ORIGINAL ARTICLES

POPULATION BASED REFERENCE INTERVALS FOR COMMON BLOOD HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN THE AKUAPEM NORTH DISTRICT

*K.A. KORAM, M.M. ADDAE[2], J.C. OCRAN, S. ADU-AMANKWAH, W.O. ROGERS[1] and F. K. NKRUMAH

Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, P. O. Box LG 581, Legon, Ghana, [1]US Naval Medical Research Unit (NAMRU) #3, Cairo, Egypt, and [2]School of Allied Health Sciences, College of Health Sciences, University of Ghana, Legon

SUMMARY
Objectives: To estimate the reference intervals for commonly used blood haematology and biochemical parameters in an adult (18-55yrs) population of residents of Mampong Akuapem.

Design: This was a population based cross sectional study of a randomly selected sample of the adult population of Mampong. The sample was selected from an updated census list of the Mampong area.

Results: Median values (95% range) for measured parameters were established as follows: Haemoglobin, (males) 14.2 g/dl (females) 12.0 g/dl Alanine aminotransferase (ALT), (female) 19.6 U/L (males) 26.1 U/L and Creatinine, (males) 108 mmol/L (females) 93 mmol/L.

Conclusion: In comparison to reference values that are commonly used in Ghana, the haemoglobulin levels from this study were lower, and liver function parameters higher. This could be a result of genetic or environmental differences and calls for the need to establish site specific reference values applicable to our population

Keywords: Reference ranges, blood chemistry, blood haematology, Ghana.

INTRODUCTION
Reference values (or normal values) for haematological and biochemical parameters are used to aid physicians to interpret results of clinical measurements. They may also be used in clinical trials as a guide to setting inclusion / exclusion criteria as well as the basis of safety monitoring for trial participants. Reference values for African populations are not readily available and the values used in Ghana are usually based on results of measurements in advanced countries, taken from the literature (of advanced countries) or from package inserts that accompany reagent kits. However, these parameters even in the healthy state are affected by several factors including age, ethnicity[1, 2] gender and environment (including altitude).[3] The few studies that have been undertaken have indicated differences in normal values of African populations, even in children and adolescents, compared to those derived from industrialized populations, especially for haematologic indices.[3] These differences may suggest the need for the development of locally derived reference values (for these parameters) to improve clinical care and also for monitoring participants in clinical trials. In establishing reference values, it is essential that the population is well defined and properly selected to be representative of that population, as the lower and upper limits of measurements are known to be more affected by the choice of the sample population, standardization of the sample collection and handling and analysis and   also the statistical analysis[4,5]. Several novel malaria vaccine candidates that have been tested in non endemic populations are entering clinical trials in endemic countries to assess safety as part of the clinical development of these products.

In Ghana, the Noguchi Memorial Institute for Medical Research (NMIMR), with support from National Institute of Allergy and Infectious Disease (NIAID) in the USA, has over the past 5 years been developing potential sites for malaria vaccine trials and it is envisaged that we will be conducting some of these trials in the near future. Prior to initiating any trial, it is imperative to establish the normal range of values for the laboratory indices that will be used to screen potential volunteers to determine if they meet the inclusion / exclusion criteria as well as assess vaccine safety in those volunteers who receive the test product. The Noguchi Memorial Institute for Medical Research is currently

Author for correspondence
KKoram@noguchi.mimcom.net

involved in preparatory studies to gather background data needed for the efficient conduct of malaria vaccine trials. We report here the results of one such study undertaken to establish the distribution of values for specific haematological and biochemical parameters in the adult population of Mampong Akuapem, a site intended for a phase I malaria vaccine trial.

MATERIALS AND METHODS
Study Site
Mampong town is located in the Akuapem North district in the Eastern Region of Ghana about 35 km north east of Accra. It lies on the Akuapem-Togo mountains range, with an elevation ranging between 381m and 487.7 m above sea level. The spoken language in Mampong is Twi, the major occupation is farming, although a substantial proportion of individuals work in the district offices, hospital and also the several schools in the district. Health services are provided by the Tetteh Quarshie Memorial Hospital, the Centre for Scientific research into Plant Medicine, and also by some private clinics and traditional healers. Malaria transmission occurs year round, and was the number one cause of hospital admission in the district for 2002[6]. It is also the leading cause of out-patient attendance at the Tetteh Quarshie Memorial Hospital[7].

The total population of Mampong is 7,733 residents in 1957 households giving an average household size of 4 persons. Females formed a slightly higher proportion of the population (54.55 %) and the population 18years and above comprise approximately 50%. We sampled from approximately 3500 individuals aged between 18 and 55 years, resident in 5 out of 10 enumeration areas chosen to represent the various sectors of the town. The list was obtained from an updated census listing prepared by the Ghana Statistical Service. Selected individuals were approached to obtain consent to participate in the study after a thorough explanation of the study had been presented to them. Those agreeing to participate were then enrolled into the study.

Study Design
This was a cross sectional study of a representative sample of the adult population of Mampong to determine reference ranges of common haematological and biochemical parameters. According to the United States National Consensus Committee on Laboratory Standards, NCCLS Guidance Document C28A2, for most analytes the lower and upper reference limits are assumed to demarcate the estimated 2.5[th] and 97.5[th] percentiles of the underlying distribution of values.[4, 8] For general standard practice, to estimate these limits with 95 % confidence a minimum sample size of 120 from each sample group (e.g. Males and Females) is

recommended, assuming the use of the nonparametric method of analysis. This minimum sample size also allows the estimation of 90% confidence interval for each reference limits. This number assumes no losses or deletion of observations, so to allow for 10 % losses, the targeted total sample size was 264 volunteers (132 males, 132 females).

Volunteer recruitment and Sampling strategy:
Previous contact with the population in Mampong facilitated the informed consent process. Recruitment efforts included discussions with opinion leaders in the community and a durbar to educate the population about the objectives of the study.

The primary aim was to get a sample that represented the target population; and to achieve that a two-stage sampling method was employed. The sampling frame was the updated list of Enumeration Areas (EA's) in the Mampong area that was used for the 2000 national population census by the Ghana Statistical Services[9]. Mampong town was divided into 3 broad areas, the northern part (around the hospital), the central part encompassing the centre of the town and the southern part. At the first stage a random sample of 5 EA's was chosen from the sampling frame. An updated listing of all households in the selected EA's was then compiled. Subsequently the list of individuals in each household was updated and those between 18years and 55 years were invited to join the survey. Each individual was approached and asked if s/he would like to participate in the study and enrolment continued until at least 264 participants (132 females and 132 males) had been sampled. An *a priori* sampling method was used where individuals had to meet well-defined exclusion/inclusion criteria before being selected as a referent individual.

Consenting individuals were screened with a history and physical examination to rule out any obvious intercurrent disease. Females had a urine dipstick examination for the presence of β-HCG hormone. Participants were included in the study only if they were aged between 18 and 55 years, generally of good health with no evidence of a chronic or acute illness on examination, resident in Mampong and agreed to take part as evidenced by the signing of the consent form. Females had to be non pregnant as evidenced by the absence of β-HCG hormone in a freshly voided urine sample. Those clinically assessed to be healthy were then asked to donate a sample of venous blood (maximum of 10mls) and a sample of urine. The urine was tested on site and the blood sample was collected in EDTA and Serum separator tubes, transported to NMIMR in cool boxes for haematological and biochemical analysis. The haematological analyses were

performed within 4 hours of blood draw. Serum was separated from the blood after centrifugation and stored between 2^0C and 8^0C before analysis, which was completed within 24 hours of blood draw.

Laboratory assays
The following assays were performed on each blood specimen: The haematological assays were performed using the automated haematology analyser KX-21, (Sysmex Coporation, Kobe, Japan) and these included: WBC and differential counts, MCV, MCH and MCHC, RBC, Haemoglobin concentration , Haematocrit and Platelet count.

The following biochemical parameters were determined using the autohumalyser 900S Plus (Human GmbH, Diagnostica Worldwide, Germany): liver enzymes including Alanine aminotransferase, Aspartate aminotransferase (AST), Serum Bilirubin (total and direct) and indicators of renal function including, Urea and Creatinine and serum electrolytes (K^+ and Na^+) concentrations. In addition all samples were screened for Haemoglobin S (HbS) by electrophoresis and thick and thin blood smears made and examined for the presence of malaria parasites by microscopy. All the assays were performed according to Standard Operating Procedures (SOPs) written and maintained in the clinical laboratory of NMIMR.

Data Management and Analysis
Data entry and analysis were done using Epi Info 2002 (CDC) and STATA 8.0 (Stata Corp, College Station, Tx). This is a descriptive study and reference intervals were determined using the methods described in the NCCLS guidelines (NCCLS 1992)[4]. This assumes no prior distribution of the data. Non-parametric methods were used to determine the 2.5^{th} and 97.5^{th} centiles and the respective 90% confidence limits around these estimates using the binomial approximation. The under-

lying assumption is that the 2.5^{th} and 97.5^{th} centiles will contain 95% of the distributions of normal values in the reference population. Extreme values were handled by the method described in the NCCLS guidelines by doing a range check and the methods of Dixon (Dixon's Test)[4]. Briefly, the extreme values were retained in the distribution if the D/R < 0.33 where D is the absolute difference between the most extreme distribution and the next value and R is the Range (max – min).

The reference intervals were calculated separately for men and women for analytes that were expected to be distributed differently between males and females on physiological grounds, or where there is a statistically significant difference between the mean values for males and females, and the difference is clinically significant[4,10].

Ethics
This was a minimal risk study and was conducted in accordance with the protocol and Good Clinical Practices to ensure protection of all aspects of the ethical rights and welfare of research participants. The study followed the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH Topic E 6 Guideline for Good Clinical Practice. The study was approved by the Institutional Review Board of Noguchi Memorial Institute, the IRB of NMRC and the Study Review Committee of DMID /NIAID.

RESULTS
Background characteristics
A total of 400 individuals aged between 18 and 55 years were randomly selected from the sampling frame and were invited to take part in the study. Of these 356 (89%) consented to take part.

Table 1a Reference haematological and biochemical intervals for males

	N	Median	2.5th percentile (90% CI)	97.5th percentile (90% CI)
Hematology				
-Haemoglobin (g/dL)	142	14.2	11.7 (11.2, 12.3)	16.5 (16.3, 18.0)
-Haematrocrit (%)	142	44.1	37.1 (34.3, 38.0)	51.4 (49.9, 59.0)
-Platelet (x10^9/L)	142	209	97 (57, 130)	356 (318, 469)
Liver Function				
-Alkaline Phosphatase (U/L)	140	193.6	123.6 (106.1,128.7)	478.9 (379.0, 536.1)
-ALT (U/L)	141	24.3	11.6 (9.9, 12.3)	53.1 (45.8, 65.1)
-AST (U/L)	139	31.3	18.7 (15.5, 20.4)	65.0 (54.2, 92.2)
Renal Function				
- Creatinine (mmol/L)	142	105.4	81.4 (79.0, 84.5)	141.2 (135.5, 147.9)

The major reason given for non participation was travel out of the study area at the intended time of blood sampling (in more than 85% of those not being able to participate).

(14.0, 14.4) (Figure 1b) than in females 12.0g/dL (11.8, 12.2). The median Hb concentration for males was 14.2g/dL and the corresponding reference interval 11.7 – 16.5 g/dL.

Table 1b Reference haematological and biochemical intervals for females

	N	Median	2.5th centile (90% CI)	97.5th centile (90% CI)
Haematology				
- Haemoglobin (g/dL)	155	12.0	9.1 (7.4, 10.2)	14.0 (13.6, 14.4)
- Haematrocrit (%)	155	37.7	29.1 (25.5, 32.4)	43.6 (42.8, 45.2)
- Platelet (x10^9/L)	155	233	118 (82, 138)	385 (359, 629)
Liver Function				
- Alkaline Phosphatase (U/L)	154	171.8	98.2 (92.2, 104.5)	316.4(294.0, 376.9)
- ALT (U/L)	152	18.0	9.5 (6.7, 10.2)	39.2 (35.2, 44.4)
- AST (U/L)	152	25.7	15.5 (14.8, 17.6)	46.5 (37.0, 80.3)
Renal Function				
- Creatinine (mmol/L)	154	91.0	69.8 (68.9, 74.9)	120.5 (115.3, 139.6)

Of the 356 individuals, 313 (87.9%) were seen at the appointed time and of these 298 (95.2%) qualified to participate.   Of the 15 volunteers disqualified, 7 (46.7%) were disqualified because they were pregnant (positive for β-HCG hormone), 3 each (20%) for hypertensive heart disease and being outside the age range and 2 (13.3%) because someone else had signed their consent forms for them.

The resultant study population comprised 156 females (52.3%) and 142 males (47.7%) with the females slightly older [mean age (s.d.) = 34.3 (10.7) yrs] than the males [mean age (s.d.) = 31.8 (11.4) yrs].   The prevalence of sickle cell trait (AS) and HbC trait (AC) was 20.1 % and 6.8 % respectively, with no significant difference between males and females. Prevalence of sub-clinical *P. falciparum* parasitaemia was 8.7%.
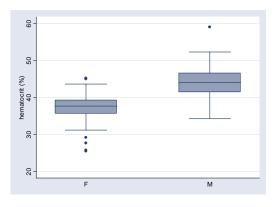
Urinalysis (by dipstick)
Analysis of freshly voided urine showed that the urine appeared cloudy more frequently in females (24.4%) compared to males (6.4%), [p<0.05]. Protein was detected in approximately 20% of study participants, all positive results being in trace quantities only. Traces of blood were detected in approximately 10% of individuals. Although the frequency of occurrence was higher in females (12.9%) than in males (6.3%) the difference was not statistically significant. Glucose was detected in trace quantities in less than 2% of study participants.

Haematological Parameters
Box plots for haemoglobin, haematocrit and platelet counts by gender are shown in Figs 1a-1c. As expected, mean (95% CI) haemoglobin (Hb) concentrations were significantly higher in males, 14.2 g/dL

For females the corresponding values were median 12.0g/dL and reference interval, 9.1 – 14.0 g/dL (Tables 1a and 1b).
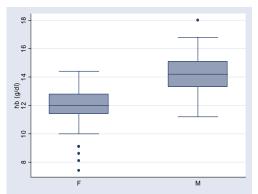
Mean corpuscular volume (MCV), Mean corpuscular heamoglobin concentration (MCHC) and Red blood cell (RBC) all differed by gender. Platelet counts were higher in females, mean (95%CI) = 239× 10^3 /μl (227 × 10^3, 250 × 10^3) than in males, mean (95% CI) = 213 × 10^3 /μl (203 × 10^3, 223 × 10^3) (Figure 1c).

Means (95% CI) for Females and Males = 37.5 (36.9, 38.0) and 44.0 (43.4, 44.7) respectively, p = 0.001
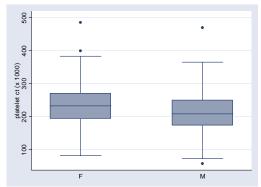**Figure 1a** Distribution of Haematocrit

The reference interval for Platelet count were reported as 118-385 × 10^3, median 233× 10^3 (female)(Table 1b) ; 97-356× 10^3, median 208× 10^3 (male) (Table 1a). The White Blood Cell (WBC) count was not significantly different for males and females. The median WBC count was estimated to be 5.6x10^9/L with a reference interval of 3.4 – 8.8x10^9/L. (Table 2)

Means (95% CI) for Females and Males = 12.0 (11.8, 12.2) and 14.2 (14.0, 14.4) respectively, p = 0.0001

Figure 1b Distribution of Hb Concentration

Means (95% CI) for Females and Males = 239 (227, 250) and 213 (203, 223) respectively, p = 0.0012
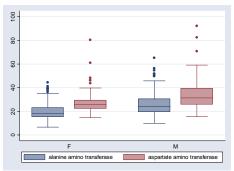
Figure 1c Distribution of Platelet count

Biochemical parameters

The concentrations of the liver enzymes, ALT, AST and Alkaline Phosphatase and serum Creatinine were significantly higher in males than in females and based on this analysis, separate reference intervals for males and females were reported for these analytes.

Mean (95% CI) concentration of ALT in females was 19.6 IU/L (18.5, 20.7) compared to 26.1 IU/L (24.4, 27.8) in males, (Fig. 2a) the reference values for ALT were 11.6 – 53.1 U/L median = 24.3 U/L (male) (Table 1a), 9.5 – 39.2 U/L, median = 18.0 U/L (female)

The mean (95% CI) concentration of AST for females was 26.8 (25.5, 28.1) compared to 34.2 (32.2, 36.2) for males (figure 2a). The reference values for AST were (male) 18.7 – 65.0U/L median = 31.3U/L (Table 1a) and (female) 15.5 – 46.5 U/L, median = 25.7 U/L (Table 1b).
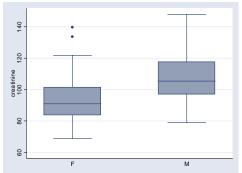
For serum Creatinine, the respective mean concentrations in males and females were 108 U/L (105, 110) and 93 U/L (91, 95) (Fig. 2b).

Means (95% CI) for Females and Males; ALT = 19.6 (18.5, 20.7) U/L and 26.1 (24.4, 27.8) U/L respectively, p = 0.001. AST, = 26.8 (25.5, 28.1) U/L and 34.2 (32.2, 36.2) U/L respectively, p= 0.0001
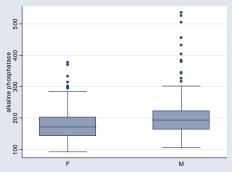
Figure 2a Distribution of ALT and AST

The median Creatinine concentrations for males and females respectively were 105mmol/L and 91mmol/L and the respective reference intervals were 81 – 141mmol/L and 70 – 121mmol/L (for males and females) (Tables 1a and 1b).

Means (95% CI) for Females and Males = 93 (91, 95) U/L and 108 (105, 110) U/L respectively, p = 0.001

Figure 2b Distribution of serum creatinine

The mean (95% CI) concentration for Alkaline Phosphatase was 180 (171, 188) and 210 (197, 223) respectively for females and males (Fig. 2c). The reference interval for Alkaline Phosphatase was; (male) 124 – 479 U/L, median = 194 U/L (Table 1a) and (female) 98 – 316 U/L, median = 172 U/L (Table 1b).

Means (95% CI) for Females and Males = 180 (171, 188) U/L and 210 (197, 223) U/L respectively; p = 0.0002

Figure 2c Distribution of Serum Alkaline Phosphatase

Table 2 Combined reference haematological and biochemical intervals for indices with similar distribution between male and female

	N	Median	2.5th centile (90% CI)	97.5th centile (90% CI)
Haematology				
-WBC (x10^9/L)	297	5.6	3.4 (3.0, 3.7)	8.8 (8.4, 9.4)
-Lymphocytes (%)	297	48.2	27.5 (22.5, 31.7)	66.5 (64.5, 68.7)
-Mixed cells (%)	293	10.2	4.2 (3.6, 5.1)	25.3 (21.1, 28.3)
-Neutrophils (%)	297	40.5	24.4 (21.8, 26.0)	60.7 (58.9, 70.2)
Liver Function				
Direct Bilirubin (mg/dL)	297	0.2	0	0.6 (0.5, 0.7)
Total Bilirubin (mg/dL)	291	0.4	0.1 (0.1, 0.1)	1.4 (1.1, 1.9)
Albumin (g/dL)	297	5.6	4.6 (4.0, 4.7)	6.8 (6.5, 6.9)
Urea & Electrolytes				
Blood Urea (mmol/L)	294	4.4	1.7 (1.3, 2.0)	7.2 (6.9, 8.2)
Potassium (mmol/L)	291	3.8	3.1 (3.0, 3.2)	4.6 (4.5, 5.0)
Sodium (mmol/L)	291	141	138.0 (137, 138)	146 (145, 146)

The distribution of Bilirubin, Albumin and Urea did not differ significantly between males and females. Mean (95% CI) Total Bilirubin concentration was 0.5 mg/dL (0.4, 0.5), median = 0.4mg/dL, reference interval 0.1–1.4mg/dL (Table 2). Mean (95% CI) for Serum Albumin was 5.6g/dL (5.6, 5.7), median = 5.6g/dL, reference interval 4.6 – 6.8g/dL (Table 2). Mean (95% CI) Urea was 4.4 mmol/L (4.2, 4.6), median = 4.4mmol/L, reference interval 1.7 – 7.2mmol/L (Table 2).

DISCUSSION

The results of our survey indicate there are significant differences in the reference values for males and females in the following parameters of the adult population of Mampong; hemoglobin, heamatocrit, platelet, Alkaline phosphatase, Alanine transaminase (ALT), Aspartate transaminase (AST) and Creatinine (Tables 3a and 3b).

The gender differences seen in heamatologic indices is a well established fact and has been similarly reported in other studies, men having a higher hemoglobin and heamatocrit level compared to women.[3,11,12,13]

Females had a higher platelet count compared to men, comparable to a study which looked at ethnic and sex differences in WBC and platelet counts[12,14]. Based on the results from this study, the haemoglobin level for a healthy male adult living in Mampong should be between 11.7 – 16.5 dl/g and that for a female, 9.1-14.0 dl/g.

These values are lower than the reference values quoted in the accompanying manual of the heamatology analyzer used in the study, meaning a percentage of these adults could be misclassified as having a low

haemoglobin level using the reference values that are often quoted. Evidence from other studies have shown that haematological indices are significantly lower in populations of African origin as compared to standard reference values quoted in the literature for industrialized countries, often applicable to Caucasians[3,11]. This difference could be due to environmental or genetic factors or a combination of both or to several other factors. Such differences indicate the need to develop reference values that are appropriate for the applicable population.

The transaminases, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are usually used as markers of hepatocyte injury and therefore are usually tracked in vaccine or drug trials for evidence of hepatic damage resulting from the drug or vaccine. They also are used to identify acute or persistent hepatic damage resulting from hepatitis. ALT and AST all showed a significant difference in the reference values for male and females, and the range was wider, unlike the often quoted values that is the same for males and females. The upper limits for the reference values for the liver function tests were higher than the quoted values. This finding is similar to that of a study in an Urban South Indian community where gender differences were seen in the distribution of ALT and AST. The upper limit for AST was found to be up to 44 U/L in men and up to 39 U/L for women, as opposed to up to 37 U/L used in the lab. That for ALT was 86 U/L for men and 75 U/L for women compared to 65 U/L used in the laboratory.[15] Wide variations are known to occur in the transaminases and differences are also known to occur between males and females between whites and non whites. The reasons for these differences are not known and it is possible that in our part of the world, occult hepatic insults from sub clini-

cal viral infections or the usage of herbal preparations may contribute to the differences seen between the values reported here and those in standard clinical texts. This has important implications for vaccine and other drug trials because using the quoted reference values without comparing it to reference intervals applicable to that population, volunteers could have been classified as having some liver dysfunction when indeed they may have a normal liver function for that population. This could lead to anxiety over volunteer laboratory results and unduly high reportage of adverse events among other things.

Diet, physical environment and socio-economic conditions all affect the physiology of a population, and hence measures of 'normal' physiological functions are expected to differ from population to population. It is obvious from this study that there is the need to establish reference values that are applicable to specific populations rather than take a set of reference values determined for one population and apply it to another population , especially so for clinical trials.

The reference values obtained from this study will guide us in screening and monitoring volunteers during the malaria vaccine clinical trials in Mampong. These values can also be embraced as guidelines in clinical management of patients at the Tetteh Quarshie Memorial Hospital in Mampong.

ACKNOWLEDGEMENT

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