ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCES
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ESTABLISHMENT OF HEMATOLOGICAL PARAMETERS REFERENCE INTERVALS FOR APPARENTLY HEALTHY ADOLESCENTS IN ASELLA TOWN, SOUTHEAST ETHIOPIA.

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This is to certify that the thesis prepared by **Berhanu Dibaba**, entitled: ***Establishment of hematological parameters reference intervals for apparently healthy adolescents in Asella town, South East Ethiopia*** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations

AAU	Addis Ababa University
BMI	Body mass index
CI	Confidence interval
CLSI	Clinical and Laboratory Standards Institute
DC	Direct current
EDTA	Ethylene diamine tetra-acetic acid
HBsAg	Hepatitis B virus surface antigen
HCV Abs	Hepatitis C virus antibodies
ISO	International Organization for Standardization
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MXD	Mixed value
PDW	Platelets distribution width
P-LCR	Platelet larger cell ratio
RDWCV	Red blood cell distribution width by coefficient of variation
RDWSD	Red blood cell distribution width by standard deviation
RI	Reference interval
SLS	Sodium lauryl sulfate
SOP	Standard Operating Procedure
SPSS	Statistical Package of Social Sciences
WHO	World Health organization

Abstract

Background: -Physicians' medical decisions are based on information provided by laboratory reports in the form of a reference interval (RI) or medical decision limit. The interval serves as a health-related standard with which to compare an individual test result. However, the lack of bringing up as its own standard, local reference values has been a problem facing hematological practice in our country.

Objective: To establish hematological parameters reference intervals for apparently healthy adolescents (12-17) in Asella town, South East Ethiopia from January to March 2020 GC.

Method: A total of 342 apparently healthy adolescents aged 12–17 years living in Asella town and fulfilling the eligibility criteria were recruited for this cross-sectional descriptive study using a systematic random sampling method. Data including socio-demographic characteristics were collected using a structured questionnaire. The hematological analysis was performed using Sysmex KX-21 3 Diff analyzer. Data was entered and analyzed using SPSS version 21. The non-parametric Mann-Whitney u test was used to compare the parameters between genders. The 97.5th percentile and 2.5th percentile as the upper and Lower Reference limit in favor of the adolescent population were determined.

Result: The study observed statistically significant mean differences between males and females in Hemoglobin (HGB), Hematocrit (HCT), Mean cell hemoglobin concentration (MCHC), Mean Cell volume (MCV), absolute lymphocyte number (LYM#), Red Blood Cell (RBC), and Red blood cell distribution width by the coefficient of variation (RDWCV). Whereas the mean values of other Hematological parameters like WBC PLT and RWDSD have no significant difference between both sex (p value>0.05). Hence the reference interval of White Blood Cells (WBC), Platelet (PLT) and Red blood cell distribution width by the standard deviation (RWDSD) in adolescents were (3.410. 9x103/ul), (153.9-390x103/ul) and (36.8-47.1fl) respectively. The current established reference intervals show higher proportions out of range values when compared to the existing reference intervals.

Conclusion: Some hematological parameter showed a significant difference in the mean values among data sets for HGB, RBC, MCV and MCHC across gender (p value<0.05) in which females having lower levels than males. The newly established hematological parameter reference intervals by this study were different from the existing reference values.

Keywords: *adolescent, Hematological parameter, reference interval*

1. Introduction

1.1 Background

Approximately 80% of Physicians' medical decisions are based on information provided by laboratory reports. However, a test result by itself is of little value, unless it is reported with the appropriate information for its interpretation. Typically, this information is provided in the form of a reference interval (RI) or medical decision limit (1). Reference interval (RI) is an interval indicate the population serviced by the laboratory correctly contains most of the subjects with comparable characteristics to the reference group and excludes the others as defined by the Ceriotti (1). The intervals are derived from a healthy population. The limiting values being a sign of a specified percentage especially central 95% of values from a healthy reference population with 90% confidence can define reference intervals Statistically (2,3).

Calculating the 2.5^{th} And 97.5^{th} percentiles of test results in the central 95% distribution model are used to determine the reference limits. So, the interval where 95% of test results within healthy individuals occur are typically defined the reference intervals and used to interpret laboratory test results and defines the common range of values from healthy individuals. Reference intervals are of great relevance as a guide in the clinical management of patients (4,5).

Published papers by the International Federation of Clinical Chemistry (IFCC) recommend that each laboratory follow defined procedures to produce its reference values and produce the theory of reference interval from 1987 to 1991. According to this publication's recommendation, every country must establish RIs for the healthy population (6-8). Also, the Clinical and Laboratory Standards Institute (CLSI) provides guidelines for verifying and establishing reference intervals and recommends that RIs should be produced for each region and specific populations.

According to this guideline, the direct approach or traditional approach is used to establishing reference intervals. In this process, individuals from a population (the reference population) are selected for sampling based on defined criteria (9). Furthermore, the ISO 15189:2012 requires that reference interval should be reviewed at regular intervals and also when there is any change in laboratory technology(10). Unfortunately, laboratories do not follow these recommendations because of their difficulty, large time consumption, and expensiveness (11). Several years ago Caucasian populations have established their hematological reference interval (12).

Most currently used hematological reference values in Africa are originated from data acquired on populations living in European countries (13). However, many authors recently tried to develop hematological parameters reference values for African countries, even though different related factors such as age, altitude, geographic origin, ethnic origin and sex, are some differences between studies to another. The few studies with African populations show differences in normal values when compared with populations in developed countries(11).

In Ethiopia, there is no nationally established RI for hematological parameters even if there are heterogeneous population and diverse geographic variability in the country, even though in some populations few attempts have been made to determine hematological parameters RI (14,15). Moreover, currently adopted hematological parameters RIs are from textbooks that are not representative of the populations (16,17).

Childhood and adolescence are characterized by a dynamic period of development and growth in an individual, which need special hematological consideration for several reasons. For instance, hemopoietic (red) marrow occupies the entire capacity of the bones at birth through adolescence whereas in old age there is increased replacement with fatty marrow(18). This may affect the hematological parameters and result in different reference ranges for different age groups. Thus, knowledge of the normal blood values through this dynamic period of growth and development in an individual is required for correct interpretation of a particular disease condition.

The hematological parameter reference interval derived locally may be favorable or advantageous for producing a desired interpretation and subsequently for improving the health care quality. The reference interval serves as a health-related standard with which to compare an individual test result (19). Besides, it prevents unneeded treatment, follow-up investigations, and mismanagement of patients and also keeps out selection bias and misclassification of adverse events in clinical trials (20,21). Therefore, this study was aimed at establishing normal reference values of hematological parameters for healthy adolescents in the Arsi zone, Asella town southeast Ethiopia. Findings from this study will provide reference values for clinicians to serve as a guide in deciding the health status during clinical assessments in screening for diseases, assessing disease treatment and progression response, particularly for pediatric hematologists.

1.2 Statement of the problem

In Africa normal ranges of Hematological parameters are adopted from data gathered form populations living in Europe and North America which are available in textbooks or guidelines provided by the laboratory test kit manufacturers and equipment. These values are not exactly alike to the reference interval for populations in the disease endemic trial area (18-20). Hematological parameter reference intervals currently adopted from textbooks in Ethiopia are referring mainly to the Caucasian subjects (22). Thus, non-Ethiopian reference values might be the misleading interpretation of the laboratory results needed for appropriate patient's diagnosis, follow-up, and treatment correctly (14). However, this is fulfilled by the knowledge of normal reference intervals that have been established in local settings due to different climate, socioeconomic status, living style, and genetic makeup than using values from other western countries or other areas of the same country (23).

Similar reference intervals used in interpreting test results for both adolescent and adults. But adolescent have significant differences in physiology and metabolic state, physical size, organ maturity, bodily fluid compartments, and immune and hormone responsiveness when compared to adults. Also, there is disease susceptibility difference such that some diseases prevail more in children than adults. Hence, separate reference intervals may be inevitable for children of distinct age groups and/or genders (16). Other factors influence hematological reference intervals including age, sex, altitudes, genetics, intense exercise, and other socio-demographic variables (6).

Absence of suitable locally derived reference interval is a challenge in interpreting results for decision-making and other patient management, whereas the use of proper reference interval may increase the risk of unneeded additional investigations, failure to discover underlying disease and patient mismanagement (23). Since information is rare concerning reference intervals of the hematological parameter for adolescence in the laboratory, this study was carried out to set up hematological reference ranges in apparently healthy Adolescence in Arsi zone, Asella town which is located at southeast Ethiopia by following the procedures desirable by the IFCC, CLSI guidelines in 2008 (24).

1.3 Significance of the study

Estimating Hematological reference values from apparently healthy adolescent is essential for accurate interpretation of hematological test results for that population, due to various physiological and environmental factors such as age, sex, exercise, altitude, genetic background and dietary pattern that affect the hematological values but there is the dearth of comprehensive reference values for adolescent in Ethiopia.

In general use, hematologic results values established with adult populations are used to interpret adolescent hematological parameter test results. Therefore, establishing their RIs used the laboratory for accreditation. Interpreting by appropriate RIs helps reducing workload due to repeating falsely high or low flags comparing to manufacturer kit result interpretation. It also helps physicians to decide confidently on the result interpreted and delivered from the laboratory.

Similarly, Asella town adolescent age groups get representative hematological parameter reference intervals for correct interpretation of a particular disease condition and subsequently community gets improved health care quality. In general, since there is a shortage of information regarding normal hematological parameter reference values for Ethiopian adolescence this study can serve as the baseline for another person with an interest like researchers, and policymakers.

2. Literature review

2.1 Reference intervals concept

The CLSI/IFCC, C28-A3 document explains in detail on how to choose Reference individuals. This reference sample group formed from reference individuals for the measurement of the values from the reference population (8, 25). The reference limits obtained from reference distribution calculated through statistical analysis define as the RIs, usually 95% interval to mark out the specific subject. RIs can be determined using direct methods based on a healthy population ideally (26). The reference population the upper limits estimate as 97.5th percentile whereas lower limits roughly calculated as 2.5th percentile of the test results distribution. During the calculation of RIs from healthy people, 5% flagged as abnormal of all results and fallout of the reference interval reported. There is the presumption that some observed values after mathematical transformation follow the Gaussian distribution or "normal" probability distribution in the parametric calculation method (6).

C28-A3 recommends the non-parametric RIs calculation method, but recent IFCC, C-RIDL study compared the RIs calculated by the parametric and non parametric methods and concluded that results of the two methods are very close. Thus, parametric methods can also be used as a first choice(27). Outlier detection and exclusion have great significance to acquire reliable RI. Outlier were determined by visual inspection data and evaluate as Dixon proposed by the D/R method. D: the absolute value of the difference between the possible outlier and continue value, R: the observations entire range. If the D/R ratio is greater than 1/3, the outlier is discarding able. However, the D/R method is not quick to detect when there is more than one outlier (28). The Horn using the Tukey method is a highly complex method, which includes Box-Cox transformation of the data to secure Gaussian distribution by recognizing the outlier in inter quartile ranges (IQR: Q3-Q1; Q3: upper quartile, Q1: lower quartile,). At levels of < Q1 - 1.5 IQR and / or > Q3 + 1.5 IQR, discard the outlier (29,30).

Grouping children into 12–17 years of age groups (male and female) was performed based on similar studies before and recommendations to establish sex-specific reference values during the pubertal stage (31). Results from the Questionnaire can be used in the posterior or priori sampling approach. When a posteriori sampling is used, exclusion criteria are applied after the sampling

whereas in prior sampling is used exclusion criteria applied before collecting the sample. But, Both of these methods are known as direct sampling which is the advised method for acquiring information on healthy subjects (30,32).

2.2 Reference interval studies

Minor studies carry out on hematologic parameters in Africa over the last period of ten years have bright differences from those of Caucasian populations (31-34). Similar to Some studies conducted in Asian countries indicated lower values compared with those from populations in North American or European countries (23,35). Due to many reasons, African countries RIs indicated different values from those western countries or quoted in the accompanying manual (20). The values show significantly higher WBC count, Neutrophil counts, PLT counts, HCT, MCHC, MCH, and HGB and significantly lower mononuclear and lymphocyte percentage Compared to black communities (33).

In Ghana a study using the existing RI reported a fallacy in using previous RI as it is conflicting to the locally generated one, pointing that based on the reference interval existing in the study area up to 53% of potential study participants would have been announced as having deviating results from normal or having unfavorable events since locally derived values have lower RI(33).The Ugandan children reported values for PLT counts 237 (126 - 376/uL), Hgb10.8 (8.8 - 12.5g/dl), HCT 31.8 (25.9 - 36.3%), and MCV 72.9 (60.7 - 82.8fL) was less than the conventional RI of Caucasian children150 - 400/uL, 12.0 - 18.0g/dl, 36.0 - 54.0%,76 - 96fL, respectively (20).

A study conducted on reference interval for childhood in Ghana middle belt on Hematologic Parameters(19)revealed that Red blood cell parameters (HGB, HCT, RBC count) are on the lower side when compared with cut-off values used by the WHO, which is synonymous with numerous studies that achieve permanent acceptance of hematological parameter reference interval for the child in Africa, like Tanzania (31) and Uganda (35).

According to these studies, adolescent males have higher values than females for the indicated parameters. Comparison of hemoglobin concentration reference values for children in Kintampo, Ghana were (5-12 years old 9.1-13.5g/dL,13-17years old Male10.4-14.8g/dL,13-17 years old female 9.4-14.2 g/dL), and Tanzania (5-12 years old 10.3-14.7 g/dL 13-17 years old Male 10.8-17.0 g/dL, 13-17 years old female g/dL 10.0-14.9 g/dL), Uganda (5-12 years old 10.0-13.7 g/dL,

13-17 years old Male 11.2-15.9 g/dL, 13-17 years old female 9.9-14.5 g/dL). These values were lower than developed countries or the reference intervals recommended by WHO (lower limits 5-12 years old 11.5 g/dL, 13-17 years old Male 13.0 g/dL, and 13-17 years old female 12.0 g/dl(19).

The study conducted on children and adolescence in Nigeria (Port Harcourt) observed some differences in hematological parameters reference range between African and Caucasian population, most notably in HGB, HCT, PLT, WBC count, and Neutrophil value. Among the adolescent ages between the 14 and 17 years, Nigerians had a lower reference limit with a WBC count of 3.0×10^9/L which is comparatively lower than those established in the European reference range but does not suggest leucopenia (36).

A study recruiting Tanzanian children and adolescent in Kilimanjaro Region from December 2006 through March 2008 showed that children aged 13 to 17 years had the following hematologic reference interval for HGB male (10.8–17.0g⁄ dl), Female (10.0–14.9g⁄ dl), HCT male (33.0–48.1%), female (30.8–44.7%), PLT count male (119–458x10^9⁄ l) and Female (107–482x10^9⁄ l). These values showed some variation compared to the Ugandan children which had HGB male (11.2–15.9g⁄dl), female (9.9–14.5g⁄dl), HCT male (32.3–45.5%), female (28.1–42.4%), PLT count (110–327x10^9⁄ l), female (124–353x10^9⁄ l). But, European reference values HGB male 13–16g⁄ dl, female 12–16g⁄dl, HCT male 37–49%, female 36–46%, and PLT count 150–400x10^9⁄ l for both sex was higher than Ugandan and Tanzanian children hematological reference intervals (20, 31).Moreover, the median and 95% RI of, HGB for these age groups were 14.1 g/dl (12.0-19.6 g/dl) for males and 14.0 g/dl (11.5-15.9g/dl) for females as indicated by a study conducted in children from southwest Ethiopia(37)were also higher than a report from Tanzania but lower than those of developed countries (31).

A typical study performed between January and September 2002 in a rural parish in eastern Uganda revealed significant differences in hematologic parameters by age and gender (35). RBC counts, HGB, HCT, levels, and MCV were increased with age until age 13 years whereas values were significantly higher among male adolescents than females. Significant differences by gender were not detected for any of the indices for children younger than 12 years of age. On the other hand, WBC reference interval: (median: 8.9, 5.9 - 14.3× 10^3/uL) for Iganga children were higher compared to the Caucasian normal reference value (4.5 - 11.0×10^3/uL) (35).

2.3 Factor affecting reference intervals

A study in western Kenya detected lower HGB, HCT, RBC, mean cell volume (MCV), Neutrophil, but elevated Eosinophil compared with the U.S derived reference ranges underscoring for determining population-specific local RI. A study from Zimbabwe has shown that textbook RI established from American and European populations had a minimal resemblance to the Zimbabwean population (38).There are significant variations in genetic makeup, diet, and environmental factors, which make the need for appropriate local reference intervals to facilitate patient/participant evaluation and management is imperative (34).

Factors such as age, sex, nutritional status, and environmental factors, especially altitude and pathogens, affect hematological parameter reference intervals. Various studies have been done on children and adolescents of different ages, and significant differences have been reported in different population 's gender subgroups (39,40). For most African countries hematological indices were lower than those established from the North American population. A significant variation was noticed among gender in the hematological parameter. An American study targeting African Americans and Non-Hispanic whites report mean HGB intervals lower in females than in males but no significant differences were noted for WBC, HCT, and MCV, an observation which is at variance with the Kenyan study (34).

However, statistically significant sex differences were noticed from 14 years in which the levels were lower in girls than boys. Earlier reports among Ugandan(35) and Saudi children (41) and Japanese (42) children are in agreement with this report. Kenyan (32)study showed significant differences in RBC, HGB, and HCT by gender, with females having lower values than males which are similar to Hematological study in Ghana (33).

A study in Zimbabwe revealed no difference between men and women until the age of 14 in platelet count but, subsequently, men had steadily fewer platelets than women. The number of PLT becomes lower quickly in childhood, becomes stable in adulthood and when further compared with early infancy it decreases with aging by 35% in men and 25% in women. But, in adolescents, under 15 years of age, there was no variation in platelet count of male and female 298 vs. 299x10^9/L, female adolescent median white blood cell 3.31 x10^9⁄l, red blood Cell 4.16 x10^{12}⁄l and PLT count was 214 x10^9⁄l (38).

Females lower limit (2.5th percentile) with 90% CI are white blood cell 3.31 (3.02 - 3.74cells/mm^3), red blood Cell 4.16 (3.98 - 4.26x 10^6/µL), PLT count 214 (183- 223) and 97.5th percentile (90% CI) for white blood cell count 9.84 (9.20 - 10.7cells/mm^3), red blood Cell 5.83 (5.53 - 6.18x 106/µL), PLT count median 476 (438 - 561) respectively. Whereas for male the 2.5th percentile (90% CI) were: white blood cell 3.25 (2.95 - 3.48), red blood cell 4.47 (4.36 - 4.58x 10^6/µL), PLT count median186 (168 - 204x 10^3/µL) 97.5th percentile (90% CI) 8.64 (7.55 - 9.91 x10^3/µL), red blood cell 6.47 (6.22 - 6.60x 10^6/µL) PLT count 415 (388 - 631x10^3/µL (38).

In the same way, studies in Port Harcourt Nigerian adolescents on determining reference value for hematological parameters observed several differences when compared to previously adopted reference values from Caucasians. similar to what has been reported from other studies from East and West Africa, particularly the variations of parameters such as comparison of mean and 95th percentile reference ranges of WBC (× 10^9/L) male 5.4 ±2.6 (2.9–9.0) female5.6 ±2.3 (3.0–9.9), Hgb(g/dl) male13.5±1.1(13.0–16.0) and female12.9±1.2(12.0–15.0) HCT(%) male 41.0±2.1 (40.0–49.0) and 39.0 ±2.1 (37.0–47.0) female, PLT (× 10^9/L) male 269 ±98 (98–427) female 285 ±111(100–443) were seen across gender (36).

Investigation of hematologic parameters in a number of studies shows significant gender differences in RBC parameters (RBC, HGB, HCT, and MCV) which are consistent with previously established evidence that men have higher values than females (23,37). Significantly PLT ranges were contrary lower in males as compared to females while no significant differences were reported in other parameters (47).

Just as the immune system produces and full-grown with age (16) different hematological parameters change with age (43). Adolescents and the older male population had significantly higher values for RBC, HGB, and HCT than young males (34). A cohort study involving 8089 individuals show exerting relation that RDW, MCV increase extending upwards from the entire age range. In accordance with convention, RDW increased by 6%, MCV increase by 6.6% and RDW-SD increased by just about 15%, in age class from youngest to oldest (44). Similarly, studies in the Saudi and Ugandan children reported that HGB, HCT, MCV, and MCH levels increased significantly while WBC decreased, MCHC remained constant from age 2 to 15 years whereas some values were similar to Caucasians. But others have values that are intermediate between African children and Caucasians according to age (40,45).

A cross-sectional study conducted in southwest Ethiopia on children revealed that the reference range of RBC, WBC, and PLT count was (4.26-5.99 × 10^{12}/L), (4.00-11.67 × 10^9/L), and (188.00-463.50×10^9/L) respectively, which showed significant differences across all age groups. Differences in lymphocyte among age groups were noted and suggested the need to develop age-specific reference ranges (37). The red cells and hemoglobin content gradually rise to adult levels by the age of puberty (46).

The environmental factors like altitude also influenced hematological parameters value. Due to descending in barometric pressure with the instance of increasing altitude, unique stress at high altitude is hypobaric hypoxia and consequently fewer oxygen molecules in a breath of air as compared with sea level (47). In children (boys and girls) living at 5500, HGB increases by 11% when compared with children living at 4355m above sea level, and in adults, HGB increases by 9.6% for the same altitude (48).

Nutrient requirements differ with age, sex, and physiological condition as measured by body mass index (BMI). BMI ‘s advantage is that weight and height are easily and accurately measured. Variations of hematological parameters between severely undernourished (BMI<16) and adequately nourished (BMI≥16) were recorded. The PLT count was found to be significantly higher in the undernourished subjects as compared to the adequately nourished counterparts (49).

3. Objectives

3.1 General Objective

To establish hematological parameters reference intervals for apparently healthy adolescents aged 12-17 years in Asella town, South East Ethiopia from January to March 2020 GC.

3.2 Specific Objectives

To determine sex-specific hematological parameters reference interval for apparently healthy adolescents in Asella town, South East Ethiopia from January to March 2020 GC.

To compare hematological parameters reference intervals of this study with currently available in Clinical use in Asella town, South East Ethiopia from January to March 2020 GC.

4. Hypotheses

There is no significant difference between reference intervals of commonly used hematological parameters for apparently healthy adolescents (12-17yrs) versus currently applicable reference value in the laboratory setting.

5. Materials and methods

5.1 Study area

The study was carryout at Asella town found in the Arsi zone Oromia Region about 175 kilometers from capital city Addis Ababa. This city has a latitude and longitude of 7°57′N 39°7′E, with an elevation of 2,430 meters above sea level. According to the 2007 Ethiopian census report, Asella has a total population of 101,739, and almost half of them 51,159 (50.5%) are males. There are 23,215 households under 8 kebeles. The majority of the inhabitant, (67.43%) are followers of Ethiopian orthodox Christianity, while 22.65% population were Muslim and around 8.75% population were protestant (50).The health care coverage of this town were One governmental and two private Hospital, two health center and more than fifteen private clinics available in Asella town.

5.2 Study design and Period

A cross-sectional descriptive study design was employed from January to March 2020 GC in Asella town to establish the reference intervals of hematological parameters for apparently healthy adolescent's resident in Asella town, South East Ethiopia.

5.3 Population

5.3.1 Source population

All adolescent individuals who live in Asella town were the source population.

5.3.2 Study population

The study population for this study was selected apparently healthy individuals from adolescent's age between 12-17 years who live in Asella town; those who fulfill the eligibility criteria at the time of the study period were the study population.

5.4 Eligibility criteria

5.4.1 Inclusion criteria

The inclusion criteria were being residents of Asella town, apparently healthy, 12-17 years age individuals give assents and get their Parent to provide informed consent by completing written consent forms to participate in the study voluntarily and also those residing in Asella town for 5years.

5.4.2 Exclusion criteria

The following criteria were used to exclude participants from the study:

Those with detectable blood-borne infections such as HIV, syphilis, or hepatitis B and C and positive for intestinal parasites.

A participant with interview has evidence of fever with any history of chronic or acute illnesses such as hypertension, diabetes mellitus, bleeding, and bleeding disorders.

Females who were menstruating, pregnant, or lactating at the time of the study

Those who were taking medications for any medical condition

Those who were their body temperature are greater than 37.5 degree centigrade.

5.5 Study Variables

5.5.1 Dependent variables

Reference range of hematological parameters

5.5.2 Independent variables

Sex, Age, Nutrition status

5.6 Measurement and Data collection

5.6.1 Sample size determination

Clinical Laboratory Standards Institute (CLSI) guidelines recommended that the best way to establish an RI is to assemble samples from an adequate number of the competent reference subject to establish a minimum of 120 individual for analysis, by the nonparametric method, for each partition, so the least 120 individuals are the smallest amount sample size required to determine 90% confidence intervals for the 95th percentile reference limits (2.5th and 97.5th percentiles) (51). However, in proportion to existing large-scale studies in other African countries, about 30% did not make competent for RI determination for various reasons when tested for common viral infections and inflammation(52). Based on this finding171 individual were enrolled in each partition of adolescent age 12-17 Years male and female. Thus, a total of 342 individuals participated in the study.

5.6.2 Sampling method

A systematic random sampling method was employed to recruit a total of 342 apparently healthy adolescents aged between12- 17 years. The 3 kebeles were selected from 8 kebeles and the total population of selected kebeles was divided by sample size. K was obtained by dividing the total households from the sample size. The number between 1 to k^{th} was randomly selected and continues sampling by (A+K) ^{the} house where A was randomly selected number of houses of the starting point. The selected kebeles house number were already adjusted which taken from kebeles data and in those eligible participants were not found in the target house the second house number were used to pick study individual.

$$K=9653/342=28$$

Therefore, the sample size was distributed to kebeles (from Welkessa 2611 households 92 participants, Burkitu 3470 households 123 participants and Hunde Gudina 3572 households 127 participants) total population by probability proportionate to size.

5.7 Data collection and test procedures

Data were assembled using a structured questionnaire on Socio-demographic characteristics (age and sex) and nutritional status of the study subjects. Measurements like mid-upper arm circumference (MUAC), weight, height, and blood pressure were carried out. Three milliliters of blood samples were collected by venipuncture using either the antecubital vein or the dorsal vein with a vacutainer system (Becton Dickinson Biosciences) into EDTA anticoagulant tubes for hematological parameter and five milliliters to serum separating tube for screening, HBsAg, HIV, HCV, and syphilis as well as clinical chemistry profile (for a separate study). Stool and urine samples were collected in leak-proof clean containers, and labeled with a unique identification number and transported at a suitable temperature to Asella referral and teaching hospital for analysis. Screening for HBsAg, HCV, HIV and syphilis using rapid tests as well as stool examination, Urinalysis, pregnancy tests for females and blood film examination were carried out following standard operating procedures (SOPs).

5.8 Principles of hematological analysis

The Sysmex KX-21N CBC analyzer (Sysmex Corporation Kobe, Japan) handles approximately 60 samples per hour and allows us to specify Seventeen parameters in two independent measurement methods. These are the impedance method for determining the WBC, RBC, and PLT data and the colorimetric method for determining the HGB. This instrument automatically dilutes whole-blood sample of 50μL in the CBC/3-part differential mode, lyses, count and enumerates a printout result of absolute numbers of WBC(10^3/μL), RBC(10^6/μL), PLT(10^3/μL), LYM (10^3/μL, NEU(10^3/μL), HGB(g/dL), HCT (%), MCV (fl), MCH (pg), MCHC (g/dL), RDW -SD (%), RDW-CV (%), PDW (%), MPV(fL), P-LCR and the percentage of the LYM, the NEU and that of MXD cell consisted of MON, BAS and EOS(53).

5.8.1 Impedance and colorimetric method Principle

The aspirated whole blood is measured to predetermined volume and diluted at a specific ratio and is passed gradually through a confined space of transducer called the aperture. On both sides of the aperture, there is an electrode between which flows DC current is applied that produce Electrical resistance or impedance. As the cells pass through the aperture and this change in voltage generates a pulse. The amplitude and size of the pulse depend on the cell volume and the number of pulses is proportional to the number of cells counted whereas the volume or size of the cell determined from the voltage pulse size. Hemoglobin is measured using the Cyanide Free hemoglobin sodium lauryl sulfate (SLS) method in the hemoglobin flow cell; hemoglobin is oxidized and binds to sodium lauryl sulfate forming a stable SLS-hemoglobin complex, which is measured photo metrically at 555NM.

5.9 Data quality assurance

The questionnaire was translating from English to Amharic and Afaan Oromo version by the well experienced linguistic person. Pre-testing of the questionnaires was done on 5% of individuals in the selected kebeles. The data collector were two laboratory technologist and one health extension worker and Training for data collectors was given by the investigator and the consistency of the data was also checked.

Pre analytical phase

The pre-analytical feature must be taken into careful thought in the implementation of a RI study include biological and methodological factors. The biological factors include sampling time relating to biological rhythms and physical activity. Whereas methodological factors put into consideration sample collection techniques with a tourniquet, the 70% alcohol used to rub during sample collection to reduce contamination, sampling equipment, type of additives, specimen handling, transportation, time and speed of centrifugation, and storage conditions. The anthropometrical measurements qualities were maintained by managing the additional things that increase or reduce falsely the measurements. For reproducibility and standardization, it is extremely important that the pre-analytical feature is accurately described and defined as the pre-analytical phase is known to have the highest errors in the total test process (51). Thus, blood samples were collected between 8:00 - 11:00 am from study participants. All procedures complied with SOPs.

Analytical phase

Known three-level whole-blood controls were run in parallel with specimen tests to assure satisfactory accuracy and precision. Reproducibility and reliability level of the test was run within company precision limit in another way in the event that the daily commercial controls or in-house prepared controls failed, testing was suspended until evaluated. All measurements were performed the same day, within 4hrs of collection. -80^{o}C has been used to store the serum sample until analyzed.

Post analytical phase

The result interpretation, archiving and specimen retention after the analytical phase was handled as a laboratory quality management system. SOP s was followed at all phases.

5.10 Data analysis and interpretation

Data analysis was performed using the computer software SPSS version 21. Standard deviation Mean, and median, was calculated for each hematological parameter and the 95th percentile reference ranges determined by using 2.5th and the 97.5th percentiles for lower reference limit and upper reference limit using the Mann Whitney U test. The upper and lower limits RI with 90% confidence interval were also calculated. The outlier had been removed using an inter quartile and visual data inspection.

5.11 Ethical considerations

The study protocol was officially accepted as satisfactory by the departmental research and ethics committee of the Addis Ababa University Health Science Medical Laboratory Sciences Department. A support letter was written by the Health Research and Postgraduate director's office of Arsi University to the concerned body including woreda health bureau and kebeles to obtain permission. Participants provided written informed assent and consent from parents or guardians before the study procedure start.

All specimens were labeled with a unique study identification to analyze only for the intended purposes. Study participants were informed about the aim of the study, how confidentiality is maintained, and their right to leave from the study anytime they want. All measurements were done by hospital laboratory staff members those are trained and fully licensed to run investigation then participants got their results for free and those their result deviating from normal find were advised through the health extension workers and linked to Asella referral and teaching hospital for treatment.

5.12 Dissemination of Result

The study result will be presented to the Addis Ababa University, Health Sciences College, Medical Laboratory Science Department and then disseminated to the Federal Ministry of Health, Oromia regional Health Bureau, Arsi University, Arsi zone health bureau, Asella town administration, and other concerned bodies. Moreover, the information will be presented in different national and international scientific seminars and workshops. A manuscript will also be prepared and submitted to peer-reviewed journals for possible publication. The data will be applicable in local studies and clinical care that is an improvement over the current practice of using reference values derived from elsewhere.

5.13 Operational Definitions

Adolescent: A young child who is in the process of developing from child to adult aged between 12-17 years old.

Hematological parameters were WBC differential, WBC, RBC, PLT, HGB, HCT, RBC indices, RDW, PDW, MPV, and P-LCR

Reference population: the population from where individuals are selected for sampling based on defined criteria.

Direct sampling: Selection of results from a reference population by predetermined criteria, which are independent of the measured of interest.

Reference interval: Reference interval is the interval between, and including, two reference limits (2.5^{th} and 97.5^{th} percentile for apparently healthy individuals.

5.14 workflow

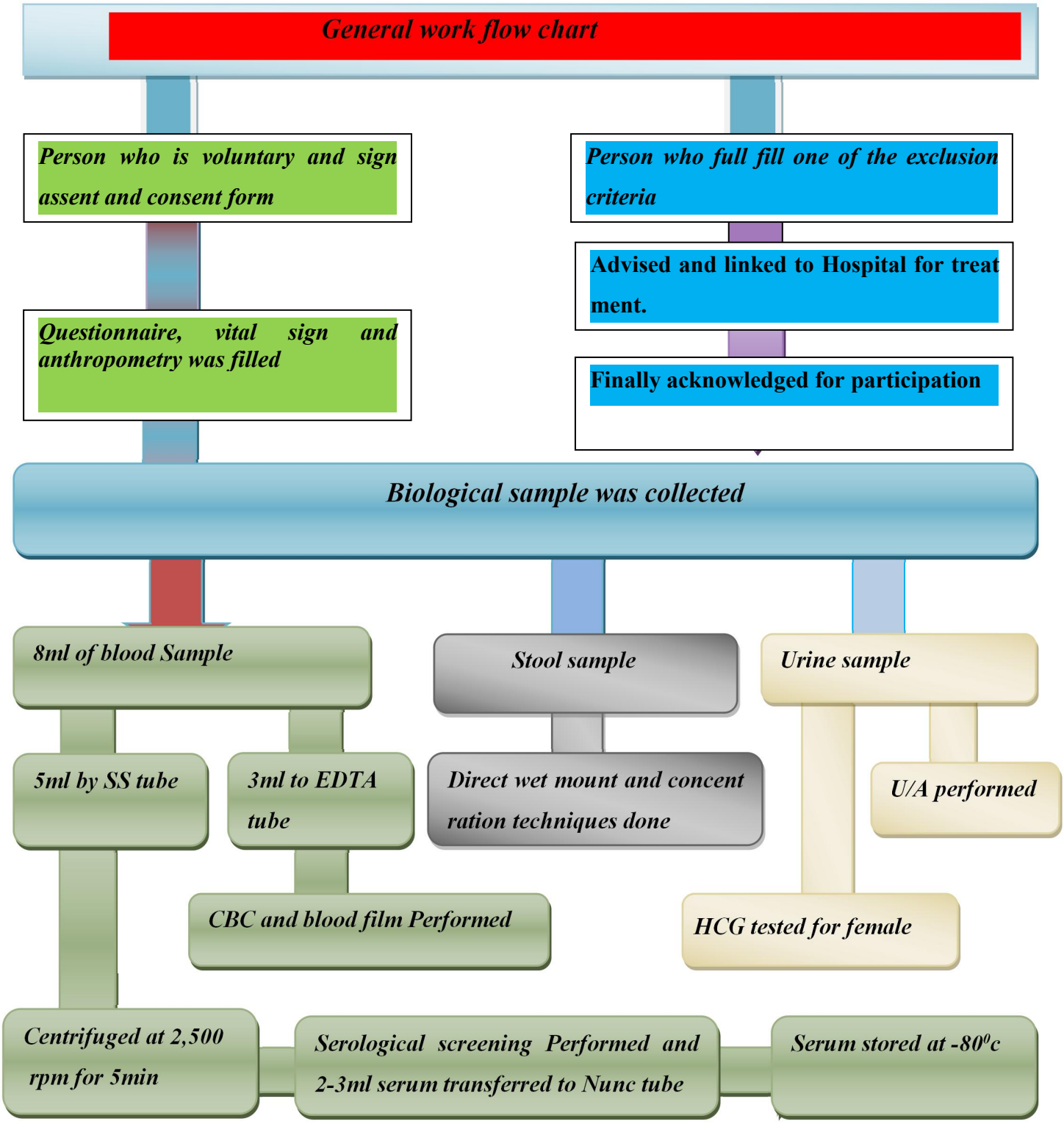

Figure 1 General work flow charts.

6. Results

From total of three hundred forty-two (342) volunteers participated through the study period to establish hematological parameter reference interval for adolescents in Asella town, about, 17.8% (61/342) individual were excluded from the study due to 8.5% (29/342) study participant was drug intake for any illness in the last three months, 4.38% (15/342) individual was respondent history of common disease such as hospital admission 1.75%(6/342), chronic gastric 1.75% (6/342), Allergy 0.3% (1/342) and bleeding disorder 0.6% (2/342) and 4.97% (17/342) individual due to positive fo r screening such as HIV 0.3%(1/342), HCV 0.3%(1/342), HBsAg 1.2%(4/342), fecal examination 1.5%(5/342) and Urinalysis 1.75% (6/342), but hemoparasite examination from smear and HCG test for a female age 15 and above did not have positive finding. As a result, they were excluded from the study and linked to Hospital for treatment.

The remaining 281 volunteer's data were analyzed. Fifty-point two percent 50.2% (141/281) male and 49.8% (140/281) females were included in the analysis of hematological parameters for reference interval establishment. Then test dependent outlier exclusion was done by visual data inspection and interquartile. Finally, sample size for each parameter varies as indicated on table 3.

6.1 Sociodemographic and behavioral characteristics

The mean and median age of apparently healthy adolescents constituted in this study population were 14.4 and 15 years respectively. The age range of the participants was 12-17 years. Of the total, 49.5% of the participants were less than 15 years old. In terms of their educational status 76.9.1% (216/281) of the study participants were on primary education level while 1.8% (5/281) was illiterate. The majority of these study participants were orthodox Christian followers and almost all of them reported no smoking and alcohol use. The study participant's Socio demographic characteristics are pointed in Table 1.

Table 1:Socio demographic characteristics of Asella town adolescents from January to March 2020.

Variables	Category	Frequency	percentage
Age	12-14	139	49.5%
	15-17	142	50.5%
Sex	Female	140	49.8%
	Male	141	50.2%
Educational status	Illiterate	5	1.8%
	Primary (1-8)	216	76.9%
	Secondary (9-12)	60	21.4%
Religion	Orthodox Christian	157	55.9%
	Muslim	86	30.6%
	Protestant	34	12.1%
	Catholic	3	1.1%
	Others	1	0.4%
Ethnicity	Oromo	196	69.8%
	Amhara	62	22.1%
	Gurage	6	2.1%
	Tigre	3	1.1%
	Silxe	2	0.7%
	Sidama	1	0.4%
	Mixed	11	3.9%

6.2 Nutritional habit of the study participant

As shown in Table 2, the study participants often consume cereals 62%, Legumes 45.9% and vegetables 57.3% more than once per day and, 64.4% take Tea and coffee, 42.7%. Roots like potato, enset, cassava were taking Once per day. Whereas 73.4% of study participant take in meat, 56.6% fruit, and 58.7% egg occasionally. However, 10.3% of study participants have never consumed milk, and Egg. Similarly, 5.8% of respondents did not have physical exercise habits.

Table 2: Asella town adolescent's nutritional habit from January to March 2020.

Variables	More than once/day	Once/day	Occasional	Never
Cereals	174 (62.0%)	103 (36.6%)	4 (1.4%)	0
Legumes	129 (45.9%)	130 (46.3%)	20 (7.1%)	2 (0.7%)
Root and tubes	117 (41.7%)	120 (42.7%)	42 (14.9%)	2 (0.7%)
vegetables	161 (57.3%)	59 (21.3%)	57 (20.3%)	3 (1.1%)
Fruits	95 (33.8%)	8 (2.8%)	159 (56.6%)	19 (6.8%)
Meat	38 (13.5%)	20 (7.1%)	206 (73.4%)	17 (6.0%)
Milk and product	77 (27.4%)	62 (22.1%)	113 (40.2%)	29 (10.3%)
Egg	49 (17.5%)	39 (13.8%)	164 (58.4%)	29 (10.3%)
Tea and coffee	45 (16.0%)	181(64.4%)	37 (13.2%)	18 (6.4%)

6.3 Test of Data Normality distribution

As the figure two indicated below the data normality distribution for a selected hematological parameter like WBC, HGB, RBC and PLT showed that the data distribution was Normal which follows Gaussian distribution or "normal" probability distribution in the parametric calculation method. However, non-parametric RIs calculation method planned depending on C28-A3 recommendation and recent IFCC, C-RIDL study compared the RIs calculated by the non-parametric and parametric methods bring results of the two approaches are very near to each other, thus non parametric calculation methods used for these RIs establishment to ensure the reliability of the study results.

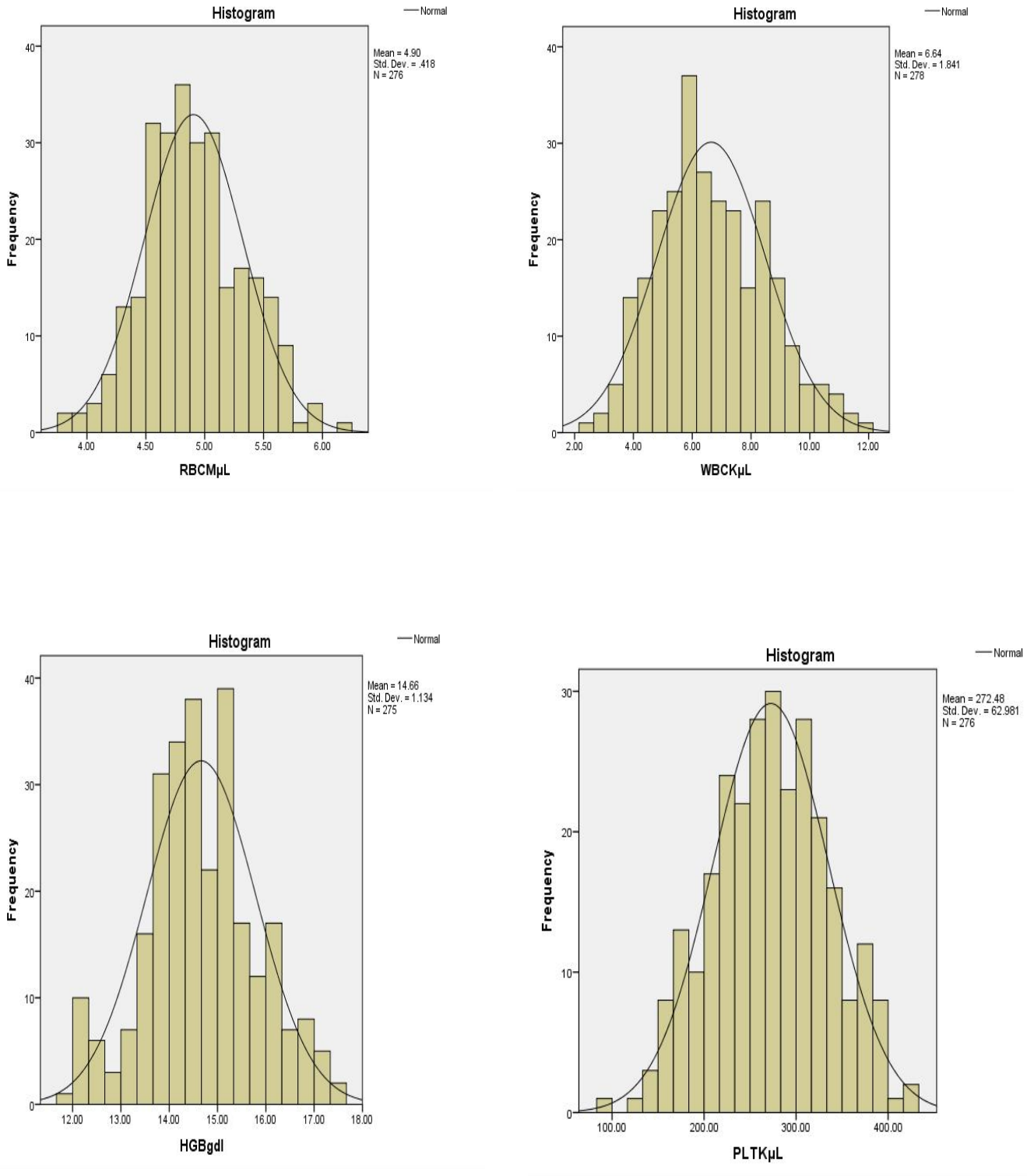

Figure 2 Data distribution of selected hematological parameters of study participants.

6.4 Hematological parameters Reference Interval by sex partition

Hematological parameter reference interval has been established among sex partition for Asella town adolescents using the Mann-Whitney U test. A significant difference was perceived in the mean values of some hematological parameters between the two sexes statistically. RBC ($p=0.01$), HGB ($p=0.016$), MCV ($p= 0.023$) and MCHC ($p<0.001$). Thus, the sex-specific reference interval for RBC (3.9-5.4), HGB (12.1-16.4) is the reference interval for females and RBC (4.1-5.8), HGB (12.3-17.2) for male. Whereas the mean of other Hematological parameters like WBC ($p=0.624$), PLT ($p=0.845$), and RWDSD ($p=0.604$) showed no statistically significant difference between both sexes ($p>0.05$). Due to this WBC (3.4-10.9), PLT (153.9-390), RWDSD (36.8-47.1), PDW (9.9-17.4), and MPV (8.9-12.6) were taken as a common reference interval for the male and female adolescent age group. The detail Reference interval other hematological parameter were indicated on the following table (Table 3).

Table 3: Hematological parameter reference intervals for male and female adolescents of Asella town from January to March 2020.

Parameters	Sex	N	min	max	median	Mean±2SD	95%(RI)		90% Confidence		P-value
							2.5%	97.5%	lower	upper	
RBC (10^6/ul)	female	135	3.8	5.6	4.8	4.8±0.7	3.9	5.5	3.8-4.0	5.4-5.6	0.010*
	male	141	4.1	5.9	4.9	5.0±0.9	4.1	5.8	4.0-4.2	5.7-5.9	
HGB (g/dl)	female	137	12.0	16.9	14.5	14.4±1.9	12.1	16.4	12.0-12.2	16.3-16.5	0.016*
	male	138	11.9	17.5	14.7	14.9±2.5	12.3	17.2	12.1-12.5	17.0-17.4	
HCT (%)	female	134	34.6	49.9	41.8	41.5±5.1	34.3	46.4	34.9-35.7	46.0-46.8	0.532
	male	137	34.0	49.9	41.6	42.2±7.0	35.3	48.7	33.8-34.8	48.2-49.2	
	combine	271	34.0	49.9	41.7	41.8±6.2	35.1	48.2	34.7-35.3	47.9-48.5	
MCV (fl)	female	138	75.4	94.6	85.8	84.8±7.5	77.5	93.5	77.4-78.4	92.9-93.9	0.023*
	male	140	76.3	94.7	84.3	85.8±7.7	77.9	93.9	77.0-77.9	93.4-94.4	
MCH (pg)	female	137	26.3	33.9	30.0	30.0±3.1	26.9	33.8	26.7-27.1	33.5-34.0	0.274
	male	138	26.1	33.9	29.9	29.9±3.2	26.9	33.8	26.7-27.1	33.5-34.0	
	combine	275	26.1	36.2	30.0	30.0± 3.2	27.0	33.6	26.8-27.2	33.4-33.8	
MCHC (g/dl)	female	137	33.1	37.2	34.9	35.0±1.6	33.4	36.8	33.3-33.5	36.7-36.9	<0.001*
	male	139	32.7	37.0	35.3	35.3±1.7	33.7	36.7	33.6-33.8	36.6-36.8	
RDWSD (fl)	female	138	35.8	47.3	41.6	41.6±5.0	37.0	47.0	36.6-37.3	46.6-47.3	0.604
	male	139	35.3	47.7	41.7	41.5±5.4	36.0	47.3	35.5-36.2	47.1-47.5	
	combine	274	35.3	47.7	41.6	41.6±5.2	36.8	47.1	36.5-37.5	46.8-47.3	
RDWCV (%)	female	139	11.3	14.7	12.7	12.8±1.6	11.5	14.6	11.4-11.6	14.5-14.7	0.033*
	male	138	11.5	18.0	13.0	13.0±1.7	11.6	14.8	11.5-11.8	14.7-14.9	

WBC (10^3/ul)	female	139	3.2	11.7	6.5	6.7±3.5	3.7	11.0	3.5-4.0	10.8-11.2	0.624
	male	139	2.4	11.2	6.5	6.6±3.8	3.1	10.3	2.8-3.4	10.0-10.6	
	Combine	278	2.4	11.7	6.5	6.6±3.7	3.4	10.9	3.2-3.6	10.7-11.2	
LYM%	female	140	15.5	65.0	39.2	39.8±23.7	17.2	64.2	15.6-18.8	62.6-65.8	0.216
	male	141	12.1	67.8	37.6	38.0±24.8	17.1	64.7	15.5-18.7	63.0-66.2	
MXD%	female	135	3.6	18.8	9.1	9.6±6.7	4.5	17.6	4.1-4.9	17.5-17.9	0.140
	male	140	3.8	18.6	10.2	10.1±6.6	4.1	17.2	3.6-4.5	16.7-17.7	
	combine	275	3.6	18.8	9.8	9.8± 6.6	4.2	17.4	3.9-4.5	17.1-17.7	
NEU%	female	140	19.1	79.7	50.9	50.0±27.2	23.8	77.6	21.9-25.7	75.7-79.5	0.294
	male	141	20.7	83.7	52.6	51.7±28.0	23.5	77.2	21.6-25.4	75.3-79.1	
	combine	281	19.1	83.7	51.1	50.8±	23.9	77.0	22.0-25.8	75.1-78.9	
LYM# (10^3/ul)	female	137	1.1	4.1	2.3	2.5±1.1	1.5	3.8	1.4-1.6	3.7-3.9	0.028
	male	139	0.9	4.2	2.3	2.3±1. 4	1.2	3.9	1.1-1.3	3.8-4.0	
MXD# (10^3/ul)	female	136	0.2	1.4	0.6	0.6±0.5	0.3	1.2	0.2-0.4	1.1-1.3	0.893
	male	136	0.2	1.2	0.6	0.6±0.4	0.2	1.2	0.1-0.3	1.1-1.3	
	combine	272	0.2	1.4	0.6	0.6±0.4	0.3	1.2	0.2-0.4	1.1-1.3	
NEU# (10^3/ul)	female	136	0.9	7.4	3.0	3.4±3.1	1.0	7.1	0.8-1.2	6.9-7.3	0.168
	male	139	0.7	7.5	3.4	3.5±3.2	0.9	7.2	0.7-1.2	7.0-7.4	
	combine	274	0.7	7.5	3.2	3.4±3.2	1.0	7.2	0.7-1.2	7.0-7.4	
PLT (10^3/ul)	female	137	131	417	274	272±113	155	383	147-163	375-391	0.845
	male	139	96	432	268	271±137	141	398	144-163	380-399	
	combine	276	96	432	271	272±126	153	390	147-160	383-396	
PDW (fl)	female	135	9.6	17.7	12.6	12.9±3.4	9.8	17.1	9.6-10.0	16.9-17.3	0.963
	male	135	9.3	17.9	12.4	12.9±3.6	9.9	17.5	9.7-10.1	17.2-17.7	

	combine	270	9.3	19.1	12.6	12.9±3.6	9.9	17.4	9.7-10.1	16.9-17.3	
MPV (fl)	female	140	8.4	13.1	10.6	10.6±1.8	8.9	12.5	8.8-9.0	12.4-12.6	0.566
	male	138	8.3	13.1	10.5	10.5±1.9	8.8	12.6	8.7-8.9	12.5-12.7	
	combine	278	8.3	13.1	10.5	10.6±1.9	8.9	12.6	8.8-9.0	11.5-12.6	
P-LCR (%)	female	140	12.6	47.6	28.9	29.4±14.6	15.6	45.0	14.6-16.6	44.0-46.0	0.692
	male	138	13.4	48.8	29.1	29.0±15.2	15.4	46.1	14.3-16.4	44.0-46.0	
	combine	278	12.6	48.8	28.9	29.1±14.8	15.6	45.1	14.9-16.3	44.4-45.8	

6.5 Comparison of current Established Reference interval and company Reference range

Compared to the old reference intervals from company to the current established reference intervals upper and lower limits, there were higher proportions of out of range values observed for RBC 12(8.6%), HGB 17(12.3%), HCT 10(7.2%), MCHC 15(10.8%), WBC 17(12.2%), absolute Neutrophils 24(17.2%) and MPV 70(50.7%) in males. In females, a higher proportion with out of range values was observed for Lymphocyte percentile 67(46.3%), Neutrophils percentile 31(22.3%), MCHC 30(21.6%) absolute lymphocyte 22(16.1%), MPV 22(15.6%) and WBC13 (9.4%).

The proportion of the male study participants with values outside the Upper reference limits was observed for RBC 10(7.1%), HGB 17(12.3%), MCHC 13(9.4%), Lymphocyte percentile 57(40.7%), absolute Lymphocyte18(12.9%)and MPV 67(48.5%) had the greatest proportion of participants with values above the upper reference limits of the old reference intervals and female had the greatest proportion of participant with values below the lower reference limits of the old reference intervals on WBC 10(7.2%), Neutrophil percentile 28(20.1%), absolute Neutrophil 22(16.2%) and RDWCV 6(4.3%) as indicated on the table below (Table 4).

Table 4; Comparison of Current RI and Company reference range with the proportion out of range from January to March 2020.

Parameter	Sex	Current RI		Company RI		Proportion out of range		Total proportion out of range
		lower	Upper	lower	upper	Lower	Upper	
RBC (10^6/ul)	M	4.1	5.8	4.2	5.6	2(1.5)	10(7.1%)	12(8.6%)
	F	3.9	5.5	4.1	5.3	2(1.5%)	3(2.2%)	5(3.7%)
HGB(g/dl)	M	12.3	17.2	12.5	16.1	0	17(12.3%)	17(12.3%)
	F	12.1	16.4	12.0	15.0	0	3(2.2%)	3(2.2%)
HCT (%)	M	35.3	48.7	36.0	47.0	1(0.7)	9(6.5%)	10(7.2%)
	F	34.3	46.4	35.0	45.0	0	7(5.2%)	7(5.2%)
MCV (fl)	M	77.9	93.9	78.0	95.0	2(1.4)	3(2.4)	5(3.8%)
	F	77.5	93.5	78.0	95.0	0	3(2.2)	3(2.2%)
MCH (pg)	M	26.9	33.8	26.0	32.0	3(2.2)	11(8.0)	14(10.2%)
	F	26.9	33.8	26.0	32.0	2(5.4)	12(8.8)	14(14.2%)
MCHC(g/dl)	M	33.7	36.7	32.0	36.0	2(1.4%)	13(9.4%)	15(10.8%)
	F	33.4	36.8	32.0	36.0	1(0.7)	29(20.8%)	30(21.6%)
RDWSD (fl)	M	36.0	47.3	NA	NA	NA	NA	NA
	F	37.0	47.0	NA	NA	NA	NA	NA
RDWCV (%)	M	11.6	14.8	11.8	15.6	1(0.7%)	1(0.7%)	2(1.4%)
	F	11.5	14.6	11.8	15.6	6(4.3%)	3(2.2%)	9(6.5%)
WBC (10^3/ul)	M	3.1	10.3	4.5	13.5	15(10.8%)	2(1.4%)	17(12.2%)
	F	3.7	11.0	4.5	13.5	10(7.2%)	3(2.2%)	13(9.4%)
LYM (%)	M	17.1	64.7	20.0	40.0	6(4.2)	57(40.7)	63(44.9%)
	F	17.2	64.2	20.0	40.0	3(2.1)	64(44.2)	67(46.3%)
MXD (%)	M	4.1	17.2	4.0	18.0	0	2(1.4)	2(1.4%)
	F	4.5	17.6	4.0	18.0	2(1.5)	2(1.5)	4(3%)
NEU (%)	M	23.5	77.2	40.0	80.0	26(18.4)	0	26(18.4%)
	F	23.8	77.6	40.0	80.0	28(20.1)	3(2.2)	31(22.3%)
LYM#(10^3/ul)	M	1.2	3.9	1.0	3.0	0	18(12.9)	18(12.9%)
	F	1.5	3.8	1.0	3.0	3(2.2)	19(13.9)	22(16.1%)
MXD#(10^3/ul)	M	0.2	1.2	0.2	1.6	0	3(2.2)	3(2.2%)

	F	0.3	1.2	0.2	1.6	0	1(0.7)	1(0.7%)
NEU#(10^3/ul)	M	0.9	7.2	2.0	7.0	22(15.8)	2(1.4)	24(17.2%)
	F	1.0	7.1	2.0	7.0	22(16.2)	0	22(16.2%)
PLT (10^3/ul)	M	141	398	140	385	0	1(0.7%)	1(0.7%)
	F	155	383	140	385	3(2.2)	0	3(2.2%)
PDW (fl)	M	9.9	17.5	NA	NA	NA	NA	NA
	F	9.8	17.1	NA	NA	NA	NA	NA
MPV (fl)	M	8.8	12.6	7.2	10.4	3(2.2)	67(48.5)	70(50.7%)
	F	8.9	12.5	7.5	11.5	2(1.4)	20(14.2)	22(15.6%)
P-LCR	M	15.4	46.1	NA	NA	NA	NA	NA
	F	15.6	45.0	NA	NA	NA	NA	NA

7. DISCUSSION

Clinical laboratory values provide important data to assess the health of an individual, interpret data in research, and screen participants in clinical and vaccine trials by interpreting those using locally adopted reference intervals. In the absence of such derived reference values, clinicians and researchers used reference values adopted from North American or European populations. Existing studies indicate that such values vary with age, ethnic origin, socio-demographic characteristics, and environmental context which need to establish Reference interval at each laboratory (9,21). However, in Ethiopia Reference Interval used in clinical practice for monitoring of patients is provided by CBC analyzer's Company which is adopted from elsewhere. Similarly, Arsi University Asella referral and teaching hospital laboratory used CBC Analyzer Company provided reference interval (Sysmex KX-21N) for patient management. Therefore, this was the first community-based study on the Adolescent age group established Hematological parameter Reference interval in Asella town.

In this study no indicative significant difference in hematological parameters like WBC, HCT, MCH, MPV PLT, PDW, Lymphocyte percentile, MXD percentile value, Neutrophil percentile, absolute MXD value, absolute Neutrophil, RDWSD and P-LCR between males and females. However, this study had significant gender differences in RBC, HGB, MCV, MCHC, absolute lymphocyte number, and RDWCV (p-value <0.05) which are consistent with previously established evidence that females have lower values than males for these parameters(37). The finding of higher values in males than in females agrees with the well-known fact of low values in females than males. Menstrual blood loss in females and biological as well as physiological factors such as the influence of the hormone androgen on erythropoietin explains the difference between females and males(55).

The lower limit of the 95th percentile reference interval of the current study for HCT, Lymphocyte percentile, MXD value percentile, Neutrophil percentile and RDWCV in both sexes, were lower than studies conducted in South West Ethiopia. While the MCH and MCHC of both sexes lower limits were higher than the above-mentioned studies. Moreover, in this newly established study, female participants had a similar lower limit for mean platelet volume and absolute Neutrophil

with studies conducted in Northern Ethiopia (54). On the other hand, the lower limit for WBC, absolute lymphocyte, and absolute MXD value of female were higher than lower limits of a study done in Ethiopia (37).

The central 95% confidence interval mean and median, of the current study as compared with other studies at a specific age group in Ethiopia is displayed in Table 5. The mean WBC of the current study was higher than the mean WBC of Northern Ethiopia but the median WBC of southwest Ethiopia was higher than the currently established reference interval. Whereas the current male HGB (12.3-17.2) reference interval was lower than the HGB (12.04-19.6) reference interval of the male in southwest Ethiopian.

Comparing these newly established RI values with those obtained from neighboring regions, the lower and upper limits for the hematological ranges obtained from this study population were slightly higher. For example, the WBC (3.4-10.9) reference interval is higher than those derived from Kenya, Nigeria, Tanzania, and Zimbabwe, lower than European. Whereas current established HGB values were higher than those from other Africans as well as American/European values. Variation among some hematological parameters was noted as shown in Table 5 below.

The differences between the current and other studies may be due to differences in many factors such as methodology, type of study population used, and environmental factors among others, and the instrument used for analysis like convenience sampling technique, For Sysmex XS-500i hematology analyzer, was used for this study. On other hand, the upper limit of RBC and HGB of males was lower than the study conducted on the Northern(54) and Southwest Ethiopia(37) this can be due to altitude can also affect these hematologic parameters and has been associated with reduced red blood cell components (22) which may account for differences in reference values in the Ethiopian studies.

The proportion of misclassified hematological parameter by using company derived values both on the upper and lower limit side were analyzed by comparing to the currently established reference intervals. Accordingly, there were higher proportions of out of range values observed for RBC 12(8.6%), HGB 17(12.3%), HCT 10(7.2%), MCHC 15(10.8%), WBC 17(12.2%), absolute

Neutrophils 24(17.2%) and MPV 70(50.7%) in males. In females, a higher proportion with out of range values were observed for Lymphocyte percentile 67(46.3%), Neutrophils percentile 31(22.3%), MCHC 30(21.6%) absolute lymphocyte 22(16.1%), MPV 22(15.6%), WBC13 (9.4%), MXD Value percentile 4(3%) and PLT 3(2.2%).

When out of range values were disaggregated by lower versus upper limit out of range, the greatest proportion of the male study participants with values outside the upper reference limits of reference intervals given by the company was observed for RBC 10(7.1%), HGB 17(12.3%), HCT 9(6.5%), Lymphocyte percentile 57(40.7%), and absolute Lymphocyte 18(12.9%). Such a proportion of individuals could have been categorized as being abnormal or having erythrocytosis and Lymphocytosis (39) if they were judged by the existing reference interval(53) But the high result could be due to the high altitude of Arsi Zone at which Chilalo mountain covers half of the town.

This misclassification implies the assessment of the health condition of our athletes. Considering that many of the elite athletes of Ethiopia are coming from this locality, it underscores the need for population-specific reference intervals for the athletes. In this regard, about 11 % of iron overload as judged by international reference interval had been reported in Ethiopian athletes(56) further supporting the finding of higher RBC and HGB upper limit in the current study. Relatively less proportion of females judged so for RBC, HGB, and HCT while a large proportion (44.2%) of females could have been flagged by the instrument as having Lymphocyte % above the upper limit given by the company. This large proportion difference may due to difference in lifestyle, nutritional status or geographical difference between reference populations.

The newly established 95% Reference interval of WBC in this study likely to be lower than those from Caucasian populations(12). previously Similar findings have been detected in healthy adolescent populations in Kenya(32), Nigeria(36), Zimbabwe (38), and Tanzania (31). However, the absolute MXD value and absolute Neutrophil of the female upper limit were slightly elevated than Europeans. This is in line with a study of the Nigerian where the absolute Neutrophil of both sexes lower and upper limit was increased than the European lower and upper limit reference

interval (32).

The differences have been noted for absolute platelet counts with lower values in the upper limit for both females and males of this study participant when compared to the Western populations(39) which is consistent in several African studies(20, 33). The causes are, however, not known but factors such as dietary, environmental, and genetics have been indicated as the probable cause(17). The upper limit for platelets in the current study was also lower compared to the values reported for Zimbabweans (38) and that from Southwest Ethiopia (36). The variation within Ethiopia signifies that the country has a heterogeneous population and each locality should determine its reference interval.

Table 5 *Comparison of mean, median, and 95% reference interval value of Current study with other studies.*

Parameters	sex	Current study			Northern Ethiopia Mekelle (54)			Southwest Ethiopia (37)		Kenya (32)		Zimbabwe (38)		Nigeria (36)		Tanzania (31)	USA (12)
		Mean	Median	95%(RI)	mean	Median	95%(RI)	Median	95%(RI)	Median	95%(RI)	Median	95%(RI)	mean	95%(RI)	95%(RI)	95%(RI)
RBC (10^6/ul)	F	4.8	4.8	3.9-5.5	4.9	4.9	4.3-5.7	4.9	4.3–5.6	4.8	3.5-6.1	4.8	4.8-5.8	NA	NA	NA	4.0-5.2
	M	5.0	4.9	4.1-5.8	5.3	5.2	4.6-6.9	5.0	4.1–6.5	5.3	4.0-6.4	5.2	4.4-6.4	NA	NA	NA	4.5-5.9
HGB (g/dl)	F	14.4	14.5	12.1-16.4	13.9	14.0	12.0-15.4	14.0	11.6–15.9	13.2	10.7-15.7	13.6	11.1-15.7	12.9	12.0-15.0	10.0-14.9	12.0-16.0
	M	14.9	14.7	12.3-17.2	14.8	14.7	12.6-17.6	14.1	12.0–19.6	15.1	12.2-18	14.4	12.1-17.4	13.5	13.0-16.0	10.8-17.0	13.0-16.0
HCT (%)	F	41.5	41.8	34.3-46.4	43.2	43.3	38.0-47.0	41.4	35.9-46.9	41.6	29.0-54.8	41.4	34.6-46.7	39.0	37.0-47.0	30.8-44.7	36.0-46.0
	M	42.2	41.6	35.3-48.7	45.4	44.8	40.0-55.0	41.4	35.6-55.2	46.1	39.6-53.0	42.5	36.1-49.7	41.0	40.0-49.0	33-48.1	37.0-49.0
MCV (fl)	F	84.8	85.8	77.5-93.5	88.0	88.0	80.0-98.0	83.2	74.5-91.0	85.2	59.6-109	84.4	68.7-96.9	NA	NA	62.2-94.5	80.0-100.
	M	85.8	84.3	77.9-93.9	86.7	86.7	76.0-94.0	82.3	75.0-93.0	85.8	65.5-106.3	81.3	70.1-93.2	NA	NA	63.2-91	78.0-98.0
MCH (pg)	F	30.0	30.0	26.9-33.8	28.3	28.6	24.5-30.8	28.0	25.1-30.8	30.6	19.6-30.5	28.3	22.1-32.0	NA	NA	NA	NA
	M	29.9	29.9	26.9-33.8	28.3	28.3	23.5-31.5	27.9	25.2-31.0	28.7	23.4-34.0	27.4	22.5-30.9	NA	NA	NA	NA
MCHC (g/d)	F	35.0	34.9	33.4-36.8	32.0	32.2	30.4-34.2	33.8	32.1-35.4	31.9	23.5-31.9	33.0	29.8-35.8	NA	NA	NA	NA
	M	35.3	35.3	33.7-36.7	32.6	32.5	30.1-35.8	34.0	32.1-36.2	32.5	29.4-35.6	33.9	30.3-36.1	NA	NA	NA	NA
RDWSD (fl)	F	41.6	41.6	37.0-47.0	NA	NA	NA	NA	NA	NA	NA	41.8	36.6-52.9	NA	NA	NA	NA
	M	41.5	41.7	36.0-47.3	NA	NA	NA	NA	NA	NA	NA	40.9	35.4-50.7	NA	NA	NA	NA
RDWCV (%)	F	12.8	12.7	11.5-14.6	13.8	13.7	13.0-16.0	13.7	12.3-15.9	NA	NA	NA	NA	NA	NA	NA	NA
	M	13.0	13.0	11.6-14.8	14.3	14.1	13.0-16.0	13.8	12.7-16.0	NA	NA	NA	NA	NA	NA	NA	NA
WBC (10^3/ul)	F	6.7	6.5	3.7-11.0	6.3	5.9	3.4-11.0	7.0	3.7-11.4	5.0	2.5-7.7	5.9	3.3-9.8	5.6	3.0–9.9	3.2-10.3	4.5-13.0
	M	6.6	6.5	3.1-10.3	5.8	5.4	2.9-10.5	7.0	4.0–11.7	5.4	2.8-8.2	5.0	3.2-8.6	5.4	2.9–9.0	3.2-10.3	4.5-13.0
LYM (%)	F	39.8	39.2	17.2-64.2	39.6	37.5	20.6-61.2	NA	NA	NA	NA	43.4	28.4-65.0	NA	NA	NA	NA
	M	38.0	37.6	17.1-64.7	38.9	38.5	18.8-63.4	NA	NA	NA	NA	46.8	27.7-62.6	NA	NA	NA	NA

MXD	F	9.6	9.1	4.5-17.6	11.4	10.5	4.8-22.7	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
(%)	M	10.1	10.2	4.1-17.2	16.3	14.4	7.2-36.8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NEU (%)	F	50.0	50.9	23.8-77.6	49.0	50.6	24.8-71	NA	NA	NA	NA	43.4	24.0-61.2	NA	NA	NA	NA
	M	51.7	52.6	23.5-77.2	44.8	45.5	23.7-64.7	NA	NA	2.1	0.3-4.3	38.8	23.3-58.5	NA	NA	NA	NA
LYM#	F	2.5	2.3	1.5-3.8	2.4	2.3	1.1-3.7	1.1	1.4-4.4	2.2	0.9-3.7	2.6	1.4-3.9	4.0	1.2–6.0	1.4-4.2	1.5-4.5
(10^3/ul)	M	2.3	2.3	1.2-3.9	2.1	2.0	1.2-3.3	1.0	1.5-4.2	2.1	1.5-3.5	2.2	1.3-3.9	3.0	1.1–3.2	1.4-4.2	1.5-4.5
MXD#	F	0.6	0.6	0.3-1.2	0.7	0.6	0.2-1.6	NA	NA	NA	NA	NA	NA	NA	NA	0.2-2.5	0.6-1.5
(10^3/ul)	M	0.6	0.6	0.2-1.2	1.0	0.8	0.3-3.8	NA	NA	NA	NA	NA	NA	NA	NA	0.2-2.5	0.6-1.5
NEU#	F	3.4	3.0	1.0-7.1	3.2	3.0	1.0-6.9	3.1	1.0-7.0	2.3	0.5-4.5	2.5	1.1-5.6	4.0	2.4–7.2	0.9-4.6	1.5-6.0
(10^3/ul)	M	3.5	3.4	0.9-7.2	2.7	2.4	0.9-6.7	3.3	1.3-7.4	2.1	0.3-4.3	1.9	1.0- 3.9	4.0	1.9–7.0	0.9-4.6	1.5-6.0
PLT	F	272	274	155-383	294	288	151-462	321	197–460	233	134–439	312	214-476	285	100-443	107-482	150-400
(10^3/ul)	M	271	268	141-398	261	261	138-364	326	158–469	224	103–386	280	186-415	269	98-427	119-458	150-400
PDW (fl)	F	12.9	12.6	9.8-17.1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	M	12.9	12.4	9.9-17.5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
MPV (fl)	F	10.6	10.6	8.9-12.5	10.7	10.7	8.9-12.8	NA	NA	NA	NA	10.0	8.4-11.2	NA	NA	NA	NA
	M	10.5	10.5	8.8-12.6	10.7	10.6	8.6-13.3	NA	NA	NA	NA	10.1	8.6-12.3	NA	NA	NA	NA
PLCR	F	29.4	28.9	15.6-45.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
(%)	M	29.0	29.1	15.4-46.1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Key: F=Female, M=Male, NA =Not available, RI= Reference interval

8.Strength and Limitation

8.1 Strength

The study was more Representative for all Asella town adolescents since it is a community-based study. Novel COVID19 pandemic disease created some inconvenient while processing sample screening test but the difficulty solved by patience and screen for all medical conditions that might have influenced the laboratory results were done on the time plan. Besides, hematological analysis is done in a Laboratory that repeatedly passed the one world Accuracy External quality assessment (EQA).

8.2 Limitation

The study has certain limitation and the fundamental limitation of the study were Chronic disease screening like erythrocyte sedimentary rate and C-reactive protein test were not done for the study participant due to research budget shortage.

9. Conclusion and Recommendation

9.1 Conclusion

Some hematological parameter showed a significant difference in the mean values among data sets for HGB, RBC, HCT, MCV and MCHC across gender in which females having lower levels than the males. The newly established hematological parameter reference intervals by this study were different from the existing reference values. The WBC parameters showed lower levels as compared to those on currently used Reference interval from the company. Platelet parameters varied across gender females showing higher lower limit values than the males and male upper limit higher than the female upper limit. Variations are also noted for percent Neutrophil and lymphocytes.

9.2 Recommendation

The study recommends using this locally derived Hematological parameters reference interval for Asella town adolescents for treatment monitoring, general health assessment, and efficient implementation of clinical trials. Particularly for better patient management and result interpretation, Asella referral and teaching hospital and surrounding health centers laboratory are recommended to use this reference interval to interpret hematological parameter test result of the adolescent age group. Because, the reference intervals need to be adjusted to suit the local populations and enable the laboratory to effectively use the blood cell analyzer avoiding unnecessary flagging. It also helps to provide a better service to clinicians who could use these results to provide better care to patients or for proper client management and use in clinical trials.

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Annex I: Information sheet in English Version

Principal Investigator: Berhanu Dibaba (BSc, MSc candidate)

Title of the Research Project: Establishment of hematological parameters reference intervals for apparently healthy adolescents in Asella town, South East Ethiopia.

Name of the Organization: Addis Ababa University, College of Health Sciences Department of Medical Laboratory Sciences

Introduction

You are invited to participate as a study subject in a research conducted by MSc candidate, from Addis Ababa University. Your participation is voluntary. The research teams will include one principal investigator, two ad-visors; from Addis Ababa University Hematology department. Please take as much time as you need to read or listen to the information sheet.

Purpose of the Research Project

We are asking you to take part in this study because we will try to establish hematological parameters reference intervals for apparently healthy adolescents in Asella town, South East Ethiopia.

Procedures and the expected participation

If you are willing to participate, you need to understand the purpose of the study and give your consent. Not only this but also a specimen collected from you was used for the research purpose, and the results of your sample were exposed to some concerned professional staff as it is needed. The required clinical sample was collected by a medical laboratory technologist. Then, you are requested to give your consent to the sample collector. After consent, a sample will be collected. Moreover, there was a face-to-face interview for additional questions which will take about 10minutes.

Potential risks and Discomforts

During the collection of specimens from you, appropriate precaution was taken and all samples were collected by medical laboratory technologists. If anything happened, appropriate medical care was provided to you.

Confidentiality

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study was kept in a locked file cabinet, or be protected by a password on the computer only

accessible to personnel involved in the study. There is no sensitive issue that you were asked related to your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential.

Potential benefits to subjects and/or to the society

You will not receive any payment for your participation in this research study as compensation. However, you will get the results for free. Also, the result of the study was beneficial for the establishment of hematological parameters reference intervals for adolescents. Hence, you are indirectly benefiting other patients and society in this respect.

Participation and Withdrawal from the Study

Participation is voluntary and you have the right not to participate in this study. You may withdraw at any time and place without consequences of any kind. You may also reject to give any sample. You can ask any questions regarding this study and you have a right to get a laboratory diagnosis result free.

Contact information

If you have any questions about this study you can contact the following principal investigators and advisors for further information.

Berhanu Dibaba Phone: 0913340003 Email: getberhanud@gmail.com

Dr. Aster Tsegaye Phone: 0911696085 E-mail: tsegayeaster@yahoo.com

Annex II: Information sheet in Amharic Version

ለጥናቱ ተሳታፊዎች መረጃ:

የጥናቱ ዋና ተመራማሪ **ብርሀኑ ዲባባ፡**

የጥናቱ ርዕስ:-

እድሜያቸው ከ12 እና ከዚያ በላይ ለሆኑት የጤናማ ሰው ደም ውስጥ የሚገኙ የክሊኒካል ላቦራቶሪ ሄማቶሎጂካል ምርመራዎች መጠንን ሪፈረንስ ኢንተርቫል መስራት፡፡

ተቋማት: አዲስአበባዩኒቨርሲቲጤናሳይንስኮሌጅየሕክምናላቦራቶሪሳይንስት/ክፍል

መግቢያ

በአዲስአበባዩኒቨርሲቲጤናሳይንስኮሌጅየሕክምናላቦራቶሪሳይንስት/ክፍልበማስተርስድግሪተማሪየመ መረቂያጥናትላይእንዲሳተፉተጋብዘዋል፡፡እባክዎበዚህጥናትለመሳተፍከመስማማትዎበፊትከዚህቀጥሎ የሚገኘውንምንባብበጥሞናያንብቡናግልፅያልሆነሎትንማንኛውንምሀሳብይጠይቁ፡፡

የምርምርጥናቱአላማ

እድሜአቸው አስራሁለት ዓመት ና ከዚያበላይ ለሆኑ የጤናማ ሰው ደም ውስጥ የሚገኙ የክሊኒካል ላቦራቶ ሪ

ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል መስራት፡፡

የጥናቱአካሄድ

በጥናቱለመሳተፍከተስማሙየጥናቱአባል/አባላት 10ደቂቃየሚወስድጥያቄይጠይቁዎታል፡፡ክብደት፣ቁ መት፣የክንድእናየደምግፊትልኬትይወሰዳል፡፡ሽንትናአይነምድርበምንሰጠውእቃእንድትሰጡንእንጠይቃ ለን፡፡በተጨማሪም10ሚሊሊትር (አንድ የሾርባ ማንኪያ የሚሆን) በንፁህቫኩቴይነርብልቃጥእናመርፌ እንቀዳለን (6) ሚሊሊትር በባዶቲዩብ፣4 ሚሊሊትር ደ እንዳይረጋ የሚያደርግ ንጥረ ነገር፣ኢዲቲኤ፣ባለበት ቲዩብ)፡፡የሄማቶሎጂ፣ሴሮሎጂ፣ፓራሲቶሎጂ ምርመራዎችን እናካሂዳለን፡፡

የጥናቱተሳታፊለመሆንየሚጠበቅበዎትምንድንነው?

በዚህጥናትለመሳተፍየሚስማሙከሆነናሙናዎለጥናቱእንዲሚዉልመስማማትይጠበቅብዎታል፡፡ከተወ ሰውናሙናላይየሚገኝውመረጃዎችከዚህአባላትውጪለሚገኙናለስራውአግባብነትላላቸዉሰዎችቢነገርየማይ ቃወሙመሆኑንመስማማትይጠበቅብዎታል፡፡ይሁንእንጂይህአይነቱመረጃየርስዎንማንነትየሚገልጡመ ረጃዎችንማለትምስም፤አድራሻናየስልክቁጥርየመሳሰሉትንመረጃዎችንአይጨምርም፡፡ይልቁንምለዚህአገልግ ሎትብቻየሚውልእርስዎንለማወቅየሚያስችልመለያቁጥርጥቅምላይእንዲዉልይደረጋል፡፡፡በተጨማሪምስለር

ስዎአጠቃላይየጤናሁኔታለሚቀርቡሎትአንዳንድተጨማሪጥያቄዎችመልስመስጠትይኖርብዎታል።በጥናቱለ መሳተፍየሚስማሙከሆነየስምምነትቅፅላይበጹሁፍወይምበጣትፊርማማስቀመጥይጠበቅቦዎታል።

በዚህጥናትመሳተፍየሚያስከትላቸዉችግሮችምንድንናቸዉ?

ናሙናበሚሰበሰብበትወቅትምንምአይነትየከፋችግርአያጋጥምዎትም።ሆኖምግንናሙናውንለመሰብሰ ብልምድያለዉባለሙያስለሚመደብናአስፈላጊዉየጥንቃቄእርምጃስለሚወሰድየህመምስሜትአይኖር ም።

የህክምናመረጃበሚስጥርተጠብቆመቆየትየሚችለዉእንዴትነዉ?

ስለራስዎየሰጡትማንኛዉምመረጃናከተወሰደዉናሙናላይየተገኘዉየላቦራቶሪዉጤትየሚዉለዉለጥናቱአላ ማብቻነው።ይህንማህደርሊያገኙየሚችሉትየተወሰኑየጥናቱተባባሪሰዎችብቻናቸው።ከዚያምበላይስለእር ስዎያለውንማንኛውንምመረጃየተለየየይለፍቃልባለውየኮምፒውተርየመረጃማህደርውስጥእንዲቀመጥይደ ረጋል።

በዚህጥናትመሳተፍየሚያስገኛቸውጥቅሞችምንድንናቸው?

ይህጥናትየማስተርስዲግሪመመረቂያእንደመሆኑመጠንበዚህጥናትበመካፈልዎበገንዘብየሚያገኙትጥቅ ምባይኖርምከጥናቱበሚገኝውውጤትግንተጠቃሚነዎት።የእርሶዎተሳትፎየእርስዎንናየወገንዎትንየደምምር መራሊኬትንለማከታተልከፍተኛጥቅምይኖረዋል።

በዚህጥናትተሳታፊየመሆንዎመብቶችምንድንናቸው?

በዚህጥናትመሳተፍሙሉበሙሉበእርስዎፈቃደኝነትየተመሰረተበመሆኑበማንኛውምሰዓትናቦታየማቋረጥሙ ሉመብትየተጠበቀከመሆኑምበላይእራስዎንከጥናቱበማግለልዎምክንያትየሚቀርብዎትምንምአይነትየሆስፒ ታልአገልግሎትአይኖርም።ከዚህምበተጨማሪጥናቱንበተመለከተማንኛውንምአይነትጥያቄየመጠየቅናገለጻየ ማግኘትመብትአለዎት።የላብራቶሪምርመራውጤቱንምበነጻማግኘትይችላሉ።ነገርግንእርስዎበሚሰጡንመረ ጃየችግሩንስፋትለመከላከልእናለመቆጣጠርጠቃሚስለሆነለሚቀርብልዎትጥያቄቀጥተኛመልስይሰጡንዘንድ በታላቅአክብሮትእንጠይቃን፡

ጥያቄ ካለኝ ወይምችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህንንጥናትበተመለከተወይምከዚህጥናትጋርበተዛመደመልኩስለሚያጋጥሙድንገተኛአደጋዎችወይም ጥያቄካለዎትበሚመለከተውአድራሻይጠቀሙ።

ብርሃኑድባባ ሞባይል፡ +251-913-340-003 ኢሜል፡getberhanud@gmail.com

ዶ/ር አስቴርፀጋዬ ሞባይል፡ +251-911-696-085 ኢሜል፡ tsegayeaster@yahoo.com

Annex III: Information sheet in Afaan Oromoo Version

Oddeeffaannoo Hirmaattottaa qoraannoo kaanafi kennaammu Qoraataan qoraannoo kanaa obboo Birhaanu Dibaaba

Mataa dureen qoraannoo kana: Giddu galeessummaa baayina dhiiga hemaatoloojjii fi kilinikaal keemistriinamaa fayyaa umriin isaa 12-17 magaaala Asallaa bu ‘uurreessuu.

Yuunvaarsitii finfinneetti koolleejjii fayyaa Diipaartmeenti Meedikaal Laboratorii saayinsii

Seensaa:

Qoraannoo baarattootni Yuunvaarsittii finfiinnee koolleejjii fayyaa Diipaartmeenti Meedikaal Laboratorii saayinsii Digrii lammaaffa (Maasteris) eebifaamuuf gaaggeessaan irraati akka hirmaataan carraan isiinif kennaame jiraa. Qoraannoo kanaa irraati hirmaachuuf waalgaluu keessaan dursitaan oddeeffaannoo armaan gadii kanaa xiyyeeffaanan akkaa dubistaani fi gaaffii dhimmaa qoraannoo kanaa ilaalchisee isiinit umaame kamuu akka gaafataan kabaajadhaan isiini gaafanna.

Kaayyoon Qoraannoo kanaa:

Dhigaa namaa fayyaa umrii isaa kudhaa lamaafi sanaa olii keessaati gidduu galeessumaan hangaa argaamaa qoraannoo kilinkaal Laboraatorii reefireensi inteervaali bu ‘uurreessuufi wantoota Quulqullinaa qoraannoo laboratorii ittiin to‘aatanihoojaachuudha.

Haala Qoraannoon itti addeemsifaamu:

Qoraannoo kanaa keessaatti hirmaachuuf yoo murteessitaan namoon qoraannoo kanaa kan gaaggeessan gaaffiii daqiqaa kudhaan (10) fudhaatu isiin gaafatani ulfaatina dheerina, dhiibbaa dhigaa keessaan safaaruun dhigaa milileetirii kudhaan (10ml) siin irraa fudhaachun milileetiri jaha (6ml) tubii homaa hin qabneeti isaa hafee ammo waan dhignii akka hin ragaane godhuu

EDTA tuubit naquun meeshaa ficaanii fi udaan itti fidaan isiinif kennamaa. Qoraannoolee akka hemaatolojjii, seeroolojjii, paraasaytolojjii fi kilinkal keemistrii irraatti addeemsifaama

Qoraannoo kanaa irraati hirmaachuuf waantotaa namaarraa egaamu:

Qoraannoo kanaa irraati hirmaachuuf yoo fedhaan ta ‘ee sammuudni keessaan qoraannoof akka oluu heyaamama ta‘uu qabduu. Akkasumaasi bu ‘uudhan qoraannoo kanaara argaammuu akkumaa barbaachisuma isaatti qamaa addaa addaa yoo qaaqaabe kaan hin mormiine yoo ta‘ee.Haa ta‘uu malee oddeeffaannoo eenyuummaa keessaan ibsuu kaneen akka maqaa teessoo fi bilbilaa keessaan kan daabarsinee hin keeninee ta‘uu ibsaa. Koodii dhimmaa kanaaf oluu qofaa kan fayyaadamnu ta ‘aa. Daabalataanis dhimmaa fayyaa keessaan ilaalchisee gaaffii isaani gaafannuu deebii keessaan

nuuf laachuutu isiiniraa eegamaa.

Qoraannoo kanaarrati hirmaachuun miidhaa qaqaabsisaa?

Yeroo saammudni fudhaatamuu miidhaan isiinira gaahuu hin jiruu. Ogeessii sammuudaa kanaa funaanu gahuumsaa fi muxxaannoo kan qabuu waan ta ‘ee hordooffii barbaachisaa waan godhuuf dhuukkubiin siniiti dhagahamuu hinjiruu.

Bu’aan qoraannoo kana

Oddeeffaannoo isiin irraa guraamesi ta ‘ii bu‘aan qoraannoo saammuda keessaan kaan oluu dhimmaa qoraannoo kaannaa qofaafi. Gaalmee kanaasi ilaalu kaan daanda ‘aan qamaa qoraannoo kanaa gaaggeessuu qofa. Oddeeffaannoo keessaan komputeeraa keessaa gaalchuun akka qamaa birootif hin saxilaamne passwordii gaargaraan cufaamee kayyaama.

Qoraannoo kanaarratti hirmaachuuni bu’aa maal argaamsisaa?

Qoraannooni kuun masteersii digrii eebifaamuuf kan rawwaatamuu waan ta ‘ee kaaffaltiin asirraati hirmaachuu keessaanif kafaalamu hin jiruu. Haa ta ‘uu malee bu‘aa qoraannoo kanaarra argaammuun fayyaadamoo tatuu. Hirmaannaan keessaanin isiinis ta ‘ee laammiin keessaani hammaa qoraannoo dhigaa laboraatorii bu‘uurreffamee irraa fayyaadamoo ta‘uu.

Mirgii qoraannoo kanaarrati hirmaachuu maal fa’aa?

Qoraannoo kanaarrati hirmaachuu fedhaa keessaani irraatti kaan bu ‘urreefame waan ta‘ee yeroo barbaadaanitti qoraannoo kanaa addaan kutaani bahuuf mirgaa guutuu qabduu. Kanaan waaliqabate taajaajille hospitaala isiinraa hafuu tokkoole hin jiraatuu. Qoraannoo kanaa ilaalchise gaaffii kamuu gaafaatani ibsaa gahaa argaachuu mirgaa guutuu qabduu. Bu ‘aa qoraannoo labooraatorii kaaffalti malee argaachuufilee mirgaa ni qabduu. Haa ta ‘uu malee oddeeffaannoon isiini nuuf keennitaan hammaa rakkoo hir‘iisuu ykn dhabaamsisuuf waan nuu gaargaarruf gaaffiiwwaan gafaatamtaanif deebii sirrii akkaa nuuf deebistaan kabaajaadhan isiini gafaanna.

Gaaffii yoon qabaadhee ykn ammoo yoo raakkoon naa muddatee maal gochuun qabaa?

Qoraannoo kanaa ilaalchisee ykn qoraannoo kanaan waaliqabate rakkoon tasaa isiini mudaate yokaan gaaffiin yoo jiraate teessoo armaan gaadi faayyaadama.

Birhaanuu Dibaaba mobayila +251-913-340-003 Email: getberhanud@gmail,com

Dr. Aster Tsagaaye mobayila +251-911-696-085 Email: tsegayeaster@yahoo.com

Annex IV: Assent form for children aged 12-17 years

I have read the information above, or it has been read to me. I have been allowed to ask questions and my questions have been answered to my satisfaction. I voluntarily assent that I would participate in this study provided my parents/guardians to give their consent.

To be a participant in this study I understand that I give stool, urine, and blood sample and understand that I have the right to withdraw from the study at any time.

Print name of participant, date, and signature or thumb impression of participant

_____ _____/_____/_____(dd/mm/yy)

If illiterate;

Print name of independent literate witness, date, and signature of witness (if possible, this person should be selected by the participant and should have no connection to the research team)

_____ _____/_____/_____(dd/mm/yy)___

Phone number(parents/guardians) _____

Print name of researcher, date, and signature of researcher

_____ _____/_____/_____(dd/mm/yy)___

Annex V: Assent form for children aged 12-17 years' Amharic version

ከላይ የተገለፀውን መረጃ አንቢብያለሁ/ተነቦልኛ ጥያቄ ለመጠየቅ እድል ተሰጥቶኝ ጠይቄ በሚያረካ መልኩ ተመልሶልኛል ። በዚህ ጥናት ለመሳተፍ በፍቃደኝነት ተስማምቻለሁ።

የአይነምድር የሽንት እና የደም ናሙና ለመስጠት እና በዚህ ጥናት ተሳታፊ ለመሆን ፤እንዲሁም በማንኛውም ሰአት ከጥናቱ ለመውጣት መብት እንዳለኝ ተረድቻለሁ ።

የተሳታፊ ስም፤ቀን እና ፊርማ(አሻራ)ከዚህ በታች ይፃፍ

ስም ----------ቀን----- ----ዓም ------

ያልተማሩ ከሆነ።የተማሩ ገለልተኛ ሰው ስም ቀን እና ፊርማ(ከተቻለ ይህ ሰው በተሳታፊው ቢመረጥና ከተመራማሪው አባላት ግኑኝነት የሌለው ቢሆን)

ስም ---------- ቀን --------- ዓም ------

የተመራማሪው ስም ፤ቀንና ፊርማ

ስም---------- ቀን --------- ዓም ------

ስልክ ቁጥር +251-913-340-003

Annex VI: Assent form for children aged 12-17 years Afaan Oromoo version

Uunka Waaligaltee ijoollee waggaa 12-17 Jiraanif

Oddeeffaannoo armaan olitti ibsamee duubiseen jira ykn naaf duubisaanitti jiruu. Carraan gaaffii gaafachuleen naaf kennamee gafaadhe deebii gahaa argadheen jiraa. Kanaaf qoraannoo kanaa keessaatti hirmaachuuf fedhaa koon waaligaleenjiraa. Qoraannoo kanaa irraattii hirmaachuuf Saammuudaa qoraannoo kanaaf oluu kan akka uudaan, fincaan fi dhigaa kan kennuuf akkasumaas yeroon barbaaddeeti qoraannoo kanaa keessaa bahuu mirgaa guutuu qabaachuu koo hubaadheen jira.

Maqaan guuyyaa fi maallattoo namaa hirmaatuu kanaa gaaditti baarreesaa.

Maqaa_____Guuyyaa_____Maallaattoo_____

Hirmaataan/ttuun kaan hin baaraattiin yoo ta'aan

Ragaa namaa baraatee kan namaa hirmaattuun filaatameefi haariiroo hirmaattaa/hirmaattuu waalin kan hin qabne maqaa, guyyaa fi maallaattoo isaan kaanaa gadditti baarreesaa.

Maqaa_____Guuyyaa_____Maallaattoo_____Bilbiilaa_____

Maqaan guuyyaa fi maallattoo namaa hirmaatuu kanaa gaaditti baarreesaa.

Maqaa_____Guuyyaa_____Maallaattoo_____

Annex VII: Consent form (English version)

Informed consent (study participants)

Name of main researcher: Berhanu Dibaba, MLS; AAU. Advisors/Co-investigators: Aster Tsegaye (MSc, PhD)

Jemal Alemu (MSc, PhD candidate) Name of institute: AAU and AHRI

Funded by: AHRI and AAU

Reviewed by: DREC (AAU)

RESEARCH TITLE: Establishment of hematological parameters reference intervals for apparently healthy adolescent ‘s in Asella town, South East Ethiopia.

Name:_____Age:_____sex____kebele____ Address_____phone_____Serial number:

If you agree that your child takes part, please read this form and sign the consent sheets at the end.

I have read, or it was read to me, the information sheet concerning this study and I understand what was required of me if I take part in the study.

I am aware of the possible risk and benefits of this study.

I know that being in this study involuntary.

I understand that at any time I may withdraw from this study without giving a reason and without affecting my normal care.

My questions concerning this study have been answered

I know that there is no special payment for being participating in the study

I agree to take part in this study. Name:_____Signature:_____Date:_____

The participant is unable to sign. As a witness, I confirm that all the information about the study was given and the participant consented to take part.

Signature_____ Date_____

We thank you for consenting to take part in the study.

Annex VIII: Consent form (Amharic version)

መለያ ቁጥር----------

ጥናቱን በሚያካሂዱት ሰዎች ስለጥናቱ በቂ መረጃ ተሰጥቶኛል። የዚህ ጥናት አላማም የደም ህዋሳት ሪፈረንስ ቫልዩ ማወቅ ነው ።ከልጄ/ከኔ የሚወሰደው የደም የሰገራ የሽንት ናሙና ላይ ምን አይነት የጤና ጉዳት የማያስከትል መሆኑን ተረድቻለሁ። እንዲሁም በጥናቱ ለመሳተፍ ፍቃደኛ ካልሆንኩ በጥናቱ ለመሳተፍ እንደማልገደድ ነገር ግን በዚህ ጥናት በመሳተፌ ሳይንሳዊ እውቀት ጠቃሚ መረጃ ማበርከትና ወደፊት በዚህ ዙሪያ ለሚሰሩ ስራዎች መሰረት የሚሆን መረጃዎችን መስጠት እንደምችል ተረድቻለሁ።በዚህ ጥናት ተሳታፊ በመሆን የሚከፈል ምንም ክፍያ አለመኖሩን ተረድቼ ጥናቱ ላይ ለመሳተፍ የተስማማሁ መሆኔን በፌርማዬ አረጋግጣለሁ።

የተሳታፊ ፊርማ ---------- ቀን----------

የጥናት አድራጊ ፊርማ---------- ቀን----------

Annex IX: consent form in AFaan Oromoo version

Maqaa namaa qoraannoo ademsisuu Berhanu Dibaba, MLS; AAU.

Goorsa qorannoo kanaa(Advisors) Aster Tsegaye (MSc, PhD) fi Jemal Alemu (MSc, PhD candidate)

Maqaa qamaa qoraanno gaaggeessu (Nameofinstitute): AAU and AHRI

Qamaa bajaata qoraanoo kana rammadee (Fundedby): AAU

Qamaa qoraannoo kana hordoffuu (Reviewed by): DREC (AAU)

Mataa duree qoraannoo kana: Giddu galeessummaa baayina dhiiga hemaatoloojjii namaa fayyaa umriin isaa 12-17 magaaala Asallaa bu ‘uurreessuu.

Maqaa:_____Umrii:_____Salaa:_____Gandaa:_____

Address_____phone_____tartibaa lakk:_____

Qoraannoo kanaa irraatti muccaa/yyoon keessaan akkaa hirmaattuu yoo fedhii qabattaan barreeffamaa armaan gadii dubisuun unkaa waali galtee qophaa ‘ee kanaa irraati mallaatteessa.

1 Oddeeffannoo dhimaa qoraannoo kanaa ibsuu duubisee ykn naaf duubiffammee qoraannoo kanaa irraattii yoo hirmaatee/ttee maalituu akka irraa baarbachisuu hubaadheen jiraa.

Bu ‘aasi ta‘ee miidhaa qoraannoo kanaa hubaadheen jira.

Qoraannoo kanaarrattii hirmaachuun fedhiidhaan akkaa ta ‘ee hubaadheen jira.

Yeroon barbaadaameti qoraannoo kanaa keessaa bahuu yoo barbaadaan Sabaabaa tokkoo malee bahuu akkaa dandaa ‘aan hubaadherra.

Qoraannoo kanaa ilaalchisee gaaffiin qabuu kamiyuu naaf deebi ‘ee jiraa

Kaafaaltiin qoraannoo kanaarraati hirmaachuuf namaa kafaalamu kamuu akkaa hin jiree naan beekaa.

Qoraannoo kanaarrati akka hirmaatu/ttuuf heyyaamaama ta ‘eerraa.

maqaa: _____mallaattoo:_____Guyyaa: _____

Hirmaattaan/ttuun waaligaltee kanaa mallaatteessuu waan hin daandeenyeef ani qoraannoo kanaa ilaalchisee oddeeffannoo gahaa nuuf kenamuu ragaa bahee qoraannoo kanarraatti akka hirmaattaanif waligaaltee koo kennee jiraa.

Maallaattoo:_____Guyyaa:_____

Qoraannoo kanaarraatti akka hirmaataanif heyaammama waan taatanif galaattoommaa.

Annex X: Questionnaires English version

Questionnaires to be filled by health professionals

Part I. General information

Code Number_____Region_____Zone__ Woreda_____/ city/_subcity_Kebele_

Part II. Personal information

1. Age (in years)_____

2. Sex_____

3. Place of Birth_____

4. For how long (years) did you live in the birth place?_____

5. How long do you live in this specific area? (If different from the birthplace)____years

No.	Questions	Responses
	Part III. SOCIO-DEMOGRAPHIC INFORMATION	
6.	Educational status	1. Illiterate 2. Read and write 3. Primary (1-8) 4. Secondary (9-12) 5. College diploma/degree and above
7.	Occupation	1. Student 2. Housewife 3. Government employee 4. Private employee 5. Farmer 6. Others(specify)___
8.	Marital status	1. Single 2. Married 3. Divorced 4. Widowed 5. Not applicable(adolescent)

9.	Religion	1. Orthodox Christian 2. Muslim 3. Protestant 4. Catholic 5. Others (Specify)_
10.	Ethnicity	_____If mixed, specify_____
11.	Father ‘s Educational Level	1. Illiterate 2. Read and write 3. Primary (1-8) 4. Secondary (9-12) 5.College diploma/degree and above
12.	Mother ‘s Educational Level	_____
13.	Father ‘s Occupation	_____
14.	Mother ‘s Occupation	_____
15.	Monthly income (in birr collected from salary, rent, and other income)	_____Birr
16.	Family Size (Number of People)	_____
17.	Source of water	1. Pipe 2. Spring water 3. Well water 4. River 5. Other sources (specify____)
18.	Type of house	1. Mud 2. Cement 3. Wood 4. Bricks 5. others/specify___
19.	Presence of or contact with Pet animals (e.g. Cat, Dog)	1. Yes 2. No
20.	Presence of domestic animals	1. Yes 2. No

21.	Did you take any type of drug for any illness for the last three months?	1. Yes 2. No	
22.	If yes to Q29, what type of drug? (more than one answer possible)	Anti-protozoa Anti-helminthic Anti-allergy Birth control pills Anti-bacterial Anti-TB Other (specify_____)	
History of common diseases			
23.	History of diabetes	1. Yes	2.No
24.	History of Hypertension	1. Yes	2.No
25.	History of Hospital Admission for the last 1 year	1. Yes	2.No
26.	History of Surgical procedure for the last three years?	1. Yes	2.No
27.	History of chronic gastritis	1. Yes	2.No
28.	History of Malaria for the last 6 months	1. Yes	2.No
29.	History of TB for the last two years	1. Yes	2.No
30.	History of Cancer	1. Yes	2.No
31.	History of Cardiac illness	1. Yes	2.No
32.	History of Bleeding disorders	1. Yes	2.No
33.	History of allergy	1. Yes	2.No
34.	History of Wheezing	1. Yes	2.No

Part V. Nutritional habit and your lifestyle

No.	**Food type**	Once/day	More than Once/ day	Occasionally (holidays)	Never
35.	Roots and Tuber like Potato, sweet potato, Enset,Cassava)				
36.	Legumes (Beans,peas,pea etc)				
37.	Cereals (Corn,Teff,Wheat, etc)				

38.	Vegetables (Tomato, cabbage, etc)				
39.	Fruits (Orange, banana, etc)				
40.	Meat (including poultry, fish, etc)				
41.	Milk and Milk products (Butter, yogh urt, cheese, etc)				
42.	Egg				
43.	Tea and/or coffee				

Part V. Lifestyle/Habit Continued…

44.	Do you have a Fasting habit?	1 Yes	2. No
45.	If Yes, how is your fasting habit?	1. Eating vegetable food-only 2. Complete abstinence from food then eating all kinds of food 3. Complete abstinence from food then eating vegetable food-only.	
48	Did you eat undercooked/raw meat?	1 Yes	2 No
49	Alcohol	1 Yes	2 No
50	Khat	1 Yes	2 No
51	Cigarettes	1 Yes	2 No
	Part VII. Anthromphotrics		
52.	Height (in cm)	_____	
53.	Weight (in kg)	_____	
54.	MUAC (in cm)	_____	
55.	Blood pressure (mm Hg)	_____	
56	Temperature (in degree centigrade)	_____	

Thank you for your cooperation

Interviewer Name_____ Signature_____

Annex XI: Questionnaire Amharic version

በጤና ባለሞያዎች የሚሞላ ቃለ መጠይቅ

መመሪያ፡በቅድሚያይህንንቃለመጠይቅለመሙላትለሰጡንጊዜናትብብርአድናቆቴንእገልፃለሁ።የዚህቃ
ለመጠይቅአላማ-በላቦራቶሪውስጥየጥራትመመርመሪያንጥረነገርእናየጤናማሰውደምውስጥየሚገኙየ
ሄማቶሎጂናየክሊኒካልኬሚሰትሪምርመራዎችመጠንሪፈረንስኢንተርቫልእድሜአቸውአስራሁለትዓመት
ናካዚያበላይ ለሆኑኢትዬጵያውያንላመስራት-መረጃለመሰብሰብነው።የዚህጥናትሃሳቡንያመጡትየጥናቱ
ዋናተመራማሪበአዲስአበባዩኒቨርስቲየህክምናላቦራቶሪትምህርትክፍልተባባሪፕሮፌሰርየሆኑትዶ/ርአስቴ
ርፀጋዬሲሆኑየኢትዮጵያህክምናላቦራቶሪማህበርያስተዳድረዋል።

የጥናቱንወጪየሸፈነውየፌደልሳይንስናቴክኖሎጂሚኒስቴርነው።ስለሆነምየእርስዎቅንትክክለኛመልስበ
ሰዓቱመስጠትየዚህንጥናትስኬትይወስናል።አስራአምስትየሚሆኑተቋማትማለትምዩኒቨርስቲዎች፣ሪጅና
ልላቦራቶሪዎች፣እናብሄራዊየደምባንክአገልግሎትጥናቱንለመደገፍዝግጁነታቸውንገልፀዋል።ስለሆነምይ
ህንንቃለመጠይቅሃቀኝነትናሃላፊነትበተሞላውመንገድእንዲሞሉበትህትናእጠይቃለሁ።

አመሰግናለሁ!!!

ክፍል1. አጠቃላይመረጃ

ኮድ_____ክልል_____ዞን_____

ወረዳ_____ከተማ/ክፍለከተማ_____ቀበሌ_____

ክፍል2. የግልመረጃ

1. እድሜ _____
2. ጾታ _____
3. የትውልድቦታ _____
4. በትውልድቦታዎለምንያህልጊዜኖረዋል?_____
5. አሁንያሉበትቦታለምንያህልጊዜኖረዋል? (ከትውልድቦታዎየተለየከሆነ)_____ዓ.መ

ቁጥር.	ጥያቄ	ምላሽ
	ክፍል3. ማህበራዊ ናኢኮኖሚያዊ መረጃ	
6.	የትምህርትደረጃ	1. ያል ተማሩ 2. ማበብ ና መፃፍ 3. አንደኛ ደረጃ (1-8) 4. ሁለተኛ ደረጃ (9-12 5. ዲፕሎማ/ዲግሪ እና ከዚያ በላይ
7.	ሥራ	1. ተማሪ 2. የቤት እመቤት 3. የመንግስት ሠራተኛ 4. የግል ተቀጣሪ 5. ገበሬ 6. ሌላ ካለ ይግለፁ__
8.	የ ጋብቻ ሁኔታ	1. ያላገቡ 2. ያገቡ 3. የተፋቱ 4. ባል/ሚስትየሞተባቸው 5. አይመለከታቸውም(ህፃናት
9.	ሃይማኖት	1. ኦርቶዶክስክርስቲያን 2. ሙስሊም 3. ፕሮቴስታንት 4. ካቶሊክ 5. ሌላ ካለ ይግለፁ___
10.	ብሄረሰብ	_____ድብልቅ ከሆኑ ይግለፁ____

11.	የአባትየትምህርትደረጃ	1. ያልተማሩ 2. ማንበብናመፃፍ 3. አንደኛ ደረጃ (1-8) 4. ሁለተኛ ደረጃ (9-12) 5. ኮሌጅዲፕሎማ/ዲግሪእና ከዚያበላይ
12.	የእናትየትምህርትደረጃ(ከተ/ቁ14 ይምረጡ)	_____
13.	የአባትሥራ	1. ተማሪ 2. የቤትእመቤት 3. የመንግስትሠራተኛ 4. የግልተቀጣሪ 5. ገበሬ 6. ሌላካለይግለፁ
14.	የእናትሥራ(ከተ/ቁ16 ይምረጡ)	_____
15.	ወራዊገቢ(በብርከደሞዝ፣ኪራይ፣እናሌሎችገቢዎች)	_____ ብር
16.	የቤተሰብብዛት	_____
17.	የውሃምንጭ	1. ቧንቧ 2. የምንጭ 3. የጉድጓድ 4. የወንዝ 5. ሌላካለይግለፁ___
19.	የቤትውስጥለማዳእንስሳመኖርወይምንክኪ ለምሳሌድመት፣ውሻ)	1. አለ 2. የለም
20.	የቤትእንስሳትመኖር	1. አለ 2. የለም
21.	ባለፉትሶስትወራትለማንኛውምዓይነትህመም ማንኛውንምዓይነትመድሃኒትወስደኃል?	1. አዎን 2. የለም

22.	ለተራቁጥር29 መልስዎወስጃለሁከሆነየትኛውንዓይነት መድሃኒትነውየወሰዱት? (ከአንድበላይመልስይቻላል)	1. ፀረ-ፕሮቶዞአ 2. ፀረ-ሄልሚንትስ 3. ፀረ-አለርጂ 4. የወሊድመከላከያኪኒን 5. ፀረ-ባክቴሪያ 6. ፀረ-ቲቢ 7. ሌላካለይግለፁ___	
	የሚከተሉት የህመም ዓይነቶች አሞዎት ያውቃል?		
23.	የስኳር ህመም?	1.አዎን	2. የለም
24.	የደም ግፊት ከፍ ማለት?	1.አዎን	2. የለም
25.	ባለፈው1 ዓመት ሆስፒታል ተኝተው ያውቃሉ?	1.አዎን	2. የለም
26.	ባለፉት3 ዓመታት የቀዶህክምና ተደርጎልዎ ያውቃል?	1.አዎን	2. የለም
27.	የቆየየ ጨጓራ ህመም አለብዎት?	1.አዎን	2. የለም
28.	ባፉት6 ወራት የወባ ህመም አጋጥሞዎት ያውቃል?	1.አዎን	2. የለም
29.	ባለፉት2 ዓመታት የቲቢ ህመምኖሮዎት ያውቃል?	1.አዎን	2. የለም
30.	ካንሰር ህመም	1.አዎን	2. የለም
31.	የልብህመም	1.አዎን	2. የለም
32.	የመድማትችግር/ህመም	1.አዎን	2. የለም
33.	አለርጂ(የሰውነትመቆጣት)	1.አዎን	2. የለም
34.	የመተንፈስችግር(ሲተነፍሱሲርሲርየሚልድምፅ)	1.አዎን	2. የለም

ክፍል5. የ አመጋገብ እና የህይወት ልምድዎ

የሚከተሉትን የምግብ ዓይነቶች ምንያህልጊዜ ይመገቧቸዋል? (“Ö “ይህን ምልክት ያስቀምጡ)					
ተ/ቁ	የምግብዓይነት	በቀንአንድጊዜ	በቀንከአንድጊዜ በላይ	አልፎአልፎ	ተጠቅሜአላውቅም
35.	ሥራሥር(ድንች፤ስኳርድንች፣እንሰት፣ካሳቫ ወዘተ)				

36.	አዝርት፡ባቄል፣አተር፣ሽንብራ ወዘተ)				
37.	ጥራጥሬ (በቆሎ፣ጤፍ፣ስንዴ)				
38.	አትክልት: ቲማቲም፣ጎመን፣ወዘተ)				
39.	ፍራፍሬ (ብርትኳን፣ሙዝ፣ወዘተ)				
40.	ሥጋ(የዶሮ፣የአሳንጨምሮ)				
41.	ወተትና የወተት ተዋፅዎ እርጎ፣ቅቤ፣አይብ፣ ወዘተ)				
42.	እንቁላል				
43.	ሻይ እና/ወይም ቡና				
44.	የ መፆም ልምድ አለዎት?		1. አዎን 2. የለም		
45.	መልስዎአዎንከሆነ፣የመፆምልምድዎእንዴትነው		1. አትክልቶችን ብቻ መመገብ 2. በአጠቃላይ ከምግብ መታቀብ ከዚያ ምያገኙትን መመገብ 3. በአጠቃላይከምግብመታቀብከያም አትክልቶችን መመገብ		
46.	የሰውነትእንቅስቃሴየማድረግልምድአለዎት?		1. አዎን 2. የለም		
47	መልስዎአለኝከሆነበሳምንትለምንያህልጊዜይንቀሳቀሳሉ		_____		
48	በደንብያልበሰለወይምጥሬሥጋይመገባሉ?		1. አዎን 2. የለም		
49	አልኮል		1. አዎን 2. የለም		
50	ጫት		1. አዎን 2. የለም		
51	ሲጋራ		1. አዎን 2. የለም		
ክፍል 6. **ክብደት፣ቁመት፣የክንድናየደምግፊትልኬት**					
52.	ቁመት		_____		
53.	ክብደት		_____		

54.	የክንድመሃለኛውክፍልዙሪያው(MUAC)	_____
55.	የደምግፊት(በሚሊሜትርሜርኩሪ)	_____
56	የሰውነት ሙቀት ልኬት(በዲግሪ ሴንቲ ግሬድ)	_____

ስለ ትብብርዎ እናመሰግናለን

ቃለ መጠይቅ የተደረገበት ቀን ----------

ቃለ መጠይቁን ያካሄደው ስው ስምና ፊርማ ----------

Annex XII: Questionary Afaan Oromoo version

Gaaffii fi deebiiOgeessaFayyaatiinGuutamu

Qajeelfama: Kaayyoon gaaffii kanaLaabooratoorii keessati qulqulinaa wantoota sakata ‘aamufi dhiiga ijoollee fayyaa bulessa ta‘ee tokko keessati kan argamu kilinikaal Hemaattoloojjii fi keemistirii ilaalchisee hanga argama isaa giddu-galessan jirata magaala Assalaa umurii waggaa 12-17 ta‘aanif hojjaachuuf data funaanuu dha. Yaad - rimeen qorannoo kana kan burqisiisan yunivaaristii Finfinneetti gosaa barnoota medical laboratorii tti gargaraa poroffesera kan ta‘aan Dr.AsterTsaagayee ta‘uu ibsaa gaaffilee kana itti gaafatamummaa fi amanamumman akka guuttanii kabajaan isin gaafanna.

Galatooma!!!

Kutaa 1ffa. Odeeffannoo Waliigalaa

1 Lakkofsaa Koodi_____Ganda_____ Lakkofsa bilbilaa_____

Kutaa 2ffa. Odeeffannoo Dhuunfaa

2 Umurii (Waggaadhaan)_____Saala _____

3 Iddoo dhaloota keessaan _____

4 Turtii Iddoo dhaloota keessan jiraataan (waggadhan) _____

5 Turtii iddoo ammaajiraatani (yoo iddoo dhalootan alaa jiraatan ta‘ee) _____

Lakk.	Gaaffii	Deebii
	Kutaa 3ffaa. Halaa Odeeffannoo ummaataa	
6	Haala Sadarkaa barumsa	1. Kan hin baraatin 2. Duubisuu fibaareessu 3. Sadaarka tokkoffaa (1-8) 4. Sadaarka Lammaaffaa (912) 5. Dipilooma/Digiriifi sana olii
7.	Haala hojii	1. Baraataa 2. Manakeessaa 3. Mootummaa 4. Dhuunfaa 5. Kaan biroo___

8.	Halaa fudhaa fi Herumaa	1. Hin funne/hinheruumne 2. Fudhee/heruume 3. Hiikee 4. Kaadhimaame 5. Hin genyee (daa'imaa)
9.	Ammaantaa keessaan	1. kiristaana ortoodoksii 2. Muusilimaa 3. Porotestaanti 4. Katoolikii 5. kan biroo_____
10.	Saabbummaa	_____makkaa yoo ta'ee ibsi ____
11.	Saadarkaa barnoota abbaa keessaan	1. kaan hinbaraatin 2. Duubisuufibaarreessu 3. Sadaarkaa tokkooffa (1-8) 4. Sadaarkaa lammaaffa (9-12) 5. Kolleejji dipilooma ykn digirii
12.	Saadarkaa barnoota hadhaa	_____
13.	Hoojii abbaa keessaan	_____
14.	Hojii Hadhaa keessaan	_____
15.	Gaalii ji'aati argaatani	_____Qaarshii_____
16.	Baayinaa maattii	_____
17.	Bishaan dhugaati eessaa fayyaadamtuu	1. Bombaa 2. Rooba 3. Boollaa 4. Lagaa 5. kan biroo
18.	Maannii keessaan maalin ijaaramee	1. Dhoqeen 2. Boollokeetin 3. Muukaan 4. Xuubiidhaan 5. Kan biroo___

19.	Beeylaada manaa qabduu ?	1, Eyyeen 2. Lakkii
20.	Beeylaada manaatin taphaatu (tuuxuxuu)	1. Eyyeen 2. Lakkii
21.	Ji‘oota saddeen darbaanif qorichii fudhate jira.	1. Eyyeen 2. Lakkii
22.	Deebiin Gaaffii 29 eyyeen yoo ta‘ee qorichaa gosaa kamittii?(Deebiin tokkoo olii ni dandaa‘ama)	1. Antiyii-pirotozuwwaa 2. Antiyii-helmeentikii 3. Antiyii-allaarjiiki 4. Qorsa Quusaanamaatti 5. Antiyiibaacteriyyaa 6. Antiyii-TB 7. Kanbiroo
	Kutaa 4ffaa Haala dhukkubaa waaligala	
23.	Dhukkuubaa suukaraa qabdaa	1. Eyyeen 2. Lakkii
24.	Dhukkuubaa dhiibbaa dhigaa qabdaa	1. Eyyeen 2. Lakkii
25.	Waggaa tokkoo keessaa hospitaala ciistee jirtaa	1. Eyyeen 2. Lakkii
26.	Waggaa saddeen keessaa qamaa baqaafattee jirta	1. Eyyeen 2. Lakkii
27.	Dhukkuubaa garaachaa qabda	1. Eyyeen 2. Lakkii
28.	Ji‘aa jahaan darbee keessaa buusaa dhukkubsatee	1. Eyyeen 2. Lakkii
29.	Waggaa lamaan keessaa daraamyoo sombaan qabaamtee jirtaa	1. Eyyeen 2. Lakkii
30.	Kaanseerii dhukkubsattee bektaa	1. Eyyeen 2. Lakkii
31.	Dhukkuubaa onnee dhukkuubsatee	1. Eyyeen 2. Lakkii
32.	Dhukkuubaa dhiguu dhukkuubsatee	1 .Eyyeen 2.Lakkii
33.	Dhukkuubaa Allaarjiikkii dhukkuubsate	1. Eyyeen 2. Lakkii
34.	Dhukkuubaa sirnaa hargaansuu dhukkuubsate	1. Eyyeen 2. Lakkii

Kutaa 5ffaa Amalaa nyaataafi haala jireenyaa

Gosotaa nyaataa armaangadiakkamiinnyaataa?(mallattoo **“X “** ka’ii)

	Gosaa nyaata	Guyyatti altokko	Guyyatti al tokko oli	Darbee darbee	Hin fayyadamnu
35.	Nyaata hidda fi jirmaa Mosee,Mixaaxisaa				
36.	Dheedhii (Baqelaa, Ataraa)				
37.	Callaa (Boqqollo,Xaafii,Qamadii)				

38.	Kuduraa (Timaatimaa,raafuu)				
39.	Fudura (Burtukaana, Muuza)				
40.	Foon (kan lukkuu,qurxummii)				
41.	Annaaniifi bu'aa isaa Itittuu Dhadhaa)				
42.	Killee				
43.	Shaayii fi ykn Bunaa				

Haala jireenyaa /barmaata itti fufaa…

Kanneen armaan gadii yeroo hamaam fayyaadamtaa ?

44	Sommaa ni somtaa?	1. Eyyee 2. Lakkii
45.	Eyyeen yoo ta'ee soomma akkami?	1. Nyaata kuduraafi fudura qofa nyachu 2. Nyaata irraa turuun sana bodaa gosaa nyaata hunda nyachuu. 3. Nyaata hundaa irraa turuun sana booda kuduraafi fudura qofa nyaachuu.
46.	Jaajjabinaa qamaa ni hojataa?	1. Eyyee 2.Lakkii
47.	Eyyeen yoo ta'ee torbaanit si'aa meqaa hojaata	_____
48.	Foonii dheedhii nyaata	1. Eyyee 2. Lakkii
49.	Alkoolii Dhuguu	1. Eyyee 2.Lakkii
50.	Jimmaa Alanfachu(qamaawuu)	1. Eyyee 2.Lakkii
51.	Tamboo afuufuu	1. Eyyee 2.Lakkii
	Kutaa 6ffaa lakkoftuu ulfaatina, dheerina, fi dhiibba dhiigaa	
52.	Dheerina (Seentimeetiriin)	_____(cm)
53.	Ulfaatinaqaama (Kiiloogiramaan)	_____(kg)
54.	Maawakii (Seentimeeteriin)	_____(cm)
55.	Dhiibbadhiiga (miilimeetirmeerkurin)	_____(mmHg)
56.	Hamma Hoo'ina qama (digrii centigireedidhan)	_____ (oC)

Waan hirmaattaanif guddaa galaatooma!

Maqaa namaa gaafatee _____

Maallaattoo_____Guuyyaa_____

Annex XIII: Standards operator Procedures (SOPS)

Urine Reagent strip procedure

Dip the test strip in the urine specimen. Remove the test-strip immediately and let the excess urine drain off on a paper towel, or tap the edge of the strip.

Read the color change

Report the result according to the color chart provided by the manufacturer.

Always read the test strip in good white light and ignore color developing on the test area after the period specified as the reading time of the test.

Be careful not to wet the reagent strip excessively. So that the acid buffer from the protein area runs into the pH area, causing an orange discoloration.

Urine Microscopy procedure

Mix the urine specimen

Transfer about 10 ml of urine into a labeled centrifuge tube.

Centrifuge the specimen at a medium speed (from 1500 – 2000 rpm) for 3-5minutes

Discard the supernatant by quick inversion of the tube.

Re suspend the sediment that is at the bottom of the tube, by tapping the tube by your fingers

Take the sediment by Pasteur pipette from the tube and transfer a drop into the clean and dry slide.

Apply a cover slide on the urine sediment that is on the slide.

Put on the microscope and look under the 10x objective of the microscope.

Then after looking through the low power objective, change the objective into 40x objective.

Then report what you get under low power and high power objective on the laboratory request form.

Procedure for stool examination using wet mount preparation

Instruct the participant how to collect the stool in the labeled, clean, and dry leak-proof container.

Receive the sample and check with participant Id and observe the appearance of the stool and record.

Place a drop of fresh physiological saline on one end of a slide and a drop of iodine on the other end.

Using a wire loop or piece of stick, mix a small amount of specimen, about 2 mg, (matchstick head amount) with the saline, and a similar amount with the iodine.

Make smooth thin preparations.

Cover each preparation with a 22x22 cover glass.

Examine systematically the entire saline preparation for larvae, ciliates, helminths eggs, cysts, and oocysts.

Use the 10x objective with the condenser iris closed sufficiently to give a good contrast.

Use the 40x objective to assist in the detection and identification of eggs, cysts, and oocysts.

Always examine several microscope fields with this objective before reporting No parasites found.

Use the iodine preparation to assist in the identification of cysts

Report the presence of larvae, ciliates, helminths eggs, cysts, and oocysts

The procedure of formal-ether concentration method

Using a rod or stick, emulsify an estimated 1 g (pea-size) of stool in about 4 ml of 10% formol water contained in a screw-cap bottle or tube.

Add a further 3–4 ml of 10% v/v formol water, cap the bottle, and mix well by shaking.

Sieve the emulsified stool, collecting the sieved suspension in a beaker.

Transfer the suspension to a conical (centrifuge) tube made of strong glass, copolymer.

Add 3–4 ml of diethyl ether or ethyl acetate.

Stopper the tube and mix for 1minute.

With a tissue or piece of cloth wrapped around the top of the tube, loosen the stopper (considerable pressure will have built up inside the tube).

Centrifuge immediately at 750–1,000 g (approx. 3000 rpm) for 1minute.

Using a stick or the stem of a plastic bulb pipette, loosen the layer of fecal debris from the side of tube

Invert the tube to discard the ether, fecal debris, and formol water. The sediment will remain.

Return the tube to its upright position and allow the fluid from the side of the tube to drain to bottom.

Tap the bottom of the tube to re suspend and mix the sediment.

Transfer the sediment to a slide, and cover with a cover glass.

Using the 10x objective with the condenser iris closed sufficiently to give a good contrast.

Use the 40x objective to examine small cysts and eggs.

To assist in the identification of cysts, run a small drop of iodine under the cover glass. Although the motility of Strongyloides larvae will not be seen, the non-motile larvae can be easily recognized.

If required, count the number of each species of egg in the entire preparation. This will give the approximate number per gram of stool.

TEST PROCEDURE, MATERIALS AND PRINCIPLE OF HIV TEST.

Principle of HIV test

The Chembion HIV ½ STAT-PAK employs a unique combination of a specific antibody binding protein, which is conjugated to colloidal gold dye particles, and HIV ½ antigens, which are bound to the membrane solid phase. The sample is applied to the sample (S) well followed by the addition of running buffer. The buffer facilitates the lateral flow of the released product and promotes the binding of antibodies to the antigens. If present the antibodies bind to the gold conjugated antibody binding protein in a reactive sample, the dye conjugated-immune complex migrates on the nitrocellulose membrane and is captured by the antigen immobilized in the test (T) area producing a pink /purple line.

MATERIALS PROVIDED

- 20 STAT-PAK individually punched to test devices
- 1 HIV running buffer(3.5ml)
- 20 disposable 5µl sample loops
- 1 product insert
- Clock or timer

PROCEDURES

1 Remove the chemboi HIV ½ STAT-PAK test devices from its pouch and place it on a flat surface

2 Label the test devices with patient name or identification number

3 Touch the 5µl sample loop provided to the specimen, allowing the opening of the loop to fill the liquid.

4 Holding the sample loop vertically, touch it to the sample pad in the center of the SAMPLE(S) well of the devices to dispense 5µl of the sample (serum, plasma and whole blood)

5 Invert the running buffer bottle and hold it vertically over the sample well and add 3 drops of buffer slowly in to sample well.

6 Read the test result 15 minutes after the addition of the running buffer**.**

TEST PROCEDURE, MATERIALS AND PRINCIPLE OF HBsAg TEST

Principle of HBsAg tests

The HBsAg rapid test is a lateral flow chromatographic immunoassay based on the principle of the double antibody sandwich techniques. The membrane is pre coated with anti-HBsAg antibodies on the test line region of the test. During testing hepatitis B surface antigen in the whole blood, serum or plasma specimen reacts with the particle coated with anti-HBsAg antibody.

MATERIALS PROVIDED

- The HBsAg test cassete containing anti-HBsAg antibody particles and anti-HBsAg antibody coated on the membrane.
- Disposable specimen dropper
- Package inserts
- Buffer
- Centrifuge for plasma and serum
- Clock or timer

TEST PROCEDURE

1. Remove the test device from the foil pouch and use it as soon as possible
2. Place the test device on a clean and level surface. And hold the dropper vertically and transfer 3 drops of serum or plasma to the specimen well of the test devices.
3. Wait for the red line to appear. The result should be read at 15 minutes.

TEST PROCEDURE, MATERIALS AND PRINCIPLE OF HCV ANTIBODY TEST

Principle of HCV Antibody tests

Rapid HCV antibody test employees chromatographic lateral flow devices in a cassette format. Colloidal gold conjugated goat anti-human IGM and mouse anti-human Ig Gare dried and immobilized on the fiber glass strip. HCV antigen are immobilized at the test zone (T) and goat anti mouse IgG antibody are immobilized at control zone (C).When the sample is added, it migrates by capillary diffusion rehydrating the gold conjugated .If present in sample, HCV antibody will bind the gold conjugated anti-human IgG and/or IgM forming complexes. These complexes will continue to migrate along the strip until the Test (T) zone where they captured by the HCV antigen to form a visible red line. The unbound gold conjugate will continue to move and bind with goat anti-mouse IgG at the control zone (C) forming a visible red line. If no antibody in the sample, only a red line is appeared at the control zone, which indicates the validity of the test.

MATERIALS NEEDED

Rapid HCV anti body test
Sample buffer
Instruction for use
Centrifuge
Clock or timer

TEST PROCEDURE

1 Allow the test strip and sample to reach room temperature

2 Open the pouch, take out the test strip and transfer pippet

3 Use the transfer pipette to draw up the sample,

4 Dispense one drop specimen to the sample pad and wait a few seconds until the sample is completely absorbed by sample pad.

5 Add one drop sample buffer to the sample pad

6 Read sample result at 20 minutes.

TEST PROCEDURE, MATERIALS AND PRINCIPLE OF SYPHILIS ANTIBODY TEST

Principle of syphilis Antibody test

The syphilis rapid test is a lateral flow chromatographic immunoassay based on the principle of the double antigen sandwich techniques. In this test syphilis recombinant antigen is immobilized in the test line region of the strip in the test device. After the specimen is added to the specimen well of the device, it reacts with the syphilis recombinant antigen coated particles in the test. This mixture migrates chromatographically along the length of the test strip and interacts with the immobilized syphilis antigen. if the specimen contains syphilis antibodies, a colored line will appear in the test line region indicating a positive result. If the specimen does not contain syphilis antibodies, a colored line will not appear in the test line region, indicating a negative result. to serve as procedure control, a colored line will always appear in the control line region, indicating that proper volume of specimen has been added and membrane wicking has occurred.

MATERIALS NEEDED

- The syphilis Ab rapid test kits
- Specimen collection container
- Centrifuge for serum
- Disposable specimen dropper
- Clock or timer

TEST PROCEDURE

1. Remove the test device from the foil pouch and use it as soon as possible
2. Place the test device on a clean and level surface. And hold the dropper vertically and transfer
3. drops of serum or plasma to the specimen well of the test devices.
4. Wait for the red line to appear.
5. The result should be read at 15 minutes.

Principle of Hematological Assay

A complete blood count (CBC) and differential was performed on the blood sample, using Sysmex KX-21N, an automated 3-part differential hematology analyzer. The machine automatically dilutes whole-blood sample of 50 ml in the CBC/Differential mode, lyses and directly measures the WBC, RBC, HGB, HCT, and PLT, LYM #, MXD #and NEUT #. The remaining parameters are calculated or derived, MCV, MCH, MCHC, MPV, RDW-CV and RDW-SD, and differential percentages LYM%, MXD%, NEUT%. Sysmex KX-21N count RBC, WBC and PLT using electronic resistance detection.

Its principle was impedance principle which was based on the detection and measurement of changes in electrical resistance produced by a particle suspended in a conductive liquid as it is drawn through a small aperture. A blood sample is diluted in saline, which is a good conductor of electrical current. DC current is applied between the two electrodes. Electrical resistance or impedance occurs as the cells pass through the aperture causing a change in voltage. The change in voltage generates a pulse. Each cell momentarily increases the electrical resistance between two electrodes. The amplitude and size of the pulse depends on the cell volume.

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university, and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Berhanu Dibaba (B.Sc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: Aster Tsegaye (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Mr. Jemal Alemu (MSc, PhD candidate)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.