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RESEARCH ARTICLE

Haemoglobin levels and growth of South African infants aged 6–12 months exposed to maternal HIV infection

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Introduction Anaemia is a significant public health concern in women of reproductive age and children under five years old, particularly in high HIV-prevalent settings.
Objective This study analysed differences in haemoglobin levels and growth between human immunodeficiency virus (HIV)-exposed-but-uninfected (HEU) and HIV-unexposed-uninfected (HUU) infants, and further determined correlations between haemoglobin levels and growth at 6, 9, and 12 months in the Siyakhula study.
Results At 6, 9, and 12 months postpartum, the maternal mean haemoglobin levels and anaemia status differed significantly by HIV status ($p < 0.05$), while HEU and HUU infants showed similar mean haemoglobin levels and anaemia status. Anaemia was prevalent in HEU infants at 6 (27%), 9 (33%), and 12 (30%) months. The HEU infants had lower weight-for-age Z-scores (WAZ) and mid-upper-arm circumference-for-age Z-scores (MUACZ) than HUU infants at 6, 9, and 12 months ($p < 0.05$). At 12 months, HEU infants had lower mean weight-for-length Z-scores (WLZ) than the HUU infants (-0.2 ± 1.2 vs. 0.2 ± 1.2; $p = 0.020$). The HEU infants had a higher prevalence of stunting at 6 months (16% vs. 8%; $p = 0.044$), and less breastfeeding at 9 (36% vs. 57%; $p = 0.013$) and 12 (25% vs. 48%; $p = 0.005$) months than the HUU infants. In HEU infants, positive correlations were found between infants' haemoglobin levels and WAZ ($p = 0.039$), LAZ ($p = 0.007$), and MUACZ ($p = 0.039$) at 9 months, and with WAZ ($p = 0.018$) and WLZ ($p = 0.017$) at 12 months, while negative correlations were found between infant haemoglobin levels and any breastfeeding practices at 6, 9, and 12 months ($p = 0.026$; $p < 0.001$; $p = 0.036$).
Conclusion Maternal HIV infection can negatively impact infant growth, and anaemia remains a public health concern in South Africa.

Keywords: anaemia, anthropometry, growth, haemoglobin, HIV exposure, infants, nutrition

Introduction

Anaemia is a global public health concern affecting women of reproductive age and children under five years.[1] In 2020, one in three women of reproductive age suffered from anaemia.[2] A high prevalence of anaemia is found in pregnant women living with HIV in South Africa.[1] Anaemia can lead to poor birth outcomes owing to the impact of iron transfer from the mother to the fetus.[3,4] A haemoglobin level of < 11 g/dl is used to classify anaemia in 6–12-month-old infants and < 12 g/dl for non-pregnant women.[5–10] Low haemoglobin levels restrict oxygen transportation,[2,11] reduce physical and mental capacity, increase infection risk, and lead to poor growth in children.[12,13] Anaemia in children has multiple causes, including prematurity, low birthweight, chronic infections, diarrhoea, and undernutrition.[10,14–18]

The transition from exclusive breastfeeding to complementary feeding in infants from six months of age increases the risk of anaemia due to low iron content in breast milk.[2,19] Infants require an intake of 11 mg of iron daily[20] but struggle to meet this, especially in low- and middle-income countries.[21] In 2019, high rates of anaemia were reported globally (40%), in Africa (60%), and in South Africa (44%) in children under the age of five years.[13] Poor nutrition in the first 1 000 days increases the risk of undernutrition and anaemia in children,[22,23] leading to growth failure, underweight, and stunting, as reported in South Africa,[21] Ethiopia,[24] and India.[25]

Anaemia is more prevalent in people living with HIV owing to the reduced red cell production and survival.[2,26] In 2022, 39 million people worldwide were living with HIV, with 54% being women of reproductive age and 23% being South African women.[27,28] Improved access to antiretroviral treatment has led to many infants being exposed to maternal HIV infection but remaining HIV-uninfected (HEU) since the decreased vertical transmission of HIV.[27] Anaemia is reportedly more prevalent in HEU infants than in HIV-uninfected (HUU) infants.[28,29] In Ethiopia, anaemia odds were 2.54 times higher for infants born to mothers living with HIV (MLWH) than those born to mothers not living with HIV (MnLWH).[30] Anaemia has been linked to growth failure in children, with lower length-for-age Z-scores in HEU infants compared with HUU infants.[29,31–34] Longitudinal studies on haemoglobin levels and growth outcomes in high HIV-prevalence settings like South Africa are lacking.[27,28,35] Available research includes studies from other

African countries, single cross-sectional analyses,[30,36,37] and lack of a comparison group.[37–39] Therefore, this study aimed to compare the haemoglobin levels and growth of HEU and HUU infants, and further determined correlations between haemoglobin levels and growth at 6, 9, and 12 months.

Methods and materials

Study design, setting, and participants

The sub-study embedded in the Siyakhula study had a repeated cross-sectional design, with the overall study aim to better understand how the in-utero and early postnatal environments, altered by maternal HIV-positive status, would influence infant growth trajectories and cognitive development, and alter their immune development and function irrespective of the infants' HIV status. The study was conducted at the Research Centre for Maternal, Fetal, Newborn, and Child Health Care Strategies, at Kalafong Provincial Tertiary Hospital, Gauteng province, South Africa, as described elsewhere.[31,40,41] The sub-study aimed to compare the haemoglobin levels and growth of HEU and HUU infants, and further determined correlations between haemoglobin levels and growth at 6, 9, and 12 months, and included 181 mother–infant dyads with birth, 6, 9, and 12 months' follow-up data, as summarised in Figure 1.

Data collection

Maternal demographic information, including age, marital status, education, employment, living conditions such as access to running water, toilet facilities, and electricity, socioeconomic background, lifestyle, current habits such as the maternal use of tobacco and alcohol, as well as HIV status, were recorded. In addition, infants' background information and feeding practices were also recorded. All MLWH self-reported the use of ART throughout and after pregnancy, with the first-line regimen at the time of study a once-daily fixed-dose combination of tenofovir, emtricitabine, and efavirenz. Data were collected by trained fieldworkers in the participants' preferred local language using a structured questionnaire.[31] Subsequently, a structured infant and mother follow-up questionnaire was used to record the mother–infant dyads' information and measurements.

Haemoglobin levels

The HemoCue® device is a point-of-care test commonly used to measure haemoglobin and can assist in diagnosing anaemia. Capillary blood samples were collected by trained research staff at postpartum visits (6, 9, and 12 months) in mothers and their infants. A lancet was used to prick the finger and the first two or three drops of blood were wiped away before filling the microcuvette with a single millilitre of blood; the microcuvette was then inserted into the portable photometer (Hb 201^{+}; HemoCue®, Angelholm, Sweden) device, recommended for use in both clinical and survey settings in resource-limited locations for determining the prevalence of anemia.[6,42] A haemoglobin level cut-off of < 11.0 and < 11.9 grams per decilitre (g/dl) was used to classify anaemia in infants and non-pregnant women, respectively.[5–10]

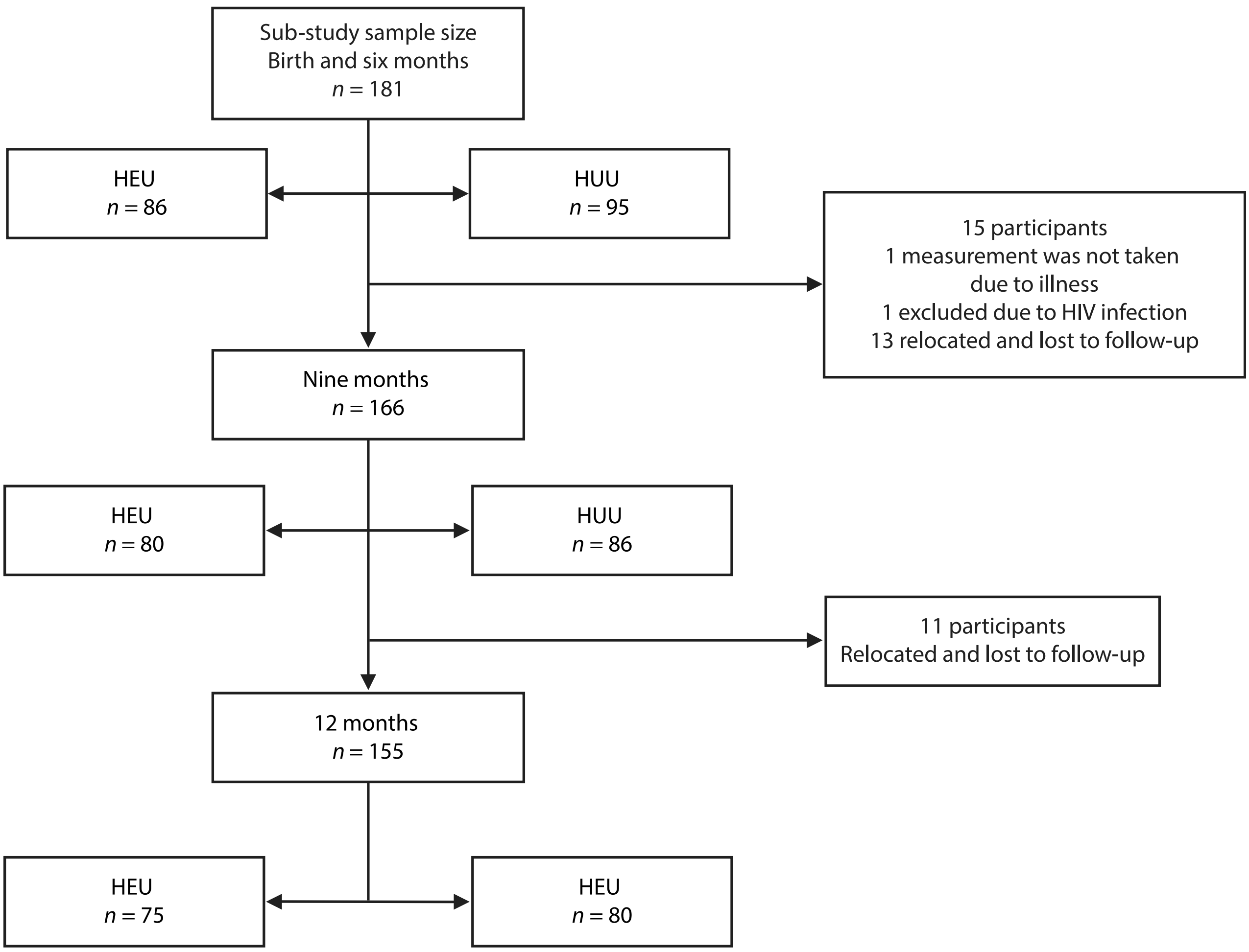

Figure 1: Sub-study flow diagram of participants at 6, 9, and 12 months. Abbreviations: HEU: HIV-exposed-uninfected; HUU: HIV-unexposed-uninfected

Anthropometric measurements

Infant anthropometry was measured at birth, 6, 9, and 12 months, which included the length, weight, head circumference (HC), and mid-upper-arm-circumference (MUAC) (the latter from 6 months onwards). A mechanical infantometer (Seca 416, Hamburg, Germany), calibrated digital scale (Seca 354, Germany), and non-stretchable tape measures (KDS measure, model F10-02DM 2 m, Kyoto, Japan) were used to collect the length, weight, MUAC, and HC of the infants, respectively.

The infants' Z-scores for weight, length, MUAC, and HC were computed using the WHO Anthro software v3.2.2 (https://www.who.int/tools/child-growth-standards/software) with gestational age correction and standardised for age and sex.[43] Stunting, underweight, and wasting were nutritional classifications defined as Z-scores less than −2 SD for LAZ, WAZ, and WLZ, respectively, and overweight as a WLZ more than +2 SD of the reference data median values.

Maternal body size was assessed using weight, height, and MUAC measurements, and the body mass index (BMI) was calculated: weight (kg)/height $(m)^2$. All anthropometric measurements were taken twice, as per the International Society for the Advancement of Kinanthropometry (ISAK) guidelines. The two measurements were recorded, and the mean then used for data analysis.[44] If the two weights differed by more than 0.05 kg or the HC or MUAC differed by more than 0.5 cm, a third measurement was taken. The two closest values were used to calculate the mean, which was used in the analysis.

Data processing and statistical analysis

Descriptive data analysis was used to present continuous data such as maternal age, haemoglobin levels, anthropometric measures, and Z-score indices for the study population. The normality of the data was determined using histograms and the Shapiro–Wilk test. The age, weight, length, HC, and MUAC are examples of continuous variables with normally distributed data that were reported as mean and standard deviation. In the case of non-normally distributed data, the median and interquartile range (IQR) were reported. All categorical variables, such as HEU status, employment status, prematurity, and low birthweight, were presented as frequencies and percentages.

The independent *t*-test (normally distributed) or the Mann–Whitney *U* test (non-normally distributed) was used to compare the data from HEU and HUU infants for continuous variables, and the Pearson chi-square test was used to compare categorical variables. The relationship between haemoglobin levels and growth parameters (infant Z-scores) and further potential confounders was examined using the Pearson correlation coefficient. The Statistical Package for the Social Sciences was used for all statistical analyses (Version 28, IBM Corp, Armonk, NY, USA), and the level of significance was set at $p < 0.05$.[16]

Ethical considerations

Ethical approval for the Siyakhula study was obtained from the University of Pretoria, Faculty of Health Sciences Research Ethics Committee (FHSREC) (Ref no. 294/2017). Additional ethical clearance for this sub-study was obtained from the Faculty of Natural and Agricultural Sciences Ethics Committee and FHSREC in the same university (Ref no. NAS063/2020). Before data collection, all pertinent information was disclosed to the mothers, who then gave their informed consent on behalf of both themselves and their infants, and the study was conducted following the principles of the Declaration of Helsinki.

Results

The sociodemographic characteristics of the MLWH and MnLWH (n total = 181; 86 (47.5%) vs. 95 (52.5%), respectively) are presented in Table 1. Significant differences were found in the mothers' age and education levels (both $p < 0.001$) between the two groups. No significant differences were found in terms of employment status, monthly household income, access to electricity, toilet facilities, and water, as well as mode of delivery ($p > 0.05$). All the MLWH were on ART, with the majority (98.8%) on tenofovir/emtricitabine/efavirenz. Most mothers initiated ART before their pregnancy (79.2%), and the mean latest CD4 count was 473 ± 273 cells/mm^3.

Table 2 lists infant characteristics at birth, with HEU infants having a lower mean WAZ (−0.7 ± 0.9 vs. −0.2 ± 1.0; $p = 0.003$) than HUU infants. Low birthweight (below 2 500 g) was more evident in HEU than in HUU infants (22.1% vs. 12.6%; $p < 0.001$). All HEU infants started with interventions of vertical transmission prevention of HIV, with 55.8% being initiated on nevirapine prophylaxis, while the remainder received dual prophylaxis with nevirapine and zidovudine (AZT).

The mothers' and infants' haemoglobin levels and anaemia status at 6, 9, and 12 months postpartum, stratified by maternal HIV status, are presented in Table 3. Significant differences were found between MLWH and MnLWH in the mean haemoglobin levels and anaemia status at 6, 9, and 12 months postpartum ($p < 0.05$), with MLWH more often anaemic at 6 (36.2% vs. 13.1%; $p < 0.001$), 9 (26.0% vs. 13.5%; $p < 0.001$), and 12 (28.6% vs. 17.9%; $p < 0.001$) months with MnLWH. No significant differences were found between HEU and HUU infants in terms of the mean haemoglobin levels and anaemia status at ages 6, 9, and 12 months ($p > 0.05$).

The maternal anthropometric measurements at 6, 9, and 12 months postpartum are presented in Table 4. The mean BMI of MLWH was significantly lower than MnLWH at 6 (25.6 ± 4.5 vs. 27.7 ± 4.7; $p = 0.011$), 9 (25.4 ± 4.5 vs. 27.9 ± 4.8; $p = 0.002$), and 12 (26.0 ± 4.5 vs. 28.2 ± 4.9; $p = 0.007$) months. The mean MUAC was significantly lower in MLWH than in MnLWH at 12 months ($p < 0.05$).

The correlation between haemoglobin levels, Z-scores indices, and confounding factors in HEU and HUU infants at 6, 9, and 12 months are presented in Table 5. At 9 months, infants' mean haemoglobin levels had positive correlations with the mean WAZ (r = 0.3, $p = 0.039$), LAZ ($r = 0.3$, $p = 0.007$), and MUACZ ($r = 0.3$, $p = 0.039$) in HEU infants but not in HUU infants, while at 12 months, positive correlations were found with WAZ ($r = 0.3$, $p = 0.018$) and WLZ ($r = 0.3$, $p = 0.017$). In HEU infants, haemoglobin levels had significant negative correlations with any breastfeeding practices at 6 ($r = −0.3$, $p = 0.026$), 9 ($r = −0.4$, $p < 0.001$), and 12 months ($r = −0.3$, $p = 0.041$). Haemoglobin levels in HUU infants at 6 months showed a significant positive correlation with maternal haemoglobin levels at the same visit ($r = 0.3$; $p = 0.028$).

Supplementary materials

The infants' anthropometric measurements, Z-score indices, and nutritional categories at 6, 9, and 12 months according to their HEU status are shown in Supplementary Table 1.[31] The mean weight of HEU infants was significantly lower than that of HUU infants at 6 months (7.3 ± 0.9 kg vs. 7.8 ± 1.0 kg; $p = 0.001$), and WAZ (−0.6 ± 1.1 vs. 0.1 ± 1.2; $p < 0.001$) than that of HUU infants. The WAZ and LAZ were significantly lower in

Table 1: Maternal characteristics stratified by HIV status[45]

Factor		MLWH $n = 86$	MnLWH $n = 95$	p-value
Age (years), mean ± SD		36.9 ± 8.6	31.3 ± 6.3	< 0.001*
Age (years), n (%)	20–29	11 (12.8)	39 (41.1)	< 0.001*
	30–39	55 (64.0)	45 (47.4)	
	≥ 40	20 (23.2)	11 (11.5)	
Education, n (%)˜	Formal education‡	55 (66.3)	31 (33.0)	< 0.001*
	Completed secondary schooling	19 (22.9)	39 (41.5)	
	Tertiary education	9 (10.8)	24 (25.5)	
Employment, n (%)	Yes	41 (49.4)	43 (45.7)	0.738
Monthly income of the household (ZAR), n (%)	Don't know §	20 (23.3)	18 (18.9)	0.282
	R0–R4 000	29 (33.8)	27 (28.4)	
	R4 001–R8 000	21 (24.4)	29 (30.5)	
	More than R8 001	16 (18.5)	21 (22.2)	
Child support grant, n (%)	Yes	64 (74.4)	74 (77.9)	0.583
Marital status, n (%)	Single/divorced/widowed	60 (72.3)	74 (78.7)	0.412
	Married/cohabiting	23 (27.7)	20 (21.3)	
Access to water, n (%)	Communal tap	21 (25.3)	19 (20.2)	0.534
	Inside yard	42 (50.6)	46 (48.9)	
	Inside house	20 (24.1)	29 (30.9)	
Access to electricity, n (%)	Yes	79 (91.9)	90 (94.7)	0.437
Access to toilet, n (%)	None ¶	2 (2.4)	0 (0)	n/a
	Pit latrine	29 (34.9)	31 (33.0)	0.816
	Flush toilet	52 (62.7)	63 (67.0)	
Smoking, n (%) ¥	Yes	3 (3.5)	3 (3.2)	n/a
Drinks alcohol, n (%) ¥	Yes	12 (14.0)	12 (12.6)	0.793
Mode of delivery, n (%)˜	Vaginal delivery ¦	49 (57.0)	65 (68.4)	0.196
	Caesarean section	37 (31.6)	30 (31.6)	
Obstetric history median [IQR]	Gravidity	3 [2, 4]	3 [2, 3]	0.024 *
	Parity	2 [1, 3]	2 [1, 2]	0.031 *
	Previous pregnancy losses ¢	0 [0, 1]	0 [0, 1]	0.647

Abbreviations: MLWH: mothers living with HIV; MnLWH: mothers not living with HIV; ZAR: South African rand; SD: standard deviation; IQR: interquartile range. ‡ Formal education = includes any primary and secondary schooling, but without school completion; § 'Don't know' category excluded from analysis; ¶ none: not considered in the calculation; ¥ at delivery and 6 months postpartum; ¦ includes assisted delivery; ¢ includes abortions, miscarriage, and termination of pregnancy,˜ excludes missing numbers.

Statistical analysis: to determine the difference in continuous data between MLWH and MnLWH the Mann–Whitney U test (non-normally distributed) was used; for categorical data, Pearson's chi-square test was used to determine the differences between MLWH and MnLWH; * p-value shows a significant difference of < 0.05.

HEU than HUU infants at 9 months ($p = 0.003$ and $p = 0.023$). At the same time, the WLZ of HEU infants was lower than HUU at 12 months ($p = 0.020$). The rates of stunting were significantly higher in HEU compared with HUU infants (16% vs. 7%; $p = 0.044$) at 6 months. Maternal haemoglobin levels, anaemia status, and anthropometric measurements at antenatal care visits and delivery, according to HIV status, are presented in Supplementary Table 2. MLWH had a significantly higher mean weight than MnLWH (69.1 ± 11.1 kg vs. 65.1 ± 11.7 kg; $p = 0.009$) at the first-trimester antenatal care visits. Supplementary Figure 1 shows the breastfeeding practices of HEU vs. HUU infants at 6, 9, and 12 months. Both HEU and HUU children had similar percentages of breastfeeding at 6 months, but there were significant differences at 9 (35.6% vs. 57.3%; $p = 0.013$) and 12 months (24.7% vs. 48.0%; $p = 0.005$).[31]

Discussion

Haemoglobin levels and anaemia of mothers and their infants

Anaemia is a significant public health concern in women of reproductive age, pregnant and postpartum women, young children, and HEU infants. The MLWH had lower haemoglobin levels but a higher prevalence of anaemia than MnLWH, with similar findings reported in other studies.[46,47] Anaemia is common in MLWH and increases the risk of having children with anaemia.[48] The haemoglobin levels of HEU and HUU infants were similar at all visits, which contrasts with a Ugandan study that found lower haemoglobin levels in HEU children from 6 months, possibly due to smaller sample sizes (HEU [$n = 25$]) vs. (HUU [$n = 291$]).[49] We found a higher prevalence of infant anaemia than reported globally (39.8%%), but lower than in the African (60.2%) region.[50] The prevalence of anaemia was similar at all visits. Nevertheless, HEU infants had higher percentages at 6 (26.5% vs. 18.2%), 9 (33.3% vs 28.8%), and 12 (29.7% vs. 16.9%) months, compared with HUU infants, with similar findings previously reported in Mozambique[51] and Uganda.[52]

ART regimen and maternal HIV status

No correlation was found between HEU infants' haemoglobin levels and MLWH at 6 months postpartum, possibly owing to improved maternal health due to high coverage of ART in the setting. The missing differences in our study may be explained

Table 2: Characteristics of HIV-exposed and unexposed infants at birth

Factor		HEU infants *n* = 86	HUU infants *n* = 95	*p*-value
Gestational age (weeks), mean ± SD †		38.2 ± 1.5	38.3 ± 1.8	0.293
Premature, *n* (%)	Yes	11 (12.8)	12 (12.6)	0.974
	Very preterm (28–< 32 weeks)	0 (0)	1 (1.1)	n/a
	Moderate preterm (32–< 34 weeks)	0 (0)	1 (1.1)	
	Late preterm (34–< 37 weeks)	11 (12.8)	10 (12.6)	
Low birthweight, *n* (%)‡		19 (22.1)	12 (12.6)	< 0.001*
Infant sex, *n* (%)	Female	32 (37.2)	44 (46.3)	0.276
APGAR score, median [IQR]	1 minute	8 [8, 9]	9 [9, 9]	0.145
	5 minutes	9 [9, 9]	9 [9, 10]	0.199
Body measurements, mean ± SD	Weight (g)†	2 844 ± 490	3 058 ± 507	0.005*
	Length (cm)	49.1 ± 4.1	49.9 ± 3.4	0.184
	Head circumference (cm)	33.8 ± 1.8	34.5 ± 1.6	0.013*
Z-scores indices, mean ± SD †	Weight-for-age	−0.7 ± 0.9	−0.2 ± 1.0	0.003*
	Length-for-age	0.6 ± 1.4	0.7 ± 1.5	0.804
	Head circumference-for-age	0.3 ± 1.3	0.7 ± 1.2	0.038*

Abbreviations: HEU: HIV-exposed-uninfected (born to mothers living with HIV); HUU: HIV-unexposed-uninfected (born to mothers not living with HIV); n/a: not applicable (no comparisons were performed as one of the groups had less than five counts leading to volatile results); g: gram; cm: centimetre; IQR: interquartile range; SD: standard deviation. ‡ Low birthweight is classified as infants born with a weight of less than 2500 g; § the birth Z-score indices are sex-normalized and were computed using INTERGROWTH-21st software (https://intergrowth21.ndog.ox.ac.uk/). Statistical analysis: to determine the difference in continuous data between mothers living and not living with HIV, the independent-*t*-test (normally distributed) and Mann–Whitney *U* test († non-normally distributed) were used; for categorical data, Pearson's chi-square test was used to determine the differences. * *p*-value shows a significant difference of < 0.05.

by the fact that the majority of MLWH were on tenofovir/emtricitabine/efavirenz and not AZT, which has been shown to increase the prevalence of anaemia in MLWH[53], as supported by studies in Botswana[36] and Ethiopia.[54] The use of ART increases the haemoglobin levels and decreases the prevalence of anemia[55], even in the case of maternal anaemia, where being enrolled in the ART programme was protective against anaemia in MLWH.[55,56] A randomised control trial conducted in three African countries (Burkina Faso, Kenya, and South Africa) also found a significant reduction in the prevalence of anaemia with the use of ART during and after pregnancy.[57]

Nutritional status and haemoglobin levels of infants

At 6 months, HEU infants had lower mean LAZ, WAZ, and MUACZ, with stunting (15.0%), underweight (8.9%), and wasting (3.7%) observed, similar to findings from Uganda.[49] In Botswana, HEU infants showed higher rates of underweight (15.6% vs. 6.9%) and stunting (15.6% vs. 7.3%) from 6 months onwards.[58] By 12 months, HEU infants in our study had lower mean WLZ but no significant differences in LAZ, consistent with findings from China.[59] No correlation was observed between haemoglobin levels and Z-scores at 6 months, aligning with Ugandan data.[60]

Feeding practices and haemoglobin levels of infants

Breastfeeding rates declined with age, particularly in HEU infants, likely influenced by cultural norms, fear, and prior formula use in HIV prevention programmes.[61] A negative correlation was found between haemoglobin levels and breastfeeding. While breast milk provides highly bioavailable iron, its quantity is insufficient after 6 months; thus, in the absence of iron-rich complementary foods, anaemia risk increases. Studies in South Africa showed similar breast milk iron concentrations at 6 and 12 months irrespective of maternal HIV status, supporting safe breastfeeding for MLWH on ART.[45,62]

Infant anaemia can result from inadequate iron intake, and inadequate iron intake due to poor-quality complementary food.[63] A South African study found that 30% of infants had never consumed meat products at 12 months[31] in the previous seven days, which are rich sources of heme-iron.[19] In Ethiopia, HEU children aged 6–24 months were not introduced to complementary foods in a timely manner and this was coupled with inadequate dietary diversity.[64] Lack of employment is a contributing factor to inappropriate complementary feeding,[65] especially in the context of maternal HIV.[66]

Policy implications

The WHO Infant and Young Child Feeding (IYCF) policy recommends the consumption of five out of eight food groups daily during the complementary feeding period;[67] however, poor dietary diversity is a concern in African infants.[45] Implementing the IYCF policy is crucial in reducing undernutrition in South Africa, especially in the HIV context.[64,67,68] Maternal nutritional status significantly impacts an infant's nutritional status within the first 1 000 days. Nutrition-specific interventions should be targeted at underweight mothers to improve their nutritional status. However, overweight and obesity are common issues, emphasising the need for healthy dietary habits and physical activity.[69,70]

Strengths and limitations

The strength of our study lies in the repeated cross-sectional analyses with known infant characteristics at baseline, as well as the availability of maternal anthropometric and haemoglobin measurements at antenatal and postnatal visits, with measurements performed by trained research staff ensuring quality control. In addition, a comparable sample size, correlating infants' haemoglobin levels and Z-score indices with maternal haemoglobin and anthropometric measurements at antenatal and postpartum visits, stratified by maternal HIV status, adds relevant information to the link between maternal and infant haemoglobin levels. However, the study has limitations, such as a relatively small sample size, lack of pre-pregnancy weight, and use of the point-of-care Hemocue® machine. Future research should examine growth and haemoglobin levels over a longer period and determine iron dietary intake in complementary foods for HEU and HUU infants.

Table 3: Haemoglobin levels and anaemia status of mothers and infants at 6, 9, and 12 months postpartum, stratified by maternal HIV status

	At 6 months postpartum			At 9 months postpartum			At 12 months postpartum		
Factor	**MLWH/ HEU $n = 86$**	**MnLWH/ HUU $n = 95$**	***p*-value**	**MLWH/ HEU $n = 80$**	**MnLWH/ HUU $n = 86$**	***p*-value**	**MLWH/ HEU $n = 75$**	**MnLWH/ HUU $n = 80$**	***p*-value**
Maternal haemoglobin levels (g/dl), mean ± SD	12.6 ± 1.6	13.2 ± 1.6	0.027 *	12.6 ± 1.3	13.3 ± 1.2	< 0.001 *	12.6 ± 1.5	13.1 ± 1.3	0.030 *
Maternal anaemia, *n* (%)[†]	25 (36.2)	8 (13.1)	< 0.001 *	19 (26.0)	10 (13.5)	< 0.001 *	20 (28.6)	14 (17.9)	< 0.001 *
Infant haemoglobin levels (g/dl), mean ± SD	11.9 ± 1.4	11.8 ± 1.2	0.996	11.7 ± 1.5[‡]	11.8 ± 1.4[‡]	0.440	11.9 ± 1.2 [‡]	11.6 ± 1.2 [‡]	0.089
Infant anaemia, *n* (%) [†]	18 (26.5)	10 (18.2)	0.276	23 (33.3)	21 (28.8)	0.556	19 (29.7)	10 (16.9)	0.096

Abbreviations: HEU: HIV-exposed-uninfected, HUU: HIV-unexposed-uninfected; Hb: haemoglobin, MLWH: mothers living with HIV; MnLWH: mothers not living with HIV; g: gram; dl: decilitre. Mothers *n* with available Hb levels at 6 months: MLWH = 69 and MnLWH = 61; at 9 months: MLWH = 73 and MnLWH = 74; at 12 months: MLWH = 70 and MnLWH = 65; infants *n* without missing Hb levels at 6 months: HEU = 55 and HUU = 68; at 9 months: HEU = 69 and HUU = 73; at 12 months: HEU = 59 and HUU = 64.

[†]World Health Organization anaemia cut-offs: non-pregnant women: < 11.9 g/dl; infants from 6–59 months: < 10.9 g/dl.

Statistical analysis: to determine the difference in continuous data between MLWH and MnLWH, independent-*t*-test (normally distributed) and Mann–Whitney *U* test ([‡] non-normally distributed) were used; for categorical data, Pearson's chi-square test was used to determine the differences in MLWH and MnLWH; * *p*-value shows a significant difference of < 0.05.

Table 4: Maternal anthropometric measurements at 6, 9, and 12 months postpartum, stratified by maternal HIV status

	6 months postpartum			9 months postpartum			12 months postpartum		
Factor	**MLWH $n = 86$**	**MnLWH $n = 95$**	***p*-value**	**MLWH $n = 80$**	**MnLWH $n = 86$**	***p*-value**	**MLWH $n = 75$**	**MnLWH $n = 80$**	***p*-value**
Weight (kg), mean ± SD	65.3 ± 12.8	68.8 ± 11.7	0.079	65.0 ± 12.7	69.2 ± 12.1	0.043*	65.6 ± 13.1	69.9 ± 12.5	0.056
Height (cm), mean ± SD	159.5 ± 6.0	157.4 ± 4.8	0.010*	159.6 ± 6.3 [†]	157.5 ± 5.7 [†]	0.028*	158.8 ± 6.2	157.7 ± 5.1	0.506
BMI (kg/m^2), mean ± SD	25.6 ± 4.5 [†]	27.7 ± 4.7 [†]	0.011*	25.4 ± 4.5 [†]	27.9 ± 4.8 [†]	0.002*	26.0 ± 4.5	28.2 ± 4.9	0.007*
Underweight, *n* (%)[‡]	5 (6.9)	1 (1.4)	n/a	5 (7.0)	1 (1.4)	n/a	2 (2.9)	1 (1.5)	n/a
Normal weight, *n* (%)[§]	29 (40.3)	20 (27.0)	0.425	29 (40.8)	21 (28.4)	0.099	29 (42.6)	18 (26.9)	0.225
Overweight, *n* (%) [¶]	27 (37.5)	32 (43.2)	0.034*	26 (36.6)	34 (45.9)	0.024*	22 (32.4)	29 (43.3)	0.004*
Obese, *n* (%)[¥]	11 (15.3)	21 (28.4)	0.056	11 (15.5)	18 (24.3)	0.184	15 (22.1)	19 (28.4)	0.399
MUAC (cm), mean ± SD	29.9 ± 4.1	30.7 ± 3.8	0.220	29.6 ± 3.9	30.7 ± 3.7	0.073	29.9 ± 3.9	31.4 ± 3.4	0.021*
Normal MUAC, *n* (%)[¦]	67 (94.4)	73 (97.3)	0.367	68 (94.4)	69 (98.6)	0.182	65 (97.0)	66 (100)	0.157

Abbreviations: MLWH: mothers living with HIV; MnLWH: mothers not living with HIV; BMI: body mass index; MUAC: mid-upper-arm circumference; SD: standard deviation.

BMI classifications [‡] underweight category < 18.5 kg/m^2, [§] normal weight = 18.5–24.9 kg/m^2, [¶] overweight = 25.0–29.9 kg/m^2 and [¥] obese ≥ 30.0 kg/m^2; [¦] mid-upper-arm-circumference normal > 23 cm; n/a: not applicable (no comparisons were performed as one of the groups had less than five counts leading to volatile results).

Statistical analysis: to determine the difference in continuous data between MLWH and MnLWH, the independent-*t*-test and Mann–Whitney *U* test ([†] non-normally distributed) were used; and for categorical data, Pearson's chi-square test was used to determine the differences in MLWH and MnLWH; * *p*-value shows significant difference of < 0.05.

Table 5: Correlation between infant haemoglobin levels, Z-scores indices, and confounding factors in HEU and HUU infants at 6, 9, and 12 months

		Infant haemoglobin (g/dl) level at 6 months				Infant haemoglobin (g/dl) level at 9 months				Infant haemoglobin (g/dl) level at 12 months			
		HEU		HUU		HEU		HUU		HEU		HUU	
Factors		*r*	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value
Infants													
Z-scores †	Weight-for-age	0.2	0.102	0.2	0.115	0.3	0.039 *	0.1	0.268	0.3	0.018*	0.0	0.978
	Length-for-age	0.0	0.971	0.3	0.068	0.3	0.007 *	0.1	0.279	0.1	0.522	0.0	0.754
	Weight-for-length	0.1	0.297	0.0	0.801	0.0	0.939	0.1	0.344	0.3	0.017*	0.0	0.357
	Head circumference-for-age	−0.1	0.275	0.1	0.733	0.2	0.222	−0.2	0.176	0.0	0.830	0.0	0.732
	Mid-upper-arm-circumference-for-age	0.2	0.076	0.3	0.047*	0.3	0.039 *	0.0	0.934	0.2	0.142	0.0	0.442
Any breastfeeding at the visit		−0.3	0.026*	0.1	0.678	−0.4	< 0.001 *	−0.1	0.232	−0.3	0.036 *	0.0	0.890
Maternal													
Age at delivery		0.0	0.946	−0.1	0.345	0.1	0.681	0.1	0.422	−0.1	0.556	0.0	0.697
Haemoglobin levels	First trimester	0.2	0.094	0.1	0.540	0.0	0.787	0.2	0.054	0.0	0.875	0.1	0.619
	Second trimester	0.0	0.833	0.0	0.896	0.1	0.682	0.0	0.826	−0.2	0.178	0.1	0.577
	Third trimester	0.0	0.978	−0.2	0.343	−0.2	0.141	0.0	0.923	−0.2	0.224	0.2	0.141
	Delivery	0.1	0.498	0.1	0.369	0.1	0.363	0.0	0.763	0.1	0.383	0.1	0.415
	6 months postpartum	0.1	0.271	0.3	0.028 *								
	9 months postpartum					0.1	0.620	0.1	0.678				
	12 months postpartum									0.0	0.746	0.0	0.768
BMI	First trimester	0.1	0.642	0.1	0.448	0.1	0.477	0.1	0.656	0.1	0.557	0.1	0.348
	Second trimester	0.0	0.955	0.2	0.244	0.0	0.732	0.1	0.245	−0.1	0.406	0.2	0.143
	Third trimester	0.0	0.816	0.1	0.347	0.1	0.269	0.0	0.882	−0.2	0.116	0.1	0.670
	Delivery	0.1	0.642	0.2	0.185	0.1	0.409	0.0	0.934	0.0	0.905	−0.1	0.413
	6 months postpartum	0.0	0.840	0.2	0.153								
	9 months postpartum					−0.1	0.492	0.2	0.181				
	12 months postpartum									0.0	0.829	−0.3	0.032 *
MUAC	First trimester	−0.1	0.449	0.1	0.465	−0.1	0.561	0.0	0.823	0.0	0.783	0.0	0.794
	Second trimester	0.4	< 0.00 1*	0.0	0.751	−0.2	0.114	0.0	0.934	0.0	0.988	−0.4	0.002 *
	Third trimester	0.3	0.025*	0.0	0.944	0.0	0.886	0.0	0.909	0.1	0.413	−0.3	0.006
	Delivery	0.2	0.237	0.2	0.237	0.2	0.267	−0.2	0.280	0.1	0.361	−0.2	0.091
	6 months postpartum	0.0	0.752	0.1	0.543								
	9 months postpartum					0.0	0.825	0.2	0.197				
	12 months postpartum									0.0	0.887	−0.3	0.033
	Education status at 6 months	−0.2	0.181	0.0	0.830	−0.1	0.330	0.0	0.713	−0.1	0.370	0.2	0.120
	Monthly household income	−0.1	0.291	0.1	0.621	0.0	0.971	0.1	0.282	0.2	0.155	−0.1	0.568

Abbreviations: HEU: HIV-exposed-uninfected; HUU: HIV-unexposed-uninfected; BMI: body mass index; MUAC: mid-upper-arm circumference; SD: standard deviation; *r* = Pearson correlation coefficient; † Z-scores indices at age 6–12 months were computed using World Health Organization Anthro software (2010); blank space: no correlation performed at the visit; * *p*-value shows significant difference of < 0.05.

Conclusion

This study compared haemoglobin levels and growth of infants (aged 6, 9, and 12 months) by maternal HIV status. The MLWH were more anaemic than MnLWH, but infants' anaemia status did not differ. The HEU infants were more stunted than HUU infants, with haemoglobin levels positively correlating with WAZ, LAZ, and MUACZ at 9 months. While maternal HIV infection impacts infant growth, it did not affect anaemia status. Nutrition education before, during, and after pregnancy and the promotion of iron-rich food consumption are crucial for preventing anaemia and improving infant growth and development.

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Ethical statement – The Siyakhula study was approved by the Faculty of Health Sciences Research Ethics Committee (FHSREC) (Ref. no: 294/2017) of the University of Pretoria. For this study, approvals were granted by the Faculty of Natural and Agricultural Sciences and the FHSREC at the same institution (Ref. no: NAS063/2020). This study was conducted in accordance with the principles of the Declaration of Helsinki, all relevant information was shared with the mothers, and the mothers provided informed consent on behalf of themselves and their infants before data collection.

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