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# Biochemical and Hematologic Parameters for Children in the Middle Belt of Ghana

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*Abstract.* Reference values derived from developed countries are used in many countries in Africa for interpretation of laboratory results obtained during routine healthcare and clinical trials. Use of locally derived reference values has been recommended. The purpose of the study was to establish age- and sex-specific reference values for children in the middle belt of Ghana. Reference values were determined for 21 biochemical and 18 hematologic parameters by using Clinical and Laboratory Standards Institute C28-A3 guidelines in a sample of 1,442 healthy children. Hemoglobin, hematocrit, mean cell volume, erythrocytes, urea, and creatinine were lower when compared with values from northern countries but alanine aminotransferase, aspartate aminotransferase, and total bilirubin were higher. A panel of locally relevant age- and sex-specific reference values was established for commonly used biochemical and hematologic tests in children in the middle part of Ghana. This will help in interpretation of laboratory results for clinical management of patients, screening, and safety monitoring during clinical trials.

## INTRODUCTION

Locally derived biochemical and hematological reference values are essential for assessing disease and monitoring the effects of therapy during routine healthcare practice and clinical trials. It has been recommended by the Clinical and Laboratory Standards Institute (CLSI)[1] and the International Federation for Clinical Chemistry[2] that each laboratory establishes reference values appropriate for the population it intends to serve. However, biochemical and hematologic reference values used in many countries in Africa were established by using data from populations in the industrialized countries[3–5] because the process of establishing reliable local reference values is expensive and time-consuming.[1] Published literature has confirmed differences between reference values obtained for adults from the industrialized countries and those from countries in Africa.[3,6–13] Furthermore, differences also exist between reference values established in different countries in Africa.[6,8,9,14,15]

There is scarcity of comprehensive reference values for children in Africa. Values from textbooks, instrument manuals and reagent inserts that have been derived from Caucasians in industrialized countries are often used to interpret laboratory results in settings in Africa.[3,16] In some instances, biochemical and hematologic results for children are interpreted by using values established with adult populations.[17] It is, however, important to emphasize that children are not small adults and reference values derived with adult populations may not be suitable for children. Also, children are constantly changing and developing and therefore, single reference values may not be appropriate for children of all ages.[17] It therefore necessitates establishing age-specific reference values to aid appropriate interpretation of biochemical and hematologic results of children. The purpose of this study was to establish a comprehensive, age-specific reference values for biochemical and hematological tests for healthy children in the Kintampo North Municipality and Kintampo South District, both located in the middle part of Ghana.

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## METHODS

**Study site.** This cross-sectional study was conducted during September 2009–December 2010 in the Kintampo North Municipality and Kintampo South District of the Brong Ahafo Region of Ghana. The studied area is located between 7°43′N and 8°44′N and 1°25′W and 2°1′W. It lies within the forest-savannah transitional ecological zone and has an elevation ranging between 60 and 150 meters above sea level. The Kintampo Health Research Center maintains a Health and Demographic Surveillance System (HDSS) that records detailed demographics of all residents, including pregnancies, births, deaths, and migrations (in and out) at four-month intervals.[18] The HDSS is made up of a resident population of approximately 140,000 persons. All houses have been digitized to make selection and tracing of persons to their homes easy.

**Reference population.** Study communities and children from birth to 17 years of age were randomly selected from the HDSS human population by using the Visual FoxPro software. Community meetings were held to explain the objectives of the study to the community leaders and other community members.

The methods used for this study has been described.[6] In brief, persons selected through randomization were invited to a central location where individual consenting, screening, and blood collections were conducted. Inclusion into the study was based on willingness of the child and the parent/caregiver to participate in the study, demonstrated by the completion and signing/thumb printing of the consent form and willingness to provide the samples required; general good health, as determined by a clinician using medical history and physical examination; and residence in the study area (as defined by the rules and guidelines for the Demographic Surveillance System). Children with evidence of acute or chronic respiratory, cardiovascular, gastrointestinal, hepatic, or genitourinary conditions; history of blood donation/transfusion within three months preceding the survey; hospitalization within a month preceding the survey; or any other findings that in the opinion of the examining clinician may compromise the assessment of the laboratory parameters of interest in this study were excluded. Adolescent females assessed to be pregnant (either clinically or by positive urine test result: human chorionic gonadotrophic hormone test) or lactating were excluded.

**Blood collection.** A maximum of 5 mL of venous blood samples were collected from the antecubital fossa by using aseptic methods, and dispensed into $K_3$EDTA, serum separator tubes with gel, and fluoride-EDTA tubes for hematology, biochemistry and glucose analysis, respectively. Sample tubes were obtained from Becton Dickinson (Plymouth, United Kingdom). Blood samples collected in $K_3$EDTA and fluoride-EDTA were stored and transported in cold styrofoam boxes and those collected in serum separator tubes were stored in styrofoam boxes without cold packs but covered to protect samples from heat and sunlight.

**Hematologic analysis.** Hemoglobin, hematocrit, red blood cell count (RBC), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration, red blood cell distribution width, platelets, platelet distribution width, total white blood cell count (WBC), lymphocytes, monocytes, and granulocytes were measured by using validated ABX Micros 60 Hematology Analyzers (Horiba-ABX, Montpellier, France). Reagents, calibrators, and controls were obtained from the instrument manufacturer. Analysis of samples was performed within 8 hours of blood draw.

**Biochemical analysis.** Blood samples for biochemical analysis were allowed to clot for at least 60 minutes, centrifuged, and serum was collected. Serum was analyzed within 24 hours after collection. If testing was delayed, serum was stored frozen at −80°C and subjected to a single freeze–thaw cycle at the time of analysis. Eighteen tests (alanine aminotransferase [ALT], aspartate aminotransferase [AST], amylase, creatine kinase, γ-glutamyltransferase [GGT], lactate dehydrogenase [LDH], total protein, albumin, total and direct bilirubin, cholesterol, glucose, iron, triglycerides, urea, creatinine, uric acid, and phosphorus) were measured by using the Vitalab Selectra E Clinical Chemistry Analyzer (Vital Scientific, Dieren, The Netherlands). Test tubes were also obtained from Vital Scientific, The Netherlands. Reagents, calibrators, and controls were obtained from Elitech Diagnostics (Sees, France). Electrolytes (chloride, potassium, and sodium) were analyzed by using the Humalyte Electrolyte Analyzer (Human Diagnostics, Wirsbaden, Germany). Reagents were obtained from the manufacturer of the instrument. The method used for each test is shown in Table 1.

**Quality control.** Normal and abnormal controls were run daily. No analysis was performed if controls were out of range. In addition to the internal quality assessment, the laboratory participates in external quality assessments for hematology and clinical chemistry with the College of American Pathologists and the United Kingdom National External Quality Assessment Scheme. The laboratory complies with the principles of Good Clinical Laboratory Practice.[19,20] Children with abnormal clinical or laboratory test results were referred for appropriate care and treatment.

**Data management and statistical analysis.** Data were recorded on questionnaires, double-entered into a Visual FoxPro 9.0 database, and verified. Children were grouped by ages: 0.5–4 years, 5–12 years, and 13–17 years. The last age group (which represented the pubertal period) was further grouped by sex because of sex-related differences during this period.[14,21] Data analysis was carried out by using Stata 11 (StataCorp LP, College Station, TX). The 2.5th and 97.5th percentiles were determined non-parametrically as per the CLSI C28-A3 guidelines on defining, establishing, and verifying reference intervals in the clinical laboratory.[1] To obtain these intervals with 90% confidence intervals, a minimum of 120 observations were required for each parameter within each subgroup. Outliers within each subgroup were identified by using the Dixon method.[1] Briefly, the extreme values were retained in the distribution if $D/R < 0.33$, where D is the absolute difference between the most extreme distribution and the next value and R is the range (maximum–minimum). Reference values were determined separately for males, females, and for combined sexes. Differences between sexes were tested by using the Mann-Whitney test. Values defined were compared with reference values obtained from the northern industrialized countries and other countries in Africa.

TABLE 1

Laboratory analytical methods used for biochemical tests, Kintampo, Ghana*

| Test | Analytical method used |
|---|---|
| ALT and AST | IFCC modified without pyridoxal phosphate |
| Amylase | 2-Chloro-4-nitrophenyl-α-maltotrioside |
| Creatine kinase | IFCC, UV kinetic with imidazole buffer |
| GGT | L-γ-Glutamyl-3-carboxy-p-nitroanilide |
| LDH | UV kinetic, pyruvate to lactate |
| Total protein | Biuret |
| Albumin | Bromocresol green with succinate buffer |
| Total and direct bilirubin | Malloy-Evelyn modified, end-point |
| Cholesterol | Cholesterol oxidase/peroxidase |
| Glucose | Glucose oxidase/peroxidase |
| Iron | Chromazurol |
| Triglycerides | Lipase/glycerol kinase/glycerol peroxidase |
| Urea | Urease, UV kinetic |
| Creatinine | Jaffe kinetic |
| Uric acid | Uricase/peroxidase |
| Phosphorus | Phosphomolybdate |
| Electrolytes | Ion selective electrode, direct |

* ALT = alanine aminotransferase; AST = aspartate aminotransferase; IFCC = International Federation for Clinical Chemistry; GGT = γ-glutamyltransferase; LDH = lactate dehydrogenase; UV = ultraviolet.

**Ethical considerations.** Ethics Committees of the Kintampo Health Research Center, the Noguchi Memorial Institute for Medical Research, and the National Ghana Health Service approved this study. Written informed consent was obtained for each participant who was ≥ 8 years of age and from parents or caregivers before being involved in any activities in the study. The parents or caregivers of all children who were < 8 years of age provided consent.

## RESULTS

A total of 1,542 children 0.5 years (i.e., 6 months) through 17 years of age were screened for enrollment into this cross-sectional study. Of these children, 1,442 (93.5%) were eligible based on the inclusion/exclusion criteria and were enrolled into the study. Reasons for ineligibility were use of prescribed drugs within two weeks before study (44), physical signs of any chronic/acute illness (26), residence in study area for < 3 months (13), history of any chronic illness (8), hospitalization within a month preceding the survey (5), blood transfusion within three months preceding the survey (3), and refusal for blood draw (1). Data for 15 participants were not included in the analysis because hemoglobin values were < 6.0 g/dL, platelet counts were $< 50 \times 10^9$/L, or a homozygous hemoglobin S genotype was present.

The laboratory analytical methods used in the biochemical tests are shown in Table 1. Median and reference values

TABLE 2
Biochemical reference values for children 0.5–12 years of age in Kintampo, Ghana*

| | | Age groups of children | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0.5–4.9 years | | | 5–12 years | | |
| Parameters | Unit | No. | Median value | Reference value | No. | Median value | Reference value |
| Enzymes | | | | | | | |
| ALT | U/L | 491 | 20 | 7–55 | 473 | 20 | 5–53 |
| AST | U/L | 489 | 39 | 23–72 | 471 | 31 | 19–57 |
| Amylase | U/L | 466 | 53 | 12–136 | 471 | 69 | 33–133 |
| CK | U/L | 454 | 108 | 35–291 | 474 | 159 | 59–515 |
| GGT | U/L | 453 | 13 | 3–34 | 474 | 14 | 7–31 |
| LDH | U/L | 257 | 608 | 360–995 | 439 | 509 | 277–823 |
| Serum proteins | | | | | | | |
| Protein, total | g/dL | 456 | 69.7 | 56.0–87.0 | 457 | 73.2 | 54.0–87.9 |
| Albumin | g/dL | 471 | 44.3 | 35.9–50.0 | 476 | 42.6 | 34.2–49.8 |
| Metabolism | | | | | | | |
| Bilirubin, total | μmol/L | 501 | 5.8 | 1.8–21.0 | 485 | 6.7 | 1.7–18.9 |
| Bilirubin, direct | μmol/L | 439 | 1.5 | 0.4–3.6 | 392 | 2.1 | 0.6–3.9 |
| Cholesterol | mmol/L | 467 | 3.1 | 1.7–5.0 | 477 | 2.8 | 1.7–4.3 |
| Glucose | mmol/L | 501 | 4.7 | 3.2–6.8 | 473 | 4.7 | 3.5–6.2 |
| Iron | μmol/L | 407 | 10.0 | 4.2–20.1 | 456 | 9.7 | 3.88–19.0 |
| Triglycerides | mmol/L | 460 | 1.2 | 0.5–2.7 | 475 | 0.9 | 0.5–1.91 |
| Kidney function | | | | | | | |
| Urea | mmol/L | 380 | 1.8 | 1.0–4.2 | 473 | 1.9 | 1.0–4.5 |
| Creatinine | μmol/L | 483 | 33 | 17–52 | 471 | 50 | 33–74 |
| Uric acid | μmol/L | 466 | 179 | 71–340 | 464 | 170 | 72–274 |
| Chloride | mmol/L | 380 | 108 | 98–115 | 423 | 107 | 99–114 |
| Phosphorus | mmol/L | 459 | 1.71 | 1.26–2.25 | 477 | 1.42 | 1.03–1.84 |
| Potassium | mmol/L | 388 | 4.6 | 3.6–5.8 | 469 | 4.4 | 3.6–5.6 |
| Sodium | mmol/L | 379 | 143 | 131–149 | 415 | 144 | 135–151 |

*ALT = alanine aminotransferase; AST = aspartate aminotransferase; CK = creatine kinase; GGT = γ-glutamyltransferase; LDH = lactate dehydrogenase.

(2.5th and 97.5th percentiles) of the biochemical tests for children 0.5–12 years of age and 13–17 years of age are shown in Tables 2 and 3, respectively. Median values for AST, direct bilirubin, LDH, phosphorus, and potassium decreased progressively with age. Although albumin, cholesterol, and iron decreased progressively in the children, they later increased in the adolescents. Creatinine and urea increased progressively with age throughout the study. Amylase increased with age

TABLE 3
Biochemical reference values for adolescents in Kintampo, Ghana*

| | | Males | | | Females | | | Persons 13–17 year of age | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Parameters | Unit | No. | Median value | Reference value | No. | Median value | Reference value | No. | Median value | Reference value |
| Enzymes | | | | | | | | | | |
| ALT† | U/L | 157 | 23 | 10–61 | 124 | 20 | 7–48 | 281 | 22 | 8–55 |
| AST† | U/L | 157 | 30 | 18–67 | 123 | 24 | 11–49 | 280 | 28 | 14–62 |
| Amylase | U/L | 160 | 62 | 31–119 | 127 | 60 | 30–123 | 287 | 61 | 31–120 |
| CK† | U/L | 153 | 258 | 96–572 | 124 | 191 | 91–567 | 277 | 218 | 94–562 |
| GGT | U/L | 157 | 16 | 5–47 | 125 | 15 | 8–30 | 282 | 15 | 6–45 |
| LDH† | U/L | 149 | 474 | 284–751 | 86 | 415 | 212–732 | 235 | 450 | 252–737 |
| Serum proteins | | | | | | | | | | |
| Protein, total | g/L | 149 | 71.6 | 45.2–86.0 | 123 | 72.4 | 46.7–87.3 | 272 | 71.8 | 46.4–86.5 |
| Albumin† | g/L | 160 | 42.0 | 34.3–48.9 | 126 | 43.2 | 37.8–50.5 | 286 | 42.7 | 35.4–49.3 |
| Metabolism | | | | | | | | | | |
| Bilirubin, total | μmol/L | 160 | 8.4 | 3.3–22.0 | 128 | 8.0 | 3.2–21.4 | 288 | 8.1 | 3.3–21.6 |
| Bilirubin, direct† | μmol/L | 124 | 2.6 | 1.2–4.0 | 101 | 2.2 | 0.8–3.9 | 225 | 2.4 | 0.9–4.0 |
| Cholesterol† | mmol/L | 161 | 2.8 | 1.7–3.9 | 127 | 3.2 | 2.0–5.3 | 288 | 3.0 | 1.8–4.6 |
| Glucose | mmol/L | 161 | 4.9 | 3.5–6.8 | 128 | 4.9 | 3.7–6.5 | 289 | 4.9 | 3.6–6.7 |
| Iron | μmol/L | 155 | 11.8 | 4.6–23.3 | 119 | 12.0 | 3.8–23.9 | 274 | 11.9 | 4.6–23.3 |
| Triglycerides | mmol/L | 157 | 0.8 | 0.4–1.8 | 127 | 0.9 | 0.5–1.7 | 284 | 0.80 | 0.40–1.70 |
| Kidney function | | | | | | | | | | |
| Urea† | mmol/L | 160 | 2.2 | 1.0–5.5 | 127 | 1.9 | 1.0–3.6 | 287 | 2.1 | 1.0–4.5 |
| Creatinine† | μmol/L | 130 | 62 | 42–79 | 116 | 60 | 33–78 | 246 | 61 | 39–79 |
| Uric acid† | μmol/L | 159 | 211 | 79–334 | 127 | 169 | 76–285 | 286 | 186 | 78–322 |
| Chloride | mmol/L | 141 | 107 | 95–117 | 112 | 107 | 98–115 | 253 | 107 | 96–116 |
| Phosphorus† | mmol/L | 161 | 1.36 | 0.95–1.79 | 127 | 1.24 | 0.96–1.65 | 288 | 1.30 | 0.96–1.77 |
| Potassium | mmol/L | 159 | 4.5 | 3.6–5.8 | 126 | 4.4 | 3.6–6.1 | 285 | 4.4 | 3.6–5.9 |
| Sodium | mmol/L | 138 | 144 | 132–156 | 108 | 145 | 132–151 | 246 | 144 | 132–152 |

*ALT = alanine aminotransferase; AST = aspartate aminotransferase; CK = creatine kinase; GGT = γ-glutamyltransferase; LDH = lactate dehydrogenase. Adolescents are classified as children 13–17 years of age.
†Parameters with significant sex differences.

TABLE 4
Hematologic reference values for children 0.5–12 years of age in Kintampo, Ghana*

| | | Age groups of children | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0.5–4.9 years | | | 5–12 years | | |
| Parameters | Unit | No. | Median value | Reference value | No. | Median value | Reference value |
| Hemoglobin | g/dL | 499 | 10.3 | 8.0–12.7 | 484 | 11.5 | 9.1–13.5 |
| Hematocrit | % | 499 | 31.6 | 24.4–38.8 | 485 | 34.4 | 27.3–41.5 |
| RBC | $\times 10^{12}$/L | 499 | 4.38 | 3.22–5.55 | 485 | 4.36 | 3.45–5.29 |
| MCV | fl | 499 | 73 | 56–87 | 483 | 80 | 68–89 |
| MCH | Pg | 498 | 23.9 | 16.9–29.7 | 483 | 26.5 | 21.4–30.3 |
| MCHC | g/dL | 493 | 32.4 | 30.0–36.9 | 481 | 33.2 | 30.9–36.0 |
| RDW | % | 498 | 16.1 | 12.5–21.6 | 484 | 13.7 | 11.5–17.9 |
| Platelets | $\times 10^{12}$/L | 498 | 301 | 110–637 | 479 | 239 | 117–417 |
| PDW | % | 497 | 16.0 | 8.8–25.4 | 484 | 15.0 | 12.1–20.5 |
| WBC, total | $\times 10^{9}$/L | 499 | 9.8 | 5.1–17.6 | 485 | 6.6 | 4.1–11.9 |
| Lymphocytes | % | 499 | 56.8 | 34.9–75.6 | 484 | 45.6 | 29.6–62.5 |
| Lymphocytes | $\times 10^{9}$/L | 499 | 5.5 | 2.3–11.9 | 485 | 2.9 | 1.6–5.8 |
| Monocytes | % | 499 | 7.7 | 4.9–13.6 | 485 | 8.6 | 5.0–13.3 |
| Monocytes | $\times 10^{9}$/L | 499 | 0.7 | 0.2–1.0 | 484 | 0.5 | 0.2–1.1 |
| Granulocytes | % | 499 | 35.2 | 18.5–59.7 | 481 | 45.1 | 28.3–62.4 |
| Granulocytes | $\times 10^{9}$/L | 499 | 3.5 | 1.5–8.5 | 484 | 3.1 | 1.6–6.2 |

*RBC = red blood cell; MCV = mean cell volume; MCH = mean cell hemoglobin; MCHC = mean cell hemoglobin concentration; RDW = red blood cell distribution width; PDW = platelet distribution width; WBC = white blood cell.

in the children but decreased in the adolescents. Levels of ALT, GGT, total protein, and total bilirubin remained almost invariable throughout the study. Levels of CK and uric acid showed great variation, increasing in the 5–12 year subgroup and decreasing in adolescents.

Adolescent males had significantly higher levels of ALT (10–61 U/L versus 7–41 U/L; $P < 0.01$), AST (18–67 U/L versus 11–49 U/L; $P < 0.01$), CK (96–572 U/L versus 91–567 U/L; ($P < 0.01$), LDH (284–751 U/L versus 212–732 U/L; $P < 0.01$), direct bilirubin (1.2–4.0 μmol/L versus 0.8–3.9 μmol/L; $P = 0.02$), urea (1.0–5.5 mmol/L versus 1.0–3.6 mmol/L; $P < 0.01$), creatinine (42–79 μmol/L versus 33–78 μmol/L; $P = 0.01$), uric acid (79–334 μmol/L versus 76–285 μmol/L; $P < 0.01$), and phosphorus (0.95–1.79 mmol/L versus 0.96–1.65 mmol/L; $P < 0.01$) than female counterparts. However, adolescent females had significantly higher levels of albumin (34.3–48.9 g/L versus 37.8–50.5 g/L; $P = 0.01$) and cholesterol (2.0–5.3 mmol/L versus 1.7–3.9 mmol/L; $P < 0.01$) than males.

Median and reference values of hematology tests for children 0.5–12 years of age and 13–17 years of age are shown in Tables 4 and 5, respectively. There was a steady increase in the red blood cell parameters (hemoglobin, hematocrit, RBC, MCV, and MCH) with age. Conversely, platelets and total WBC counts showed a decrease with age. In adolescents (children 13–17 years of age) cohort, significantly higher values were observed for hemoglobin (10.4–14.8 g/dL versus 9.4–14.2 g/dL; $P = 0.01$)), hematocrit (31.1–45.1% versus 25.4–45.1%; $P = 0.03$), RBC (3.79–5.69 $\times 10^{12}$/L versus 3.53–5.57 $\times 10^{12}$/L; $P < 0.01$), granulocytes (33.6–64.4% versus 30.8–64.0%; $P = 0.01$), and granulocyte numbers (1.4–5.4 $\times 10^{9}$/L versus 1.6–5.2 $\times 10^{9}$/L; $P = 0.04$) in males than in females. Lower values were observed in males than in females for platelets (108–326 $\times 10^{9}$/L versus 143–390 $\times 10^{9}$/L; $P = 0.02$), PDW (11.9–20.8 versus 12.4–24.1; $P = 0.01$), and lymphocytes (26.5–56.7% versus 25.7–60.2%; $P = 0.01$). No sex differences were observed for MCV, MCH, mean cell

TABLE 5
Hematologic reference values for adolescents in Kintampo, Ghana*

| | | Male | | | Female | | | Combined | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Parameters | Unit | No. | Median value | Reference value | No. | Median value | Reference value | No. | Median value | Reference value |
| Hemoglobin† | g/dL | 161 | 12.4 | 10.4–14.8 | 128 | 12.3 | 9.4–14.2 | 289 | 12.2 | 9.5–14.4 |
| Hematocrit† | % | 161 | 37.4 | 31.1–45.1 | 128 | 36.9 | 25.4–45.1 | 289 | 37.2 | 29.4–44.9 |
| RBC† | $\times 10^{12}$/L | 161 | 4.66 | 3.79–5.69 | 128 | 4.50 | 3.41–5.40 | 289 | 4.58 | 3.53–5.57 |
| MCV | Fl | 161 | 82 | 66–92 | 127 | 83 | 67–94 | 288 | 82 | 67–93 |
| MCH | Pg | 161 | 27.2 | 21.6–31.4 | 128 | 27.2 | 20.9–35.0 | 289 | 27.2 | 21.2–32.0 |
| MCHC | g/dL | 161 | 33.1 | 30.8–35.9 | 124 | 32.9 | 30.1–37.2 | 285 | 33.0 | 30.5–36.6 |
| RDW | % | 161 | 13.7 | 11.5–16.1 | 127 | 13.4 | 11.7–16.0 | 288 | 13.5 | 11.6–16.1 |
| Platelets | $\times 10^{12}$/L | 159 | 220 | 108–326 | 125 | 232 | 143–390 | 284 | 226 | 113–363 |
| PDW† | % | 161 | 15.4 | 11.9–20.8 | 121 | 15.7 | 12.4–24.1 | 282 | 15.5 | 12.4–22.6 |
| WBC, Total | $\times 10^{9}$/L | 160 | 6.0 | 3.6–10.3 | 127 | 5.7 | 3.8–9.3 | 287 | 5.9 | 3.7–9.4 |
| Lymphocytes† | % | 161 | 41.5 | 26.5–56.7 | 128 | 44.9 | 25.7–60.2 | 289 | 43.0 | 26.6–58.9 |
| Lymphocytes | $\times 10^{9}$/L | 160 | 2.4 | 1.4–4.2 | 127 | 2.6 | 1.4–3.9 | 287 | 2.5 | 1.4–4.0 |
| Monocytes | % | 161 | 8.1 | 4.8–14.0 | 128 | 7.9 | 4.9–14.7 | 289 | 8.0 | 4.9–14.4 |
| Monocytes | $\times 10^{9}$/L | 162 | 0.4 | 0.2–1.0 | 129 | 0.4 | 0.2–0.9 | 289 | 0.4 | 0.2–0.9 |
| Granulocytes† | % | 158 | 49.2 | 33.6–64.4 | 127 | 47.1 | 30.8–64.0 | 285 | 48.2 | 31.0–64.0 |
| Granulocytes† | $\times 10^{9}$/L | 159 | 3.1 | 1.4–5.4 | 126 | 2.7 | 1.6–5.2 | 285 | 2.9 | 1.6–5.2 |

*RBC = red blood cell; MCV = mean corpuscular volume; MCH = mean cell hemoglobin; MCHC = mean cell hemoglobin concentration; RDW = red blood cell distribution; PDW = platelet distribution width; WBC = white blood cell. Adolescents are classified as children 13–17 years of age.
†Parameters with significant sex differences.

TABLE 6

Comparison of commonly used biochemical reference values for adolescents in Kintampo, Ghana, with those for Kenya and a developed country*

| Parameters | Kintampo | Kenya[23] | Developed country[22] |
|---|---|---|---|
| ALT, U/L | | | |
| M | 10–61 | 5–42 | 10–33 |
| F | 7–48 | 4–65 | 8–24 |
| AST, U/L | | | |
| M | 18–67 | 17–59 | 18–40 |
| F | 11–49 | 12–43 | 17–33 |
| Bilirubin, total, μmol/L | | | |
| M | 3.3–22.0 | 5.7–62.6 | 1.7–14.4 |
| F | 3.2–21.4 | 3.7–38.5 | 1.7-1-4.4 |
| Bilirubin, direct, μmol/L | | | |
| M | 1.2–4.0 | NA | 1.9–7.1 |
| F | 0.9–4.0 | NA | 1.7–6.7 |
| Creatinine, μmol/L | | | |
| M | 42–79 | 50–104 | 58–92 |
| F | 33–78 | 48–88 | 52–76 |
| Urea, mmol/L | | | |
| M | 1.0–5.5 | NA | 2.6–7.5 |
| F | 1.0–3.6 | NA | 2.6–6.8 |

*ALT = alanine aminotransferase; AST = aspartate aminotransferase; NA = not available.

hemoglobin concentration, red blood cell distribution width, total WBC, lymphocytes (absolute numbers) and monocytes.

## DISCUSSION

This achieved its aim of establishing comprehensive biochemical and hematologic reference values that would serve as standards for the interpretation of laboratory results for children in routine healthcare practice and screening/follow-up during clinical trials in the Kintampo area of Ghana, as well as in populations with similar profiles.

One of the difficulties when establishing reference values for children is how the population should be subdivided.[17] Grouping children into the 0.5–4 years, 5–12 years, and 13–17 years of age groups (male and female) was performed based on the subgroups used for similar studies in Uganda[15] and Tanzania[14] and also based on recommendations to establish sex-specific reference values during the pubertal stage.[21] Determination of the values by using combined sex for children < 13 years of age was based on the general absence of sex differences for the parameters in these age groups.[14,15,22]

There is a dearth of comprehensive age- and sex-specific biochemical reference values for children in Africa. Most published biochemical reference values for children in Africa usually do not cover the entire subpopulations of children (i.e., infants, pre-adolescents, and adolescents) and only a limited number of tests are presented.[4,16,23]

We have established in this study age- and sex-specific reference values for a wide range of biochemical tests, including liver, kidney and cardiac function tests, lipids, iron, and glucose. For the commonly used biochemical tests during screening/enrollment and safety monitoring of children in the Kintampo study area, higher values were obtained for ALT, AST and total bilirubin compared with values from children in the developed countries, and creatinine and urea levels were lower (Table 6). Although there are differences in the values of some of these parameters when compared with other studies in Africa,[4,14,16,23] similar patterns were observed when compared with Caucasian values.[17,22] A possible cause of higher liver enzyme levels (ALT and AST) is subclinical viral infections or the use of herbal preparations.[3] Although screening for hepatitis was not performed, prevalence of these viruses among blood donors in Ghana is 7–15% for hepatitis B virus[26,27] and 7–11% for hepatitis C virus.[26] Regarding the use of herbal preparations, it has been estimated that the

TABLE 7

Comparison of commonly used hematologic reference values for children in Kintampo, Ghana, with those for Uganda, Tanzania, and developed countries*

| Parameters | Kintampo | Uganda[15] | Tanzania[14] | WHO (lower limits)[24,25]† |
|---|---|---|---|---|
| Hemoglobin, g/dL | | | | |
| 0.5–4 years old | 8.0–12.6 | 8.8–12.5 | 8.1–13.9 | 11.0 |
| 5–12 years old | 9.1–13.5 | 10.0–13.7 | 10.3–14.7 | 11.5 |
| 13–17 years old (M) | 10.4–14.8 | 11.2–15.9 | 10.8–17.0 | 13.0 |
| 13–17 years old (F) | 9.4–14.2 | 9.9–14.5 | 10.0–14.9 | 12.0 |
| Hematocrit, % | | | | |
| 0.5–4 years old | 24.4–38.8 | 25.9–36.3 | 26.5–40.8 | 33.0 |
| 5–12 years old | 27.3–41.5 | 29.2–39.4 | 31.9–43.5 | 34.0 |
| 13–17 years old (M) | 31.1–45.1 | 32.3–45.5 | 33.0–48.1 | 39.0 |
| 13–17 years old (F) | 25.4–45.1 | 28.1–42.2 | 30.8–44.7 | 36.0 |
| MCV | | | | |
| 0.5–4 years old | 56–87 | 60.7–82.8 | 54.7–91.6 | 73 |
| 5–12 years old | 68–89 | 63.3–83.9 | 66.0–90.0 | 76 |
| 13–17 years old (M) | 66–92 | 65.0–89.5 | 63.2–91.0 | 79 |
| 13–17 years old (F) | 67–94 | 67.4–89.9 | 62.2–94.5 | 78 |
| MCH | | | | |
| 0.5–4 years old | 16.7–31.3 | NA | NA | 25 |
| 5–12 years old | 21.4–30.3 | NA | NA | 26 |
| 13–17 years old (M) | 21.6–31.4 | NA | NA | 27 |
| 13–17 years old(F) | 21.2–32.0 | NA | NA | 26 |
| RBC, $\times 10^{12}$/L | | | | |
| 0.5–4 years old | 3.22–5.55 | 3.50–5.20 | NA | 3.7 |
| 5–12 years old | 3.45–5.24 | 3.80–5.40 | NA | 3.8 |
| 13–17 years old (M) | 3.79–5.69 | 4.10–5.80 | NA | 4.2 |
| 13–17 years old (F) | 3.53–5.57 | 3.50–5.40 | NA | 3.9 |

*WHO = World Health Organization; MCV = mean cell volume; MCH = mean cell hemoglobin; NA = not available; RBC = red blood cell.
†Age ranges for MCV, MCH, and RBC = 2–4.9 years, 8–11.9 years, and 15–17 years (M and F).

first-line treatment of 60% of children with fever resulting from malaria in Ghana, Mali, Nigeria and Zambia has been the use of herbal medicine at home.[28] Low protein intake could account for the decreased levels of urea observed in this study.[29] Medical decision limits are more appropriate for some parameters (such as glucose, cholesterol, and triglyceride) for monitoring and assessment of disease than population-based reference values.[1] However, reference values defined for the healthy population of children in this study is of epidemiologic interest.[22] Higher triglyceride values for children less than five years of age could be caused by samples being collected from persons in a non-fasting state.

Red blood cell parameters derived in this study are on the lower side when compared with cut-off values used by the World Health Organization (Table 7).[24,25] This finding is synonymous with those of many studies that established hematologic reference values for children in Africa.[4,14,16,23] Factors contributing to the lower values include poor nutritional status (e.g., iron deficiency), chronic blood loss resulting from parasitic infections (e.g. hookworm and schistosomes) and hemoglobinopathies.[8,14] The finding of significant sex differences in the red blood cell parameters (hemoglobin, hematocrit, and RBC count) among adolescents is consistent with previously evidence that adolescent males have higher values than females for these parameters.[14,23] The reasons for these differences have been attributed to factors such as the influence of the androgen hormone on erythropoiesis and to menstrual blood loss in females.[8–10,15,30]

The upper limits of the platelet values for infants in this study were higher than those for infants in the developed countries. Similar findings have been observed in other countries in Africa, such as Gabon,[16] Mozambique,[4] Uganda[15] and Tanzania.[14] No clear reasons could be attributed to this finding that is in contrast with lower platelet counts reported for African adults compared with those for Caucasians.[6,30–33] However, the steady decrease in maximum platelet counts with age from birth up to adolescence is a finding that has been established in Caucasians and Africans. Malaria-related thrombocytopenia could be one of the contributing factors to low platelet counts in our study,[13,34] in addition to dietary, environmental, and genetic factors'[31,32,35] White blood cells counts for children ≤ 4 years of age in this study were comparable to those of children of the same ages in developed countries. However, lower WBC values were observed in children 5–17 years of age. Similar findings of lower WBC values in children within this age range have been reported in other studies in Africa.[14,15] This finding continues into adulthood.[6,8,35] Lower WBC counts in Africans could be caused by dietary, genetic, and environmental factors.

This study is important because it emphasizes the need for the use of locally derived reference values to be used to guide in interpreting biochemical and hematologic results in clinical practice and clinical trials and determining eligibility and reporting of adverse events during clinical trials. The use of inappropriate reference values obtained from industrialized populations would result in the exclusion of otherwise healthy participants (leading to unnecessary prolongation of clinical trials), over-reporting of adverse events during clinical trials, and inappropriate treatment of patients in routine healthcare practice.

Before enrollment and blood sample collection, the children who participated in this study were examined by clinicians to determine their health status. Only those who were found to be healthy were enrolled. However, the selection of a normal group for the determination of laboratory reference values is complex and not all medical conditions could be screened at the time of this study. Therefore, it is possible that a small proportion of the children who had minor illnesses might not have been detected. However, as much as practical, children with conditions such as signs/history of acute or chronic diseases, hospitalization within a month before the study, or sickle cell disease were not included in the analysis. Data for participants with significant abnormality were not used in the determination of the reference intervals.

The reference values developed for children in the Kintampo study area will be of immense benefit to most clinical trials requiring screening and monitoring of hematologic and biochemical parameters and for patient care in general. Compared with values for those in the developed countries, the reference values for hemoglobin, hematocrit, RBC counts, and urea are lower in the Kintampo study area. These values may be used in laboratories in other parts of Ghana and Africa after some form of validation, as recommended by the CLSI.

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