European Journal of Oral Sciences

Eur J Oral Sci 2020; 128: 46–54
DOI: 10.1111/eos.12669
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European Journal of
Oral Sciences

Association of maternal HIV-1 severity with dental caries: an observational study of uninfected 5- to 7-yr-old children of HIV-1-infected mothers without severe immune suppression

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Birungi N, Fadnes LT, Engebretsen IMS, Lie SA, Tumwine JK, Åstrøm AN; the ANRS 12174 and 12341 study groups. Association of maternal HIV-1 severity with dental caries: an observational study of uninfected 5- to 7-yr-old children of HIV-1-infected mothers without severe immune suppression.
Eur J Oral Sci 2020; 128: 46–54. © 2020 The Authors. Eur J Oral Sci published by John Wiley & Sons Ltd

Treatment programs to prevent perinatal and postnatal HIV-1 transmission have become available in sub-Saharan Africa, leading to an emerging population of HIV-1 exposed uninfected (HEU) children. Exposure to HIV-1 *in utero* and during breastfeeding may increase the risk of morbidity and mortality in HEU children. This study estimated the association of the severity of maternal HIV-1 infection as assessed by CD4 count and viral load at baseline (7 d postpartum), with dmft count of their 5- to 7-yr-old HEU offspring. A follow-up study was conducted of HIV-1-infected mother–HEU children pairs (n = 164) from the Ugandan site of the ANRS 12341-PROMISE- PEP trial (ClinicalTrials.gov, number NCT00640263). HIV-1-infected mothers were interviewed and the HEU children were examined for caries using the World Health Organization's survey methods for field conditions and the dmft index. Directed acyclic graphs and negative binomial regression were used for analyses. The prevalence of 1 or more dmft was 48%. Negative binomial regression showed no association between the dmft count and maternal CD4 counts 7 d postpartum but a 10% lower dmft count with longer breastfeeding duration was found. Maternal CD4 count at birth was not associated with the dental caries experience in uninfected children born to women without severe immune suppression, while there appeared to be a protective effect of high viral load and breastfeeding duration.

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Key words: CD4 count; DAGs; tooth decay; HIV exposed uninfected; viral load

Accepted for publication October 2019

The burden of HIV-1 infection is substantial in sub-Saharan Africa, and the absolute number of fertile (15–44 yr of age) women infected with HIV-1 continues to increase (1, 2). Infants born to mothers with HIV-1 are at risk of becoming infected with HIV-1 through direct exposure to the HIV-1 virus *in utero*, or during delivery and lactation (3). The growing availability of antiretroviral therapy during pregnancy, delivery, and breastfeeding has reduced the HIV-1 transmission rate from mother to child, making the goal of global elimination of pediatric HIV-1 infections increasingly likely (3). In fact, the number of newly infected infants has decreased by 58% worldwide, from an estimated 520,000 in 2000 to 220,000 in 2014, and 41% of this decline occurred between 2010 and 2014 (3). The focus of the '90-90-90' treatment strategy recently adopted by the Joint United Nations Programme on HIV and AIDS (UNAIDS) is drastic reduction of new infections and bending the trajectory of the epidemic (4). A consequence of this successful treatment strategy is an inevitable emerging population of HIV-1 exposed uninfected (HEU) children, particularly in sub-Saharan Africa (5). While the elimination of HIV-1 transmission from mother to child is of highest priority, the health situation of a growing population of HEU children cannot be ignored.

There is evidence suggesting that exposure to HIV-1 *in utero*, intrapartum, and during breastfeeding may confer a high risk of mortality, morbidity, and growth retardation in children, even in the absence of HIV-1 transmission (6, 7). Evans *et al* (8) suggested that the potential underlying causes of morbidity and

mortality in HEU children centered around fetal immune activation and immunological abnormalities resulting from direct exposure to HIV-1 *in utero*. Suboptimal health outcomes in HEU children have also been associated with severity of maternal HIV-1 disease, and a decline in maternal CD4 count has been associated with serious bacterial infections in their offspring (9).

Whereas the burden of oral disease in HIV-1-infected populations is well documented (10, 11), the oral health situation of the emerging population of HEU children is unclear at present. A previous study comparing oral disease among HIV-1-infected and HEU children in the USA observed higher dental caries experience among HIV-1-infected youths than among their HEU counterparts (12). Accordingly, Coker *et al*. (13) studied dental caries experience in HIV-1-infected, HEU, and HIV unexposed uninfected Nigerian children, and found that caries was most prevalent in the HIV-1-infected group and least prevalent in the HIV unexposed uninfected group. Previous studies considering the health and oral health situation of HEU children are limited by relatively small sample sizes, being cross-sectional in design, or being prospective cohort studies with only short follow-up times. Some have focused on the CD4 counts of children with HIV-1 and did not investigate HEU children in the context of maternal HIV-1 infection and its severity. The most recent studies are also complicated by exposure to maternal and infant antiretroviral therapy for preventing perinatal and postnatal transmission, making the natural history of HIV-1 exposure difficult to determine.

As the primary dentition starts to develop *in utero*, exposure to HIV-1 during pregnancy, delivery, and lactation could affect the integrity of the enamel and its subsequent resistance to tooth decay (14). Given the scarcity of literature on the oral health of HEU children and the known impact of oral diseases on the daily lives of children and their families, it is important to explore the oral health aspects of 5- to 7 yr-old HEU children to guide policy and oral health-care planning (15).

This study relies on the conceptual framework of early life-course influences on oral health in HEU children and adopts a causal approach by using directed acyclic graphs (DAGs) (16, 17). According to the life-course approach, early life circumstances (such as exposure to maternal HIV-1 infection) may impact subsequent caries development in the primary teeth, either directly or through later life-course circumstances, suggesting a gradual accumulation of exposures (18). The aim of this study was to investigate the potential association of the severity of maternal HIV-1 infection (assessed by CD4 count and viral load at 7 d postpartum) with dental caries experience of HEU children at 5–7 yr of age while adjusting for other early and later life-course circumstances. It was hypothesized that dental caries in primary teeth was more prevalent among children exposed to mothers with more severe HIV-1 infection than among children exposed to mothers with less severe HIV-1 infection at birth.

Material and methods

This observational study is a follow-up of dyads of HIV-1-infected mothers and HEU children from the Ugandan site of the ANRS121741-PROMISE-PEP trial (ClinicalTrials.gov, number NCT00640263). The ANRS 12171 PROMISE-PEP, conducted between 2009 and 2013, was a multicenter randomized controlled trial with the aim of preventing HIV transmission to offspring. The inclusion criteria were HIV-1-infected pregnant women, ≥18 yr of age, intending to breastfeed, and who were not eligible for antiretroviral therapy (for clinical reasons or because of having a CD4 count of >350 cells μl^{-1}). The aim of the trial was to compare the effect of lopinavir–ritonavir with lamivudine (given from day 7 postpartum until either 50 wk postpartum or 1 wk after cessation of breastfeeding) on the rate of HIV-1 transmission and adverse events among HIV-1-exposed infants in Africa (19). The women were recruited at 28–40 wk of gestation at antenatal clinics in four African sites: Ouagadougou in Burkina Faso; East London in South Africa; Mbale in Eastern Uganda; and Lusaka in Zambia. Their newborn infants were eligible for inclusion in the trial if they were: singletons; breastfed at day 7 by their mothers; had a negative HIV-1 DNA PCR; had received any perinatal and postnatal HIV-1 treatment; and if the mother intended to continue breastfeeding. Briefly, in the Ugandan site, 278, 7-d-old uninfected children born to HIV-1-infected women were randomly allocated to receive infant prophylaxis [either lamivudine (3TC) or boosted Lopinavir/Ritonavir (LPV/r), daily] throughout the breastfeeding period, plus or minus 1 wk, from day 7 to 50 wk postpartum. The primary outcome was mother to child HIV-1 transmission between day 7 and 50 wk postpartum, diagnosed every third month with HIV-1 DNA PCR. Detailed information on the PROMISE-PEP trial is published elsewhere (19).

In 2017, some 244 of the 278 HIV-1-infected mothers and their uninfected children were eligible for re-enrolment in the follow-up study: the PROMISE-PEP Mechanism Safety study (PROMISE-PEP M&S ANRS12341), conducted in Mbale, Eastern Uganda. The criterion for re-enrolment was being a child whose HIV-1 serostatus had remained negative since the end of the trial (50 wk postpartum). Of the 166 HEU children re-enrolled, two were excluded due to HIV-1 conversion. Thus, 67% (164/244) of the potentially traceable HEU children were followed up; 32% (n = 112) of the women originally enrolled in the trial (and their children) were lost through attrition. The eligible children were aged 5–7 yr at the 2017 follow-up (Fig. 1).

This study uses information obtained from 164 mothers with HIV-1-HEU child pairs at the 7 d postpartum recruitment interview and at 50 wk postpartum, as well as from interviews and clinical oral examinations at the follow-up study in 2017. Ethical clearance was sought from the School of Medicine Research and Ethics Committee, Makerere University (SOMREC)-REC REF 2018-030; Uganda National Council of Science and Technology (UNCST)-HS 2373; and the Regional Committee for Health and Medical Research (REK) −2017/760/REC Sør-Øst from Norway.

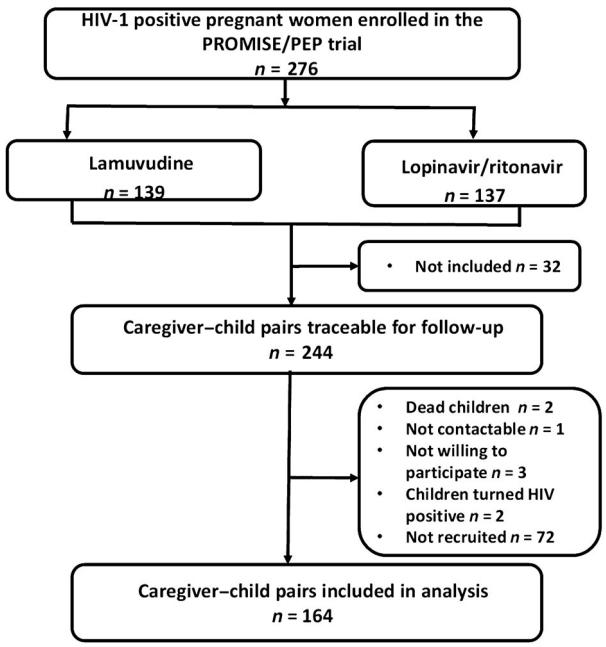

Fig. 1. Cohort flow diagram showing the participants of the 2017 follow-up

Assessment of severity of maternal HIV-1 status at 7 d postpartum and at the 2017 follow-up

The severity of maternal HIV-1 was assessed in terms of CD4 counts (at the screening visit), viral load, and clinical staging (at 7 d postpartum). The CD4 counts were determined using flow cytometry, viral load was measured using a commercial real-time RNA PCR test (Generic HIV Charge Virale; Biocentric, Bandol, France), and HIV-1 clinically staged reported according to World Health Organization (WHO) criteria (20). In the analysis, CD4 count and viral load were continuous variables. The latter was divided by 100, and the CD4 count was log-transformed. The offspring's HIV-1 status was determined using HIV-1 DNA PCR, both at 7 d postpartum and at the follow-up in 2017. A team of trained biologists, pharmacists, physicians, and counsellors collected the data.

Anthropometry at 7 d postpartum and at the 2017 follow-up

Anthropometric measurements (in terms of weight and height) were collected twice and according to WHO guidelines (http://www.who.int/childgrowth/training/en/) using a weight scale (SECA 813 mobile digital weight scale; SECA, Hamburg, Germany) and a stadiometer (SECA 213 mobile stadiometer; SECA). The measurements of height (cm) and weight (kg) were reported to the nearest decimal place. The WHO AnthroPlus 2007 software (21) was used to generate height-for-age *Z*-scores (HAZ) at the 2017 follow-up. To measure the degree of undernutrition we used low HAZ values, with HAZ values above -1 coded as 0 because the negative consequences (such as child deaths) are substantial with lower anthropometric measures (22, 23). The HAZ value at the 2017 follow-up was used as a measure of chronic undernutrition. The weight-for-age *Z*-score (WAZ) at day 7 postpartum was used as an early measure of growth.

Interviews with mothers at baseline (7 d postpartum) and at the 2017 follow-up

At baseline (7 d postpartum) and the 2017 follow-up, data were collected in the local language (Lumasaaba) using semi-structured questionnaires on case record forms (CRFs) and entered online using the electronic data-capture system Open Clinica (http://www.openclinica.com) and Epidata (www.epidata.dk) respectively. Trained research assistants carried out the interviews. The following information was assessed at baseline (7 d postpartum), and at 24 h and at 1-wk breastfeeding recalls ($\pm$ 2 days): age; sex; presence of household assets (radio, television, bicycle, motocycle/scooter, car/truck, refrigerator, computer/laptop, mobile phone, smartphone); mothers' marital status; mothers' occupation; mothers' level of education; presence of electricity in home; and whether delivery had taken place in a clinic. At the 2017 follow-up, the mothers with HIV-1 gave face-to-face interviews using semi-structured questionnaires developed and tested previously in a similar population (24). Information on child's age and sex was obtained, in addition to the following details on oral health: child's use of oral health-care services, child's sugar consumption, and child's oral hygiene behavior. Measurement details of the variables used in analyses are presented in Appendix S2.

Clinical oral examination of children at the 2017 follow-up

Two experienced and calibrated dental surgeons (NB and MM) performed the clinical oral examination. Duplicate examinations were performed 14 d apart in 26 children who were not included in the ANRS12174-PROMISE-PEP trial cohort, and the results were used to calculate inter-rater and intra-rater reliability of the recordings. Dental caries was assessed on fully erupted primary teeth using the decayed, missing, and filled teeth (dmft) index, in accordance with the WHO guidelines for field conditions ((25)). A tooth was documented as decayed if it was visually cavitated when examined using a dental mirror and a periodontal probe. A tooth was recorded as missing when it had been extracted because of caries, as confirmed by the mother. In the present analysis, the dependent variable, dental caries, was measured using the dmft index, both as a count variable and as a dichotomous variable. The count variable was the sum of the decayed, missing, or filled teeth. The dichotomized variable was categorized as absent (0) for a dmft count = 0 or present (1) for a dmft count of >0.

Directed acyclic graphs

The causal structure underlying the effect of severity of maternal HIV-1 infection (in terms of maternal CD4 count) on dental caries in the primary dentition of HEU children was visualized and evaluated using directed acyclic graphs (DAGs) (26, 27). According to the causal diagram theory, DAGs are graphical tools guided by qualitative assumptions that visualize the hypothesized causal structure underlying the exposures and outcomes under study, whilst considering potential confounders, colliders, and mediators (16, 17). A priori knowledge from theory and empirical evidence guided the relationships among the variables included in the postulated DAG, as well as directionality of the paths within the DAG. Before

being included in the DAG, the possible direct paths between variables were evaluated for plausibility based on theoretical and empirical evidence. A detailed description of the relationship between the variables in the DAG is provided in Appendix S3.

According to GREENLAND *et al.* (28), all paths having arrowheads pointing from maternal CD4 to child's dental caries (i.e., front-door paths), and all back-door paths (i.e., alternative non-causal biasing paths between exposures and outcome) not blocked by colliders (i.e., open back-door paths), were identified and selected as the variables to be included in the final regression analyses. In summary, early lifecourse factors assessed at day 7 postpartum, such as maternal CD4 count, viral load, socio-economic status, maternal education, and child's weight-for-age were assumed to influence dental caries directly or indirectly through later life course circumstances (breastfeeding duration, oral hygiene behavior, sugar consumption and height-for-age). According to the DAG depicted in Fig. 2, the front door path from maternal CD4 through socio-economic status and use of oral care services to dental caries is blocked by use of oral care services being a collider on that path. All other front door paths from maternal CD4 to dental caries through socio-economic status are open and include intermediate variables in terms of oral hygiene behavior, height-for-age at follow-up, breastfeeding duration, and sugar consumption. These later life course variables were assumed to have independent effects on the dmft count at the 2017 follow-up and were included as covariates in the final multiple regression model. Finally, the back door path from maternal CD4 through maternal viral load to dental caries is blocked by inclusion of viral load as a confounder in the final regression model.

Statistical analysis

The statistical package STATA 15 (StataCorp, College Station, TX, USA) was used for data analysis. To assess differences in baseline (7 d postpartum) characteristics between HEU children lost to follow-up and those retained in the cohort, chi-square tests were used for categorical variables and independent sample *t*-tests were used for continuous variables. The online software tool DAGITTY (http://www.dagitty.net/) checked the DAG for consistency and validity of the set of variables adjusted for in the regression (29). The likelihood-ratio test comparing negative binomial regression and Poisson models indicated that the negative binomial model was more appropriate than the Poisson model ($P < 0.001$). The dmft count was analyzed using negative binomial regression with overdispersion of the mean from the variance. Incidence rate ratios (IRRs) and their 95% CI were determined. To adjust for potential differences in loss to follow-up, an inverse probability weighted method (IPW) was applied using probit regression for binary outcomes to predict the risk of loss to follow-up based on background factors (socio-economic status, level of education, and marital status) (30). By using this method, participants are weighted by the inverse of their probability of being followed up. First of all, a probit regression analysis was conducted to identify the background factors that are associated with loss to follow-up (socio-economic status, level of education and marital status). This probability was then used to calculate the inverse probability weight by calculating the inverse of predicted scores for being lost to follow-up. The weights were included in the regression models (using the *P* weight command).

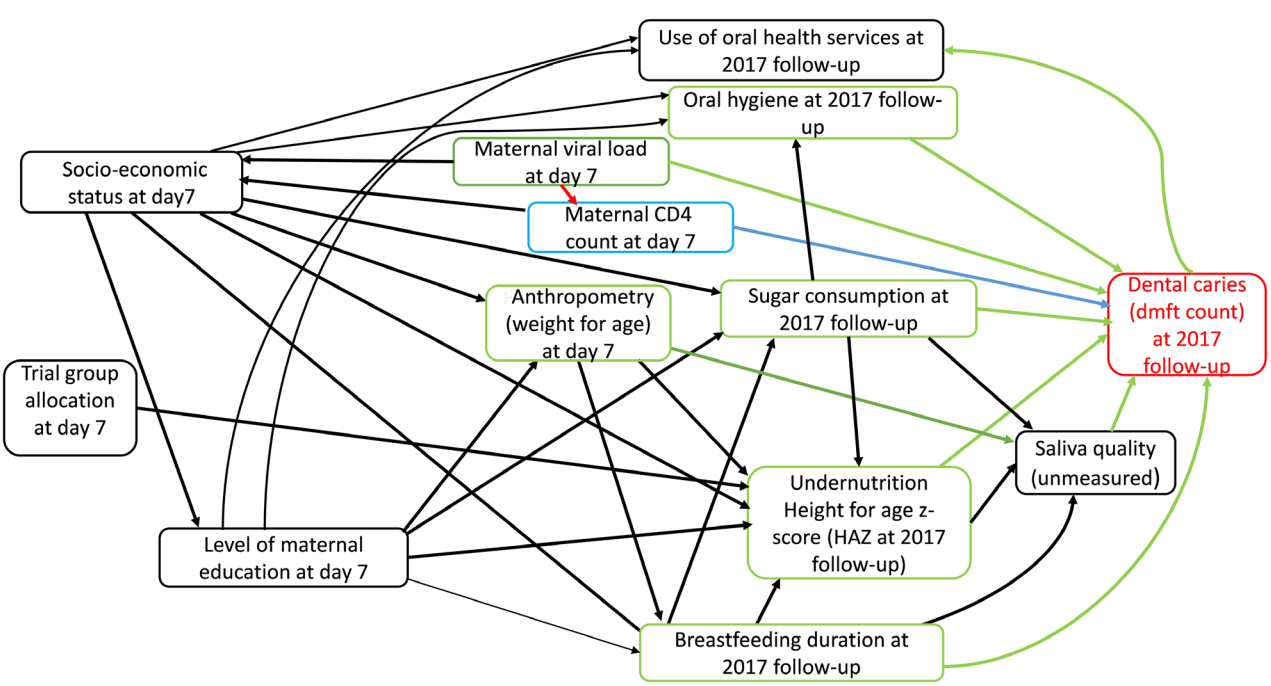

Fig. 2. Directed acyclic graph (DAG) indicating the main exposure (blue path/node; maternal CD4 count at day 7), outcome (dental caries in red font), and the covariates in the regression model with green paths [oral hygiene behavior at the 2017 follow-up; maternal viral load at day 7 postpartum; anthropometry (weight for age) at day 7 postpartum; undernutrition – height-for-age *Z*-score (HAZ) – at the 2017 follow-up; breastfeeding duration at the 2017 follow-up; and salivary quality)

Results

Inter-rater reliability analyses for assessment of dmft > 0 (caries)/dmft = 0 (no caries) across the dentition of each child yielded a median kappa [interquartile range (IQR)] of 0.7 (0.5–0.8). Intra-rater reliability analyses yielded a median kappa value (IQR) of 0.6 (0.5–0.7).

Table 1 summarizes baseline (7 d postpartum) sociodemographic and clinical characteristics of HIV-1-infected mothers and their HEU children according to follow-up status. In general, baseline characteristics did not differ between those lost to follow-up and their counterparts followed up 5–7 yr later. At baseline (7 d postpartum), the mothers who were followed up had a median age (25%–75% percentile) of 28 (23–30) years, a viral load (IQR) of 4.3 (0.8–15.4) copies × 10^3 ml^{-1}, and a CD4 count (IQR) of 524 (439–615) cells μl^{-1}. Corresponding data for the mothers lost to follow-up were age 25 (22–28) years, a viral load of 4.3 (1.5–7.6) copies × 10^3 ml^{-1}, and a CD4 count of 519 (417–639) cells μl^{-1}. Children's WAZ and HAZ were similar according to follow-up status. Boys comprised 50% of the children who were followed up and 43% of the children who were lost to follow-up.

Table 2 shows summary data of the various covariates utilized in the analysis of the 164 participants who were followed up. Most (63%) participants were in the poorest quintile. The majority (62%) of mothers had at least a primary level of education. Sixty percent of mothers had a CD4 cell count of >500 cells μl^{-1} and 26% had a viral load of >1,000 copies ml^{-1} on day 7 postpartum. A majority of the children had normal weight for their age on day 7 postpartum and normal height for their age at the 2017 follow-up. The median (IQR) child age at the 2017 follow-up was 6 (5–6) yr, with 28%, 52%, and 20% of children aged 5, 6, and 7 yr, respectively. The prevalence of dmft > 0 was 48% and the mean dmft score was 2.0 ± 3.2.

The DAG depicted in Fig. 2 informed the adjustment set of covariates and confounding variables to be included in the regression model, in terms of sugar consumption, breastfeeding duration, height-for-age, and maternal viral load. As shown in Table 3, the negative binomial regression of dental caries revealed no association between CD4 count and dmft count (IRR = 0.94, 95% CI: 0.82–1.08), whereas viral load showed a marginally significant negative association with dmft count (IRR = 0.72; 95% CI: 0.53–0.98). Regarding the effect of breastfeeding duration, the results showed a 10% lower dmft per month of breastfeeding duration (IRR = 0.87; 95% CI: 0.87–0.94), while the risk of dental caries was about twofold higher in those with good oral hygiene behavior than in those with poor oral hygiene behavior. Sensitivity analyses (using inverse probability weighting) to assess potential differences as a result of attrition, taking into account socio-economic status, level of education, and marital status, revealed similar findings as the analyses performed without taking into acount inverse probablity weighting (Table S1).

Table 1

Characteristics (grouped as continuous or categorical variables) of mothers at baseline and of children at 7 d postpartum, stratified according to follow-up status

Characteristic/Variable	Follow-up (2017, *n* = 164)	Lost to follow-up (2017, *n* = 112)
Mother's characteristics		
Continuous variables		
Mother's age (yr)	28 (23−30)	25 (22–28)
BMI (kg m^{-2})	23 (21−25)	23 (21–24)
Viral load (×1000 ml^{-1})	4.3 (0.8–15.4)	4.3 (1.5–7.6)
$CD4^+$ cells (μl^{-1}) at screening	524 (439–615)	519 (417–639)
Categorical variables		
CD4 (≤500 cells)	66 (40)	52 (46)
HIV staging at d 7 (Stage I)	150 (91)	104 (93)
ART postpartum (yes)	117 (71)	81 (72)
Detectable viral load (yes)	108 (68)	67 (61)
Arm (3TC)	84 (51)	55 (49)
Marital status (married/cohabiting)	142 (86)	84 (75)
Occupation (working)	58 (35)	40 (36)
Parity (primiparous)	32 (19)	17 (15)
Clinic delivery (yes)	130 (79)	84 (76)
Electricity (yes)	59 (36)	36 (32)
Education level		
None	7 (4)	6 (5)
Primary	65 (40)	54 (48)
End of primary	30 (18)	14 (12)
Secondary	50 (30)	31 (28)
End of secondary or greater	12 (7)	7 (6)
Wealth quintiles		
1	59 (36)	39 (35)
2	44 (27)	49 (44)
3	46 (28)	16 (14)
4	13 (8)	5 (4)
5	2 (1)	2 (2)
Child's characteristics		
Continuous variables		
Weight-for-age *Z*-score day 7	−0.1 (−0.6 to 0.4)	−0.3 (−0.9 to 0.3)
Height-for-age *Z*-score day 7	−0.3 (−0.9 to 0.4)	−0.5 (−1.1 to 0.2)
Weight for height *Z*-score day 7	−0.05 (−1.1 to 0.8)	−0.3 (−1 to 0.5)
Categorical variables		
Sex (boys)	82 (50)	48 (43)

Data for continuous variables are given as median (interquartile range) and data for categorical variables are given as *n* (%).
ART, antiretroviral therapy; BMI, body mass index.

Discussion

This study is among the first to report on the prevalence of, and early life course influences on, dental caries in the primary dentition of Ugandan HEU children aged 5–7 yr. The present findings did not reveal a link between maternal HIV-1 severity, assessed in terms of CD4 count, immediately after delivery and dmft count in HEU children at follow-up, whereas high viral load seemed to have a protective effect. The assumption of

Table 2

*Summary data, stratified according to categorical (A) and continuous (B) variables, of early life course factors in the study group followed up at 5–7 yr of age (*n = *164)*

(A) Categorical variables	Category	*n* (%)
Baseline (7 d postpartum)		
Maternal socio-economic status	Poorest (quintile 1–2)	103 (63)
	Least poor (quintile 3–5)	61 (37)
Maternal level of education	Primary/end of primary	102 (62)
	Secondary and above	62 (37)
Child's WAZ ≤2	Underweight	6 (4)
	Normal	158 (96)
Maternal CD4 count	≤500 cells μl^{-1}	66 (40
	>500 cells μl^{-1}	98 (60)
Maternal viral load	≤1000 copies ml^{-1}	122 (74)
	>1000 copies ml^{-1}	42 (26)
2017 follow-up		
Child age groups	5 yr	46 (28)
	6 yr	85 (52)
	7 yr	33 (20)
Child's HAZ ≤2	Stunted	15 (9)
	Normal	149 (91)
Sugar consumption	Low consumption	94 (58)
	High consumption	69 (42)
Oral hygiene behavior	Bad	41 (25)
	Good	123 (75)
Use of oral health services	No	155 (96)
	Yes	9 (4)
dmft score	dmft = 0	85 (52)
	dmft > 0	79 (48)

(B) Continuous variables	Median (IQR) or mean ± SD	*n*
Any breastfeeding duration at 50 (wk)	40 (37–43)	163
Any breastfeeding at 2017 follow-up (months)	9 (6–9)	164
Child age at follow-up (yr)	6 (5–6)	164
Parents age at follow-up (yr)	32 (27–32)	164
dmft score	2.0 ± 3.2	162

dmft, decayed, missing, and filled primary teeth; HAZ, height-for-age *Z*-score; WAZ, weight-for-age *Z*-score.

Table 3

Negative binomial regression of dental caries and maternal HIV-1 severity (determined according to CD4 count), adjusted for early life course variables

Variable	IRR (95% CI)
Maternal CD4 count (μl^{-1})	0.94 (0.82–1.08)
Maternal viral load (× 1000 ml−per ten time increase)	0.72 (0.53–0.98)
Weight-for-age at d 7 postpartum (WAZ)	1.12 (0.49–2.56)
Breastfeeding duration (per monthly increase)	0.87 (0.80–0.94)
Sugar consumption	
Low sugar consumption	1
High sugar consumption	1.05 (0.60–1.82)
Oral hygiene behavior	
Bad	1
Good	1.96 (1.01–3.79)
Undernutrition (degree of stunting) (HAZ)	0.79 (0.52–1.20)

HAZ, height-for-age *Z*-score; IRR, incidence rate ratio; WAZ, weight-for-age *Z*-score.

additional direct effects on dental caries from later life course variables assessed at follow-up was confirmed in that breastfeeding duration had a protective effect, whereas good oral hygiene behavior was associated with a higher incidence of dental caries – a result completely in contrast to that expected.

Use of DAGs for causal assessment has been advocated for research but is yet to be widely implemented in dentistry (31). Only a few previous studies have applied DAGs in the analyses of empirical clinical and public dental health data (16, 17). A major strength of the present study is the use of DAGs to visualize the causal structure underlying any effect of early life course HIV-1 exposure on subsequent caries experience in primary teeth and to guide the identification of appropriate confounding variables and other covariates to be included in the final regression analyses. According to the causal graph theory, variables along paths closed by collider variables (i.e., a variable that is a common effect of two other variables, such as use of oral care services), should be left unadjusted to avoid unblocking and creation of confounding bias. As shown in Fig. 2, most front door paths from maternal CD4 counts through socio-economic status were open and included potential mediating life course variables assessed at follow-up in 2017. The open biasing back door path from maternal CD4 through maternal viral load to dental caries was closed by adjusting for viral load in the final regression model (16). The sources of confounding bias, as identified by the DAGs, might not be identified using conventional statistical methods (27). However, it should be stressed that use of the DAG theory assumes that the causal structure of the underlying relationships studied is correct and hence is an argument to use different possibilities of modeling (32). Given the multifactorial and complex nature of risk factors associated with dental caries, the authors constructed the DAGs based on established risk factor models as well as previous empirical evidence. In addition, the online tool, DAGitty, was used to enhance the validity of the variables finally used in the regression models (29). Another major strength of this study is its long-term prospective design, which enhances the potential to make valid causal conclusions. As to the external validity of the present findings, it seems possible to generalize these to HEU children in the Ugandan population as the study setting mirrors the general population in the HIV-1 context. A limitation of the present study, as in most long-term prospective studies, is attrition, with only 67% of the original cohort retained because of loss to follow-up. It is unlikely, however, that the estimates in this study were affected by selection bias because inverse probability weights based on socio-economic variables were applied to adjust for potential differences and did not change the unweighted estimates. As shown in Table 1, the homogeneity observed for the majority of baseline characteristics between those retained in the cohort and those lost to

follow-up made it less possible that selection bias affected the results. A major limitation is the lack of a concomitant HIV-1-uninfected control cohort from the same setting; thus, it is difficult to infer any effect or null effect in relation to maternal HIV-1 severity. A further limitation is use of the dmft index to assess dental caries in deciduous teeth; although a standard for use in field studies, this index weighs untreated caries, missing teeth, and restored teeth similarly (33).

Although the effects of exposure to HIV-1 during pregnancy, delivery, and lactation on the postnatal development of HEU children remain uncertain, previous studies in sub-Saharan Africa have reported increased infant morbidity according to low maternal CD4 counts, which is not explained by maternal mortality (34–36). By contrast, low maternal CD4 count did not increase the IRRs of having caries in primary teeth for the HEU children investigated in this study. This study's results are at odds with those of some previous studies (35, 37–40), but in accordance with others (41, 42). The absence of an effect of maternal CD4 count on caries might be explained by the particular characteristics of the HEU children investigated, being born to mothers who did not qualify for ARV therapy during pregnancy because of less-advanced infection and high immune competency (i.e., CD4 count > 350 μl^{-1}). Moreover, HEU children participating in this study were exposed to 50 wk of prophylaxis (lopinavir/ritonavir and lamivudine) that might have protected them from immunologic deficiencies in breast milk and early infections. Theoretically, infant infections might be on the causal pathway from HIV exposure to subsequent caries in the primary dentition (43). The findings reported by Moscocki *et al*. (12), who found no association of oral disease with CD4 count and viral load among HIV-1-infected youths, are similar to those of the present study. However, in the present study we investigated the effect of maternal HIV-1 markers assessed immediately after childbirth, whereas other studies investigated those markers in HIV-1-infected children themselves. In addition, most previous studies have been of cross-sectional or short-term prospective design, making valid comparisons difficult (12, 13). No other published study assessing caries experience in primary teeth of HEU children according to maternal HIV-1 status at birth were available for comparison.

As regards early life course determinants of dental caries other than maternal HIV-1 exposure, the present findings complement those from previous studies (17, 41). Thus, the present findings suggest that dental caries experience was lower in children with longer duration of breastfeeding. This might be explained partly by a possible late first time exposure to sugar when children are breastfed, as reported previously (44). These findings corroborate recent reviews that have documented less dental caries experience with any breastfeeding duration under 1 yr (17, 41, 42). Unexpectedly, the present findings indicated that those with good oral hygiene behavior had double the risk of dental caries at age 5–7 yr. This could have been a chance finding or a result of under-powered analysis, but might also reflect that children reporting appropriate oral hygiene habits are those visiting the dentist most frequently because they are in most need for dental care.

The low uptake of dental services depicted in Table 2 may be attributed to the fact that most of the Ugandan population seeks dental services for pain and other curative reasons, although preventive oral health care is emphasized in the national oral health policy. Okullo *et al*. (45) showed that seeking dental care for toothache was associated positively with dental caries experience among adolescents in Uganda. Further work is recommended to study dental health service uptake among preschool children in the Ugandan context.

In summary, maternal CD4 count at birth was not associated with the dental caries experience in HIV-1-uninfected children born to women without severe immune suppression, while there appeared to be a protective effect of breastfeeding duration and higher viral load.

Acknowledgements – We acknowledge the participants of the study for accepting to have oral assessments. We thank the Department of Paediatrics and Child Health, School of Medicine, Makerere University, and the ANRS12341-PROMISE- PEP M&S study team. We also acknowledge the ANRS 12174 clinical trial study group for making it possible to use the baseline data in this analysis. This study used data collected during the ANRS 12174 clinical trial funded by the French National Institute of Health and Medical Research – National Agency for Research on AIDS and Viral Hepatitis (Inserm–ANRS), the European Developing Countries Clinical Trials Partnership (EDCTP; grant number CT.2006.33020.004), the Research Council of Norway (GlobVac grant number 183600), and the Total Foundation. NB also benefitted from a postdoctoral position funded from the Department of Clinical Dentistry, University of Bergen, Norway.

Conflicts of interest – None to declare.

References

1. Beyrer C, sAbdool KQ. The changing epidemiology of HIV in 2013. *Curr Opin HIV AIDS* 2013; **8**: 306–310.
2. Global UNAIDS. *Report on the Global AIDS Epidemic*. Geneva: 2013.
3. Campion EW, Luzuriaga K, Mofenson LM. Challenges in the Elimination of Pediatric HIV-1 Infection. *N Engl J Med* 2016; **374**: 761–770.
4. UNAIDS. 2016–2021 Strategy:on the fast track to end AIDS; 2016. Available from http://www.unaids.org/en/goals/unaidsstrategy
5. Mofenson LM. Editorial commentary: new challenges in the elimination of pediatric HIV infection: the expanding population of HIV-exposed but uninfected children. *Clin Infect Dis* 2015; **60**: 1357–1360.
6. Evans C, Humphrey JH, Ntozini R, Prendergast AJ. HIV-exposed uninfected infants in Zimbabwe: insights into health outcomes in the pre-antiretroviral therapy era. *Front Immunol* 2016; **7**: 190.
7. Von Mollendorf C, Von Gottberg A, Tempia S, Meiring S, De Gouveia L, Quan V, Legana S, Avenant T, Du Plessis N, Eley B, Finlayson H, Reubenson G, Moshe M, O'brien KL, Klugman KP, Whitney CG, Cohen C. Increased risk for and mortality from invasive pneumococcal disease in HIV-exposed but uninfected infants aged <1 year in South Africa, 2009–2013. *Clin Infect Dis* 2015; **60**: 1346–1356.

8. Evans C, Jones CE. prendergast AJ. HIV-exposed, uninfected infants: new global challenges in the era of paediatric HIV elimination. *Lancet Infect Dis* 2016; **16**: e92–e107.
9. Taron-Brocard C, Le Chenadec J, Faye A, Dollfus C, Goetghebuer T, Gajdos V, Labaune JM, Perilhou A, Mandelbrot L, Blanche S, Warszawski J. Increased risk of serious bacterial infections due to maternal immunosuppression in HIV-exposed uninfected infants in a European country. *Clin Infect Dis* 2014; **59**: 1332–1345.
10. Nabbanja J, Gitta S, Peterson S, Rwenyonyi CM. Orofacial manifestations in HIV positive children attending Mildmay Clinic in Uganda. *Odontology* 2013; **101**: 116–120.
11. Rwenyonyi CM, Kutesa A, Muwazi L, Okullo I, Kasangaki A, Kekitinwa A. Oral manifestations in HIV/AIDS-infected children. *Eur J Dent* 2011; **5**: 291–298.
12. Moscicki AB, Yao TJ, Ryder MI, Russell JS, Dominy SS, Patel K, McKenna M, van Dyke RB, Seage GR, Hazra R. The burden of oral disease among perinatally HIV-infected and HIV-exposed uninfected youth. *PLoS ONE* 2016; **11**: e0156459.
13. Coker M, El-Kamary S, Enwonwu C, Blattner W, Langenberg P, Mongodin E, Akhigbe P, Obuekwe O, Omoigberate A, Charurat M. Perinatal HIV infection and exposure and their association with dental caries in Nigerian children. *Pediatr Infect Dis J* 2018; **37**: 59–65.
14. Arrive E, Meless D, Anaya-Saavedra G, Gallottini M, Pinzon L, Ramirez-Amador V. The global burden of oral diseases in pediatric HIV-infected populations: a workshop report. *Oral Dis* 2016; **22**(S1): S149–S157.
15. Abanto J, Carvalho TS, Mendes FM, Wanderley MT, Bönecker M, Raggio DP. Impact of oral diseases and disorders on oral health-related quality of life of preschool children. *Community Dent Oral Epidemiol* 2011; **39**: 105–114.
16. Akinkugbe AA, Sharma S, Ohrbach R, Slade GD, Poole C. Directed acyclic graphs for oral disease research. *J Dent Res* 2016; **95**: 853–859.
17. Birungi N, Fadnes LT, Kasangaki A, Nankabirwa V, Okullo I, Lie SA, Tumwine JK, Åstrøm AN. Assessing causal effects of early life-course factors on early childhood caries in 5-year-old Ugandan children using directed acyclic graphs (DAGs): A prospective cohort study. *Community Dent Oral Epidemiol* 2017; **45**: 512–521.
18. Ben-Shlomo Y, Kuh D. A life course approach to chronic disease epidemiology: conceptual models, empirical challenges and interdisciplinary perspectives. *Int J Epidemiol* 2002; **31**: 285–293.
19. Nagot N, Kankasa C, Tumwine JK, Meda N, Hofmeyr GJ, Vallo R, Mwiya M, Kwagala M, Traore H, Sunday A, Singata M, Siuluta C, Some E, Rutagwera D, Neboua D, Ndeezi G, Jackson D, Maréchal V, Neveu D, Engebretsen IMS, Lombard C, Blanche S, Sommerfelt H, Rekacewicz C, Tylleskär T, Van de Perre P, Van de Perre P, Nagot N, Vallo R, Marechal V, Peries M, Neveu D, Foulongne V, Segondy M, Blanche S, Treluyer JM, Hirt D, Tumwine JK, Ndeezi G, Karamagi C, Musoke P, Mugaba PM, Kwagala M, Murungi J, Muweesi HN, Ninsiima E, Baryeija S, Juma F, Kata CB, Katushabe S, Meda N, Ouédraogo R, Yé D, Somé E, Traoré HA, Nadembega C, Konaté J, Zongo A, Ouédraogo A, Néboua D, Bélemviré A, Bambara A, Boncoungou J, Zoungrana D, Nikodem C, Hofmeyr J, Harper K, Jackson D, Sanders D, Singata M, Aku A, Okegbe-Eze C, Williams X, Mshweshwe N, Henge V, Gomba F, Gundu T, Khondowe O, Kankasa C, Mwiya M, Lusaka M, Chizyuka M, Phiri M, Imakando B, Musaku M, Kapasa M, Rutagwera D, Clement G, Mwaba HM, Matoba J, Siuluta C, Chola K, Mwamutanda P, Tylleskär T, Sommerfelt H, Engebretsen I, Klungsøyr J, van den Broeck J, Blume J, Rekacewicz C. Extended pre-exposure prophylaxis with lopinavir–ritonavir versus lamivudine to prevent HIV-1 transmission through breastfeeding up to 50 weeks in infants in Africa (ANRS 12174): a randomised controlled trial. *Lancet* 2016; **387**: 566–573.
20. World Health Organisation. *WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children*. Switzerland: World Health Organization Geneva, 2007.
21. World Health Organization. *WHO AnthroPlus for personal computers Manual: Software for assessing growth of the world's children and adolescents*. Geneva: WHO, 2009.
22. Briend A, van den Broeck J, Fadnes LT. Target weight gain for moderately wasted children during supplementation interventions–a population-based approach. *Public Health Nutr* 2011; **14**: 2117–2123.
23. Schwinger C, Fadnes LT, Van den Broeck J. Using growth velocity to predict child mortality. *Am J Clin Nutr* 2016; **103**: 801–807.
24. Birungi N, Fadnes LT, Okullo I, Kasangaki A, Nankabirwa V, Ndeezi G, Tumwine JK, Tylleskär T, Lie SA, Åstrøm AN. Effect of breastfeeding promotion on early childhood caries and breastfeeding duration among 5 year old children in eastern Uganda: a cluster randomized trial. *PLoS ONE* 2015; **10**: e012535.
25. WHO, Oral Health Surveys. *Basic methods*, 5th edn. Albany, NY: World Health Organization, 2013.
26. Hernan MA, Robins JM. Estimating causal effects from epidemiological data. *J Epidemiol Community Health* 2006; **60**: 578–586.
27. Williamson EJ, Aitken Z, Lawrie J, Dharmage SC, Burgess JA, Forbes AB. Introduction to causal diagrams for confounder selection. *Respirology* 2014; **19**: 303–311.
28. Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. *Epidemiology* 1999; **10**: 37–48.
29. Textor J, Hardt J, Knuppel S. DAGitty: a graphical tool for analyzing causal diagrams. *Epidemiology* 2011; **22**: 745.
30. Seaman SR, White IR. Review of inverse probability weighting for dealing with missing data. *Stat Methods Med Res* 2013; **22**: 278–295.
31. Merchant AT, Pitiphat W. Directed acyclic graphs (DAGs): an aid to assess confounding in dental research. *Community Dent Oral Epidemiol* 2002; **30**: 399–404.
32. Hernán MA, Hernández-Díaz S, Werler MM, Mitchell AA. Causal knowledge as a prerequisite for confounding evaluation: an application to birth defects epidemiology. *Am J Epidemiol* 2002; **155**: 176–184.
33. Burt BA. How useful are cross-sectional data from surveys of dental caries? *Community Dent Oral Epidemiol* 1997; **25**: 36–41.
34. Rupérez M, González R, Maculuve S, Quintó L, López-Varela E, Augusto O, Vala A, Nhacolo A, Sevene E, Naniche D, Menéndez C. Maternal HIV infection is an important health determinant in non-HIV-infected infants. *AIDS* 2017; **31**: 1545–1553.
35. Kuhn L, Kasonde P, Sinkala M, Kankasa C, Semrau K, Scott N, Tsai WY, Vermund SH, Aldrovandi GM, Thea DM. Does severity of HIV disease in HIV-infected mothers affect mortality and morbidity among their uninfected infants? *Clin Infect Dis* 2005; **41**: 1654–1661.
36. Muhangi L, Lule SA, Mpairwe H, Ndibazza J, Kizza M, Nampijja M, Nakazibwe E, Kihembo M, Elliott AM, Webb EL. Maternal HIV infection and other factors associated with growth outcomes of HIV-uninfected infants in Entebbe. *Uganda. Public Health Nutr* 2013; **16**: 1548–1557.
37. Chatterjee A, Bosch RJ, Hunter DJ, Fataki MR, Msamanga GI, Fawzi WW. Maternal disease stage and child undernutrition in relation to mortality among children born to HIV-infected women in Tanzania. *JAIDS* 2007; **46**: 599–606.
38. McHenry MS, McAteer CI, Oyungu E, McDonald BC, Bosma CB, Mpofu PB, Deathe BS, Vreeman RC. Neurodevelopment in young children born to HIV-infected mothers: a meta-analysis. *Pediatrics* 2018; **141**: e20172888.
39. Garcia-Knight MA, Nduati E, Hassan AS, Gambo F, Odera D, Etyang TJ, Hajj NJ, Berkley JA, Urban BC, Rowland-Jones SL. Altered memory T-cell responses to bacillus Calmette-Guerin and tetanus toxoid vaccination and altered cytokine responses to polyclonal stimulation in HIV-exposed uninfected kenyan infants. *PLoS ONE* 2015; **10**: e0143043.

40. Drotar D, Olness K, Wiznitzer M, Guay L, Marum L, Svilar G, Hom D, Fagan JF, Ndugwa C, Mayengo RK. Neurodevelopmental outcomes of Ugandan infants with human immunodeficiency virus type 1 infection. *Pediatrics* 1997; **100**: e5.
41. Avila WM, Pordeus IA, Paiva SM, Martins CC. Breast and bottle feeding as risk factors for dental caries: a systematic review and meta-analysis. *PLoS ONE* 2015; **10**: e0142922.
42. Tham R, Bowatte G, Dharmage S, Tan D, Lau M, Dai X, Allen CJ, Lodge CJ. Breastfeeding and the risk of dental caries: a systematic review and meta-analysis. *Acta Paediatr* 2015; **104**(S467): 62–84.
43. Lehman DA, Farquhar C. Biological mechanisms of vertical human immunodeficiency virus (HIV-1) transmission. *Rev Med Virol* 2007; **17**: 381–403.
44. Masumo R, Bardsen A, Mashoto K, Åstrøm AN. Feeding practice among 6–36 months old in Tanzania and Uganda: reliability and relationship with early childhood caries, ECC. *Acta Odontol Scand* 2013; **71**: 1309–1318.
45. Okullo I, Åstrøm A, Haugejorden O. Social inequalities in oral health and in use of oral health care services among adolescents in Uganda. *Int J Paediatr Dent* 2004; **14**: 326–335.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. ANRS 12174 and 12341 study group members.

Appendix S2. Categories of variables used in the analysis.

Appendix S3. Directed acyclic graph paths.

Table S1. Negative binomial regression sensitivity analysis (adjusted for wealth asset index, level of education and marital status) of dental caries and maternal HIV severity (CD4 count) adjusted for early life course variables.