CHILD HIV EXPOSURE AND CMV SEROPREVALENCE IN BOTSWANA: NO ASSOCIATIONS WITH 24-MONTH GROWTH AND NEURODEVELOPMENT

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ABSTRACT

Background: We sought to identify predictors of child cytomegalovirus (CMV) infection overall and by maternal HIV status, and to assess associations of child CMV status with growth and neurodevelopmental outcomes at 24 months of age in Botswana.

Methods: Data and samples were used from the Botswana-based observational Tshipidi study (2010-2014), enrolling pregnant women living with and without HIV and following their infants through 2 years of age. Child plasma samples were tested at 18 months of age for anti-CMV IgG. Associations were assessed between detectable anti-CMV IgG and growth (using the WHO Child Growth Standards) and neurodevelopment (using Bayley Scales of Infant and Toddler Development III and the Developmental Milestones Checklist) at 24 months of age.

Results: Of 317 children, 215 (68%) had detectable anti-CMV IgG at 18 months of age. Comparatively, 83% (N=178) of HIV-unexposed uninfected (HUU) children had positive CMV serology versus 47% (N=139) of HIV-exposed uninfected (HEU) children (p <0.01); 100% of HUU vs. 10.5% of HEU children breastfed. Child CMV infection was not associated with weight-for-age, weight-for-length, or length-for-age z-scores at 24 months. In HUU children, CMV infection was associated with smaller head circumference (p <0.01). No difference was observed by child CMV status in any neurodevelopmental domain at 24 months.

Conclusion: We observed high CMV seropositivity in 18-month-old children in Botswana, with higher seropositivity among breastfed (HUU) children. Positive CMV serostatus was not associated with 24 months child growth or neurodevelopmental outcomes, with the exception of smaller head circumference among HUU CMV-positive children.

Keywords: Cytomegalovirus; HIV Exposed Uninfected; HIV Unexposed Uninfected; Anthropometrics; Neurodevelopment; Children; Botswana
INTRODUCTION

Approximately one in four infants in southern Africa is born to a woman living with HIV (WLHIV), and the vast majority of these children remain HIV-uninfected as a result of programs to prevent vertical transmission (VT) of HIV. These HIV-exposed uninfected (HEU) children experience higher rates of morbidity and mortality compared with their HIV-unexposed uninfected (HUU) counterparts, as well as poorer growth and in some studies, poorer neurodevelopmental outcomes.

Mechanisms behind this increased susceptibility of HEU children to poor growth and developmental outcomes have not been fully elucidated. However, these outcomes may be caused by multiple factors including sequelae of antiretroviral (ARV) exposure in utero, exposure to maternal co-infections, and/or suboptimal immune responses, including to vaccinations. Some studies have identified an association between early infection with cytomegalovirus (CMV) and poorer growth and development in HEU children. Furthermore, a Kenyan cohort observed a six-fold increase in odd of CMV infection in HEU versus HUU infants, also indicating that CMV infection may lead to decreased CD8+ T cell activation and impaired growth.

In developing countries, most adult women are CMV-seropositive (>70%), as CMV is generally acquired early in life due to breast milk transmission and crowded living conditions. In Botswana, 96% of adults living with HIV are CMV IgG positive. These high rates of pre-existing CMV immunity result in a low incidence of acute CMV infection during pregnancy and thus low rates of congenital CMV infection in infants. However, some studies suggest that HEU infants are more likely than HUU infants to acquire congenital CMV, due to increased risk of CMV reactivation among pregnant WLHIV. Congenital CMV constitutes the most common cause of non-genetic childhood hearing loss worldwide and is a significant cause of neurodevelopmental delay. HEU infants in developing settings are also at high risk of acquiring CMV in early life, but the impact of early childhood
CMV infection on the growth and neurodevelopmental outcomes in this vulnerable population is unknown.

We sought to determine the association between child CMV infection at 18 months and 2-year growth and neurodevelopmental outcomes among HEU and HUU children who were followed in a prospective observational study in Botswana.

**METHODOLOGY**

**Study Population**

This study used existing samples and data from the “Tshipidi” study\(^29,30\). Tshipidi was a prospective observational cohort study that enrolled WLHIV and HIV-negative women who were pregnant (88%) or at/within 7 days of delivery (12%) between 2010 and 2012, and followed mother-infant pairs for two years after delivery/birth. During the study period, WLHIV in Botswana with CD4 count ≤350 cells/mL or WHO stage 3 or 4 disease were eligible for 3-drug antiretroviral treatment (ART); all others were eligible for prophylaxis with zidovudine (ZDV) during pregnancy and single dose nevirapine (NVP) during labor and delivery. WLHIV chose their infant feeding method following counseling, and were generally encouraged to formula-feed (and provided with free infant formula) according to Botswana government policy at the time. HIV-negative women were encouraged to breastfeed. HIV-1 RNA and CD4 count were measured at enrollment among WLHIV. Tshipidi study eligibility required documentation of HIV DNA PCR results for all HIV-exposed infants at birth, one month, and repeated at six and twelve months if the infant breastfed for any period. HIV testing was performed on all infants at premature discontinuation of study participation or 18 months of age (by enzyme-linked immunosorbent assay ELISA), regardless of maternal HIV or of feeding status. Children living with HIV and their mothers were excluded from this analysis.
**Patient Consent Statement**

Women provided written informed consent for study participation. Evaluation of the role of CMV infection in child outcomes was one of the pre-specified, approved objectives of the Tshipidi study.

The Botswana Health Research Development Committee and the Office of Human Research Administration at Harvard T.H. Chan School of Public Health granted ethics approvals.

**CMV, Anthropometry, and Neurodevelopmental Testing**

All children with at least one available stored plasma sample from the 18-month visit were tested for anti-CMV IgG using the CMV immunoglobulin (IgG) ELISA Kit, Trinity Biotech. The relative sensitivity and specificity of the CMV (IgG) ELISA Kit (Trinity Biotech) that was used to test CMV in infant plasma is 99.2% and 94.1% respectively. Child length, weight, and head circumference were measured by trained study staff in standardized fashion. Length was measured by two individuals with the child in recumbent position, with one person (usually the infant’s mother or caregiver) holding the child’s head at the top of an infant measuring mat and the second individual, always a trained study staff member, ensuring straight alignment of the child with extension of the legs and with heel used for the measurement at the bottom of the mat. Infants were weighed without nappies/diapers. Trained staff measured head circumference using a non-stretch measuring tape, by securely wrapping the tape around the widest possible circumference of the head. Weight-for-age, weight-for-length, length-for-age and head circumference-for-age z-scores were calculated using World Health Organisation (WHO) 2006 Childhood Growth standards, which accounts for a child’s age and sex. Neurodevelopmental assessment was performed using the Bayley Scales of Infant and Toddler Development, Third Edition (BSIDIII). Neurodevelopmental assessments were performed by research nurses who had been trained and monitored by pediatric neuropsychologists. Cognitive, receptive language, expressive language, fine motor, and gross motor domains were assessed. We also
administered the Developmental Milestones Checklist (DMC), a parent report questionnaire of language, motor, and personal/social skills developed for use in sub-Saharan Africa\textsuperscript{32,33}. Details of test adaptation, training of assessors, and monitoring of data quality have been described elsewhere\textsuperscript{29}.

**Statistical analysis**

We used descriptive statistics to summarize the frequency of characteristics according to child CMV serostatus at 18 months of age. We undertook univariable and multivariable logistic regression analyses to evaluate the association between potential predictors and child CMV serostatus at 18 months. Any covariate with $p<0.2$ in univariable analysis was included in final multivariable analysis of predictors of positive child CMV status. The association between child CMV infection status at 18 months of age and anthropometrics at 24 months of age was analyzed using linear regression using growth as a continuous variable, adjusting for covariates selected a priori (maternal education level and infant prematurity), two sample t-tests were also performed by comparing z-scores between CMV-infected and CMV–uninfected children. Neurodevelopmental test scores at 24 months of age for each of the 5 Bayley domains and DMC tests were compared by child CMV serostatus at 18 months of age using two sample t-tests (separately for each domain tested). Because maternal HIV status and infant feeding method were each important potential confounders of the relationship between child CMV status and child growth and neurodevelopmental outcomes, we conducted multivariable analyses of these outcomes adjusted for feeding method and for HIV exposure status separately; these variables could not be included in the same multivariable model due to collinearity. $P$-values $<0.05$ were considered statistically significant. Stata v14.0 was used for all analyses.
RESULTS

Enrollment, testing, and baseline characteristics

A total of 912 mothers and 910 of their live-born children were enrolled in the Tshipidi study: 454 WLHIV with 453 respective children, and 458 HIV-uninfected women with 457 respective children. Plasma samples were available for 317 (35%) children at 18 months (139 HEU and 178 HUU); all of these samples were tested for CMV IgG. The characteristics of infants whose samples were tested for CMV IgG and those that were not are shown in Table S1.

Among the 317 children and their mothers who were included in this analysis, median maternal age was 29 years (interquartile ranges: 25 and 36 years). Among the 139 WLHIV, median CD4 cell count at enrollment was 406 cells/mm$^3$ and median HIV-1 RNA was 3.2 log$_{10}$ copies/ml. Fifty-eight (42%) WLHIV took 3-drug ART during pregnancy, and the remaining 58% received zidovudine prophylaxis. Fourteen (10%) WLHIV opted to breastfeed, compared with 178 (100%) HIV-negative mothers. Median gestational age at delivery was 40 weeks (range 38-41 weeks), and was similar between WLHIV and those without HIV. The only statistically significant finding in Table 1 is that more HUU infants were likely to have CMV IgG tested positive at 18 months after adjusting for child sex, preterm birth and maternal education level.

Prevalence and predictors of positive CMV IgG at 18 months of age

Overall, 215 (68%) children tested positive for CMV IgG at 18 months. A greater proportion of HUU children had positive CMV IgG (83%) than HEU children (47%, p<0.01). Similarly, breastfed children had higher rates of CMV seropositivity (75%) compared with those who did not breastfeed (30%, p<0.01), (Table 1). Among the 14 WLHIV who breastfed, CMV seropositive children were breastfed longer than CMV seronegative children (median 26 weeks vs. 13 weeks, respectively; p = 0.20). A similar trend was observed for HUU children: CMV seropositive children breastfed for a median of 55 weeks vs. 46 weeks for children who were CMV seronegative (p=0.30). Other than breastfeeding
(and negative maternal HIV status, which was highly correlated with breastfeeding), higher maternal education was the only factor associated with positive child CMV IgG in the univariable analysis, although it did not remain a significant predictor in the multivariable model. The additional socioeconomic factors available from the original study dataset included cooking method, availability of electricity, cooking method, source of water; these showed no differences between the comparator groups, this data is not shown. Among HEU children, maternal HIV-1 RNA, CD4 cell count, and ARV regimen during pregnancy were not associated with child CMV serostatus (Table 2).

**Child CMV infection status and 24-month growth**

Child CMV seropositivity at 18 months was not associated with weight-for-age, length-for-age, or weight-for-length z-scores at 24 months (Table 3). HUU CMV-infected children had lower head circumference-for-age z-scores at 24 months compared with HUU who were CMV-uninfected (p<0.01) (Table 3). This association between child CMV status and head circumference-for-age z-score was not observed in HEU children. The mean head circumference was 48.1cm (95% CI:47.7 - 48.4) and 47.9cm (95% CI: 47.6 – 48.1) for CMV-uninfected and CMV-infected infants, respectively. Adjusting for breastfeeding status in separate analysis (data not shown) did not show any associations between CMV and all anthropometric outcomes.

**Child CMV infection status and 24-month neurodevelopmental outcomes**

Results of child neurodevelopmental testing at 24 months did not differ by CMV serostatus for any of the domains/tests in univariable analyses (Table 4), nor after adjusting for HIV exposure status or (separately) for breastfeeding status (data not shown).
DISCUSSION

Approximately two-thirds of 18-month old children in Botswana had positive CMV serology, with CMV infection strongly associated with breastfeeding. Higher proportions of children born to women without HIV were CMV-seropositive, likely due to much higher rates of breastfeeding in HUU compared with HEU children. Breastfeeding is known to be a common route of postnatal CMV infection, with longer duration of breastfeeding associated with greater risk of CMV acquisition among HEU children (a finding that we confirmed).

We found an association between child CMV infection and smaller head circumference-for-age z-scores at 24 months among HUU, but not HEU children. A similar observation was made in a Zambian cohort which showed that 18-month CMV seropositivity in children was associated with decreased head circumference as well as stunting (length-for-age z-score < -2), in both HEU and HUU children. Furthermore, a Kenyan cohort found a significant negative association between CMV viral load and weight-for-age z-score and head circumference-for-age Z-scores among HEU and HUU children. We did not measure CMV viral loads in infants for this analysis; therefore, we are unable to determine whether active CMV disease contributed to anthropometrics outcomes in our cohort. Other than head circumference, we did not observe differences in anthropometrics through 24 months of age by child CMV infection status.

Several studies have attributed poorer neurodevelopment to CMV infection and HIV exposure in children. We did not observe significant differences in 24-month neurodevelopmental outcomes in CMV seropositive vs. seronegative children. This finding is in contrast to the Zambian
study, which observed decreased psychomotor skills in CMV seropositive HEU, but not HUU, children at 18 months\textsuperscript{15}. Of note, the mental development index did not differ by CMV status. It is possible that this difference in findings could be related to a higher prevalence or degree of CMV viremia in pregnant WLHIV in the Zambian study, due to less ART coverage and hence greater immunosuppression. The Zambian study was conducted from 2005-2009 when ART coverage in the region was lower, does not provide maternal CD4 count, and ART coverage is not described. In our previous analyses of Tshipidi data, neurodevelopmental outcomes were similar between HEU and HUU children, while breastfeeding was associated with better neurodevelopmental outcomes in general\textsuperscript{29}. Furthermore, in the absence of infant CMV infection, transplacentally transferred maternal antibodies are lost by 18 months of age. Hence, the presence of CMV IgG at 18 months of life establishes that the infant is infected with CMV. This benchmark has been used in previous studies. For example, the same study in Zambia tested a total of 460 (57\%) for HCMV antibody at 18 months and assessed development using the mental development index (MDI) and psychomotor development index (PDI)\textsuperscript{15}.

Our study had several limitations. Firstly, a key limitation of this study is potential selection bias due to a relatively small proportion (~1/3) of the original cohort being included in the secondary analysis. There were significant differences in those who were included and excluded, this included breastfeeding and preterm birth status. However, we believe that this paper may be very beneficial as it is the first of its kind in the country. Moreover, we were not able to determine the timing of CMV infection in children, including whether infection was congenital or postpartum. We are also unable to comment on duration of exposure which may contribute to varying results in terms of neurodevelopment. The vast majority of infected children likely acquired CMV after delivery, given that congenital CMV infection is rare in populations with high rates of maternal CMV seropositivity prior to pregnancy, and also given the strong association between CMV and breastfeeding in our study. In addition, our sample size was relatively small and our assessments were only through 24
months of age and may not predict growth or neurodevelopment at older ages. Finally, it was not possible to separate the effects of HIV-exposure from infant feeding status, given that all women without HIV opted to breastfeed while 90% of WLHIV formula-fed their infants.

In summary, we observed a high prevalence of CMV seropositivity among children in Botswana at 18 months, especially in breastfed HUU children. Postpartum CMV infection is unlikely to explain differences in growth or neurodevelopment between HEU and HUU children, especially where exclusive formula feeding from birth is practiced and where WLHIV are receiving ART and/or who do not have advanced HIV disease. Reassuringly, for all children, CMV infection by 18 months had no apparent negative impact on growth or neurodevelopment through 24 months of age.
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Conflicts of interest: None declared

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Table 1: Maternal and child baseline characteristics by child CMV IgG status at 18 months, and factors associated with child CMV seropositivity in univariable and multivariate analyses

| Characteristic                              | Child CMV IgG serostatus at 18 months of age | Univariable analysis | Multivariable Analysis |
|---------------------------------------------|----------------------------------------------|----------------------|------------------------|
|                                             | CMV Positive (n = 215)                       | CMV Negative (n = 102) | OR (95% CI)* P value  |
| All mothers (n = 317)                       |                                              |                      |                        |
| Median maternal age in years (Q1, Q3)       | 29 (25,36)                                   | 31 (26,35)           | 0.99 (0.9 – 1.0) 0.7   |
| Mother HIV positive (n = 139)               | 66 (31%)                                     | 73 (72%)             | 0.2 (0.1 – <0.1) 0.04  |
| Mother Employed (n = 52)                    | 28 (13%)                                     | 39 (38%)             | 1.5 (0.7 – 3.0) 0.3    |
| Median Number of people living in household, median (IQR) | 7 (4.10)                                    | 6(4,9)               | 1.1 (0.98 – 0.2) 0.02  |
| Maternal Education Level (n = 314)          |                                              |                      |                        |
| None or Primary                             | 17 (8%)                                      | 16 (16%)             | 1 (ref)                |
| Secondary                                   | 166 (77%)                                    | 72 (71%)             | 2.2 (1.0 – 4.5) 0.04   |
| Tertiary                                    | 33 (15%)                                     | 13 (13%)             | 2.2 (0.8 – 5.6) 0.1    |
| Child characteristics (n = 317)             |                                              |                      |                        |
| Male                                        | 104 (48%)                                    | 59 (58%)             | 1.5 (0.91 – 2.9) 0.05  |
Median birth weight in kg (Q1, Q3) 3.1 (2.8, 3.4) 3.1 (2.7, 3.5) 1.3 (0.8 - 2.4)
Median birth height in cm (Q1, Q3) 50 (49, 52) 51 (48, 52) 0.1 (0.9 - 1.9)
Median birth head circumference in cm (Q1, Q3) 34 (33, 35) 34 (33, 35) 0.9 (0.8 - 1.3)
Low birthweight (<2.5 kg) 20 (9%) 12 (12%) 0.5 (0.02 - 1.3)
Preterm birth (<37 estimated weeks gestation) 24 (11%) 5 (5%) 2.4 (0.9 - 7.2)
Birth Defects
Yes 3 (1.4%) 0 NA
No 212 (98.6%) 102 (100%) NA
Child Ever Breastfed
Yes 161 (75%) 31 (30%) 1 (ref)
No 54 (25%) 71 (70%) 6.8 (4.1 <0.01
CI, confidence interval; CMV, Cytomegalovirus; HIV, human immunodeficiency virus; IQR, interquartile range; OR, odds ratio; Q1, 25th percentile; Q3, 75th percentile.

* Odds ratio for having characteristic if CMV IgG-positive

a Adjusted for child sex, preterm birth, maternal education level and maternal HIV status. Breastfeeding was not included in the model due to collinearity with maternal HIV status.

b Birth Defects reported: Craniosynostosis (2 infants) and bilateral supranumerary digits (1 infant)
Table 2: Characteristics of mothers living with HIV at enrollment, by child CMV IgG status at 18 months

| Characteristic | Child CMV IgG serostatus at 18 months of age | Univariable analysis |
|---------------|--------------------------------------------|---------------------|
|               | CMV Positive (n= 66) | CMV Negative (n=73) | OR (95% CI)* | P value |
| HIV Exposed Infants (n =139) | | | | |
| Median maternal baseline HIV-1 RNA (log_{10} copies/mL, IQR) , n = 122 | 3.4 (1.6 ,4.2) | 3.2 (1.6 , 4.0) | 1.1 (0.8 - 1.5) | 0.5 |
| Maternal baseline HIV-1 RNA <400cp/ml (n= 46) | 22 (33%) | 24 (33%) | 1.0 (0.99 - 1.0) | 0.4 |
| Maternal baseline CD4 (cells/mm$^3$) Median (Q1, Q3) , n = 139 | 411 (330-546) | 387(324,498) | 1.0 (0.99 - 1.0) | 0.5 |
| Type of prenatal ARVs | | | | |
| ZDV (n =81) | 39 (59%) | 42 (57%) | | |
| 3-drug ART (n = 58) | 27 (41%) | 31 (42%) | 1.1 (0.5 – 2.0) | 0.9 |

ARVs, antiretrovirals; CI, confidence interval; CMV, Cytomegalovirus; ART, antiretroviral therapy; HIV, human immunodeficiency virus; IQR, interquartile range; OR, odds ratio; Q1, 25th percentile; Q3, 75th percentile; ZDV, zidovudine.

* Odds ratio for having characteristic if CMV IgG-positive
Table 3: Effect of Child 18-month CMV serostatus on child anthropometrics at 24 months, overall and stratified by HIV exposure group

| Anthropometric Z scores at 24 months | CMV seropositive; mean (SD) | CMV seronegative; mean (SD) | Regression Coefficient (95% CI) | P value | *Regression Coefficient (95% CI) | *P value |
|--------------------------------------|-----------------------------|----------------------------|--------------------------------|---------|-------------------------------|---------|
| **All Children (n = 317)**           |                             |                             |                                |         |                               |         |
| WAZ                                 | -0.58 (1.1)                 | -0.50 (1.3)                 | 0.004 (-0.03 - 0.03)           | 0.98    |                               |         |
| LAZ                                 | -0.67 (1.4)                 | -0.58 (1.3)                 | -0.02 (-0.05 - 0.00)           | 0.10    |                               |         |
| WLZ                                 | -0.35 (1.1)                 | -0.31 (1.3)                 | 0.003 (-0.002 - 0.008)         | 0.23    |                               |         |
| HCZ                                 | 0.06 (1.2)                  | 0.13 (1.3)                  | -0.01 (-0.05 - 0.03)           | 0.6     |                               |         |
| **HEU (n = 139)**                    |                             |                             |                                |         |                               |         |
| WAZ                                 | -0.65 (1.2)                 | -0.49 (1.4)                 | -0.02 (-0.09 - 0.06)           | 0.66    |                               |         |
| LAZ                                 | -0.74 (1.4)                 | -0.45 (1.4)                 | -0.07 (-0.14 - 0.001)          | 0.05    |                               |         |
| WLZ                                 | -0.40 (1.1)                 | -0.39 (1.3)                 | 0.04 (-0.04 - 0.12)            | 0.31    |                               |         |
| HCZ                                 | 0.16 (1.2)                  | -0.08 (1.4)                 | 0.03 (-0.04 - 0.10)            | 0.38    |                               |         |
| **HUU (n = 178)**                    |                             |                             |                                |         |                               |         |
| WAZ                                 | -0.6 (1.1)                  | 0.5 (1.1)                   | -0.004 (-0.03 - 0.02)          | 0.75    |                               |         |
| LAZ                                 | -0.65 (1.4)                 | -0.85 (1.2)                 | -0.008 (-0.03 - 0.01)          | 0.46    |                               |         |
| WLZ                                 | -0.33 (1.1)                 | -0.17 (1.3)                 | 0.001 (-0.003 - 0.006)         | 0.53    |                               |         |
| HCZ                                 | 0.03 (1.2)                  | 0.73 (0.9)                  | 0.07 (-0.11 - -0.02)           | <0.0    | 0.32(-0.17 - 0.0)             | 2.00    |

CI, confidence interval; CMV, cytomegalovirus; HCZ, head circumference-for-age z-score; HEU, HIV-exposed uninfected infants; HUU, HIV unexposed uninfected infants; LAZ, length-for-age z-score; SD, standard deviation; WAZ, weight-for-age z-score; WLZ, weight-for-length z-score

* Adjusted for maternal education level, infant prematurity
Table 4: Neurodevelopmental test scores at 24 months by child CMV serostatus at 18 months

| BAYLEY III DOMAIN         | CMV Positive (n = 215) | CMV Negative (n = 102) | Unadjusted mean difference (95% CI) | P value |
|---------------------------|------------------------|------------------------|------------------------------------|---------|
| **Cognitive:**            |                        |                        |                                    |         |
|                           | 276                    | 53.1 (3.3)             | 52.9 (3.2)                         | -0.2 (-1.0 to 0.6) | 0.6     |
| **Gross Motor**           | 263                    | 52.8 (2.6)             | 52.9 (2.5)                         | 0.1 (-0.6 to 0.7) | 0.8     |
| **Fine Motor**            | 279                    | 37.2 (1.6)             | 37.2 (1.5)                         | 0.03 (-0.4 to 0.4) | 0.9     |
| **Receptive Language**    | 274                    | 20.6 (3.2)             | 20.5 (2.9)                         | -0.1 (-0.9 to 0.7) | 0.8     |
| **Expressive Language**   | 273                    | 25.2 (4.2)             | 25.1 (4.3)                         | -0.1 (-1.1 to 0.9) | 0.8     |
| **DMC DOMAIN**            |                        |                        |                                    |         |
| **Locomotor**             | 310                    | 32.1 (2.0)             | 32.3 (1.4)                         | 0.1 (-0.3 to 0.6) | 0.5     |
| **Fine Motor**            | 307                    | 19.5 (1.9)             | 19.4 (2.0)                         | -0.1 (-0.6 to 0.3) | 0.5     |
| **Language**              | 310                    | 16.3 (2.7)             | 16.3 (2.2)                         | 0.04 (-0.6 to 0.6) | 0.9     |
| **Personal-Social**       | 310                    | 44.7 (3.9)             | 44.9 (2.7)                         | 0.2 (-0.6 to 1.1) | 0.6     |

CI, confidence interval; CMV, Cytomegalovirus; DMC, Development Milestones Checklist; SD, standard deviation

Mean scores were compared using paired student t-test