milk in developed settings have better outcomes in terms of incidence of infection-related events and length of hospitalization than comparable infants fed with preterm formula.[15,16]

The purpose of the current study will be to build on the prior research reviewed above by evaluating health outcomes in an existing pilot program in Durban, South Africa. This program facilitates heat-treatment for HIV-infected mothers who are choosing to express breast-milk and exclusively breastfeed their preterm infants in accordance with WHO guidelines. We believe that documenting health outcomes in this population will enable providers and policymakers to better assess whether flash heat-treatment of

breast milk from HIV-infected mothers and its administration to a vulnerable population of infants leads to a clinically significant improvement in health outcomes compared to administration of preterm formula. Controls will include formula feeding HIV-positive women who cannot express milk or choose not to, and HIV-positive women who are exclusively breastfeeding but choosing not to heat-treat. Our hope is that this data will eventually enable HIV-positive women in resource-poor settings to make a more educated choice regarding how to most safely and conveniently nourish their children.

B. Study Design and Statistical Analysis

This will be a prospective, non-randomized intervention cohort study. HIV-infected women delivering preterm infants (born at 37 weeks or earlier) at King Edward VIII Hospital in Durban, South Africa will be invited to enroll in the study if they are 16 years of age or older, plan to stay in the study area for at least 6 months after delivery, and provide written informed consent.

All women regardless of HIV serostatus will receive post-natal counseling by a feeding counselor regarding the full range of feeding options available to them, including a demonstration of flash heat treatment and an explanation of the heat treatment's utility in the event of maternal HIV infection. Mothers and their infants will enter the respective arms of the study (heat-treated exclusive breastmilk, exclusive breastmilk, or formula feeding) based on their feeding choice, and will be able to change their feeding choice at any time. Mothers will be counseled again at the time of discharge from the neonatal intensive care unit/newborn nursery regarding their plan for feeding in their respective home environments.

The primary outcome will be the number of infection-related events (eg. diarrheal illness, pneumonia, sepsis, meningitis) occurring for each infant of an HIV-infected mother over the 6 month study period. Infection will be documented by the presence of clinical signs of a systemic inflammatory response (two elevated values in heart rate, respiratory rate, temperature, or white blood cell count) and/or positive cultures for pathogenic organisms at one or more of the following sites: blood, spinal fluid, urine, stool, pleural fluid, respiratory secretions, umbilicus, or surgical wound. Mothers will be instructed to bring their infants to clinic in the event of illness at home for effective diagnosis, and to record any symptoms occurring at home in the event that the infant cannot be brought in. Data on number of infections will be analyzed using a two-sided t test. Growth (weight, length, and head circumference; two-sided t test) and HIV seroconversion at 6 months (chi-square) will be measured as secondary outcomes. Logistic regression analysis will be conducted retrospectively to control for the potential confounding effect of seroconversion on morbidity/number of infection-related events in infants with HIV-positive mothers.

In analyzing the data, outcomes in the group of HIV-positive mothers in the HTEBM arm, the exclusive breastfeeding arm, and the formula arm will be compared. Subgroup analysis will subsequently be performed to compare health outcomes in low birth weight (< 2500g) vs. very low birth weight infants (<1500g).

We assume that in an enrollment period of 6 months in a 25 bed nursery receiving 3-4 new infants a day, we will be able to enroll 300 infants and their mothers into this study. HIV prevalence in pregnant women presenting to King Edward VIII Hospital is 50-60%. We predict that with adequate counseling and education, at least 2/3 of participating HIV-positive mothers will agree to join the HTEBM arm of the study and 1/3 of mothers will use preterm formula, whether by default or choice. Power analysis using unpaired t-test shows that this sample size is more than adequate to detect a clinically significant 20% difference in number of infections between the primary study groups (at chosen power of 80% and alpha of 0.05%).

C. Study Procedure

Maternal sociodemographic information, HIV serostatus based on routine prenatal testing, most recent CD4 cell count, and infant weight at birth will be recorded at the time of enrollment. Single-dose nevirapine will be provided for all HIV-infected women and their infants any time after first booking or at 28 weeks of gestation in accordance with current standard of care. Duration of ICU and hospital stay will be recorded in all cases. Infant DNA PCR within 48 hours of birth will be collected. Six months supply of commercial infant formula will be offered free through the KwaZulu Natal prevention of mother to child transmission (PMTCT) program; HIV-positive mothers in the formula feeding arm can choose to access this supply at any time in the child's first 12 months of life. Mothers will present for routine follow-up in neonate clinic 6 weeks after delivery and then for 2-3 monthly visits as dictated by the infant's clinical condition in accordance with current standard of care in this nursery. At every visit, study nurses will measure the infant, record any events of interest (e.g. intercurrent illness) reported by the parent, take a dried blood spot sample by heel or finger prick from the infant, and take a breast milk sample from the mother if possible. Relevant information such as whether or not a mother is also receiving highly active anti-retroviral therapy (HAART) or has a CD4 count less than 200 will be recorded.

The flash heat treatment method will involve manually expressing 50mL of breast milk into an uncovered glass peanut butter jar, which is then placed in 450mL of water in an aluminum pan. The water and milk are heated together over a high flame until the water first reaches a rolling boil. The breast milk is immediately removed from the water, covered, and allowed to cool and then cup or spoon-fed to the infant. The milk can remain unrefrigerated for up to eight hours. Field observations using local stoves, utensils, pans, and jars and varying water and milk volumes in South Africa, Kenya and Tanzania have shown that this protocol can be adapted as long as the water level is approximately two finger-widths above the milk level.[12]

D. Study Drugs

None.

E. Medical Device

None.

F. Study Questionnaires (see attached)

Qualitative questionnaires will be orally administered to mothers at the time of their infants' NICU admission (with the counseling session), at the time of discharge, and at the end of the six-month study period. Questionnaires will assess awareness of breastfeeding recommendations and anticipated feeding choice before counseling, any change in perceptions after counseling, and will elicit what prompted a particular feeding choice. The survey will also assess what factors are playing into maternal feeding choice and practice, including fear of HIV-related stigma and resources available in the home environment. Questionnaires will be administered in Zulu and English.

G. Study Participants

All mothers delivering preterm infants and admitting to the neonatal intensive care unit (NICU) at King Edward VIII Hospital in Durban will be counseled regarding their feeding choice and screened for eligibility. 300 HIV-infected mothers who are 16 years of age or older, plan to stay in the study area for at least 6 months after delivery, and are able to provide written informed consent will be enrolled and will enter one of three study arms based on their choice. The study arms are: flash heat treatment, exclusive

breastfeeding without heat treatment, or formula feeding. Infants with a birth weight lower than 700g, or greater than 2500g will be excluded from the study.

H. Participant Recruitment

All mothers delivering preterm infants at the study location will be screened for eligibility, counseled regarding their feeding choice for their infant, and invited to provide written informed consent to participate in the study in person.

I. Confidentiality

All heat treatment will occur just outside of the actual nursery and with the assistance of a nurse to ensure participant confidentiality regarding HIV status, and thereby avoid any potential stigma associated with it. All patient information will be identified with a study code number and kept in a secure locked file in the research assistant's office. Lactation counselors will not know the HIV status of study participants prior to beginning their counseling sessions. A separate project monitor will record the feeding choice of eligible mothers and inform the nursing staff of this choice.

J. Conflict of Interest

None.

K. Location

The study will be based in the neonatal intensive care unit and newborn nursery at King Edward VIII Hospital in Durban, KwaZulu Natal (KZN), South Africa. This is the second largest hospital in South Africa, performing 13,000 deliveries annually, and providing tertiary care to the entire province of KZN as well as part of Mpumalanga and the Eastern Cape. It is also the main teaching hospital for the Nelson R Mandela Medical School of the University of Natal.

L. Potential risks

There is a potential risk that heat-treatment leads to nutritional depletion of breast-milk. However, it has been demonstrated in the lab that flash heat treatment does not lead to significant drops in the amount of protein (and specifically immunoglobulin), vitamins, and bactericidal activity of the milk. There is also a potential risk of HIV transmission which needs to be explained to all study participants—while the intervention is aiming to minimize HIV transmission, and while heat treatment eliminates HIV viral load in vitro, it is not a clinically demonstrated guarantee that HIV transmission will not occur.

M. Potential benefits

This intervention has several potential benefits, such as decreased risk of HIV transmission in the HIV-positive breastfeeding group due to flash heat treatment (compared with standard of care, which is non-heat treated exclusive breast feeding regardless of HIV-status), and an immunoprotective benefit to the infant. Participants will also benefit from information and counseling about safe feeding techniques.

N. Alternative therapies

As discussed above, alternative heat treatment methodologies have not been shown to be as effective and safe as flash heat treatment.

O. Compensation

There will be no formal monetary compensation.

P. Costs to participants

There will no associated additional costs to research participants. Formula will be provided to mothers who cannot or are unwilling to express breastmilk. The flash heat treatment method requires no technical equipment in the home. No follow-up visits outside of the routine standard of care will be required.

Q. Minors as Research Subjects

Separate approval from the Department of Pediatrics Committee on Human Investigation will be obtained prior to submission for IRB review. The study will also undergo necessary review by the Ethics Committee of the Nelson R Mandela Medical School at the University of Natal in Durban, South Africa.

Study Survey

I. Pre-counseling survey

Demographic information

1. How old are you?
2. What is your first language?
3. What is the highest level of education you have obtained?

Obstetric history

4. How many pregnancies have you carried to term? Preterm?
5. How many living children do you have?
6. How many abortions? Miscarriages?
7. Did you have any complications during this pregnancy? If so, what were they?

Medical history

8. Do you have any existing medical conditions?
9. Have you ever been diagnosed with HIV?
    a. If you have been diagnosed with HIV, do you know your most recent CD4 count?
    b. Have you had any HIV/AIDS-related symptoms recently (a list of common symptoms will be provided)?
    c. Are you taking any medications for your HIV?
        i. If yes, which medications?
        ii. If no, have you taken or been offered medication in the past?
    d. Does your partner/the father/your family know about your HIV status?
    e. If no, do you want them to know about your HIV status?

Breastfeeding history

10. Have you breastfed your children in the past?
    a. If yes, for how long did you breastfed each infant?
        i. Did you face any obstacles to breastfeeding? What were they?
    b. If no, why did you choose to formula feed?

Breastfeeding attitudes

11. Do you have any worries or concerns about breastfeeding?

12. What do you think about women who do not breastfeed their babies?
13. What do you know about heat treatment of breastmilk as an option for you and your infant?
14. Does your baby's father, if he is involved, or other members of your family or community have a preference as to how you feed your child?
15. What about breastfeeding would you like to know more about (e.g. proper positioning, advantages and disadvantages to breastfeeding, etc) ?

Understanding of mother-to-child-transmission of HIV

16. What do you think about how HIV may affect your child?
17. What do you know about how HIV can spread from mothers to their children?
18. Can HIV be spread through breastmilk?
19. Should women who have HIV breastfeed?

Availability of resources

20. Where do you live?
21. How long do you expect to live in this place?
22. Do you have access to a refrigerator? Freezer?
23. Do you have electricity?
24. Are you the primary source of income for your family? If not, who is?
25. Are you employed? What do you do for work?
26. Does concern about money affect your decision on how to feed your child?

Feeding choice

27. What is your planned feeding method for this child? (Possible confounding influence of preterm status, concerns over medical condition?) If breastfeeding, how long do you plan to breastfeed?
28. What are your concerns about feeding your child?

II. Counseling session based on algorithm presented in Bland et al [Infant feeding counseling for HIV-infected and uninfected women: appropriateness of choice and practice. Bull WHO: 2007, 85(4): 289-296], modified to include explanation and demonstration of flash heat treatment.

III. Post-counseling survey

Feeding choice

29. What is your planned feeding method for this child?
30. Why do you prefer this method?
31. What concerns or questions about feeding your child do you still have?

Flash heat treatment acceptability and feasibility

32. Do you feel comfortable hand-expressing and heating your breastmilk, and then feeding your infant the milk? If no, why not?
33. Do you think members of your community or family would have any objection to the flash heat treatment? Why or why not?
34. Do you feel comfortable making the choice to feed flash heated breastmilk to your child?
35. Do you have the necessary materials and resources to do this in your home?

IV. Pre-discharge survey

Feeding choice

36. What is your planned feeding method now that you are going home?
37. Why do you prefer this method?

38. What concerns or questions about feeding your baby do you still have?

V. Exit survey

Feasibility of HTEBM and future research priorities

39. If you heat-treated your breastmilk, how many times a day were you able to do so?
40. What problems did you have with heat-treating?
41. What questions about breastfeeding and/or its relationship to HIV do you still have?

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