Haematological and Biochemical Reference Values for Healthy Adults in the Middle Belt of Ghana

David K. Dosoo¹, Kingsley Kayan¹, Dennis Adu-Gyasi¹, Evans Kwará¹, Josephine Ocran², Kingsley Osei-Kwakye¹, Emmanuel Mahama¹, Stephen Amenga-Etego¹, Philip Bilson¹, Kwaku P. Asante¹, Kwadwo A. Koram², Seth Owusu-Agyei¹*

¹ Kintampo Health Research Centre, Kintampo, Ghana, ² Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana

Abstract

Background: Reference values are very important in clinical management of patients, screening participants for enrolment into clinical trials and for monitoring the onset of adverse events during these trials. The aim of this was to establish gender-specific haematological and biochemical reference values for healthy adults in the central part of Ghana.

Methods: A total of 691 adults between 18 and 59 years resident in the Kintampo North Municipality and South District in the central part of Ghana were randomly selected using the Kintampo Health and Demographic Surveillance System and enrolled in this cross-sectional survey. Out of these, 625 adults made up of 316 males and 309 females were assessed by a clinician to be healthy. Median values and nonparametric 95% reference values for 16 haematology and 22 biochemistry parameters were determined for this population based on the Clinical Laboratory and Standards Institute guidelines. Values established in this study were compared with the Caucasian values being used currently by our laboratory as reference values and also with data from other African and western countries.

Results: Reference values established include: haemoglobin 113–164 g/L for males and 88–144 g/L for females; total white blood cell count 3.4–9.2×10⁹/L; platelet count 88–352×10⁹/L for males and 89–403×10⁹/L for females; alanine aminotransferase 8–54 U/L for males and 6–51 U/L for females; creatinine 56–119 μmol/L for males and 53–106 μmol/L for females. Using the haematological reference values based on the package inserts would have screened out up to 53% of potential trial participants and up to 25% of the population using the biochemical parameters.

Conclusion: We have established a panel of locally relevant reference parameters for commonly used haematological and biochemical tests. This is important as it will help in the interpretation of laboratory results both for clinical management of patients and safety monitoring during a trial.

Introduction

Locally relevant reference ranges for commonly used biochemical and haematological parameters are essential for screening and safety follow up of trial participants as well as for routine clinical management of patients. However, reference values being used in most laboratories in African countries have been obtained from the literature, reagent inserts accompanying the reagent kits or instrument manuals [1]. These values more often than not have been derived from Caucasian populations of industrialised countries and may not be applicable in most local settings. Factors such as age, gender [2], ethnicity [3] and environment including altitude and geo-chemicals [4] affect the measurements determined in different populations. Published literature has confirmed that many of the reference values obtained from the developed countries differ significantly from what pertains in most African localities [1,5,6,7,8]; thus making it necessary to establish locally relevant values. The Clinical and Laboratory Standards Institute (CLSI) [9] and the International Federation for Clinical Chemistry (IFCC) [10] recommend that each laboratory establishes its own reference values.

The Kintampo Health Research Centre (KHRC) located in Central Ghana has been undertaking Phase II/III drugs and vaccines trials since 2003 and plans soon to add Phase I trials in infectious diseases. Locally relevant biochemical and haematological reference values for the population are needed in determining eligibility of participants being enrolled into future studies and also for monitoring of the onset of any adverse events during a trial. Availability of such reference values in the locality would also assist physicians in the management of patients. This study was, thus, aimed at establishing reference values for commonly used haematological and biochemical parameters in the population.
Figure 1. Map of the study area.
doi:10.1371/journal.pone.0036308.g001

Figure 2. Map of study communities.
doi:10.1371/journal.pone.0036308.g002
within the Kintampo North Municipality and Kintampo South District.

Methods

Study Site

The study was carried out in the Kintampo North Municipality and Kintampo South District of the Brong Ahafo Region of Ghana (Figure 1 and Figure 2). The studied area is located between Latitudes 7°43′N and 8°44′N and Longitudes 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 m above sea level. It is made up of a resident population of about 140,000. The Kintampo Health Research Centre 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 4 monthly intervals. All the compounds have been digitized making the selection and tracing of individuals to their homes easy.

Selection of reference population

The communities and individuals who participated in this study were randomly selected from the HDSS human population using the Visual FoxPro software. Community meetings were held to explain the objectives of the study to the opinion leaders and other community members. Those selected through the randomisation were invited to a central location where individual consenting, screening and blood collections were carried out. Inclusion into the study was based on willingness of the individual to participate in the study (demonstrated by the completion and signing/thumbprinting of the willingness of the individual to participate in the study (demonstrated by the completion and signing/thumbprinting of the consent form and willingness to provide the samples required), general good health (as determined by a clinician’s medical history and physical examination, and residence in the study area for at least 3 months. Individuals with evidence of acute or chronic respiratory, cardiovascular, gastrointestinal, hepatic or genitourinary conditions, history of blood donation/transfusion within the immediate past three months, hospitalisation within the immediate past one month, or any other findings that in the opinion of the examining clinician may compromise on the assessment of the laboratory parameters of interest in this study were excluded. Those assessed to be pregnant (either clinically or by positive urine β-HCG test) and lactating mothers were all excluded.

Laboratory analysis

Venous blood samples were collected from the antecubital fossa, dispensed into a 2 ml K3EDTA, a 5 ml SST tubes with gel and 1 ml Fluoride-EDTA for haematology, biochemistry and glucose analysis, respectively. Sample tubes were from Becton Dickinson (Plymouth, United Kingdom). Haematological analysis (complete blood count with 3-part differential) was performed using previously validated ABX Micros 60 analysers (Horiba-ABX, Montpellier, France). Calibrators and controls were obtained from the instrument manufacturer. Analysis of samples was performed within 8 hours of blood draw.

Samples for biochemical analysis were allowed to clot for at least 60 minutes, centrifuged and the serum collected. Serum was analysed 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. The Vitalab Selectra E Clinical Chemistry analyser (Vital Scientifc, The Netherlands) was used to perform the analysis. Test tubes for the clinical chemistry analysis were from Vital Scientifc, The Netherlands. Reagents, calibrators and controls were from Elitech Diagnostics (Sees, France). Electrolytes (chloride, potassium and sodium) were analysed using the Humalyte Electrolyte analyser (Human Diagnostics, Germany). Reagents were from the manufacturer of the instrument.

Table 1. Haematology reference values for Kintampo.

| Analyte               | Unit     | Males N | Med  | Reference Values  | Females N | Med  | Reference Values  | Combined males and Females N | Med  | Reference Values | p-value* |
|-----------------------|----------|---------|------|-------------------|------------|------|-------------------|------------------------------|------|------------------|----------|
| Haemoglobin           | g/L      | 316     | 139  | 113–164           | 308        | 123  | 88–144            | 624             | 131  | 98–160           | <0.0001  |
| Haematocrit           | %        | 316     | 42.2 | 33.2–50.5         | 309        | 36.9 | 26.4–45.0         | 625             | 39.4 | 28.9–48.7        | <0.0001  |
| RBC                   | ×10¹²/L  | 316     | 4.84 | 3.79–5.96         | 307        | 4.32 | 3.09–5.30         | 623             | 4.57 | 3.39–5.83        | <0.0001  |
| MCV                   | fl       | 316     | 88   | 70–98             | 309        | 86   | 73–96             | 625             | 87   | 72–97            | 0.0192   |
| MCH                   | pg       | 316     | 29.1 | 22.7–33.5         | 307        | 28.4 | 22.3–33.6         | 623             | 28.6 | 22.6–33.5        | 0.1448   |
| MCHC                  | g/dL     | 315     | 33.1 | 30.6–36.0         | 305        | 33.1 | 30.4–36.5         | 620             | 33.1 | 30.5–36.2        | 0.3621   |
| RDW                   | %        | 316     | 13.6 | 11.5–16.7         | 309        | 13.4 | 11.4–16.8         | 625             | 13.5 | 11.5–16.7        | 0.2064   |
| Platelets             | ×10¹²/L  | 316     | 208  | 88–352            | 309        | 224  | 89–403            | 625             | 216  | 89–380           | <0.0001  |
| PDW                   | %        | 315     | 15.8 | 12–23.4           | 308        | 15.7 | 12.6–22.9         | 623             | 15.7 | 12.6–23.0        | 0.5511   |
| WBC, Total            | ×10⁹/L   | 311     | 5.5  | 3.5–9.2           | 309        | 5.3  | 3.4–9.3           | 620             | 5.4  | 3.4–9.2          | 0.0218   |
| Lymphocytes           | %        | 315     | 41   | 24.0–57.2         | 309        | 42.3 | 26.9–58.3         | 624             | 41.6 | 25.2–57.7        | 0.0430   |
| Monocytes             | ×10⁹/L   | 316     | 2.2  | 1.2–5.2           | 308        | 2.1  | 1.2–4.4           | 624             | 2.1  | 1.2–4.4          | 0.2046   |
| Granulocytes          | %        | 316     | 48.6 | 30.2–69.9         | 309        | 48.5 | 33.3–67.5         | 625             | 48.5 | 32.0–68.1        | 0.8643   |

Med: Median.

*Mann-Whitney test for differences in between males and females.
doi:10.1371/journal.pone.0036308.t001
Normal and abnormal controls were run daily. No analysis was done if controls were out of range. In addition to the internal quality assessment, the laboratory participates in external quality assessments for haematology and clinical chemistry both with the College of American Pathologists (CAP) and the United Kingdom National External Quality Assessment Scheme (UK NEQAS). The laboratory complies with the principles of Good Clinical Laboratory Practice [11,12]. Individuals with abnormal clinical or laboratory test results were referred for appropriate care and treatment.

Between-run precision for the analytes were assessed using at least 20 measurements made on separate days using normal control samples. The mean, standard deviation (SD) and coefficient of variation (CV) were calculated for each analyte. Coefficients of variation (CVs) were compared to those quoted in the analyser manuals and reagent inserts.

### Data Management and Statistical Analysis

Data were recorded on questionnaires, double-entered into a Visual FoxPro 9.0 database and verified. Data analysis was carried out using Stata 11 (Stata Corp, College Park, TX, USA). The 2.5th and 97.5th percentiles were determined non-parametrically. This was according to the CLSI/IFCC guidelines on defining, establishing and verifying reference intervals in the clinical laboratory [9]. To obtain these intervals, a minimum of 120 observations were required for each analyte within each subgroup. Outliers within each subgroup were identified using the Dixon method [9]. 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 combined gender. Differences between genders were tested using the Mann-Whitney test. The values defined were compared with the recommended reference values (based on a North American population) provided in the ABX Micros 60 Haematology User Manual [13] and Elitech Diagnostics chemistry reagent inserts, respectively.

### Ethical Considerations

This study was approved by Ethics Committees of the Kintampo Health Research Centre, the Noguchi Memorial Institute for Medical Research and the Ghana Health Service.

---

**Table 2. Biochemistry reference values for Kintampo.**

| Analyte (Unit) | Males | | | Females | | | Combined males and females | | |
|---|---|---|---|---|---|---|---|---|---|
| | N | Med | Reference Values | N | Med | Reference Values | N | Med | Reference Values | p-value* |
| **Enzymes** | | | | | | | | | |
| ALT (U/L) | 303 | 23 | 8–54 | 300 | 17 | 6–51 | 603 | 20 | 7–51 | <0.0001 |
| AST (U/L) | 300 | 30 | 17–60 | 288 | 23 | 13–48 | 588 | 26 | 14–51 | <0.0001 |
| ALP (U/L) | 293 | 178 | 101–353 | 302 | 155 | 82–293 | 595 | 160 | 85–241 | <0.0001 |
| Amylase (U/L) | 313 | 69 | 34–137 | 308 | 66 | 30–139 | 621 | 67 | 32–139 | 0.0089 |
| Creatine Kinase (U/L) | 302 | 249 | 93–786 | 288 | 165 | 58–476 | 590 | 190 | 66–532 | <0.0001 |
| GGT (U/L) | 302 | 21 | 9–71 | 298 | 16 | 6–53 | 600 | 19 | 7–61 | <0.0001 |
| LDH (U/L) | 275 | 421 | 274–745 | 248 | 389 | 214–688 | 523 | 406 | 223–723 | 0.0002 |
| **Serum Proteins** | | | | | | | | | |
| Protein, Total (g/L) | 296 | 72.5 | 46.7–86.4 | 282 | 73.4 | 55.2–86.9 | 578 | 72.9 | 50.6–86.7 | 0.1253 |
| Albumin (g/L) | 315 | 42.5 | 32.7–49.8 | 307 | 42.2 | 33.5–50.4 | 622 | 42.3 | 33.0–49.9 | 0.7558 |
| **Metabolism** | | | | | | | | | |
| Bilirubin, Total (μmol/L) | 310 | 11.3 | 3.8–32.0 | 305 | 9.4 | 2.7–26.6 | 605 | 9.9 | 2.9–25.8 | 0.0006 |
| Bilirubin, Direct (μmol/L) | 189 | 2.9 | 0.9–4.1 | 213 | 2.4 | 0.7–3.8 | 402 | 2.6 | 0.8–4.0 | <0.0001 |
| Cholesterol (mmol/L) | 315 | 3.2 | 1.8–5.0 | 307 | 3.5 | 2.1–5.6 | 622 | 3.3 | 2.0–5.4 | <0.0001 |
| Glucose (mmol/L) | 315 | 4.8 | 3.5–6.3 | 307 | 4.8 | 3.7–6.6 | 622 | 4.8 | 3.6–6.4 | 0.5790 |
| Iron (μmol/L) | 309 | 15.0 | 6.0–32.8 | 296 | 13.5 | 5.4–27.8 | 605 | 14.1 | 5.5–30.6 | 0.0004 |
| Triglycerides (mmol/L) | 314 | 0.9 | 0.4–2.2 | 305 | 0.9 | 0.4–2.1 | 619 | 0.9 | 0.4–2.2 | 0.1388 |
| **Kidney Function** | | | | | | | | | |
| Urea (mmol/L) | 315 | 2.7 | 0.9–6.2 | 307 | 2.5 | 0.9–5.4 | 622 | 2.5 | 0.9–5.7 | 0.0066 |
| Creatinine (μmol/L) | 312 | 85 | 56–119 | 303 | 74 | 47–110 | 615 | 80 | 49–118 | <0.0001 |
| Uric Acid (μmol/L) | 314 | 243 | 126–418 | 306 | 181 | 83–381 | 620 | 216 | 91–399 | <0.0001 |
| **Serum Electrolytes** | | | | | | | | | |
| Chloride (mmol/L) | 271 | 107 | 101–115 | 260 | 108 | 113 | 531 | 107 | 102–114 | 0.1609 |
| Phosphorus (mmol/L) | 315 | 1.1 | 0.7–1.5 | 307 | 1.1 | 0.8–1.5 | 622 | 1.1 | 0.7–1.5 | 0.0202 |
| Potassium (mmol/L) | 295 | 4.5 | 3.6–5.2 | 288 | 4.3 | 3.4–5.1 | 583 | 4.4 | 3.6–5.2 | <0.0001 |
| Sodium (mmol/L) | 276 | 144 | 135–151 | 265 | 145 | 135–150 | 541 | 144 | 135–150 | 0.1080 |

Med: Median; NA: Not available.

*Mann-Whitney test for differences between males and females.

doi:10.1371/journal.pone.0036308.t002
Written informed consent was obtained from each participant prior to involving them in the study.

Results

A total of 691 randomly selected adults made up of 351 males and 340 females between the ages of 18 and 59 years (mean = 37 years) were screened during the survey. Out of this, 625 individuals (316 males and 309 females) with a mean age of 36 years were enrolled. Tobacco use among all males screened was 64 (9.26%) and 34 (10.97%) respectively. Forty-three (6.2%) of the screened population were taking prescribed medication and were excluded from the study.

Reference Values

Median and 95% reference values (2.5th–97.5th percentiles) for Haematology and Biochemistry are shown in Tables 1 and 2, respectively. Males had significantly higher haemoglobin values of 113–164 against 88–144 g/L for females (p<0.0001), haematocrit of 33.2–50.5 against 26.4–45.0% (p<0.0001), compared to females. On the other hand, platelets were significantly higher in females with 89–403 against 88–352 ×10^9/L for males (p<0.0001), and/or enrolled was a smoker. Screened and enrolled males and females who take alcohol were 107 (9.26%) out of which 52 (8.3%) were among those enrolled. None of the females screened and/or enrolled was a smoker. Screened and enrolled males and females who take alcohol were 107 (9.26%) out of which 52 (8.3%) were among those enrolled. None of the females screened and/or enrolled was a smoker.

Table 3. Haematology out of range (OOR) values based on comparison with ABX values.

| Analyte        | Unit          | Males ABX Values | % OOR | Females ABX Values | % OOR |
|----------------|---------------|------------------|-------|-------------------|-------|
| Haemoglobin    | g/L           | 135–165          | 37.0  | 120–150           | 42.6  |
| Haematocrit    | %             | 41–50            | 41.6  | 37–45             | 53.1  |
| RBC            | ×10^12        | 4.37–5.63        | 26.6  | 3.9–5.10          | 27.7  |
| MCV            | fl            | 83–101           | 23.7  | 84–94             | 39.5  |
| MCH            | pg            | 26–34            | 14.3  | 27–34             | 25.5  |
| MCHC           | g/dL          | 32–35            | 26.4  | 32–35             | 31.8  |
| RDW            | %             | 12–16            | 13.6  | 12–14             | 43.7  |
| Platelets      | ×10^9/L       | 145–355          | 16.1  | 150–330           | 20.1  |
| PDW            | %             | NA               | NA    | NA                | NA    |
| WBC, Total     | ×10^9/L       | 4.7–9.6          | 26.1  | 4.9–12.3          | 38.9  |
| Lymphocytes    | ×10^9/L       | 23–47            | 22.9  | 19–41             | 56.3  |
| Monocytes      | %             | 3–6              | 95.9  | 2–6               | 90.1  |
| Monocytes      | ×10^9/L       | NA               | NA    | NA                | NA    |
| Granulocytes   | %             | 49–74            | 52.2  | 53–79             | 68.6  |
| Granulocytes   | ×10^9/L       | NA               | NA    | NA                | NA    |

Table 4. Biochemistry out of range (OOR) values based on comparison with values from Elitech reagent inserts.

| Analyte (Unit) | Males | Females | Elitech Values | % OOR | Elitech Values | % OOR |
|----------------|-------|---------|----------------|-------|----------------|-------|
| Enzymes        |       |         |                |       |                |       |
| ALT (U/L)      | 0–40  | 11.8    | 0–40           | 5.6   |                |       |
| AST (U/L)      | 0–46  | 14.9    | 0–46           | 5.2   |                |       |
| ALP (U/L)      | 0–270 | 16.6    | 0–240          | 10.8  |                |       |
| Amylase (U/L)  | 0–90  | 28.0    | 0–90           | 18.2  |                |       |
| Creatine Kinase (U/L) | 0–171 | 74.2 | 0–145 | 58.3 | |
| GGT (U/L)      | 0–55  | 11.4    | 0–38           | 4.9   |                |       |
| LDH (U/L)      | 235–470 | 36.6  | 235–470        | 27.6  |                |       |
| Serum Proteins |       |         |                |       |                |       |
| Protein, Total (g/L) | 60–78 | 33.8 | 60–78 | 31.2 | |
| Albumin (g/L)  | 35–52 | 4.8 | 35–52 | 5.5 | |
| Metabolism     |       |         |                |       |                |       |
| Bilirubin, Total (μmol/L) | 5–21 | 22.7 | 5–21 | 22.5 | |
| Bilirubin, Direct (μmol/L) | 0–4 | 2.1 | 0–4 | 0.5 | |
| Cholesterol (mmol/L) | 0–5 | 1.9 | 0–5 | 8.1 | |
| Glucose (mmol/L) | 4.0–6.0 | 15.3 | 4.0–6.0 | 12.0 | |
| Iron (μmol/L)  | 9.0–30.0 | 16.5 | 9.0–30.0 | 16.5 | |
| Triglycerides (mmol/L) | 0–1.7 | 6.4 | 0–1.7 | 6.2 | |
| Kidney Function |       |         |                |       |                |       |
| Urea (mmol/L)  | 2.0–7.0 | 25.0 | 2.0–7.0 | 31.6 | |
| Creatinine (μmol/L) | 71–115 | 21.6 | 53–106 | 8.9 | |
| Uric Acid (μmol/L) | 208–428 | 29.6 | 155–357 | 36.3 | |
| Serum Electrolytes |       |         |                |       |                |       |
| Chloride (mmol/L) | NA | NA | NA | NA | |
| Phosphorus (mmol/L) | 0.9–1.5 | 23.2 | 0.9–1.5 | 23.7 | |
| Potassium (mmol/L) | NA | NA | NA | NA | |
| Sodium (mmol/L)  | NA | NA | NA | NA | |

NA: Not available.

doi:10.1371/journal.pone.0036308.t003

doi:10.1371/journal.pone.0036308.t004
### Table 5. Comparison of adult haematological reference values obtain from this study against others.

| Analyte              | Unit | Present Study | Southern Ghana [1] | Kenya [16] | Eastern & Southern Africa [7] | Mbeya, Tanzania [20] | USA [14] |
|----------------------|------|---------------|---------------------|------------|-------------------------------|----------------------|---------|
| Haemoglobin (M)      | g/L  | 113–164       | 117–165             | 83–113     | 122–177                       | 137–177             | 135–175 |
| Haemoglobin (F)      | g/L  | 88–144        | 91–140              | 59–100     | 95–158                        | 111–157             | 120–160 |
| Haematocrit (M)      | %    | 33.2–50.5     | 37.1–51.4           | 40–50      | 35.0–50.8                     | 40.2–53.7           | 41.0–53.0 |
| Haematocrit (F)      | %    | 26.4–45.0     | 29.1–43.6           | 30–50      | 29.4–45.4                     | 36.2–46.8           | 36.0–46.0 |
| RBC (M)              | $10^{12}$/L | 3.79–5.96     | NA                  | 4.4–6.3    | 4.0–6.4                       | 4.41–6.27           | 4.5–5.9 |
| RBC (F)              | $10^{12}$/L | 3.09–5.30     | NA                  | 3.7–5.6    | 3.8–5.6                       | 3.84–5.59           | 4.0–5.2 |
| MCV                  | fl   | 72–97         | NA                  | 69–97      | 68–98                         | 78–98               | 80–100 |
| MCH                  | pg   | 22.6–33.5     | NA                  | 22.4–33.5  | NA                            | 23.6–33.1           | 26.0–34.0 |
| MCHC                 | g/dL | 30.5–36.2     | NA                  | 32.2–33.5  | NA                            | 30.6–34.9           | 31.0–37.0 |
| Platelets (M)        | $10^9$/L | 88–352        | 97–356              | 115–366    | 147–356                       | 150–350             | 150–350 |
| Platelets (F)        | $10^9$/L | 89–403        | 118–385             | 124–444    | 150–350                       | 151–425             | 150–350 |
| WBC, Total           | $10^9$/L | 3.4–9.2       | 3.4–8.8             | 2.8–8.4    | 3.1–9.1                       | 3.0–7.9             | 4.5–11.0 |

NA, Not Available.
doi:10.1371/journal.pone.0036308.t005

### Table 6. Comparison of adult biochemical reference values obtained from this study against others.

| Analyte              | Unit | Present Study | Southern Ghana [1] | Kenya [16] | Tanzania [20] | USA [14] |
|----------------------|------|---------------|---------------------|------------|---------------|---------|
| Sodium               | mmol/L | 135–150       | 138–146             | 141–153    | 134–143       | 136–145 |
| Potassium            | mmol/L | 3.6–5.2       | 3.1–4.6             | 3.9–5.8    | 3.8–5.5       | 3.5–5.0 |
| Chloride             | mmol/L | 102–114       | NA                  | 101–112    | 98–107        | 98–106  |
| Urea                 | mmol/L | 0.9–5.7       | 1.7–7.2             | 1.4–4.6    | 1.5–5.0       | 3.6–7.1 |
| Creatinine, M        | μmol/L | 56–119        | 81–141              | 62–106     | 48–96         | <133    |
| Creatinine, F        | μmol/L | 47–110        | 70–121              | 51–91      | 40–81         | <133    |
| ALT (M+F)            | U/L  | 7–51          | NA                  | 10–52      | 8–48          | 0–35    |
| ALT, M               | U/L  | 8–54          | 12–53               | 11–54      | 9–55          | 0–35    |
| ALT, F               | U/L  | 6–51          | 10–39               | 9–47       | 7–45          | 0–35    |
| AST, (M+F)           | U/L  | 14–51         | NA                  | 14–42      | 14–48         | 0–35    |
| AST, M               | U/L  | 17–60         | 19–65               | 15–45      | 15–53         | 0–35    |
| AST, F               | U/L  | 13–48         | 16–47               | 13–38      | 14–35         | 0–35    |
| ALP, (M+F)           | U/L  | 85–242        | NA                  | 46–158     | 30–120        |
| ALP, M               | U/L  | 101–353       | 124–479             | NA         | 45–170        | 30–120 |
| ALP, F               | U/L  | 82–293        | 98–316              | NA         | 45–155        | 30–120 |
| GGT                   | U/L  | 7–61          | NA                  | 8–108      | 1–94          |
| Bilirubin, Total     | μmol/L | 2.9–25.8      | 1.7–27.0            | 4.9–39.9   | 5.2–41.0      | 5.1–17.0 |
| Bilirubin, Direct    | μmol/L | 0.8–4.0       | 3.4–10.3            | 1.1–8.8    | 0.7–8.2       | 1.7–5.1 |
| Albumin              | g/dL | 33.0–49.9     | 46–68               | 35.8–48.1  | 35.6–50.4     | 35–55   |
| Total Protein        | g/dL | 51–87         | NA                  | 66–85      | 55–80         |
| Cholesterol          | mmol/L | 2.0–5.4       | NA                  | 2.6–5.7    | 2.5–5.5       | <5.17   |
| Triglyceride         | mmol/L | 0.4–2.2       | NA                  | 0.4–2.6    | 0.4–2.9       | <1.8    |
| Uric Acid (M)        | μmol/L | 123–418       | NA                  | 196–459    | 150–480       |
| Uric Acid (F)        | μmol/L | 83–381        | NA                  | 148–360    | 90–360        |
| Glucose (Fasting)    | mmol/L | 3.6–6.4       | NA                  | 3.1–5.7    | 2.9–5.2       | 4.2–6.4 |
| Phosphorus           | mmol/L | 0.73–1.45     | NA                  | 0.7–1.5    | 1.0–1.4       |
| Iron                 | μmol/L | 5.5–30.6      | NA                  | NA         | NA            | 0.9–27  |
| Amylase              | U/L  | 32–139        | NA                  | 38–163     | 43–164        | 60–180  |
| LDH                  | U/L  | 223–681       | NA                  | 126–264    | 127–264       | 100–190 |

NA, Not Available.
doi:10.1371/journal.pone.0036308.t006
show a comparison of haematology and biochemistry reference values established for Kintampo with values from other studies. Between-run precision for the haematology and clinical chemistry assays are presented in Tables 7 and 8, respectively. The methods used for the various clinical chemistry analytes are also shown in Table 8.

### Discussion

This study aimed at establishing haematological and biochemical reference values to serve as standards for the interpretation of laboratory results during screening and follow-ups in clinical trials and routine healthcare in the Kintampo area. The results obtained from the Kintampo area demonstrated that the red blood cell parameters (haemoglobin, haematocrit and RBC counts) were lower than values set as standards on the clinical haematology machines being used for clinical trials assessments in the study area. Such variations are expected for populations in different geographical/ecological locations; the recommendations of the manufacturers for each laboratory to establish its own reference values based upon the local population [12] has been proved beneficial. Values in the manual accompanying the haematology analyzer were defined using a population in New Jersey, USA. It is of interest to document also that the values obtained from our study were on most occasions far lower than those of other western industrialized countries [14,15]. Similar observations have been made in studies carried out in Mampong Akuapem in southern Ghana [1]; Kericho in Kenya [16]; in southern and eastern Africa [7]; in Saudi Arabia [17], Erzurum; in Turkey [18] as well as Pakistan [19]. These lower values for areas in sub-Saharan Africa have been attributed to factors such as poor nutritional status, genetic red blood cell disorders (such as sickle cell trait) or parasitic infections including schistosomiasis or malaria [7]. No differences were observed in both haemoglobin and haematocrit values obtained in this study and that of the populations of southern Ghana [1]. A similar survey in the northern part of Ghana will help inform the scientific community about how much generalization one can make using the Kintampo area study results. The haemoglobin levels were, however, higher in this survey than those obtained from other populations in Kericho, Kenya [16].

### Table 7. Analytical precision for haematology assays.

| Analyte                   | Between-Run Precision | ABX Precision |
|---------------------------|------------------------|---------------|
|                           | Mean  | SD   | CV (%) | Mean  | CV (%) |
| Haemoglobin               | 13.4  | 0.27 | 2.02   | 3.33  |
| Haematocrit               | 38.3  | 1.02 | 2.65   | 2.47  |
| Red Blood Cell Count (RBC)| 4.73  | 0.09 | 1.91   | 1.24  |
| Platelets                 | 281   | 17.75| 6.31   | 10.1  |
| White Blood Cell, Total (WBC)| 7.4   | 0.19 | 2.64   | 1.9   |

### Table 8. Methods and analytical precision for clinical chemistry assays.

| Analyte                   | Method                                      | Between-Run Precision | Producer’s Precision* |
|---------------------------|---------------------------------------------|------------------------|-----------------------|
|                           | Mean  | SD   | CV (%) | Mean  | CV (%) |
| Alanine Aminotransferase (ALT) | IFCC Modified without pyridoxal phosphate | 44.5                   | 1.5                   | 3.5    | 57     | 4.6    |
| Aspartate aminotransferase (AST) | IFCC Modified without pyridoxal phosphate | 49.5                   | 3.0                   | 6.0    | 46     | 6.3    |
| Alkaline Phosphatase (ALP)      | P-Nitrophenyl phosphate. Diethanolamine     | 160.3                  | 7.4                   | 4.6    | 33     | 5.1    |
| Amylase                     | 2-chloro-4-nitrophenyl-α-maltotrioside      | 56.3                   | 1.6                   | 2.8    | 92     | 2.5    |
| Creatine kinase, Total       | IFCC, UV Kinetic/Imidazole Buffer           | 149.4                  | 9.1                   | 6.1    | 49     | 4.4    |
| Gamma glutamyltransferase (GGT) | L-γ-Glutamyl-3-carboxy-p-nitroanilide       | 36.4                   | 1.4                   | 3.9    | 28     | 4.5    |
| Lactate dehydrogenase (LDH)   | UV kinetic (Pyruvate to Lactate)            | 309                    | 10.8                  | 3.5    | 333    | 3.9    |
| Protein, Total               | Bluret/endpoint                             | 66.7                   | 2.4                   | 3.7    | 38     | 4.7    |
| Albumin                     | Bromocresol green- Succinate Buffer         | 46.4                   | 1.3                   | 2.7    | 27     | 2.6    |
| Bilirubin, Total             | Malloy-Evelyn modified. End point           | 24.8                   | 0.9                   | 3.7    | 16.9   | 3.3    |
| Bilirubin, Direct            | Malloy-Evelyn modified. End point           | 10.1                   | 0.7                   | 7.2    | 10.9   | 3.5    |
| Cholesterol                  | Cholesterol Oxidase/Peroxidase              | 2.2                    | 0.1                   | 4.0    | 3.3    | 3.8    |
| Glucose                     | Glucose oxidase/peroxidase                  | 5.3                    | 0.2                   | 4.6    | 5.1    | 3.5    |
| Iron                       | Chromazurol                                 | 22.0                   | 1.1                   | 4.9    | 19.9   | 5.4    |
| Triglyceride                | Lipase/GX/GPO/Peroxidase/dye                | 1.1                    | 0.1                   | 4.3    | 1.3    | 4.3    |
| Urea                       | Enzymatic –UV Kinetic                      | 7.6                    | 0.4                   | 5.4    | 9.8    | 4.5    |
| Creatinine                  | Jaffé-Kinetic                              | 96.1                   | 3.8                   | 3.9    | 136    | 4.9    |
| Uric acid                   | Uricase/Peroxidase                          | 275                    | 12.5                  | 4.6    | 286    | 1.8    |
| Chloride                    | ISE, Direct                                | 107.4                  | 1.4                   | 1.3    | NP     | 2.0    |
| Phosphorus                  | Phosphomolydate formation                   | 1.3                    | 0.1                   | 4.2    | 1.44   | 2.8    |
| Potassium                   | ISE, Direct                                | 4.7                    | 0.1                   | 2.9    | NP     | 3.0    |
| Sodium                      | ISE, Direct                                | 148                    | 2.0                   | 1.3    | NP     | 2.0    |

SD = Standard Deviation; CV = Coefficient of variation; NP = Not provided.

*Chloride, Potassium and Sodium by Human Diagnostics, Germany; Others by Elitech Diagnostics, France.

doi:10.1371/journal.pone.0036308.t008
Significant gender differences were documented for the RBC parameters (haemoglobin, haematocrit and RBC), and this is consistent with an already-established knowledge that males have higher values for these parameters than females [6,15,18,20,21]. The reasons for these differences have been attributed to factors such as the influence of the androgen hormone on erythropoiesis and menstrual blood loss in females [16,21]. The demonstration of significantly higher platelet counts in females than males supports findings from previous studies [1,15,16]. Platelet and WBC values documented in this study were generally lower compared with the values in the instrument manual as well as values reported by Krata et al. [14] in the US and Wakeman et al. [15] in the UK. The values from this study were however similar to those reported in southern Ghana [1] and many other African countries [21,22,23,24]. The cause(s) of lower platelet counts in Africans is not known [6]; however, the lower platelet values in our studies could be due to genetic factors [25] or increased consumption of platelets as a result of malaria infection in our study areas [26].

The most commonly requested haematology parameters for screening/enrolment of participants and monitoring safety during clinical trials are haemoglobin, haematocrit, total WBC and platelet counts [27,28,29]. The proportion of OOR values for these four parameters ranged between 16.1% and 53%. This means based on the previous reference values used in the study area, up to 53% of potential study participants would have been declared as having abnormal results or enrolled participants would be reported as having adverse events (AEs). In the area of clinical management of patients, a patient requiring a particular treatment may be denied it whereas one who does not need treatment would end up being treated due to the use of inappropriate reference values. In other studies, the OOR values for these parameters were up to 29% [7] and 44% [20] when the locally derived values were compared with US data.

Findings of significantly higher values in males than females for the following biochemical parameters (ALT, AST, ALP, Bilirubin, CK, GGT, iron, creatinine, urea), and vice versa for uric acid is generally supported by other studies [1,16,20] as shown in Table 6. The urea levels were low when compared to the values from the reagent inserts of Elitech Diagnostics and the US values [14]. However the results from this study were comparable to those from other African countries [16,20] and from Saudi nationals [17].

Biochemical tests commonly used during screening/enrollment and safety monitoring of trial participants in the Kintampo study area are ALT, AST, Bilirubin (Total and Direct), Urea and Creatinine. The proportion of OOR values for these parameters was up to 32% in our study, compared to up to 42% in Kenya [7] and up to 81% in Tanzania [20]. The concern is about the levels of disqualification from screening/enrolment into clinical trials and mis-interpretations of AEs. Using the western values, we will report laboratory AEs in essentially normal volunteers, with the potential to ruin a trial (based on AEs) where there is no problem as such. Similar findings of higher ALT and AST values have been reported in south India [30]. Although the definite cause of higher liver enzymes in our population is unknown, there is the possibility of this being due to subclinical viral infections or the levels of usage of herbal preparations as discussed in earlier publications [1].

Screening for Hepatitis viruses was not performed in this study. However, published data on prevalence of these viruses among Ghanaian blood donors is 7 to 15% for Hepatitis B virus [31,32] and 7 to 11% for Hepatitis C virus [31]. On the use of herbal preparations, it has been estimated that the first line treatment for 60% of children with fever resulting from malaria in Ghana, Mali, Nigeria and Zambia is the use of herbal medicine at home [33].

Between-run test measures a method’s overall precision as it measures the random error inherent in the method from day to day. It takes into account variable factors such as changes in reagents, operators and ambient operating conditions. Between-run precision for haemoglobin and platelets were within precision limits quoted in the analyser manual. Although the CVs for the other analytes were higher (i.e. haematocrit 2.65 against 2.47%, RBC 1.91 against 2.14% and WBC 2.64 against 1.9%), they were within the Clinical Laboratory Improvement Act (CLIA) acceptable test performance criteria [34]. These 5 parameters are presented for haematology because they are the measured analytes from which the others are derived. Majority of the clinical chemistry tests were also within the precision limits indicated by the reagent producers. The precision of all the analytes were within the CLIA acceptable performance criteria limits. This precision data supports the reliability of the reference values established by this study.

**Conclusion**

The reference values developed for the Kintampo study area will be of immense benefit to most clinical trials requiring monitoring of haematological and biochemical parameters and patient care in general. Compared to other references, the reference values for haemoglobin, haematocrit, red blood cell counts and urea are lower in the Kintampo study area.

**Acknowledgments**

The authors would like to thank the community members of the Kintampo North Municipality and South District for volunteering to participate in this study; staff of the Kintampo Health Research Centre who supported the field work including logistics acquisition, especially Drs Ruth Owusu and Stephen Apana for clinical support, Kofi Tchum for laboratory support, Elizabeth Asini and Samuel Danso for data management and analysis; Ghana Health Service and the Noguchi Memorial Institute for Medical Research.

**Author Contributions**

Conceived and designed the experiments: DKD KPA KOK JO KAK SOA. Performed the experiments: DKD KK DAG. Analyzed the data: DKD SAE EM. Wrote the paper: DKD KPA KOK SOA. Data collection: DKA KK PB KOK EK. Revised and approved final version of manuscript: DKD KK DAG EK JO KOK EM SAE PB KPA KAK SOA.

**References**

1. Koram K, Adiakae M, Orjan J, Ado-Amankwah S, Rogers W, et al. (2007) Population based reference intervals for common blood haematological and biochemical parameters in the Ashanti north region. Ghana Med J 41: 160–166.
2. Buchanan AM, Murom TJ, Gratz J, Crippa JA, Mustyka AM, et al. (2010) Establishment of haematological and immunological reference values for healthy Tanzanian children in Kilimanjaro Region. Trop Med Int Health 15: 1011–1021.
3. Horn PS, Pesce AJ (2002) Effect of ethnicity on reference intervals. Clin Chem 48: 1802–1804.
4. El-Hazmi MAF, Warsy AS (2001) Normal reference values for the haematological parameters, red cell indices, HbA2 and Hb F from early childhood through adolescence in Saudi Arabia. Ann Saudi Med 21: 165–169.
5. Adeyita IM, Hill PC, Jeffries D, Jackson-Sillah D, Banga HB, et al. (2009) Haematological values from a Gambian cohort–possible reference range for a West African population. Int J Lab Hematol 31: 515–622.
6. Laguda EK, Mermin J, Kahiruza F, Uwasted E, Were W, et al. (2004) Population-based hematologic and immunologic reference values for a healthy Ugandan population. Clin Diag Lab Immunol 11: 29–34.
