East African Medical Journal Vol. 89 No. 6 June 2012
PREDICTORS OF INCIDENT TUBERCULOSIS IN HIV-EXPOSED CHILDREN IN TANZANIA
J. L. Finkelstein, MPH, Division of Nutritional Sciences, Cornell University, Ithaca, New York, K. P. Manji, Department of Paediatrics, C. Duggan, Department of Nutrition, Division of GI/Nutrition, Children's Hospital, Boston, MA, E. Hertzmark, Department of Epidemiology, S. Mehta, Division of Nutritional Sciences, Cornell University, Ithaca, New York, G. I. Msamanga, Department of Community Health, Muhimbili University of Health and Allied Sciences, Dar-es-Salaam, Tanzania, D. Spiegelman, Departments of Epidemiology and Biostatistics and W. W. Fawzi, Departments of Nutrition, Epidemiology and Global Health and Population, Harvard School of Public Health, Boston, MA

Request for reprints to: Dr. J. L. Finkelstein, Division of Nutritional Sciences, Cornell University, Ithaca, New York, 14853

PREDICTORS OF INCIDENT TUBERCULOSIS IN HIV-EXPOSED CHILDREN IN TANZANIA

J. L. FINKELSTEIN, K. P. MANJI, C. DUGGAN,
E. HERTZMARK, S. MEHTA, G. I. MSAMANGA, D. SPIEGELMAN and W. W. FAWZI

ABSTRACT

***Objective*: To examine the predictors of tuberculosis infection in HIV-exposed children.**
***Design*: A longitudinal cohort study nested within a randomised controlled trial.**
***Setting*: Antenatal clinics in Dar-es-Salaam, Tanzania.**
***Subjects*: Children born to 875 HIV-infected women in Tanzania.**
***Results*: A total of 82 children developed tuberculosis during the follow-up period. In multivariate analyses, HIV infection was associated with a six-fold increase in risk of tuberculosis. Breastfeeding duration, child mid-upper arm circumference, and maternal CD4 T-cell counts were inversely related to risk of tuberculosis. In HIV-infected children, greater number of people eating at the same household meal and child CD8 T-cell counts were associated with increased risk of tuberculosis; higher maternal lymphocyte counts, increased duration of breastfeeding, and lower vitamin E levels were associated with reduced risk of tuberculosis. In HIV-uninfected children, breastfeeding duration and increased child mid-upper arm circumference were associated with reduced risk of tuberculosis.**
***Conclusion*: Breastfeeding duration, HIV status, maternal and child nutritional and immunological status were important predictors of child tuberculosis. Appropriate infant feeding and nutritional interventions could represent important adjuncts to prevent tuberculosis in children born to HIV-infected women in sub-Saharan Africa.**

INTRODUCTION

One in three people are infected with *Mycobacterium tuberculosis* worldwide - with more than one new infection each second (1). Over 250,000 children develop tuberculosis and 100,000 die of tuberculosis each year (1).

Infants and young children are at increased risk of becoming infected with tuberculosis bacilli and progressing to active tuberculosis, as their immune system is less developed. Several factors may contribute to risk of tuberculosis infection in children, including HIV infection, malnutrition, socio-economic, demographic and environmental characteristics.

Studies examining risk factors for tuberculosis have predominantly focused on adults, risk factors include socio-economic and environmental conditions, such as crowding and inadequate ventilation, HIV infection, malnutrition, and other serious illnesses (2-5). However, many of these studies have been constrained by short duration of follow-up, and few studies have been conducted among children, particulary among HIV-exposed children, who may be at increased risk. Further elucidation of factors that influence risk of tuberculosis in children may assist in the prevention and appropriate diagnosis and case management.

We conducted a prospective observational analysis of predictors of tuberculosis in children born to HIV-infected women in Tanzania, a country with one of the highest burdens of tuberculosis (1).

MATERIALS AND METHODS

Study Design and Population: Participants were women and their children enrolled in the Trial of Vitamins (TOV), a randomised placebo-controlled trial conducted in Dar-es-Salaam, Tanzania (1995-1997). This study was conducted to examine the effects of daily multivitamin supplementation in HIV-infected pregnant women on the risks of mother-to-child HIV transmission, HIV disease progression, and adverse perinatal outcomes, among 1,078 HIV-infected pregnant women and their children (6, 7). The detailed design of the trial has been previously described (8).

Ethics: Informed consent was obtained from all mothers. The research protocol was approved by the Research and Publications Committee of Muhimbili University College of Health Sciences, the Ethical Committee of the Tanzanian National AIDS Control Programme, and the Institutional Review Board of the Harvard School of Public Health.

Assessment of Baseline Co-variates: Structured interviews were conducted at the baseline clinic visit between 12 and 27 weeks gestation to collect information on demographic characteristics, including maternal age, educational level, and socio-economic status, and obstetric history. Study physicians performed a complete medical examination and collected blood, urine, stool, and vaginal swab specimens to assess co-infections. HIV-1 serostatus was determined using an enzyme-linked immunosorbent assay (ELISA) (Wellcozyme; Murex Biotech), and positive results were confirmed using Western blot analysis (Bio-Rad). HIV disease stage was classified in accordance with the World Health Organisation (WHO) guidelines (9). Participants were counselled regarding the risks and benefits of infant feeding options for HIV-infected mothers, as per World Health Organisation guidelines and standard of care in Tanzania at that time.

Follow-up: Of 965 live singleton births, 875 had available information on tuberculosis status during follow-up, and were included in these analyses (Figure 1). Children were followed for a median duration of 58 months (IQR: 13-69).

Clinical evaluations were performed at monthly and interim clinic visits to evaluate maternal and child health status. A study physician examined the children and recorded clinical data. Anthropometric measurements were obtained by trained research assistants using standardised procedures and calibrated instruments.

Participants who missed a clinic visit or traveled outside of Dar-es-Salaam were followed *via* home visits, to establish maternal child health and vital status. Child mortality was defined based on mother's self-report.

Laboratory methods: Whole blood samples were collected from women at baseline, delivery, six weeks postpartum, and every six months thereafter; and from children at birth (range 0-21 days), at six weeks (range 21-49 days), and at three-monthly thereafter. Laboratory samples were tested in batch, and instruments were calibrated daily using standardised procedures.

Infant HIV status was determined from blood samples collected at birth, six weeks and three-monthly thereafter. HIV infection was defined as a positive PCR test at any age; in children ≥ 18 months of age, HIV infection was defined by a positive ELISA, and confirmed by Western blot.

Albumin was measured by an automated dye-binding method using the Hitachi 911 analyzer and Roche Diagnostics reagents (Indianapolis, IN, USA), and cholesterol levels were determined based on an enzyme and subsequent peroxidase/phenol-4-aminophenazone indicator reaction. Haemoglobin concentrations were assessed using a CBC5 Coulter Counter (Coulter Corporation, Miami) or the cyanmethemoglobin method with a colorimeter (Corning Inc, Corning, NY).

Assessment and Definition of Outcome: Tuberculosis was diagnosed in accordance with World Health Organisation criteria (10) and clinical guidelines (11, 12). Childhood tuberculosis data were collected by review of clinical files (KPM); the Paediatric Tuberculosis Score Chart (TSC), modified from Edwards *et al* (13), was used to diagnose tuberculosis. This 11-item scale (0-3 score) included the following items: duration of cough, nutritional status, tuberculosis in family, positive tuberculin skin test, enlarged painless neck glands (lymphadenopathy), night sweating or prolonged fever, spinal swelling, prolonged malnutrition and ascites or abdominal masses. Tuberculosis was defined based on a pre-determined cut off of ≥ seven for the paediatric tuberculosis score.

Statistical Analyses. We used Cox proportional hazard models to examine predictors of time to tuberculosis (14). We examined predictors of tuberculosis (n=82), and conducted additional analyses among both HIV-infected (n=226) and HIV-uninfected children (n=649). For children without the outcome of interest, follow-up ended on the date of the last visit or death. We also examined if results changed in Cox analyses among HIV-uninfected children, when we retained HIV-uninfected person-time for HIV-infected children, but censored them.

Conventional cut-offs were used to categorise risk factors, where available; otherwise, medians were used to classify variables, as is consistent with previous publications from this trial (15). Variables such as HIV infection and breastfeeding status were allowed to vary with time, and variables with univariate p-values of less than 0.20 were included in each of the multivariate regression models and retained if their p-values were less than 0.05. The final multivariate model was fully adjusted for all the univariate predictors (P<0.20) that were not retained. The missing indicator method was used to account for missing covariate data (16).

Potential predictors were also examined as continuous variables. We explored potential non-linearity of the relationships between covariates and outcomes non-parametrically, using stepwise restricted cubic splines (17, 18). A partial likelihood ratio test for non-linearity compared the model with only the linear term to the model with the linear and the selected cubic spline terms. If non-linear associations are not reported, they were not significant.

We examined the effect of regimen (multivitamins and vitamin A) assignment on incident tuberculosis in children born to the HIV-infected women enrolled in the trial. We also examined effect modification of regimen assignment by each covariate in the multivariate model (all variables with univariate P<0.20, listed in Table 2, namely: shillings spent per person per day on food, number of people eating at the same meal in the household, maternal progression to HIV Stage III/IV, maternal death, CD4 T-cell counts, lymphocytes, pathological protozoan infections, weight, mid-upper arm circumference, primiparity, systolic blood pressure; and child breastfeeding duration, serum cholesterol, serum albumin, mid-upper arm circumference, HIV infection, CD3 T-cell counts, and CD8 T-cell counts) using the Likelihood Ratio Test (LRT). If effect modification estimates are not reported, they were not significant.

Statistical analyses were performed using SAS software, version 9.2 (SAS Institute Inc., Cary, NC, US).

RESULTS

The baseline characteristics of the 875 women and children included in these analyses (Figure 1) are presented in Table 1. Baseline characteristics or multivitamin regimen assignment in this subset did not significantly differ from the original group of randomised participants.

Follow-up: A total of 82 children developed tuberculosis during the follow-up period. Two hundred and twenty six of the children (26%) became HIV-infected during the follow-up period, and 649 were HIV-uninfected (74%). Children were followed for a median duration of 58 months (IQR: 13-69).

Univariate (P<0.20) predictors of child tuberculosis are presented in Table 2. In multivariate analyses, HIV infection predicted a six-fold increase in risk of tuberculosis during follow-up (HR: 5.62, 95% CI: 3.24-9.73) (Table 2). Breastfeeding duration was associated significantly with reduced risk of tuberculosis, with a 9% reduced risk in child tuberculosis per month of breastfeeding (HR: 0.91, 95% CI: 0.87-0.94). Increased mid-upper arm circumference was associated with reduced risk of tuberculosis, with a 15% lower risk of tuberculosis per unit increase in mid-upper arm circumference (HR: 0.85, 95% CI: 0.74-0.98). Higher maternal CD4 T-cell counts also were associated with a significantly reduced risk of a child being diagnosed with tuberculosis during follow-up (HR: 0.86, 95% CI: 0.75-0.98).

There was no significant effect of regimen assignment on incident tuberculosis in children born to the HIV-infected women enrolled in the trial. However, primiparity was a significant modifier of the effect of multivitamins on the risk of tuberculosis; children born to primiparous women who received multivitamins had a 66% lower risk of tuberculosis (Primiparous: HR: 0.34, 95% CI: 0.13-0.87; CI; p-value (LRT): 0.03), compared to women who did not receive multivitamins.

In multivariate analyses among children with confirmed HIV infection during follow-up (n=226), increased number of people eating at the same meal in the household (HR: 1.20, 95% CI: 1.01-1.43) and increased child CD8 T-cell counts (HR: 1.11, 95% CI: 1.01-1.22) were associated with increased risk of tuberculosis. Increased maternal lymphocyte (HR for lymphocytes ≥ 1340: 0.46, 95% CI: 0.23-0.93) counts, increased duration of breastfeeding (HR: 0.87, 95% CI: 0.80, 0.93), and lower vitamin E levels (HR for vitamin E ≥ 9.7 μmol/L: 4.37, 95% CI: 1.88-10.15) were associated with significantly lower risk of tuberculosis (Table 3).

In multivariate analyses among HIV-uninfected children during follow-up (n=649), increased duration of breastfeeding was associated with a 9% lower risk of tuberculosis per month of breastfeeding (HR: 0.91, 95% CI: 0.85-0.96) (Table 4). Increased child mid-upper arm circumference was associated with significantly lower risk of tuberculosis during follow-up, with a 32% lower risk of tuberculosis per unit increase in child MUAC (HR: 0.68, 95% CI: 0.52-0.89). Findings were similar when we retained HIV-uninfected person-time for HIV-infected children, but censored

them.

Figure 1
Study Profile

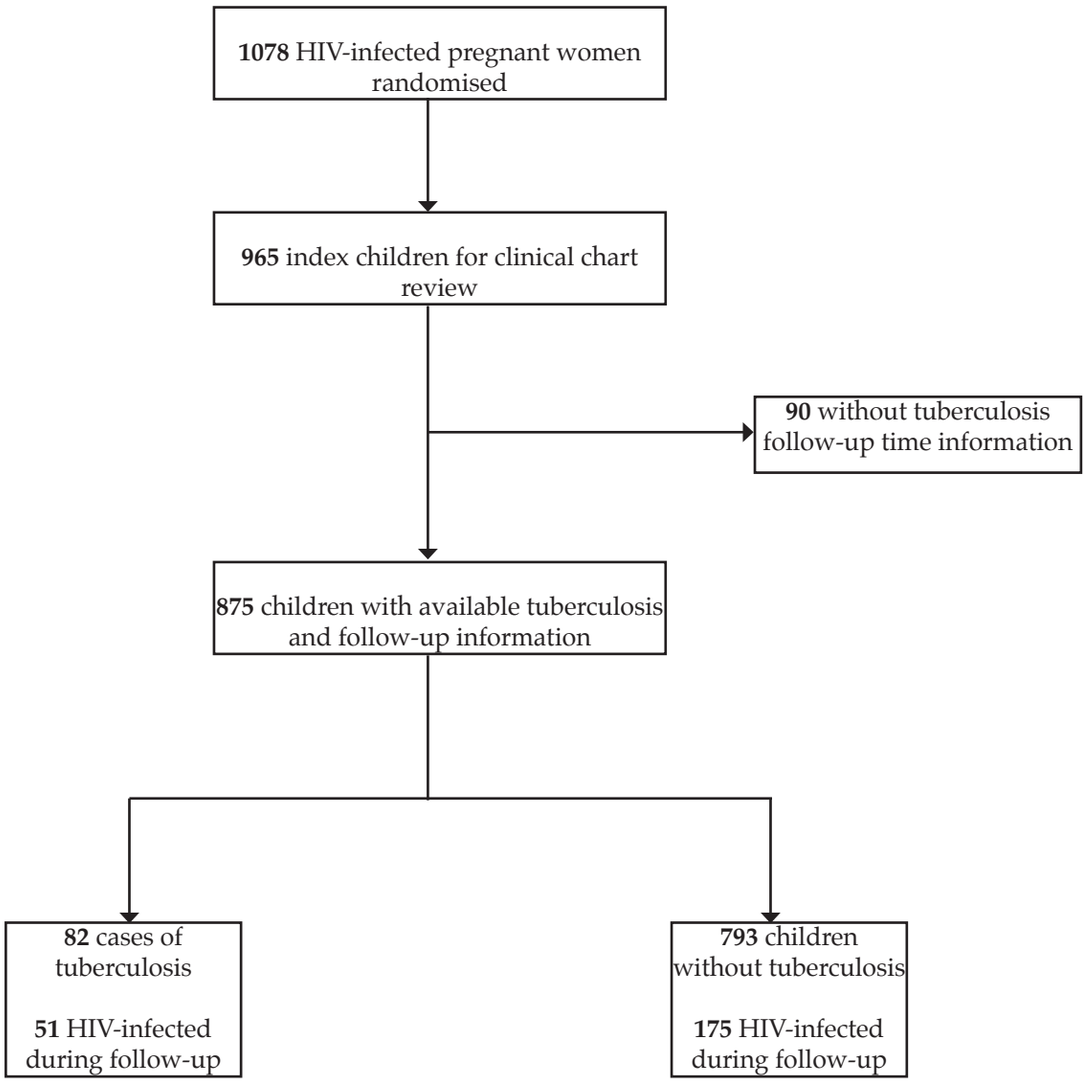

Table 1
Baseline Characteristics of the Study Population (n=875)

Variable		Mean ± SD or n (%)
Maternal		
Shillings* spent on food per person per day		526 ± 264
	< 500 Tsh	314 (40)
Maternal age (years)		25 ± 5
Married		562 (64)
Maternal Education		
Yes		813 (93)
No formal education		62 (7)
Maternal WHO HIV Disease Stage		
	1	743 (85)
	>1	132 (15)
Maternal CD4 T-cell count/μL		417 ± 194
Maternal Body Mass Index at baseline (kg/m2)		23 ± 3
Maternal Mid-Upper Arm Circumference (cm)		26 ± 3
Child		
Sex (females)		426 (50)
Birth weight (g)		3024 ± 509
	Low birth weight (< 2,500 g)	84 (11)
Small size for gestational age		83 (11)
Gestational age at birth (weeks)		39 ± 3
	< 37	207 (24)
	< 34	78 (9)
HIV-infected at birth (PCR, 0-21 days)		54 (8)
Breastfeeding duration (months)		16 ± 7

* 1US Dollar was equivalent to approximately 500 Tanzanian Shillings at the time the trial was conducted

Table 2

Predictors of Tuberculosis Incidence in Children (n=875) (n/N=82 cases / 875 children / 39,762 child-months)*

Variable[1]	Univariate Hazard Ratio (95% CI)	P-value[4]	Multivariate[2] Hazard Ratio (95% CI)	P-value[4]
Maternal[3]				
Socio-demographic				
Shillings per person spent per day on food < 500	1.43 (0.91, 2.27)	0.12		
Number of people eating at the same meal in household	1.07 (0.97, 1.17)	0.18		
Immunological				
Progression to HIV Stage III / IV or Death	0.25 (0.08, 0.78)	0.02		
HIV Stage III / IV	0.28 (0.09, 0.88)	0.03		
All-cause Death	0.31 (0.11, 0.84)	0.02		
CD4 T-cell count (per 100 cells/μL)	0.83 (0.73, 0.95)	<0.01	0.86 (0.75, 0.98)	0.02
Lymphocytes ≥ 1340 cells/μL	0.65 (0.41, 1.02)	0.06	0.65 (0.40, 1.08)	0.10
Pathological protozoan infections	1.49 (0.82, 2.69)	0.19		
Nutritional				
Weight (kg)	0.98 (0.95, 1.01)	0.11		
Mid-upper arm circumference (cm)	0.92 (0.85, 0.99)	0.03		
Other Clinical Variables				
Primiparous	1.47 (0.94, 2.30)	0.09		
Baseline systolic BP	0.98 (0.96, 1.00)	0.10		
Multivitamin Regimen				
Multivitamins	0.98 (0.64, 1.52)	0.94	0.82 (0.51, 1.30)	0.3
Vitamin A	0.79 (0.51, 1.23)	0.30	0.86 (0.53, 1.39)	0.53
Child				
Nutritional				
Breastfeeding duration (months)	0.90 (0.87, 0.92)	<0.0001	0.91 (0.87, 0.94)	<0.0001
Serum cholesterol (per 100 mg/dL, mean, 6 wks and 6 mos)	0.43 (0.20, 0.94)	0.03		
Serum albumin (per 100 IU/L, mean, 6 wks and 6 mos)	0.54 (0.34, 0.87)	0.01		
Mid-upper arm circumference, cm	0.63 (0.57, 0.70)	<0.0001	0.85 (0.74, 0.98)	0.03
Immunological				
HIV infection during follow-up	6.54 (4.18, 10.25)	<0.0001	5.62 (3.24, 9.73)	<0.0001
CD3 T-cell count (per 100 cells/μL)	1.02 (1.00, 1.04)	0.14		
CD8 T-cell count (per 100 cells/μL)	1.06 (1.02, 1.09)	<0.01	1.06 (0.99, 1.13)	0.08

[1]Predictors shown only if univariate p-value <0.20, except multivitamin regimen

[2]Predictors shown only if multivariate p-value <0.10, except multivitamin regimen

[3]Maternal values are at baseline, unless otherwise noted

[4]P-values obtained from Cox regression models

* 1US Dollar was equivalent to approximately 500 Tanzanian Shillings (Tsh) at the time the trial was conducted

Table 3
Predictors of Tuberculosis Incidence in HIV-infected Children (51 cases / 226 children / 6,214 child-months)

Variable	Child Tuberculosis (n / child-months at risk) Yes (51 / 703)	No (175 / 5511)	Univariate Hazard Ratio (95% CI)	Multivariate[2] Hazard Ratio (95% CI)	P-value[3]
Number of people eating at the same meal in the household	-	-	1.02 (0.92, 1.14)	1.20 (1.01, 1.43)	0.04
Breastfeeding duration (months)	-	-	0.89 (0.86, 0.93)	0.86 (0.80, 0.92)	<0.0001
Child CD8 cells (per 100 cells/μL)	-	-	1.05 (1.00, 1.10)	1.11 (1.01, 1.22)	0.03
Maternal Lymphocytes ≥ 1340	33 (703)	129 (5511)	0.64 (0.36, 1.13)	0.47 (0.23, 0.94)	0.03
Maternal serum vitamin E ≥ 9.7 μmol/L	74 (703)	14 (5511)	1.95 (1.02, 3.74)	3.86 (1.71, 8.74)	0.001

[1]Maternal values are at baseline, unless otherwise noted

[2]Multivariate model adjusted for all variables that had P<0.20 in univariate assessments (Table 2), including money spent on food per person per day in the household; maternal progression to HIV disease stage III/IV, death, CD4 T-cell counts, pathological protozoan infections, weight (kg), mid-upper arm circumference (cm), primiparity, systolic blood pressure, and multivitamin regimen; child serum cholesterol, albumin, mid-upper arm circumference, and CD3 T-cell counts

[3]P-values obtained from Cox regression models

Table 4
Predictors of Tuberculosis Incidence in HIV-uninfected Children (31 cases / 875 children / 33,549 child-months)

Variable	Child Tuberculosis (n / child-months at risk) Yes (31 / 635)	No (844 / 32,914)	Univariate Hazard Ratio (95% CI)	Multivariate[1] Hazard Ratio (95% CI)	P-value[2]
Breastfeeding duration (months)	-	-	0.84 (0.80, 0.88)	0.91 (0.85, 0.96)	<0.01
Child mid-upper arm circumference (cm, during follow-up)	-	-	0.47 (0.36, 0.61)	0.68 (0.52, 0.89)	<0.01

[1]Multivariate model adjusted for all variables that had P<0.20 in univariate assessments (Table 2), including money spent on food per person per day in the household, and number of people eating at the same meal in the household; maternal progression to HIV disease stage III/IV, death, CD4 T-cell counts, pathological protozoan infections, weight (kg), mid-upper arm circumference (cm), primiparity, systolic blood pressure, and multivitamin regimen; child serum cholesterol, albumin, CD3 T-cell counts, and CD8 T-cell counts

[2]P-values obtained from Cox regression models

DISCUSSION

In this study, HIV infection, duration of breastfeeding, and maternal and child nutritional and immunological status were important risk factors for tuberculosis among children born to HIV-infected women. HIV infection predicted a six-fold increase in risk of tuberculosis during follow-up, in the overall analysis; increased breastfeeding duration, child mid-upper arm circumference, and maternal CD4 T-cell counts were associated with a significantly reduced risk of tuberculosis. In analyses among children with confirmed HIV infection during follow-up, greater number of people eating at the same meal in the household and child CD8 T-cell counts were associated with increased risk of tuberculosis during follow-up. Higher maternal lymphocyte counts, increased duration of breastfeeding, and lower vitamin E levels were associated with significantly reduced risk of tuberculosis during the follow-up period. In analyses among HIV-uninfected children, increased duration of breastfeeding and child mid-upper arm circumference were associated with reduced risk of tuberculosis.

Findings are consistent with a previous analysis among mothers in the parent study, examining risk factors of tuberculosis among HIV-infected pregnant women (19); low CD4 cell count, elevated erythrocyte sedimentation rate, decreased mid-upper arm circumference, and high viral load were associated with an increased risk of tuberculosis in multivariate analyses, after adjusting for age, education, and hemoglobin concentrations.

Previous studies also have demonstrated a strong relationship between HIV infection and risk of tuberculosis. It is estimated that only one out of ten immunocompetent persons infected with tuberculosis develops active tuberculosis in his or her lifetime; whereas, one out of ten HIV-infected persons infected with tuberculosis will develop active disease every year (20). For example, in a study of risk factors for developing incident tuberculosis in HIV-infected adults from communities in Cape Town, South Africa (21), the risk of tuberculosis infection increased markedly with HIV disease progression (WHO clinical stages III and IV, RR=3.4; 95% CI: 1.8-6.4).
The relationship between malnutrition and increased risk of tuberculosis has been well-established (20, 22, 23). Mid-upper arm circumference is an excellent marker of nutritional status, particularly in children under the age of five years. The increased risk of tuberculosis with lower mid-upper arm circumference observed in our study is, therefore, consistent with *a priori* hypotheses.

Similarly, breastfeeding confers protection against child morbidity and mortality due to diarrhoea and acute respiratory infections (24-26). In a recent analysis in the same cohort of children born to HIV-infected women in Tanzania, exclusive breastfeeding was associated with lower risk for cough (RR: 0.49, 95% CI: 0.41-0.60, P <0.0001), cough and fever (RR=0.44, 95% CI 0.32-0.60, P <0.0001), and cough and difficulty breathing or refusal to feed (RR=0.31, 95% CI 0.18-0.55, P <0.0001).

Exclusive breastfeeding was also associated with lower risk of acute diarrhoea, watery diarrhoea, dysentery, fever and outpatient visits during the first six months of life, but showed no effect at six to 24 months of life (27). However, the relationship between breastfeeding and risk of incident tuberculosis has not been extensively evaluated in HIV-exposed children, particularly in developing countries, who might be at increased risk.

Our finding that lower CD4 T-cell counts were associated with an increased incidence of tuberculosis is consistent with several studies among HIV-infected adult populations (19, 28-30). For example, in a study among 715 HIV-infected women in Pune, India, baseline CD4 T-cell count <200 cells/μL was an independent predictor of incident tuberculosis (adjusted IRR: 7.58, 95% CI: 3.07-18.71). In a prospective cohort study among 804 adult factory workers (95 HIV-infected, 709 HIV-uninfected) in Ethiopia, low CD4 T-cell counts and high HIV viral load preceded the development of tuberculosis disease (29). A likely explanation for this observed relationship is that the CD4 T-cells are part of the cellular immune response, which assists with the response to tuberculosis infection and confers protection against replication of *M. tuberculosis* within macrophages (31).

In this analysis, we found that HIV-infected children born to women who were less immunologically compromised at baseline (lymphocytes ≥1340 cells/μL) had reduced risk of tuberculosis. Similarly, a study in South Africa among adults found that poor immunological status, as indicated by WHO HIV disease stage III and IV, was a significant independent risk factor for developing tuberculosis (21).

In the analysis among HIV-infected children, we found that increased maternal serum vitamin E concentrations were associated with increased risk of tuberculosis infection during follow-up. This finding is contrary to published studies; cross-sectional studies have found that tuberculosis patients suffer from multiple deficiencies in vitamins including E (32). Similarly, vitamin E supplementation to guinea pigs infected with tuberculosis resulted in restoring adequate vitamin E status, increased survival, and reduced weight loss (33). Vitamin E is responsible for improving delayed type hypersensitivity skin response, increasing IL-2 production, neutrophil phagocytosis, lymphocyte proliferation, and antibody response to T-cell dependent vaccines, and reducing production of inflammatory cytokines such as TNF-∝ and IL-6 (34, 35).

To date, there has been limited assessment of risk factors for tuberculosis among HIV-exposed children. Our analysis is distinct from previous studies due to its extensive assessment of potential risk factors, and long duration of follow-up period (58 months). Our study had few limitations. The use of the tuberculosis score chart and the retrospective assessment of tuberculosis, rather than active case finding, was a limitation in this study. Although we were able to assess TB disease status retrospectively using chart reviews, it was not possible to assess TB infection in such a manner; this remained a study limitation. Other potential risk factors for tuberculosis, such as information regarding tuberculosis contacts, were not measured because the study was not designed to obtain this information. Also, a bias may result if loss to follow-up was informative with respect to risk of tuberculosis. This analysis was conducted to examine predictors of tuberculosis among children born to HIV-infected women who were not on anti-retroviral therapy (ART); as such, findings may not be generalisable to pregnant women and their children receiving ART.

In conclusion, breastfeeding duration, HIV status, maternal and child nutritional and immunological status were important predictors of child tuberculosis during the follow-up period. Appropriate infant feeding and nutritional interventions could represent important adjuncts to prevent tuberculosis in children born to HIV-infected women in sub-Saharan Africa.

ACKNOWLEDGEMENTS

To the mothers and children, and field teams, including Physicians, Nurses, Midwives, Supervisors, laboratory staff, and the administrative staff, who made this study possible; and Muhimbili Medical Centre, Muhimbili University College of Health Sciences, and the National AIDS Control Programme in Dar-es-Salaam for their institutional support. This study was supported by the National Institute of Child Health and Human Development (NICHD R01 32257; and K24HD058795) and the Harvard School of Public Health.

AUTHOR CONTRIBUTIONS

JLF, SM, CD, EH, DS, and WWF contributed to the plans for data analysis. JLF analysed and interpreted the data, and wrote the initial draft of the manuscript. KPM reviewed clinical files to identify children with tuberculosis, and extracted relevant data. DS provided statistical guidance and helped interpret data analyses. KPM, GIM, DS, and WWF were investigators of the trial and contributed to the study design and implementation. All co-authors participated in manuscript preparation. None of the authors had a personal or financial conflict of interest.

REFERENCES

1. WHO. Global Tuberculosis Control: epidemiology, strategy, financing. Geneva: World Health Organization2009 Contract No.: WHO/HTM/TB/2009.411.
2. Cegielski, J. P. and McMurray, D. N. The relationship between malnutrition and tuberculosis: evidence from studies in humans and experimental animals. *Int. J. Tuberc. Lung Dis*. 2004; **8**: 286-298.
3. Moran-Mendoza, O., Marion, S. A., Elwood, K., *et al*. Risk factors for developing tuberculosis: a 12-year follow-up of contacts of tuberculosis cases. *Int. J. Tuberc. Lung Dis.* 2010; **14**: 1112-1129.
4. de Vries, G., van Hest, N. A., Baars, H. W., Sebekm M. M. and Richardus, J. H. Factors associated with the high tuberculosis case rate in an urban area. *Int. J. Tuberc. Lung Dis*. 2010;**14**: 859-865.
5. Wood, R., Liang, H., Wu, H., Middelkoop, K., Oni, T, Rangaka, M. X., *et al*. Changing prevalence of tuberculosis infection with increasing age in high-burden townships in South Africa. *Int. J. Tuberc. Lung Dis*. 2010; **14**: 406-412.
6. Fawzi, W. W., Msamanga, G. I., Spiegelman, D., Urassa, E. J., McGrath, N., Mwakagile, D., *et al*. Randomised trial of effects of vitamin supplements on pregnancy outcomes and T cell counts in HIV-1-infected women in Tanzania. *Lancet*. 1998; **16**: 1477-1482.
7. Fawzi, W. W., Msamanga, G. I., Spiegelman, D., Wei, R., Kapiga, S., Villamor, E., *et al*. A randomized trial of multivitamin supplements and HIV disease progression and mortality. *N. Engl. J. Med*. 2004; **351**: 23-32.
8. Fawzi, W. W., Msamanga, G. I, Spiegelman, D., *et al*. Rationale and design of the Tanzania Vitamin and HIV Infection Trial. *Control Clin. Trials*. 1999; **20**: 75-90.
9. Proposed 'World Health Organization staging system for HIV infection and disease': preliminary testing by an international collaborative cross-sectional study. The WHO International Collaborating Group for the Study of the WHO Staging System. *Aids*. 1993; **7**: 711-718.
10. Houwert, K. A., Borggreven, P. A., Schaaf, H. S., Nel, E., Donald, P. R. and Stolk, J. Prospective evaluation of World Health Organization criteria to assist diagnosis of tuberculosis in children. *Eur. Respir. J*. 1998; **11**: 1116-11120.
11. Marais, B. J., Gie, R. P., Obihara, C. C., *et al*. Well defined symptoms are of value in the diagnosis of childhood pulmonary tuberculosis. *Arch. Dis. Child*. 2005; **90**:1162-1165.
12. Marais, B. J., Gie, R. P., Hesseling, A. C., Schaaf, H. S., Lombard, C., Enarson, D. A., *et al*. A refined symptom-based approach to diagnose pulmonary tuberculosis in children. *Pediatrics*. 2006; **118**:e1350-1359.
13. Edwards, K. The diagnosis of childhood tuberculosis. *P N G Med. J*. 1987; **30**: 169-178.
14. Cox, D. Regression models and life tables. *J. Royal. Stat. Soc*. 1972; **34**:187-220.

15. Antelman, G., Smith-Fawzi, M., Kaaya, S., Mbwambo, J., Msamanga, G., Hunter, D., *et al*. Predictors of HIV-1 sero-status disclosure: A prospective study among HIV infected pregnanat women in Dar es Salaam, Tanzania. *AIDS*. 2001;**15**: 1865-1874.
16. Miettinen, O. Theoretical Epidemiology. New York: John Wiley & Sons; 1985.
17. Durrleman, S. and Simon, R. Flexible regression models with cubic splines. *Stat Med*. 1989; **8**: 551-561.
18. Govindarajulu, U. S., Spiegelman, D., Thurston, S. W., Ganguli, B. and Eisen, E. A. Comparing smoothing techniques in Cox models for exposure-response relationships. *Stat Med*. 2007; **26**: 3735-3752.
19. Venkatesh, P. A., Bosch, R. J., McIntosh, K., Mugusi, F., Msamanga, G. and Fawzi, W. W. Predictors of incident tuberculosis among HIV-1-infected women in Tanzania. *Int. J. Tuberc. Lung Dis*. 2005; **9**: 1105-1111.
20. Marais, B. J. Tuberculosis in children. *Pediatr Pulmonol*. 2008; **43**: 322-329.
21. Wood, R., Maartens, G. and Lombard, C. J. Risk factors for developing tuberculosis in HIV-1-infected adults from communities with a low or very high incidence of tuberculosis. *J. Acquir. Immune. Defic. Syndr.* 2000; **23**: 75-80.
22. Grange, J. M., Gandy, M., Farmer, P. and Zumla, A. Historical declines in tuberculosis: nature, nurture and the biosocial model. *Int. J. Tuberc. Lung Dis.* 2001; **5**: 208-212.
23. Marais, B. J., Obihara, C. C., Warren, R. M., *et al*. The burden of childhood tuberculosis: a public health perspective. *Int. J. Tuberc. Lung Dis*. 2005; **9**: 1305-1313.
24. Kuhn, L., Stein, Z. and Susser, M. Preventing mother-to-child HIV transmission in the new millennium: the challenge of breast feeding. *Paediatr. Perinat. Epidemiol*. 2004; **18**:10-16.
25. Nicoll, A., Newell, M. L., Van Praag, E., Van de Perre, P. and Peckham, C. Infant feeding policy and practice in the presence of HIV-1 infection. *Aids*. 1995; **9**: 107-119.
26. Effect of breastfeeding on infant and child mortality due to infectious diseases in less developed countries: a pooled analysis. WHO Collaborative Study Team on the Role of Breastfeeding on the Prevention of Infant Mortality. *Lancet*. 2000; **355**: 451-455.
27. Mwiru, R. S., Spiegelman, D., Duggan, C., Peterson, K., Liu, E., Msamanga, G., *et al*. Relationship of exclusive breast-feeding to infections and growth of Tanzanian children born to HIV-infected women. *Public Health Nutr.* 2011; **16**: 1-8.
28. Grantm , A. D., Djomand, G., Smets, P., Kadio, A., Coulibaly, M., Kakou, A., *et al*. Profound immunosuppression across the spectrum of opportunistic disease among hospitalized HIV-infected adults in Abidjan, Cote d'Ivoire. *AIDS*. 1997; **11**: 1357-1364.
29. Wolday, D., Hailu, B., Girma, M., *et al*. Low CD4+ T-cell count and high HIV viral load precede the development of tuberculosis disease in a cohort of HIV-positive Ethiopians. *Ethiop. Med. J.* 2003; **41 Suppl**: 67-73.
30. Gupta, A., Nayak, U., Ram, M., Bhosale, R., Patil, S., Basavraj, A., *et al*. Postpartum tuberculosis incidence and mortality among HIV-infected women and their infants in Pune, India, 2002-2005. *Clin. Infect. Dis.* 2007; **45**: 241-249.
31. Narain, J. P., Raviglione, M. C. and Kochi, A. HIV-associated tuberculosis in developing countries: epidemiology and strategies for prevention. *Tuber. Lung Dis*. 1992; **73**: 311-321.
32. Panasiuk, A. V., Penenko, O. R., Kuz'menko, I. V., *et al*. [Vitamin E and its structural analogs in tuberculosis]. *Ukr Biokhim Zh*. 1991; **63**: 83-88.
33. Pushkareva, V. D. [The effect of vitamin E on experimental tuberculosis in guinea pigs]. *Probl. Tuberk.* 1968; **46**: 67-70.
34. Meydani, S. N., Meydani, M., Blumberg, J. B., *et al*. Vitamin E supplementation and in vivo immune response in healthy elderly subjects. A randomized controlled trial. *Jama*. 1997; **277**: 1380-1386.
35. Wang, Y., Huang, D. S., Wood, S. and Watson, R. R. Modulation of immune function and cytokine production by various levels of vitamin E supplementation during murine AIDS. *Immunopharmacology*. 1995; **29**: 225-233.