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Haematological and biochemical reference values of Gambian infants

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Abstract

OBJECTIVE To establish haematological and biological reference values for Gambian infants.
METHODS Basic haematological and biochemical indices were analysed in blood samples obtained from healthy infants from Sukuta in the Western Division of The Gambia. The 2.5 and the 97.5 centiles for these indices were estimated.
RESULTS Reference ranges for haematological and biochemical indices were determined. Haemoglobin, total white cell count (WBC) and platelet levels decreased with age ($P < 0.001$), whereas most of the white cell count subsets except monocytes did not vary with age. Potassium and alkaline phosphatase fell significantly with increasing age ($P < 0.001$; $P < 0.001$), whereas urea and creatinine rose with increasing age ($P = 0.002$; $P < 0.001$, respectively).
CONCLUSION Our set of haematological and biochemical reference values for healthy infants in The Gambia differs from values in other settings, thus underscoring the importance of establishing region-specific paediatric reference ranges to ensure optimal patient management and evaluate the impact of interventions in clinical research.

keywords reference values, haematological, biochemical, Gambian infants

Introduction

Haematological and biochemical indices are important health indicators widely used in clinical practice. Accurate and appropriate reference ranges are crucial for the interpretation of the normalcy of results. Moreover, these reference ranges are also useful in determining safety of investigational products in intervention trials. Indeed, increasing numbers of clinical trials of vaccines against infectious diseases are being conducted in African infants for which determinants of safety will include the degree of deviation of the haematological and biochemical indices from the reference ranges following vaccination. Unfortunately, the reference ranges of haematological and biochemical indices used for infants in many African countries including the Gambia are those obtained from industrialised countries (Tugume *et al.* 1995; Karita *et al.* 2009). Given that these laboratory parameters might be influenced by differences in genetic, environmental, dietary and social factors (Saxena & Wong 1990; Bain 1996; Taylor *et al.* 1997; Quinto *et al.* 2006; Adetifa *et al.* 2009) using reference values derived from another population might lead to a wrong interpretation, which may influence the outcome. For instance, haematological and biochemical parameters of children residing in areas where infectious diseases and parasitic infestations are prevalent, and those living in high altitudes, are likely to be significantly different from those living in other areas (Onwukeme & Olomu 1991; Bain 1996; Evans *et al.* 1999). In the Gambia, the prevalence of haemoglobinopathies, helminthic and HIV infections is low (Facer & Brown 1979; UNAIDS 2008). However, malaria is endemic but transmission is seasonal (Ceesay *et al.* 2008).

A few studies designed to determine reference ranges in African populations have focused mainly on adults and children older than 1 year (Quinto *et al.* 2006; Adetifa *et al.* 2009). There is a paucity of reference range information in infancy and early childhood, a period characterised by rapid physical growth and high dietary requirements that could impact on the blood indices. These early periods of life require specific haematological consideration for several reasons (Odunukwe & Akanmu 2004; Lewis 2006). First, enzyme activities and foetal haemoglobin (HbF) content of the circulating red blood cells in the first months of life are different from that in later life, and the responsiveness of the haemopoietic

system of the newborns and infants is not the same (Buseri & Jeremiah 2010). Second, like all other systems, the haematopoietic system of the infant and the child undergoes development modification and growth; this is adapted to meet the requirements of these phases of life (Lewis 2006; Buseri & Jeremiah 2010). Third, all human bones at birth through to adolescence contain haemopoietic marrow which is subsequently replaced with fatty marrow with increasing age (Odunukwe & Akanmu 2004; Buseri & Jeremiah 2010). Haematological parameters may be influenced by these resulting in different reference ranges for different age groups (Miller 1995). Thus, knowledge of normal reference intervals for routine laboratory tests during this dynamic period of growth and development in infancy and early childhood is a requirement for correct interpretation of these tests. We therefore aimed to determine the normal reference intervals for common laboratory tests in Gambian infants aged 3–10 months.

Materials and methods

Study population

This survey took place at the Sukuta Health Centre, a peri-urban settlement in the western region of The Gambia between 2006 and 2010. The Gambia is on an altitude of about 33 m above sea level (Central Statistics Department TG 2008). Briefly, mothers who brought their infants for regular Expanded Programme on Immunization (EPI) vaccines at the Infant Welfare Clinic of Sukuta Health Centre were invited to participate in the study. At screening, apparently healthy children with no clinically significant acute or chronic illness (as determined by history and full physical examination conducted by two medical doctors: AAO and MOA) were enrolled into the study after written consent was obtained. None of these children presented with clinical malaria or diarrhoea during the visit or the week before the visit. Blood samples were obtained for haematology and biochemistry. The samples were also checked for malaria parasites.

Anthropometry

Weight was measured using a digital weighing scale (Soehnle-Waagen GmbH & Co.KG) and assessed to the nearest 100 g and length to the nearest millimetres with a Harpenden Infantometer (Chasmors, UK). Underweight was defined as weight for age below minus two standard deviations from median of reference population, stunting as height for age below minus two standard deviations from median of reference population, wasting as weight for height below minus two standard deviations from median of reference population. The nutritional status was determined using the Nutchildren nutrition software [EPI INFO version 3.4; Centres for Diseases Control and Prevention (CDC), Atlanta, GA, USA](Centres for Diseases Control & Prevention (CDC)) from which weight-for-age (WAZ), height-for-age (HAZ) and weight-for-length (WHZ) *Z*-scores were generated. WAZ, HAZ and WHZ *Z*-scores < -2 were considered as underweight, stunted and wasting, respectively (WHO 1995).

Venepuncture

Venepuncture was performed with the study child sitting on the mother's lap. Using a 21G hypodermic needle, 500 μL of blood was collected into a microtainer® (Becton, Dickinson (BD), Oxford, England) tube containing dipotassium ethylenediaminetetraacetic acid (EDTA), and 1 ml was collected into a BD Vacutainer®(BD) containing lithium heparin. The samples were mixed thoroughly by gently inverting them eight times and transported to Medical Research Council Unit (MRC) clinical laboratories within 2 h of collection.

Laboratory tests

Haematological indices were measured in the routine laboratory using the CELL-DYN 3700 sample loader (ABBOTT, USA) according to the manufacturer's instructions. The system performs a complete automated blood cell analysis and measures up to 23 parameters in whole blood. This study measured haemoglobin (g/dl), red blood cell count ($\times 10^{12}$/l), total and differential white blood cell count ($\times 10^{9}$/l), platelets ($\times 10^{9}$/l) and mean corpuscular volume (fl).

Biochemical indices of the blood were measured using the VITROS 350 Analyzer (Ortho Clinical Diagnostics, USA). This is a dry versatile chemistry system that uses VITROS MicroSlide technology to perform a number of discrete tests on serum or plasma samples. A daily quality control is performed on the equipment to ensure that intraday variability is minimised and results generated are correct.

All procedures were performed according to good clinical laboratory practice (GCLP) using appropriate working instructions and standard operating procedures by trained laboratory staff of the Medical Research Council Unit, The Gambia.

Ethical approval for the study was given by the Gambia Government/Medical Research Council Joint Ethics Committee.

Statistical methods

Data were double-entered into a Microsoft ACCESS database and validated. Anthropometric measurements were processed using the Nutchildren nutrition software [EPI INFO version 3.4; Centres for Diseases Control and Prevention (CDC), Atlanta, GA, USA] to generate weight-for-age (WAZ), height-for-age (HAZ) and weight-for-height (WHZ) *Z*-scores.

We estimated the unconditional size 95% reference intervals (USRI) using mixed effects normal linear regressions, with unstructured variance–covariance matrix structure, to account for within subject correlation due to repeated sampling over time and allow for separate intercepts and slopes. The target age group for most vaccine trials is infants, which coincides with a time of rapid haematological and biochemical change; hence, we classified our study participants into 2–5 months group and 6–10 months group, to assess for differences in the two age groups.

Transformations of outcomes and age. Both the outcome variables and age were transformed (Pan & Goldstein 1998) before fitting the models to improve the normality of residuals and linearity of their associations, respectively. The summary of transformation powers are presented in Table 1 below.

If outcome values cannot be zero, such variables were transformed using powers estimated from Box–Cox regression conditional on cubic polynomial function of age while controlling for sex, nutritional status *Z*-scores and dummy variables representing all the subjects.

Square root and shifted logarithm transformations were considered if the outcome values can be zero like monocytes, basophils and eosinophils. Depending on the preferred power of transformation, the following function was used:

$$T(\text{outcomes}) = \begin{cases} (\text{outcome})^{p} \text{if outcome cannot be 0 and} \, p \neq 0 \\ \log_e (\text{outcomes}) \text{ if outcome cannot be 0 and} \, p = 0 \\ \log_e (K + \text{outcome}) \text{ OR} \\ \text{squareroot (outcome) if outcome can be a zero} \end{cases}$$

where

- T(outcome) represents the transformed outcome values
- P is the power of transformation
- K is a constant value added to the outcome values to make them >0.
- $\log_e$ is the natural logarithm (base = 2.7182818)

Fractional polynomials were applied to estimate the suitable powers for the linearising function of age applied on a normal regression of each transformed outcome conditional on age while controlling for sex, nutritional status *Z*-scores and dummy variables representing all the subjects (fixed effect model). Models with best powers of age were selected using likelihood ratio tests (LRT) (StataCorp 2011). Second-degree polynomial transformations were applied if statistically significantly better than first-degree polynomials. Further, other first-degree polynomials were used only if significantly better than 1 (no transformation).

Using the best powers from the fractional polynomial procedure, new variables (T) were generated from the age linearising functions (Royston 1995) as follows:

$$\text{Fractional polynomial with 1 degree: } T = (\text{Age})^{p} \quad (1)$$

Fractional polynomial with 2 degrees: $T =$

$$\begin{cases} (\text{Age})^{p1} + \left(\frac{\beta 2}{\beta 1}\right)(\text{Age})^{p2} \text{If} \, p1 \neq p2 \\ (\text{Age})^{p1}\left(1 + \left(\frac{\beta 2}{\beta 1}\right) \text{In (Age)}\right) \text{If} \, p1 = p2 \end{cases} \quad (2)$$

Table 1 Transformation powers and coefficients

Power transformations				
Outcome		Age function		
Values	Power	Power	Coefficient1	Coefficient2
Alanine transaminase	0.5	1	−0.060	na
Aspartate transaminase	0.5	1	0.032	na
Alkaline phosphatase	1	−1	314.972	na
Potassium	ln	1	−0.012	na
Chloride	−2.8	−2	0.000	na
Urea	ln	−2 −2	5.665	−6.393
Creatinine	ln	3 3	−0.004	0.002
Bilirubin	0.3	−1 −1	1.676	−2.277
Haemoglobin	1.3	1	−0.269	na
WBC	0.4	−2	−0.896	na
MCV	3.3	−2	7237231.484	na
Monocytes	0.5	1	0.017	na

$$(\text{Age})^p = \text{In}\,(\text{Age}) \text{ if } p = 0 \quad (3)$$

where

- p and ($p1$, $p2$) are the best estimates of the powers of age from fractional polynomial regression with degrees one and two, respectively.
- β and ($\beta 1$, $\beta 2$) are the estimated coefficients of age functions in the fractional polynomial regression.

Then, associations of each outcome with age function (T), sex and nutritional status indicator variables were mutually assessed by Wald tests after mixed effects linear regression analyses.

Exclusion criteria from analysis. Severely malnourished children (nutritional status Z-score< -3) were dropped from the entire analysis. Extreme outliers (three times the IQR below the 25th percentile or above the 75th percentile) were recorded as missing values and excluded from a particular analysis without dropping the entire record. Further outliers were identified and dropped from a particular model if their standardised residuals (lowest level) were less than -3 or >3.

Estimation of USRI. The 95% reference intervals were estimated as mean ± 1.96*standard deviation. The mean function was estimated from the best linear unbiased predictions (BLUPs) fitted values after each mixed effect model, a combination of the fixed effect and random effect components.

Standard deviations were estimated using the following function (Royston 1995)

$$\text{SD} = \sqrt{\left\{\sigma_\mu^2 + T^2 * \sigma_\beta^2 + 2 * T * \sigma_{\mu\beta} + \sigma_\varepsilon^2\right\}}$$

where

- SD = standard deviation
- T = transformed function of age
- σ_μ^2 = variance for the random intercept component
- σ_β^2 = variance for the random coefficient component
- $\sigma_{\mu\beta}$ = covariance between the random intercept and coefficient components
- σ_ϵ^2 variance of the fixed component of the model

The estimated reference intervals were then back-transformed to the original scale of the outcome accordingly.

Results

Participant characteristics

A total of 717 blood samples were processed, of which 21 were excluded from analysis because the children were severely malnourished (Z-scores ≤ -3). Ten of these samples belonged to infants that were in the 2–5 months group, and 11 were in the 6–10 months group. Of the 675 samples analysed, 362 (52.0%) were from boys. The characteristics of the study participants are shown in Table 2. As explained above, for haemoglobin, platelets and MCV, 9, 3 and 5 observations, respectively, were considered as outliers in the 2–5 months group and 4, 6 and 7 observations, respectively, in the 6–10 months group. Similarly, for alanine transaminase, potassium and urea, 4, 3 and eight observations, respectively, were considered as outliers in the 2–5 months group and 5, 2 and 6 observations, respectively, in the 6–18 months group. These outliers were excluded from further analysis to determine reference intervals.

Twenty (2.9%) participants were underweight, 27 (3.9%) were stunted and 17 (2.5%) were wasted using the Nutchildren nutrition software [EPI INFO version 3.4; Centres for Diseases Control and Prevention (CDC), Atlanta, GA, USA], but they were not excluded from the analysis as there were no significant differences between their values and those of the apparently normal infants. There were 458 (65.8%) participants in the 2–5 months group and 238 (34.2%) in the 6–10 months group. There was no significant sex difference between the number of infants 2–5 months group and the 6–10 months group (boys, 51.8% *vs.* 48.7%; $P = 0.562$).

Table 2 Characteristics of the study participants

	n (%)
Sex	
Male	362 (52.0)
Females	334 (48.0)
Age (months)	
Median (IQR)	4.39 (3.1–8.9)
2–5 months	458 (65.8)
6–10 months	238 (34.2)
Weight (Kg)	
Median (IQR)	7.2 (6.4, 8.1)
Length (cm)	
Median (IQR)	63.0 (60.5, 68.0)
Weight-for-age *z*-score	
Normal	671 (97.1)
Underweight	20 (2.9)
Height-for-age *z*-score	
Normal	662 (96.1)
Stunted	27 (3.9)
Weight-for-height *z*-score	
Normal	671 (97.5)
Wasted	17 (2.5)

Haematological indices

The estimates of 2.5 and 97.5 centiles for infants from 2 to 10 months of age are displayed to show the trend (Figure 1). The estimated intervals for the haematological indices varied with age (Table 3a). Haemoglobin, white cell count (WBC), mean corpuscular volume (MCV) and monocytes had statistically significant differences with age ($P < 0.001$; $P < 0.001$; $P < 0.001$; $P = 0.028$), while only haemoglobin and MCV showed differences with sex ($P = 0.002$; $P < 0.001$). Haemoglobin (Hb) and MCV values declined significantly with increasing age. Total white cell count (WBC) varied with age ($P < 0.001$), but most of the white cell subsets did not. In both age groups, MCV levels were higher in girls than boys ($P < 0.001$). By contrast, there was no significant difference in total white cell count or platelets by sex. Children who were wasted had lower values of total white count than those who were not ($P = 0.015$). The haematological parameters of the infants in this study tended to be lower than those of Caucasian counterparts except for the values for lymphocytes (Table 3) (El-Hazmi & Warsy 2001; Lewis 2006).

Biochemical indices

The estimated intervals of the biochemical indices are shown in Table 3b. Potassium, alanine transaminase (ALT), bilirubin and alkaline phosphatase (ALP) decreased significantly with increasing age ($P < 0.001$). In contrast, urea, chloride, creatinine and AST increased with age ($P = 0.002$; $P < 0.001$, $P < 0.001$, $P < 0.002$, respectively; Figure 2). There is no sex disparity in the levels of urea, creatinine, potassium, AST, ALT or bilirubin. Boys had a higher level of ALP than girls ($P < 0.001$), while girls had a higher level of chloride than boys ($P = 0.002$). Children who were wasted had lower values of potassium than those who were not ($P = 0.001$). Similarly, children who were wasted, stunted or underweight had lower levels of creatinine than those who were not ($P = 0.006$; $P = 0.002$; $P = 0.005$).

All other biochemical and haematological parameters were similar in children who were underweight or stunted compared to those with normal weight or length (data not shown).

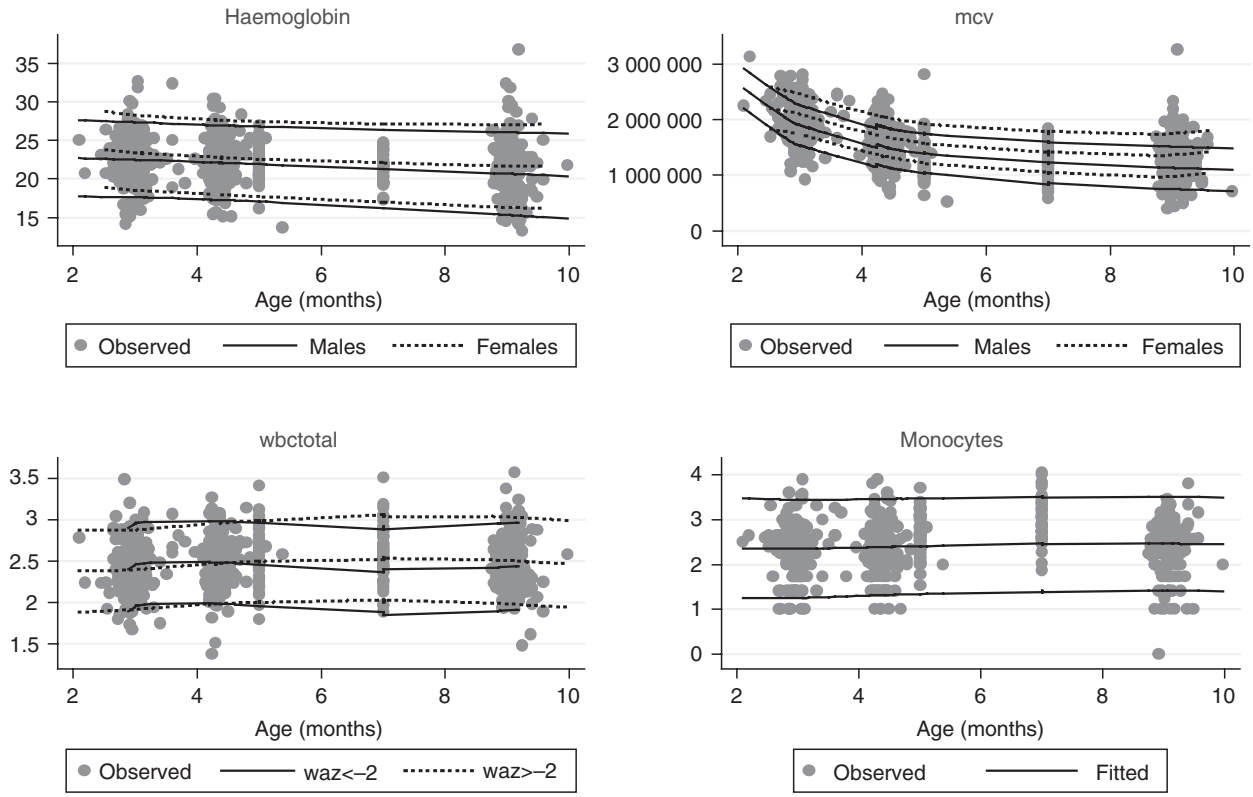

Figure 1 Reference ranges for major haematological indices according to age. The central 95% of the values obtained from infants across the ages are displayed for haemoglobin, white cell count, mcv and monocytes.

Table 3 Reference ranges of the (a) haematological and (b) biochemical parameters of the participants by age, sex and nutritional status and comparison of reference values in Gambian and Caucasian infants

(a)

Parameter (units)	Age		Western children*
	2–5 months	6–10 months	Infants
Haemoglobin (g/dl)			9.0–14.0
Male	9.0–12.7	8.2–12.3	
Female	9.3–12.9	8.6–12.6	
WBC ($\times 10^9$/L)			5.0–19.5
Normal	5.6–15.4	4.8–14.6	
Wasted	5.4–14.7	5.7–16.3	
MCV (fl)			70.0–86.0
Male	70.2–80.6	59.8–74.6	
Female	73.4–83.4	64.4–77.4	
Platelets ($\times 10^9$/L)	135.5–708.9		84.0–478.0
Neutrophils (%)	10.9–61.0		54.0–62.0
Lymphocytes (%)	33.6–82.5		25.0–33.0
Monocytes (%)	1.7–11.9	2.1–12.4	3.0–7.0

(b)

Parameter (units)	Age		Western children
	2–5 months	6–18 months	Infants
Potassium (mM)			3.5–6.0
Normal	3.9–5.3	3.4–5.1	
Wasted	4.0–5.4	3.5–5.3	
Chloride (mM)			98.0–106.0
Male	99.5–109.6	98.4–109.2	
Female	100.2–110.6	99.1–110.2	
Urea (mM)	0.8–2.5	0.9–2.4	1.8–6.4
Creatinine (μM)			18.0–35.0
Normal	13.3–33.2	14.9–29.7	
Wasted	13.1–32.6	17.3–30.9	
AST (U/L)	27.6–139.1	30.8–137.6	35.0–140.0
ALT (U/L)	7.8–35.3	7.5–29.6	5.0–45.0
ALP (mM)			145.0–420.0
Male	203.3–466.4	185.1–388.1	
Female	173.6–437.8	160.0–362.0	
Bilirubin (mM)	2.0–9.4	1.9–8.8	0.2–1.0

*Kliegman *et al.* (2004), Glader (2004).

Models of other variables did not show significant deviance from linear models. Table 3 shows the estimated reference intervals for the haematological and biochemical indices in Gambian infants.

Discussion

We have established a set of haematological and biochemical reference values for a healthy population of very young children in western Gambia. The values of the haematological parameters are lower than those for Caucasians of the same age group, a finding consistent with other African studies (Lugada *et al.* 2004; Quinto *et al.* 2006; Kibaya *et al.* 2008; Karita *et al.* 2009). Furthermore, they are similar to the values reported among Tanzanian children of the same age group, but lower than those of Nigerian children (Buchanan *et al.* 2010; Buseri & Jeremiah 2010). These differences may be due to diversity in the types of laboratory methods used. It is striking that Tanzanian infants had similar haemoglobin levels as Gambian infants despite wide differences in altitude in the two countries and also different methods of analysis used in the two studies. The total WBC in our study varied with age, which is consistent with reports from other developing countries (Lugada *et al.* 2004; Adetifa *et al.* 2009). Nigerian infants of similar age group had lower WBC than the infants in this study, while levels among Caucasian infants are higher (Onwukeme & Olomu 1991; Kliegman *et al.* 2004). On the other hand, all the white cell subsets except monocytes did not vary with age, a finding which is in contrast to what was reported among Nigerian and Tanzanian children (Buchanan *et al.* 2010; Buseri & Jeremiah 2010). The percentages of neutrophils among the Gambian infants are similar to those of Western infants except that the Gambian values extended to much lower values (Kliegman *et al.* 2004). This may be due to higher exposure to infections in Gambian infants.

Haemoglobin levels are generally higher at birth and progressively decline with age, a phenomenon known as physiologic anaemia (Glader 2004). A number of factors, including a developmental switch from foetal to adult haemoglobin synthesis, increase in body size and reduced folic acid and iron in the diet contribute to this decline in haemoglobin with age (Glader 2004). That Nigerian infants have higher haemoglobin and lower WBC values may be explained by the difference in the manual methodology used in their study compared with the automated machine in ours (Buseri & Jeremiah 2010). On the other hand, this difference may be real, underpinning the importance of generating local reference ranges. Children with haemoglobinopathy, especially sickle cell anaemia, have lower levels of haemoglobin than those without. The prevalence of sickle cell anaemia in the Gambia is low but higher in Nigeria (Bain 2006; McLean *et al.* 2009).

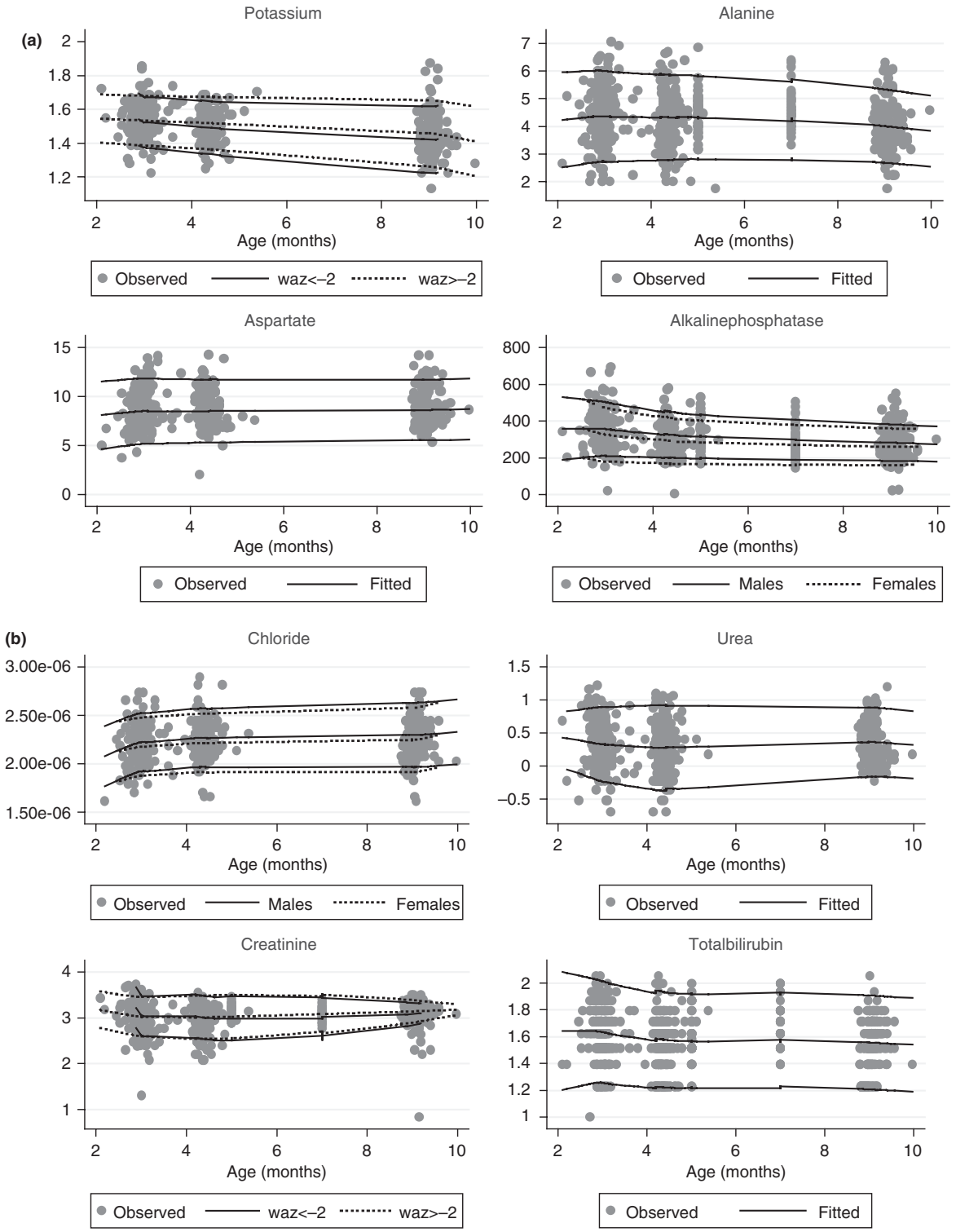

Figure 2 Reference ranges for major biochemical indices according to age. The central 95% of the values obtained from infants across the ages are displayed for (a) potassium, ALT, AST and ALP; (b) chloride, urea, creatinine and bilirubin.

The platelet counts in our study were lower than those reported among Western populations, and accordingly the reference ranges extended to much lower values among our study infants. Unlike other similar studies in African population (Adetifa *et al.* 2009) which reported a decrease in platelet count with increasing age, we did not observe this trend in our study population. This may be explained by the difference in ages of the population studied; others studied children 1 year of age and older with wider variance in age, whereas we evaluated only children up to 10 months old.

Previous studies have shown that genetic factors contribute to the majority of the variance in haematological parameters (Dal Colletto *et al.* 1993; Evans *et al.* 1999). The differences between our findings and those reported from another West African country underscore the evidence that laboratory reference values from one population should not be applied universally, even when countries share similar characteristics or geographical conditions.

Sex differences described in haemoglobin levels in older children (Taylor *et al.* 1997; Adetifa *et al.* 2009; Buseri & Jeremiah 2010) were also noted in our study on a younger population. The reason for this is not clear although early life differences in sex hormone levels may play a role (Andersson *et al.* 1998). Apart from haemoglobin concentration, our study did not find any sex differences for the total WBC and the white cell subsets. Furthermore, similar platelet levels by sex have been reported in other studies (Taylor *et al.* 1997; Odunukwe & Akanmu 2004), comparable to what we found.

In our study, we found no difference in the haemoglobin and MCV of children who were underweight or wasted and those who were not. This is similar to findings reported among older children and adults in the Gambia (Adetifa *et al.* 2009). However, infants who were wasted had lower WBC compared with those who were not, albeit not to significant levels. Deficiency of trace elements is commonly seen in malnourished infants and this is closely associated with thymic hypofunction which invariably contributes to low WBC (Mocchegiani *et al.* 1989; Rink & Gabriel 2000).

The values of the biochemical indices in our study are similar to those obtained in Western populations. We did not observe sex disparity in the levels of potassium, urea, creatinine, AST and ALT. These findings contrast with some studies where sex differences were observed for creatinine values, although these included older children whose hormonal changes might influence these indices (Erasmus *et al.* 1997; Quinto *et al.* 2006). Furthermore, the high AST levels in younger infants in our study have been reported in other studies (Bugeac *et al.* 2007).

Our data underpin the importance of using locally derived reference values to determine wellness and normality in every setting (Kibaya *et al.* 2008; Karita *et al.* 2009). With increase in the number of clinical trials related to infectious diseases being undertaken in sub-Saharan Africa, ensuring that adverse events due to laboratory abnormalities are correctly reported is important. Given the differences observed, the use of externally derived reference values has implications such as inappropriate interventions that could be fatal, the exclusion of many otherwise healthy infants from participating in studies and misinterpretation of the safety of an intervention. This non-inclusion of otherwise healthy individuals translates into unnecessarily increased time and cost, but more importantly, it prevents healthy individuals from participating in clinical trials and ultimately makes trial results less generalisable to the healthy population being studied (Kibaya *et al.* 2008).

Although we made effort to collect relevant health data from all participants and carried out necessary physical examinations and anthropometric measurements, it was not possible to screen for all medical conditions that might have influenced haematological and biochemical parameters.

In conclusion, this study has provided a set of haematological and biochemical reference values for healthy infants in the Gambia. The differences with values in other settings underscore the importance of needs-driven and evidence-based region-specific paediatric reference ranges that would enhance optimal patient management and evaluating the impact of interventions in clinical research.

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