LABORATORY REFERENCE INTERVALS FOR ROUTINELY ANALYZED IRON PROFILE PARAMETERS IN ADULT POPULATION AGED 18-64 YEARS, IN UASIN GISHU COUNTY, KENYA

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DEDICATION

To my wife Metrine Julie and to my son Joe Muuo, for the encouragement and love, and to my late mom Dorothy Kasimu who passed on while I was undertaking this journey, for the encouragement for me to begin this journey.

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LIST OF ABBREVIATIONS AND ACRONYMS

BMA	Bone marrow aspiration
HBsAg	Hepatitis B surface antigen
DMT1	Divalent metal-ion transporter-1
DNA	Deoxyribonucleic acid
EpoR	Erythropoietin receptor
HCV	Hepatitis C virus
HH	Hereditary hemochromatosis
HIV	Human immunodeficiency virus
HTF	Human transferrin
FPN 1	Ferroportin 1
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine.
IRPS	Iron regulatory proteins

ISO	International Organization for Standardization
KNH	Kenyatta National Hospital
MCHC	Mean cell heamoglobin concentration
MCV	Mean cell volume
Mmol/L	millimole per liter
MTRH	Moi Teaching and referral hospital
NCCLS	National Committee for Clinical Laboratory Standards
nm	Nanometers
Ph	Hydrogen ion concentration
QC	Quality control
RI	Reference interval
r.p.m	Revolutions per minute
TFR	Transferrin receptor

SOPs	Standard operating procedures
TAT	Turn around time
TIBC	Total iron binding capacity
µl	Microlitre
UIBC	Unsaturated iron binding capacity
VDRL	Venereal Disease Research Laboratories

DEFINITIONS

Early Adulthood: Adulthood stage between the ages of 18 and 34 years.

Early Middle Age: Adulthood stage between the ages of 35 and 44 years.

Late Middle Age: Adulthood stage between the ages of 45 and 64 years.

Late Adulthood/geriatric: Adulthood stage people 65years and older.

Observed value: The value of a particular type of quantity, obtained by observation or measurement of a test subject which is to be compared with reference values; reference distributions, reference limits, or reference intervals.

Reference distribution: This is the distribution of reference value and involves reference sample group and adequate statistical methods.

Reference individual: A candidate chosen for testing using predetermined criteria.
Reference interval: Also commonly referred to as reference range or normal range

Reference limits: A value derived from the reference distribution and used for descriptive purposes.

Reference population: The selected population that the reference individuals belonged to.

Reference sample group: A group of selected reference individuals from a reference

the population on which the reference intervals are determined.

Reference value: The value obtained by the observation or measurement of a particular type on a reference individual.

ABSTRACT

Reference intervals are regarded as golden tools used to guide clinicians to correctly diagnose and monitor diseases, assess patients' responses to therapy, and forecast any probable risk factors. However, reference intervals vary largely across different populations due to multiple factors. To address these issues, the Clinical Laboratory Standards Institute (CLSI) advocates for the use of locally established and verified RIs. The literature on iron profile RIs for adults in Kenya remains scanty, and no known publications or research has been done for the Uasin Gishu population. The interpretation of these results is currently based on the manufacturer's provided RIs, derived from other regions. This study was aimed at establishing and verifying normal reference intervals for four routinely analyzed iron profile parameters for the adults in Uasin Gishu County, Kenya. The study was a cross-sectional population study and was carried out at Moi Teaching and Referral Hospital, with 290 healthy participants enrolled for the study using the multi-stage sampling technique. Using a fully automated clinical chemistry and immunochemistry analyzer (Cobas 6000 series), the levels of iron and unsaturated iron binding capacity were analyzed using the ferrozine method, while ferritin and transferrin were analyzed based on the immunoturbidimetric method. Reference values were determined by using the non-parametric method at a confidence interval of 95%, this entailed computing the 2.5^{th} and 97.5^{th} percentiles as the lower and upper limits, respectively. Sigma diagnostics method was used for the verification of the established reference intervals. Statistical analysis was performed using IBM SPSS version 23.0. The established reference intervals were as follows: Ferritin: males: 28.41–425.60 ng/mL, females: 11.15–155.87 ng/mL; Iron: males: 8.27–32.02 umol/L, females: 5.21–25.45 umol/L; Transferrin: males: 2.13–4.01 g/L, females, 2.08–4.04 g/L; UIBC: males, 20.50–64.08 umol/L, females: 20.07–64.21 umol/L. Comparisons between genders for significant differences were done using Mann-Whitney U tests, revealing statistically significant differences in ferritin and iron levels with p=<0.001. Conversely, transferrin and UIBC exhibited no significant gender-based differences with p values of 0.829 and 1.00 respectively. Notably, the manufacturer's given RIs were verified, with the majority aligning with the established RIs except for iron values in women. Despite this, the RIs showed a broader interval spread between the upper and lower limits, which is clinically significant. In conclusion, the established reference intervals are sex, and age specific for the adult population in Uasin Gishu County, Kenya. These intervals are crucial for accurate clinical diagnosis and effective patient management.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information of the study

Iron profile tests is an array of tests that offer useful information regarding the blood levels of many iron-related factors. These tests aid in the diagnosis and follow-up of illnesses involving iron metabolism and the body's capacity to carry and store iron, such as anemia, hereditary hemochromatosis, iron excess, iron deficiency, and other abnormalities of iron metabolism (Pagani *et al.*, 2019). Iron profile tests are designed to augment other laboratory tests, such as the heamatological tests and bone marrow biopsy, with the aim of offering accurate, scientific-based patient care. Iron profile tests are easily performed, less invasive, and are more routinely used in the diagnosis and therapy monitoring of iron related diseases compared to other diagnostic tests, such as bone marrow aspiration (BMA) (Pfeiffer & Looker, 2017).

As with any other clinical laboratory test results, iron profile results will not be useful to either the clinician and the patients unless reported with, and interpreted with reference intervals/normal ranges (Katayev *et al*., 2010). Reference intervals (RIs) are golden tools frequently employed in the process of interpreting and making medical diagnoses, therapeutic management choices, or other physiological evaluations. Reference intervals are crucial because they provide medical healthcare professionals with a baseline to evaluate a patient's health, identify potential illnesses, and reach a decision-making point

between those with pathological conditions and those who are normal. However, the use of unverified reference intervals from other regions is not recommended as reference intervals vary significantly in different locations due to factors such as race, genetics, diet, altitude, and age, among others. This not only contravenes the clinical laboratory standard institute (CLSI) recommendations but can also bring about accuracy in diagnosis and treatment (Rappoport *et al.,* 2018). Typically, the normal reference interval for a given test is harboured on the results that are observed in 95% of the healthy reference population (Jones & Barker, 2008; Xia *et al* 2016; Ozarda, 2020). These reference intervals, when applied to a broader population served by the laboratory, accurately comprise the majority of individuals who share similar characteristics to the reference group. (Miller *et al*., 2016).

The reference interval indicates the range limits of results expected for a given condition. Generally, when a patient's test results fall within the reference interval, it signifies that they are within the range expected for a healthy individual of the patient's age and sex. Conversely, results that fall outside the reference interval may indicate the presence of a medical issue or the need for further testing. While the term “reference range” is sometimes used, it is more accurate to refer to it as the “reference interval” because “range” implies the absolute maximum and minimum values, whereas “RI” encompasses the expected variation (Higgins, 2009). Ideally, reference intervals are established based on data from a healthy reference population. However, in certain situations, these intervals can be obtained from persons with other physiological or pathological conditions, and can serve various purposes. In all cases, the reference values allow observed data to be compared with reference data from a defined population (Sikaris, 2014).

The interpretation of clinical chemistry tests relies heavily on the reference intervals (RIs), which represent the expected range of values for specific medical conditions. These RIs can vary significantly between different population groups due to factors such as heredity, age, sex, race, nutrition, climate, and altitude (Rappoport *et al.,* 2018). Genetic variations within populations can directly impact reference intervals, as these changes might results in differences in the distribution of certain results within a given group. Consequently, using reference intervals from books or other sources can be unreliable, as they may not accurately reflect the local population's characteristics. Therefore, each laboratory should establish its own reference intervals tailored to its specific context, aligning with the local population it serves (Ozarda *et al*, 2018). The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and the Clinical and Laboratory Standards Institute (CLSI), leading organizations in clinical laboratory medicine, recommend that laboratories follow standardized procedures to determine local reference intervals for clinical chemistry tests (Ozarda, 2016). This guidance discourages sole reliance on manufacturer-supplied reference interval values or those from the literature (PetitClerc & Solberg, 1987; Solberg & PetitClerc, 1988; Solberg & Stamm, 1991). Of note, studies around the globe, including Africa and some parts of Kenya, have shown disparity in reference values between the manufacturers'-provided values and those obtained from the local population. Studies that have been done currently in different parts of the world, Kenyan regions included, have shown these differences in reference values. For instance, in Rwanda, the North Rift Valley, Taita Taveta, and Kisumu in Kenya, some clinical chemistry parameters have shown a significant difference between reference values

provided by reagent manufacturing companies and those obtained from the local populations served in these regions (Juma *et al*., 2012; Gitimu, 2015; Rutayisire, 2017; Sing'oei *et al*., 2021). Despite many studies on reference intervals for other biochemistry parameters that have been widely done in the country, few studies have been on iron profile parameters reference intervals done in Kenya have remained scanty, with the only known study done in Nairobi by Omuse *et al* in 2020.

Dependable and precise reference intervals (RIs) for clinical laboratory investigations are a fundamental part of the correct interpretation of medical laboratory test results (Ozarda, 2016). Locally established reference intervals are considered to represent of the local population medical ranges, which can be relied on as this relies on the central 95% of the local healthy population. When establishing reference intervals, the various factors that lead to fluctuations must be taken into account (Ozarda, 2020; Villanova, 2000).

The establishment of reference intervals is costly, time-consuming, and logistically challenging, and for these reasons, many medical laboratories in many African countries, Kenya included, have not established their own reference intervals and rely on foreign reference intervals (Matt, *et al.,* 2022). Most of these reference intervals are provided by the test reagent manufacturers that are primarily based in America, Europe, and Asian countries, or those published in medical laboratory textbooks (Kamali *et al*., 2008). With the diversity of instrumentation, methodologies, test reagents, and populations as a requirement, moderate to large-sized clinical laboratories must determine locally their reference values (Gitimu, 2015).

Globally, the standardization of reference intervals has been of great concern to the various clinical laboratories and medical bodies as a whole, such as the CLSI, as this could play an important role in the consistency of diagnosis and treatment. This would ensure uniformity and comparability of test results across different laboratories and regions, regardless of where the measurements are conducted. This standardization would ensure that test findings are consistent and comprehensible on a global scale (Plebani & Lippi 2022). This would see the use of the same criteria in patients' diagnosis, which could lead to high quality assurance, improved patient care, patients' mobility, interoperability, and telemedicine, whereby standardized reference intervals allow cross-border patients and data sharing and internationalized interpretation of such results (Zlata *et al.,* 2016). However, this standardization faces several challenges, including population variability, genetic variations, differences in testing methodologies, age and gender variations, regional and geographic influences, technological advancements, and cultural and ethical differences. These challenges, coupled with limited resources, make achieving standardized reference intervals on a global scale a complex endeavour (Rappoport *et al*., 2018). Due to a lack of harmonization to test the same parameters, various laboratories and healthcare systems may employ various methodologies and assays for the same analyte, resulting in discrepancies in reference intervals resulting from differences in equipment, reagents, and measurement methods (Armbruster & Donnelly 2016; Zlata *et al.,* 2016). Regional, geographic, and environmental factors such as altitude, temperature, and dietary practices have a direct impact on reference intervals; all these factors have also affected efforts towards achieving global standardization. (Rappoport *et al.,* 2018). Technological

developments, with new testing techniques and tools, are made to guarantee accuracy and relevance; hence, this may require frequent updating of the reference intervals, further destabilizing the goal of global harmonization of the reference intervals. Lastly, cultural and ethical variations may have an effect on the gathering and analysis of health information. The make-up of reference populations as well as the levels deemed normal or abnormal may be impacted by these variables. All these issues, coupled with limited resources, make it difficult to develop and validate standardized reference intervals as a very large sample size will be needed to accommodate participants from all continents, regulatory disparities, as well as the standardization of all equipment and test methodologies, well-equipped laboratories, and knowledgeable employees, which might be difficult to achieve across the world (Vesper *et al.,* 2016). Due to these challenges faced in the globalization exercise, the Clinical and Laboratory Standards Institute (CLSI) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), which provide guidelines for establishing and harmonizing these intervals, also put more emphasis on the development of local population reference intervals that will serve them more accurately. To encounter the impediments that can occur due to the observed discrepancies in reference intervals in different locations, each laboratory should establish its own reference intervals tailored to its specific context, aligning with the local population it serves.

1.2 Problem statement

Due to the variability in RIs among different population, the use of unverified foreign reference intervals, this may lead to misinterpretations of test results. This inaccuracy risks misdiagnosis, unnecessary investigations, and inappropriate treatments for patients. This also increases risks and costs due to, delayed dx or over diagnosis & overtreatment. The use of unverified RIs not only bring about inaccuracy in diagnosis but also is non-Compliance to the CLSI recommendation specifically on clause CLSI C28-A3, for every laboratory to locally establish its own RIs, or verify reference intervals from foreign origin, Since the introduction of iron profile tests at MTRH, the facility has depended on unverified RIs provided by the manufacturer.

Globally, there has been a surge in diseases linked to iron deficiency or iron overload. Conditions such as cancer, liver diseases, and anemia, among others, often require frequent blood transfusions. This in turn has increased the demand for iron profile tests, which has been accelerated by ffrequent blood transfusions. Frequent blood transfusion, lead to the accumulation of iron; every transfused pint of blood contains around 200–250 mg of iron. Unlike dietary iron, which is controlled by the body's absorption systems, iron from transfused red blood cells is directly deposited in the body. This demands continuous monitoring of iron levels (Rasel M, *et al* 2024).

In Kenya, the incidence of cancer, in particular, increased significantly from 2012 to 2018, with annual cases rising from 37,000 to 47,887 (Jani *et al*., 2021). This surge has increased the demand for iron tests at Moi Teaching and Referral Hospital (MTRH), one of the largest

cancer treatment centers in the country. Iron profile test interpretation at MTRH, traditionally relies on the manufacturer's provided reference intervals. However, scientifically, it is proven that reference intervals are dependent on various factors such as diet, age, sex, race, geographical areas, altitude, and genetics (Rappoport *et al.*, 2018). Relying on reference intervals provided by reagent manufacturers from distant regions poses significant challenges. Using these non-local reference intervals for interpreting clinical chemistry parameters may lead to misdiagnosis, exposing patients to unnecessary risks and increasing disease management costs. Therefore, there is a critical need for verified, accurate, reference intervals to ensure accurate diagnosis and overall patient management. (Juma *et al*., 2012; Gitimu 2015; Rutayisire 2017; Sing'oei *et al*., 2021).

In accordance with regulatory and ethical concerns as stipulated by the Clinical and Laboratory Standards Institute's (CLSI) C28-A3 recommendation, every laboratory is advised to establish its own local reference intervals or verify the provided reference intervals by the manufacturer's before being employed for use in the local population, a recommendation that is reiterated as well by the reagent manufacturing companies (Dosoo *et al*., 2014; Rappoport *et al*., 2018). Since various countries have different healthcare practices, disease prevalence rates, vaccination policies, and other contextual factors, using foreign reference intervals may not account for these factors/variables, potentially affecting the normal interpretation of test results. This study aimed at establishing iron profile reference intervals that can be used locally for the interpretation of iron indices at MTRH.

1.3. Objectives

1.3.1 General objective

To establish laboratory reference intervals for routinely used iron profile parameters in the adult population aged 18–64 years in Uasin Gishu County, Kenya.

1.3.2 Specific objectives

i. To establish the normal laboratory reference interval values of serum ferritin, for the adult male and female population aged 18–64 years in Uasin Gishu County.

ii. To establish the normal laboratory reference interval values of serum iron, for the adult male and female population aged 18–64 years in Uasin Gishu County.

iii. To establish the normal laboratory reference interval values of serum transferrin and unsaturated iron binding capacity (UIBC) for the adult male and female population aged 18–64 years in Uasin Gishu County.

1.4 Research questions

I. What are the reference intervals for serum iron in the healthy adult population aged 18–64 years in Uasin Gishu County?

II. What are the reference intervals for serum ferritin in the healthy adult population aged 18–64 years in Uasin Gishu County?

III. What are the reference intervals for transferrin and unsaturated iron binding capacity in the healthy adult population aged 18–64 years in Uasin Gishu County?

1.5 Justification

This study addresses the lack of reliable iron profile RIs for the local population as unverified reference intervals has been in use, this will help to improve the accuracy in diagnosis and treatment outcomes.

Previous studies on commonly used biochemical markers, such as lipid profiles, renal function tests, and liver function tests, conducted in the North Rift region at MTRH (Juma *et al.,* 2011), showed significant differences in reference intervals between those supplied by manufacturers and those established for the region. This emphasizes the need for context-specific reference values due to the limitations of using foreign RIs in local populations (Juma *et al*., 2012; Karla, 2014 ; Gitimu, R. 2015; Rutayisire, 2017; Rappoport et al., 2018 ; Sing'oei *et al*., 2021)..

This study is in tandem with the CLSI recommendations, that each medical laboratory establish their reference intervals or validate the supplied reference intervals on their local population before using those from different regions.

Global surge of certain disorders has increased the demand for iron profile tests, highlighting the need for locally established iron profile reference intervals which are deemed to be more accurate and reliable as they reflect the true characteristics of the local population served. Reference intervals vary in different populations due to many factors and due to these differences, patients' results can lead to misdiagnosis, and incorrect treatment. Misdiagnosis does not only affect the health of the patients, but also has some

social and economic implications, including turn around time (TAT) and cost due to overdiagnosis or overtreatment.

MTRH lacks locally established and verified reference intervals for the newly introduced iron profile tests establishing and verifying these reference intervals is necessary to improve patient management and care. The choice of MTRH as the analysis center driven out of the fact that been one of the main cancer treatment centers in the country and there has been a high demand for iron profile tests, which are essential for diagnosis, monitoring disease progression, and assessing treatment success.

Iron profile tests are routinely used as a less invasive alternative to bone marrow biopsy (BMA), which is more complex. These tests can be repeatedly used to evaluate patients' responses to iron-related therapies.

The choice of adults aged 18–64 years for this study is based on previous research on cancer cases at both Kenyatta National Hospital (KNH) and MTRH, which indicated that 70% of these cases were reported in adults aged 25–64 years (Macharia *et al*., 2019).

Factors such as sex, age, environmental variation, altitude, and diet can cause significant variation in reference intervals (Dosoo *et al*., 2014; Rappoport *et al*., 2018).

This study will as well add data on iron profile RIs for adults in UG county and Kenya as none exists for the north rift region of Kenya.

1.6 Significance of the study

The information generated from the study can lead to improved accuracy in the interpretation of iron profile results in the region. Locally obtained reference intervals provide an accurate representation of the population being served. This study's findings will inform policy makers at both MTRH and the Ministry of Health about the need to establish local reference intervals for iron and other biochemical parameters. Additionally, the results of this study can guide other researchers who aim to establish reference values for various parameters, in accordance with the requirements of NCCLS (2000).

1.7 Conceptual framework

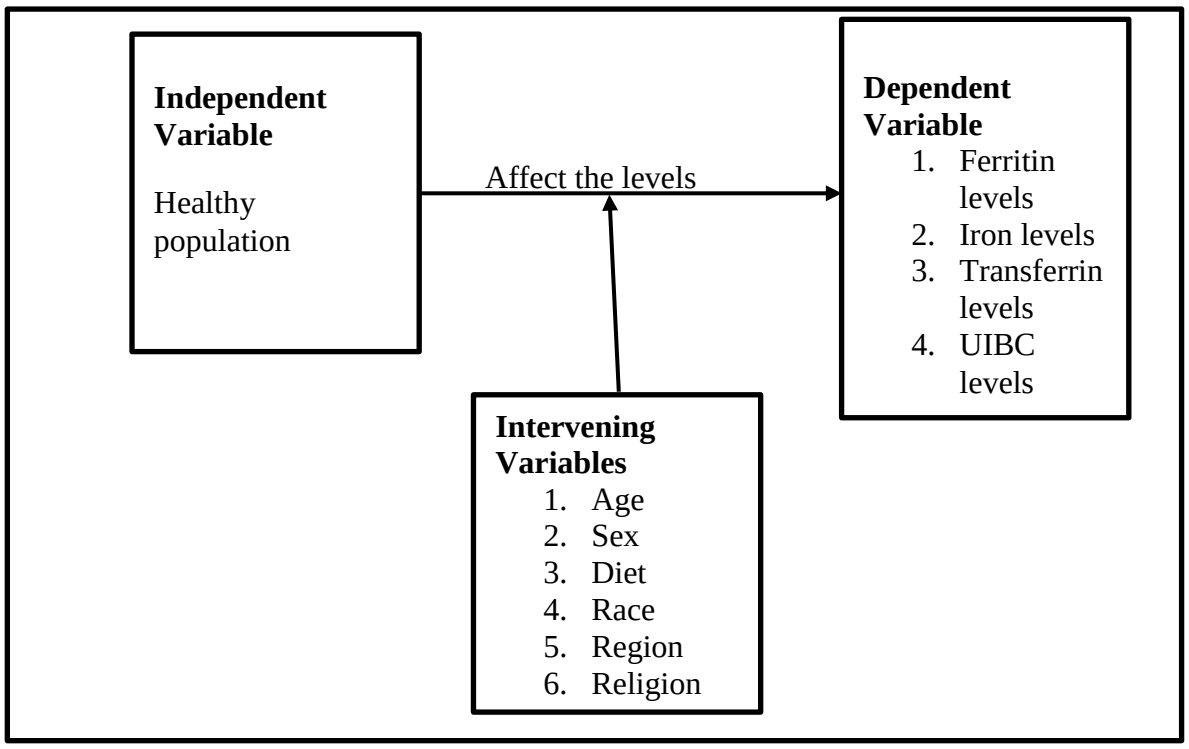

Figure 1. 1: Conceptual framework

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Use of reference intervals

Reference intervals (RIs) are a set of numerically quantified values of analytes obtained from a group of persons in a defined state of health. RIs remain important tools used in medical decision-making or for interpreting the findings of clinical laboratory tests. Purposefully, reference intervals are used for determining a medical diagnosis, choosing a treatment plan, and doing additional physiological analyses (Rutayisire, 2017; Martinez-Sanchez *et al*., 2021). These ranges describe the distribution of test parameter values of apparently healthy persons in a particular population and are used as health status indicators (Ayemoba *et al.*, 2021). According to the International Federation for Clinical Chemistry (IFCC) and the Clinical and Laboratory Standards Institute (CLSI), reference intervals represent the central 95% of laboratory test results from a healthy reference population (Ozarda · 2016, Timbrell N. 2024).

The interval values are circumscribed by the reference limit values at certain selected percentiles. The choice of a boundary value determines the trade-off between clinical sensitivity and specificity. The use of a low cut-off increases the sensitivity and the specificity decreases, while a high cut-off increases the specificity and the sensitivity decreases (Simon & Boring, 1990; Ekelund, 2018). Most commonly, reference intervals refer to the interval values containing 95% of the healthy reference population. The central 95% values are located between the 2.5^{th} and 97.5^{th} percentiles, respectively, and are commonly defined as the mean ± 1.96 standard deviations of the reference population.

As reference intervals differ in populations due to genetics, age, sex, race, diet, climate, and altitude influences, the Clinical and Laboratory Standards Institute (CLSI) and the International Federation for Clinical Chemistry (IFCC) emphasize that every laboratory establishes local reference intervals appropriate for the population (Solberg & Stamm, 1991; Rappoport *et al*., 2018). In African laboratories, the commonly used biochemical reference intervals are those supplied by reagent companies, and these are established using data from populations in the reagent manufacturing countries, especially America, Europe, and Asia (Henny *et al.*, 2000).

However, the research done worldwide and published literature confirmed the differences between reference values obtained for adults from manufacturing countries as well as those from different countries and regions in Africa (Dosoo *et al*., 2014). Research' done in Africa , East Africa and particularly in Kenya on the establishment of reference intervals for various biochemical parameters are such as those carried out by researchers such as Rutayisire and Alice Juma, among others (Juma *et al.*, 2012; Gitimu, 2015; Rutayisire, 2017; Sing'oei *et al*., 2021).

2.2 Iron profile

Iron profile tests are a collection of blood tests performed in clinical chemistry laboratories to assess the body's iron stores in the blood or serum levels. The iron profile includes tests such as serum iron, transferrin, transferrin saturation, ferritin, unsaturated iron binding capacity (UIBC), and total iron binding capacity (Pfeiffer & Looker, 2017). Iron profile has played a vital role in clinical medicine in the evaluation of various pathological conditions such as iron-loading anemias, hereditary hemochromatosis (HH), transfusional iron overload, and iron deficiency anemia (Pagani *et al.*, 2019). Iron profile tests can be used to differentiate between these ailments

as well as serve as guides in the management of these diseases (Peng & Uprichard, 2017). Iron, as an essential mineral, plays an integral part in the formation of hemoglobin (Faruqi & Mukkamalla, 2022). Iron profile tests are used as markers that help to identify iron deficiency and iron overload and monitor response to treatment (Melio, 2022).

2.2.1 Serum Iron

Serun iron is an important mineral that is essential for various body functions, essentially as a component of hemoglobin, among other functions. Iron as well partakes in several metabolic activities, such as the transfer of oxygen, the movement of electrons, and DNA synthesis. As part of the body's homeostasis, iron is tightly regulated since excess amounts of iron can form free radicals that can cause tissue damage (Abbaspour *et al*., 2014). In normal body homeostasis, the right amount of iron is desirable; low iron levels result in iron deficiency anemia; and high iron levels lead to iron overdose. Several factors may lead to low and high iron levels. Low iron levels can result from blood loss, a poor diet, or a lack of iron absorption from food. Young children, pregnant women, or those who might have prolonged menstrual periods are at increased risk of having too little iron (Coad & Pedley, 2014; Warner & Kamran, 2022).

In the body, iron homeostasis depends on a complex mechanism based on the principle of feedback between the human body's need for iron and intestinal absorption, since there is no physiological mechanism for the excretion of excess iron (Penkova & Ivanova, 2019). Duodenal enterocytes absorb dietary iron, whereas reticuloendothelial macrophages in the bone marrow, liver, and spleen store iron derived from destroyed erythrocytes. The hormone hepcidin is the central regulator of universal iron homeostasis in the body, The hormone modulates plasma iron levels via interaction

with ferroportin1. Ferroportin is an iron-export transmembrane protein found in macrophages and enterocytes, it facilitates the export of iron from the enterocyte, and it acts as the iron efflux pump (Knovich *et al*., 2009). Under non-pathological conditions, iron, hypoxia, inflammation, and erythropoiesis control Hepcidin levels. When there are high tissue iron requirements, hepcidin concentrations are low and consequently high for low tissue iron requirements. Therefore, low hepcidin levels favor bone marrow iron supply for hemoglobin synthesis and red blood cell production (Pagani *et al.,* 2019). The hepcidin hormone is primarily produced by the liver; hence, the liver is the major site for systemic iron regulation. Hepcidin is a negative regulator of iron absorption and recycling; this is achieved by its capacity to bind the cellular iron exporter ferroportin 1 (FPN 1) and cause its internalization and lysosomal degradation by the enterocytes and macrophages, thus leading to a reduction of iron efflux from target cells and serum iron levels (Wallace, 2016; Chambers *et al*, 2023). Any impaired regulation of hepcidin hormone production results in a diversity of iron disorders, such as hereditary hemochromatosis (HH) and iron-loading anemias. Hereditary hemochromatosis is brought about by the mutation of the Homeostatic Iron Regulator (HFE) gene, a genetic disorder manifested by extreme intestinal absorption of dietary iron, resulting in an uncontrolled increase in total body iron stores. (Nemeth & Ganz, 2009; McDowell *et al*., 2022). In pathological conditions, iron uptake in the gut and iron recycling by macrophages are usually blocked by high levels of hepcidin. These conditions cause iron-restricted erythropoiesis and anemia.

Iron in the diet is found in two forms: heme as ferrous (Fe^{2+}) or non-heme as ferric (Fe^{3+}) forms. Heme iron (from animals) is absorbed more efficiently than non-heme iron (from plants). Non-heme iron is absorbed in the duodenum after acidic gastric secretions enzymatically reduce it to

enhance iron solubility. Soluble inorganic iron is absorbed in the proximal duodenum by divalent metal-ion transporter 1 (DMT1) and exported into circulation through FPN1 (Coffey & Ganz, 2017). Roughly 2 mg of iron is absorbed every day in the duodenum and proximal jejunum and transported across the apical membrane of enterocytes by DMT1. Iron is carried in the circulation attached to transferrin; in cases of high iron levels and upon saturation of the transferrin, iron can also occur in an unbound state as in iron loading conditions (Anderson & Frazer, 2017). Transferrin bound to iron is taken up by transferrin receptor 1 (TFR1) found on cell surfaces through receptor-mediated endocytosis. On endocytosis, the endocytic vesicles get acidified, facilitating the release of iron. On iron release, the apotransferrin is reprocessed back to the cell surface and released by TFR1 (Wallace, 2016).

Iron must be converted to Fe^{3+}, before being transported out of the cell by hephaestin or ceruloplasmin, which are active proteins within the intestine that have ferroxidase activity ($Fe^{2+} \rightarrow Fe^{3+}$) (Knovich *et al.*, 2009). Fe^{3+} when bound to transferrin, is soluble and non-reactive, thus permitting it to enter the circulatory system. Cellular iron concentrations are modulated by the iron regulatory proteins (IRPs), IRP1 and IRP2 (Anderson & Frazer, 2017). Red blood cell hemoglobin controls most of the iron, which is recycled through erythrophagocytosis by reticuloendothelial macrophages, which are the main iron stores besides the liver hepatocytes (Dev & Babitt, 2017).

Iron overload and toxicity are harmful to the body tissues, as they can cause damage in the Fenton reaction, whereby a reaction occurs between iron (II) (Fe^{2+}) and hydrogen peroxide (H_2O_2) producing highly reactive and destructive hydroxide (OH^-) and hydroxyl radicals that cause oxidative harm to vital macromolecules such as DNA, proteins, lipids, and antioxidants (Coad & Pedley, 2014). Excess iron deposits cause numerous destructions to body tissues, especially the

heart, liver, and endocrine tissues. Liver damage can lead to cirrhosis, chronic liver disease, hepatocellular carcinoma, and the pancreas (Belhoul *et al*., 2012; McDowell *et al*., 2022). Low levels of iron may lead to undiagnosed and untreated iron-deficiency anemia that can cause severe complications such as fatigue, headaches, dyspnea on exertion, heart problems, restless legs syndrome, pregnancy complications, and stunted growth in children. (Warner & Kamran, 2022).

The reference intervals that were used for this study were those provided by the reagent manufacturer, who also recommends each laboratory establish its own reference locally (Roche 2018).

2.2.2 Serum Ferritin

A blood ferritin test is done to measure the levels of ferritin, a protein that primarily stores intracellular iron and releases it in a measured fashion. Ferritin has a role in keeping iron in a soluble and non-toxic form and also serves as a cushion against iron deficiency and iron overload in humans. It converts Fe^{2+} to Fe^{3+}, sequestering and circulating iron into its mineral core and storing it. This process takes place in normal iron homeostasis and in pathologic processes (Knovich *et al.*, 2009; Arosio *et al*., 2017).

Principally, ferritin is used as an indirect marker of the total body iron stores. The ferritin test is an important test for iron metabolism diagnostics, monitoring of iron therapy, the determination of iron stores, and the differential diagnosis of anemia. The amount of serum ferritin plays a crucial role in both iron excess and deficiency and in the diagnosis and management. Changes in ferritin levels are commonly experienced in cases associated with disturbances in iron homeostasis (Gulhar *et al*., 2022). Ferritin, as an acute-phase protein, has a role in a horde of other

circumstances, associated with both acute and chronic inflammation, neurodegenerative diseases, and malignant diseases. These conditions include malignancy, chronic kidney disease, rheumatoid arthritis, and other autoimmune diseases (Wang *et al.,* 2010). The pathophysiological role of ferritin elevation during these conditions is to sequester iron to hinder microbial iron scavenging and raise it to reduce free iron accessible to tumor cells or pathogens (Gulhar *et al*., 2022).

Serum ferritin is the most important and the utmost frequently done clinical test in combination with iron parameters to measure the iron status in patients, although the bone marrow biopsy iron staining technique is known to be the gold standard for iron deficiency diagnosis but is a delicate invasive test to carry out (Daru *et al*., 2017). Low serum ferritin is exceedingly specific for iron depletion, with only two other conditions known to lower serum ferritin: hypothyroidism and ascorbate deficiency (Knovich *et al*., 2009; Wopereis *et al.*, 2018). In iron overload diagnosis and treatment, ferritin plays a major role, and successful management is done through therapeutic phlebotomy while monitoring ferritin levels. In other conditions that cause storage of higher levels, this could likewise point to conditions such as rheumatoid arthritis, liver disease, other inflammatory conditions, and hyperthyroidism. Lower levels of ferritin than normal suggest iron deficiency conditions (Peng & Uprichard, 2017). For this research, the reference intervals that were used are the ones provided by the Roche reagent manufacturer; the company recommends the establishment of local RIs by each laboratory (Roche 2018).

2.2.3 Transferrin

Transferrin tests are done to directly measure the levels of iron in the blood and correspondingly test the body's ability to transport iron in the blood *(*Claise *et al*., 2022). Transferrin is a

glycoprotein only produced in the liver and released into circulation (Luck & Mason, 2012). Functionally, the work of the transferrin is to bind free Fe^{3+}, chelate free toxic iron, and prevent the development of reactive oxygen species. Transferrin's role in the primary immune system is to sequester iron, hence impeding the survival of bacteria (Ogun & Adeyinka, 2022).

Transferrin binds iron from food intake and iron recycled by macrophages, transporting it to the cells that will use it and internalizing it via transferrin receptor 1 (TfR1) (Tolosano, 2015). The specific transferrin receptor (TFR) binds strongly to the iron-containing HTF in the blood. After endocytosis of the transferrin bound to iron, endosome vesicle acidification enables the release of Fe^{3+} from HTF through TFR (Luck & Mason, 2012).

In normal biological conditions, the buildup of iron in parenchymal tissues is restricted by plasma transferrin functioning as a signal to the hepatocyte and the erythroblast to regulate hepcidin secretion. Its concentration in serum is affected by the cellular iron requirements and the erythroid production rate. In times of pathological conditions that make erythropoiesis, plasma, and tissue iron inefficient, the supplementation of transferrin normalizes erythropoiesis and restores normal iron sensing (Tolosano, 2015). Augmented transferrin plasma levels are often linked with iron deficiency anemia in women during pregnancy and with the use of oral contraceptives. In conditions characterized by high levels of transferrin, this is an indication of low iron, which means there is less iron attached to transferrin, allowing for a high number of circulating non-bound iron transferrin, a marker of iron deficiency anemia. In such conditions, the compensatory homeostasis is done by the liver, which boosts the synthesis of transferrin, allowing transferrin to bind to more iron and transport it to the cells. (Jain *et al*., 2011; Ogun & Adeyinka, 2022). Causes of low levels of transferrin are observed in liver damage, kidney injury that leads to loss of transferrin in urine,

and genetic atransferrinemia, a condition associated with mutations resulting in the absence of transferrin that leads to hemosiderosis in the heart and liver, hence heart and liver failure (Ogun & Adeyinka, 2022).

For this research, the reference intervals that were used are those that were provided by the reagent manufacturer, who as well recommend each laboratory establish its own reference locally (Roche 2018).

2.2.4 Iron binding capacity (IBC)

This is an important test useful in the diagnosis of iron deficiency anemias, iron overload conditions, and other iron-related metabolic conditions. Iron binding capacity is the measure of transferrin's capability to bind with iron. This is categorized into two categories: total iron binding capacity (TIBC) and unsaturated iron binding capacity (UIBC). TIBC measures the total amount of iron that can be bound by transferrin in the blood, while the UIBC test determines the reserve capacity of transferrin, that portion of transferrin that is not yet saturated with iron (Faruqi & Mukkamalla, 2022).

As transferrin is the main iron-binding protein, IBC tests are utilized as an indirect measure of transferrin availability and the quantity present to bind to iron. UIBC is a test to measure the unoccupied binding sites of transferrin's binding sites with iron, which serves as the reserve iron-binding capacity. The UIBC test is done to complement other serum iron measurements to aid in the categorization of individuals with iron-related conditions (deficiency or overload) and as a marker for altered iron metabolism. Transport of iron in plasma occurs as ferric ions attached to apotransferrin (transferrin). At normal conditions, an ordinary person has roughly one-third of

transferrin's binding capacity utilized, with the remaining portion referred to as the unsaturated iron binding capacity (Raoof & Abdalah, 2020; Whitten, 2021). The depletion of iron stores, in turn, raises transferrin levels in the blood, as only a few percentages of the transferrin are saturated with iron. Consequently, the transferrin present in serum has an extra binding capacity, which is the unsaturated iron-binding capacity. Low UIBC indicates that transferrin is highly saturated with iron, a finding consistent with hereditary hemochromatosis, while high UIBC levels indicate that transferrin is lowly saturated with iron and are a suggestion of iron deficiency syndrome. (Kundrapu and Noguez, 2018).

2.2.5 Iron deficiency anemia (IDA)

Iron deficiency anemia (IDA) is the most common cause of anemia worldwide and is characterized by microcytic and hypochromic erythrocytes in peripheral smears. By definition, anemia is referred to as a condition whereby hemoglobin (Hb) levels deviate to below two standard deviations of the mean for the age and gender of the patient (Warner & Kamran, 2022). As a result of blood loss, decreased iron intake, impaired iron absorption, or increased demand, this leads to depletion of iron stores and anemia. WHO describes anemia as a condition where blood heamoglobin levels are below 130 g/L in men and 120 g/L in women, serum ferritin is less than 30 ug/L, elevated transferrin levels, reduced serum iron, and transferrin saturation (TSAT) of less than 20%. (Kumar *et al*., 2022). Normal red blood indices for adults are as follows: RBC: for the adult population. Males: 5 to 6 million cells/mcL; females: 4 to 5 million cells/mcL; Hb: adult population: Males: 14 to 17 gm/dL, females: 12 to 15 gm/dL, hematocrit (HCT), adult males: 41% to 50%, adult females: 36% to 44% (Hb and HCT levels vary with altitude). Mean corpuscular volume (MCV) is 80 to 95 femtoliters, mean cell heamoglobin (MCH) levels are around 27 to 33

picograms (pg) per cell in adults, and mean cell haemoglobin concentration (MCHC) in adults is 33.4–35.5 grams per deciliter (g/dL).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study site

The study was carried out in Uasin Gishu County, part of the north rift region, Kenya. The County is a highland plateau with altitudes falling gently from 2,700 metres (8,900 ft) above sea level to about 1,500 metres (4,900 ft) above sea level. The laboratory analysis was conducted at the Moi Teaching and Referral Hospital Clinical Chemistry Laboratory. The hospital is located along Nandi Road in Eldoret Town, Uasin Gishu County, Kenya.

3.2 Study design

The study design was a cross-sectional study. The study was carried out at a one-time point over a short period of time.

3.3 Study population

The participants for the study population consisted of healthy volunteers', aged 18–64 years old, who consented to take part in the study. The participants had lived in the county for at least six months and above and were drawn from the six subcounties of Uasin Gishu County.

3.3.1. Inclusion criteria

Subjects who participated in the study were healthy males and females aged 18 to 64 years who underwent a screening procedure that involved a physical examination and a review of medical history in order using questionnaire to rule out anyone with known illnesses or conditions that

could affect iron metabolism. The participants were free from chronic diseases chronic illnesses such as hypertension, chronic renal failure, tuberculosis, diabetes mellitus, HIV, hepatitis B, hepatitis C, and syphilis. Since this is an iron study, the participants had to have Hb levels of 12.5 to 17.2 g/dL and 12.1 to 15.1 g/dL for males and females, respectively, the participants also had lived in Uasin Gishu County for not less than 6 months.

3.3.2 Exclusion criteria

Subjects that never met the inclusion criteria were excluded; these included pregnant females and individuals who were on any form of medication, such as those on iron supplements, oral contraceptives, and iron chelators. Individuals who had recently donated blood in the previous 3 months and smokers were excluded from the study as well. Smokers were exempted from the study as there is substantial evidence that cigarette smoking causes iron dysregulation (Zhang *et al*., 2019). Alcoholics were excluded as well, as alcohol use is known to dysregulate hepcidin production in the liver and is an underlying cause of iron overload (Harrison-Findik, 2009).

3. 4 Sample size determination

Sample size was calculated using the G power 3.1.9.7 sample size calculator at a two-tail error probability of 0.05 and a power of 0.95 for the two groups giving a total of 298 (Figure 3.1). A total of 321 volunteers were recruited, and out of these 290 samples met inclusion criteria for the study. The number met the CLSI procedure guidelines, which recommends a minimum of 120 people per group (male and female) as per CLSI C28-A3C (Horowitz *et al*., 2008).

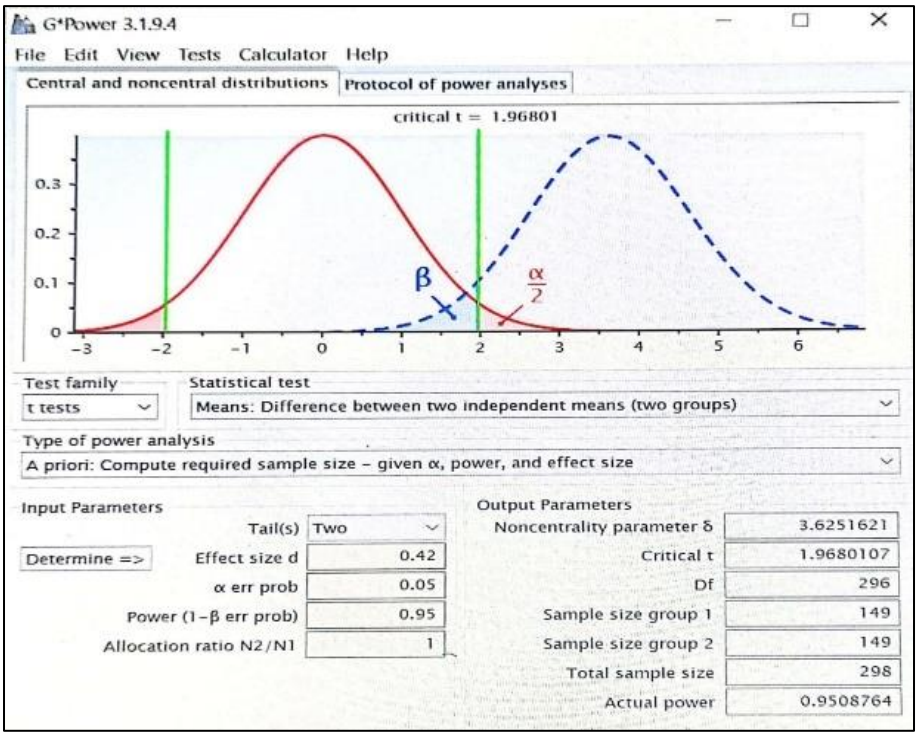

Figure 3 2: G power sample calculation

3.5 Sampling design

The participants were selected based on a multistage cluster sampling technique. These entailed the selection of the participants geographically from the six sub-counties of Uasin Gishu. Since the county's social and economic activities are almost evenly distributed, in every subcounty, 2–3 wards were randomly picked to represent the subcounty, depending on the subcounty's population. A total of 15 wards were picked out of the 30 wards in the county, and participants were selected in the hierarchy of population within the wards. Turbo, which had the highest population, had 3 wards selected, followed by Soy Subcounty with 3 wards, Kapseret Subcounty participants from 3 wards, Moiben Subcounty participants were drawn from 2 wards, Kesses Subcounty participants were from 2 wards, and lastly, Ainabkoi Subcounty was represented by participants from 2 wards.

In the wards after mobilization, the participants were placed in strata according to their sex. In the clusters, systematic random sampling was done to choose the participants. The selection of the participants here was at a regular interval after randomly selecting the starting point.

3.6 Sample handling

3.6.1 Sample Collection

Venous blood was drawn from the antecubital fossa, either from the cephalic, median cubital, or basilic veins, using a 5 ml syringe, and at least 4 ml of blood were collected and transferred into labeled plain vacutainers with the participants' unique coded number, sex of the participant, and age.

3.6.2 Specimen Transportation

Specimens collected from the subjects were transported to the analyzing laboratory in ice-packed cool boxes within one hour from the collection center.

3.6.3 Processing and Storage of the Samples

Blood was left to clot, and the clotted samples were centrifuged at 3000 rpm for five minutes at room temperature (20°C–28°C) to get the serum. Using a Pasteur pipette, the serum was aliquoted into well-labeled cryovials. At the analytical center, the samples that were not analyzed immediately were stored at 4–8 °C for a maximum of a maximum of 7 days, or stored at -20 °C for those that were to take longer periods. The samples were analyzed on a Cobas® 6000 series chemistry analyzer, which is a fully automated clinical chemistry analyzer by Roche Diagnostics.

3.7 Analytical Methods

3.7.1 Serum iron determination

3.7.1.1Test principle.

Serum iron, after conversion to ferrous iron, is reacted with ferrozine to form a colored complex whose absorbance can be measured photometrically at a wavelength of 552/659 nm. Iron under acidic conditions, iron is liberated from transferrin to apotransferrin and ferric. Ascorbate causes the reduction of ferric (Fe^{3+}) to ferrous ions (Fe^{2+}), which then react with FerroZine to form a colored complex. The color intensity produced is directly proportional to the iron concentration. This is an endpoint absorbance calculation and is determined by monitoring the increase in absorbance at a wavelength of 552/659 nm.

Transferrin-Fe complex $\xrightarrow{\text{pH < 2.0}}$ apotransferrin + ferric ion (Fe^{3+})

Ferric iron (Fe^{3+}) $\xrightarrow{\text{Ascorbate}}$ Ferrous iron (Fe^{2+})

FerroZine + Ferrous iron (Fe^{2+}) $\longrightarrow$ Colored complex

3.7.1.2 Test procedure

The aliquoted serum sample was pipetted into a well-labeled Cobas sample cup using a micropipette, at least 150 µL in volume, and placed in a sample rack. The sample racks were then inserted into the machine sample rack holding bay. Test ordering was done from the test panel display, and the start button was switched on. The results automatically displayed for validation and auto-printed (ROCHE 2019).

3.7.2 Unsaturated iron binding capacity determination

3.7.2.1 Test principle

This was based on the FerroZine method by the measurement process of excess ferrous iron (Fe^{2+}). A known amount of ferrous (Fe^{2+}) is added to the specimen. In an alkaline environment, the Fe^{2+} is converted to the ferric ion (Fe^{3+}), which binds to the unbound endogenous transferrin. The unbound Fe^{2+} in the reagent then reacts with the FerroZine reagent to form a colored compound. This is an inverse outcome: the colored intensity is directly proportional to the unbound excess Fe^{2+} and indirectly proportional to the unsaturated iron binding capacity (UIBC). This is determined by measuring the increase in absorbance at a wavelength of 552 nm. The greater color development indicates the transferrin is more highly occupied by iron (low UIBC). Lesser color development indicates that the transferrin has more binding capacity available (high UIBC).

$$\text{Transferrin} + Fe^{2+} \xrightarrow{\textbf{Alkaline buffer}} \text{Transferrin} - Fe^{3+} + Fe^{2+}\ (\text{Excess})$$

$$\text{Ferrous iron } (Fe^{2+}) + 3\ \text{FerroZine} \longrightarrow \text{Colored complex.}$$

3.7.2.2 Test procedure

The aliquoted serum samples were pipetted into a well-labeled Cobas sample cups using a micropipette, at least 150 µL in volume, and placed in a sample rack. The sample rack was then inserted into the machine sample rack holding bay. Test ordering was done from the test panel, and the start button is switched on. The results are automatically displayed for validation and auto-printed (ROCHE 2019).

3.7.3 Serum transferrin determination

3.7.3.1 Test principle

This is a particle-enhanced immunoturbidimetric assay of human soluble transferrin receptor agglutinate coated with latex particles coated with anti-soluble transferrin receptor antibodies. This is an antigen/antibody complex, and the precipitate is determined photometrically at 583 nm.

3.7.3.2Test procedure

The aliquoted serum sample was pipetted into a well-labeled Cobas sample cup using a micropipette, at least 150 µL in volume, and placed in a sample rack. The sample rack was then inserted into the machine sample rack holding bay. Test ordering was done from the test panel, and the start button is switched on. The results are automatically displayed for validation and auto-printed (ROCHE 2019).

3.7.4 Serum ferritin determination

3.7.4.1 Test principle

The method for measurement of on the Cobas® 6000 is based on the immunoturbidimetric, human ferritin agglutinates with latex particles coated with anti-ferritin antibodies. The precipitate is determined turbidimetrically at 570/800 nm.

3.7.4.2Test procedure

The aliquoted serum sample was pipetted in a well-labeled Cobas sample cup using a micropipette, at least 150 µL in volume, and placed in a sample rack. The sample rack was then inserted into the

machine sample rack holding bay. Test ordering done from the test panel and the start button is switched on. The results automatically displayed for validation and auto printing (ROCHE 2019).

3.7.5 HIV, Hepatitis B and C and VDRL screening

The tests for the above were done based on a chemiluminescence immune assay (CLIA) technique using the Liaison XL machine.

3.7.5.1 Test Principle

An immune complex is formed in the presence of complimentary antigens and antibodies; the paratope of the antibody binds to the epitope of the antigen, hence forming the complex. The estimation of the levels of the immune complex is done by the use of labeled antibodies, which form the basis of CLIA. The tests were done using a fully automated immunoassay analyzer, LIAISON® XL. Manufactured by DiaSorin Ltd., Italy.

3.7.6 Heamoglobin levels testing

The participants heamoglobin level determination was done using HemoCue system that utilizes the principle of oxidation of heamoglobin to hemiglobin by sodium nitrite and the subsequent conversion of hemiglobin to hemiglobinazide by sodium azide, with the absorbance read at 565nm and 880 nm.

3.8 Quality management

This was done to ensure that the results gotten from the study were of high quality, accurate, and dependable. All the recommended quality management activities were employed, such as internal

quality control (IQC). This was done as per the laid-down standard operating procedures (SOPs) for all pre-analytical, analytical, and post-analytical. In the analytical stage, we made sure that the machines were calibrated and that all controls ran and passed. The SOP procedures for running the samples were adhered to ensure that the correct results were produced. In the post-analytical stage, we ensured that the results were well handled and secured to avoid mix-ups.

3.8.1 Machine calibration and Quality control.

Calibrators and Controls safeguard the dependability and consistency of test results and help improve laboratory analytical performance by ensuring the reliability and consistency of assay results. The calibrators ensure the precision of test equipment to reduce any measurement ambiguity, and they also customize the systems to an established reference method. They quantify and control errors or uncertainties within measurement procedures to an acceptable level on the machines. Calibration of the machine was done before use; a calibrator for automated systems (C.F.A.S.) was used. This is a lyophilized calibrator based on human serum. The aliquoted calibrator was put in a specimen cup, placed in calibrator racks, and put into the machine. It was run and compared with previously run calibration to ensure that it passed as per the calibration curve.

Controls check the standardized reagents' and calibrators' recovery levels. Controls for Iron, transferrin, and UIBC, PreciControl ClinChem Multi 1 (PCC1) as normal, and PreciControl ClinChem Multi 2 (PCC2) as pathological controls, were used. For ferritin, Precinorm protein and Precipath protein were used for normal and pathological controls, respectively. The controls were

aliquoted to the sample cups, placed in their respective positions in the control rack, and ran in the machine. It was made sure that all controls passed as per the requirement.

3.8.2 Analytical method validation

To ensure that the results gotten from this research were of high quality, reliable, and consistent, the various analytical methods were validated before carrying out the tests. The following analytical method validation procedures were carried out: tests for method accuracy, method comparison, precision, detection limit, repeatability, specificity, quantitation limit, ruggedness (intermediate precision), linearity, range, and robustness (Rao, 2018; Sankar & Babu, 2020). Selectivity or specificity when it comes to an analytical method is done to test the method's capacity to measure precisely an analyte in the presence of interferences that may be expected to be present in the sample matrix (Roy, 2018). Precision tested the closeness of agreement among individual test results when the procedure was applied repeatedly to numerous samplings. The accuracy of the methodology was checked by testing known concentrations of the analytes to see the degree of agreement of the results produced with the true value. As part of ensuring that all tests done are reliable, the MTRH laboratory participates in proficiency testing (PT) with the various accredited EQA bodies, such as the HuQAS (Human Quality Assessment Services), BIO-RAD, and Kenya External Quality Assessment Scheme (KNEQAS), among other registered bodies.

Nevertheless, the methods used in analyzing the iron profile using the Cobas 6000 series in this study had been validated and documented by a branch of the Centers for Disease Control and Prevention (CDC), namely the National Health and Nutrition Examination Survey (NHANES),

which is a program of the National Center for Health Statistics (NCHS). Precision and method comparisons have been conducted and documented by Roche. Method comparison for the Cobas 6000 series was done by having the results compared with those assayed using the Roche/Hitachi 917 analyzer, the Cobas Integra 400 analyzer, and the Cobas Integra 700 analyzer (Roche 2018).

3.8.3 Interferences or limitations

For this study, all factors leading to analytical interference were checked and, where possible, tested. Analytical interferences lead to the deviation of the analysis from the true value of the analyte. The causes of these deviations are due to the presence of some endogenous or exogenous substance. These substances bring about physical interference (light absorption) and chemical interference in the sample being analyzed and affect the outcome of the overall results. The main cause of interference is seen in samples that show significant hemolysis, icterus, and lipaemia. This interference visually changes the normal appearance of serum, which is supposed to be a clear, straw-colored liquid, to a different color depending on the interfering substance.

Lipemia indicates presence of an abnormally high concentration of emulsified fat in the blood, making plasma or serum turbid and opaque. The accumulation of these lipoproteins makes the Lipemic serum sample appear milky white. The icteric serum is caused by the presence of excess bilirubin in the bloodstream, and the sample visually appears more yellowish. A physical and visual examination of the samples was done, and the samples that were suspected to be hemolyzed, lipemic, or icteric were tested for their levels. Those samples that exceeded the minimum allowed indexes were exempted from the study.

3.9 Estimation of the reference intervals

The collected serum samples were analyzed for serum iron, serum ferritin, serum transferrin, and unsaturated iron binding capacity levels. The assays were performed with adherence to the manufacturer's methodology and as per the laboratory standard operating procedures (SOPs). After measuring their levels, the reference intervals were calculated as per the CLSI guide lines. The determination of outliers was based on the Tukey's fences method, which is based on the interquartile range (IQR) to detect outliers. The outliers were values below Q1−1.5×IQR or above Q3+1.5×IQR. The interquartile range (IRQ) is the third quartile figure minus the first quartile figure (Q3 - Q1).as depicted by Dima Diachkov (2024)

3.9.1 Estimation of Serum Iron, ferritin, transferrin and unsaturated iron binding capacity levels.

The determination of serum iron and unsaturated iron binding capacity was done based on the Ferrozine method, and the determination of ferritin and transferrin was done based on the immunoturbidimetric assay method using the automated Cobas 6000 series (Roche Diagnostics, Mannheim, Germany). The aliquoted serum samples were pipetted into well-labeled Cobas sample cups using a micropipette, containing at least 150 uL in volume, and placed in a sample rack. The sample rack was then inserted into the machine sample rack holding bay, and test ordering was done from the test panel by selecting the intended tests. The results, when ready, were automatically displayed for validation and auto-printing.

3.9.2 Calculation of the reference intervals

The reference value calculations were done using the nonparametric approach at a confidence interval of 95%, this was due to the fact that our variables were not normally distributed. The non-parametric approach entailed choosing the lower and upper reference bounds as being the values that fall at the 2.5 and 97.5 percentiles of the population, respectively, at a confidence interval of 95% as per the CLSI guidelines for the determination of reference intervals (NCCLS 2000). Reference intervals were taken as the values that were in between and including the two reference limits.

3.10 Reference range tolerance limit verification

Tolerance limit verification is a crucial method used in ensuring that quality and consistency of laboratory testing methods are maintained, and as well as means to test the viability of reference intervals and ensuring that they are in compliance with the laid down guidelines by the relevant bodies like the international federation for clinical chemistry (IFCC) and the clinical and laboratory standard institute (CLSI). This is usually done to make sure that the targeted population for the specified reference intervals by a certain laboratory test or clinical assessment is appropriately represented (LaValley C. and Graff P. 2022). It entailed determining whether the reference interval is sufficiently wide and comprehensive to account for any normal variation that may exist in a population. For the computation of the established reference intervals for the healthy population of Uasin Gishu County, reference range tolerance limit verification was done according to the Sigma Diagnostics method (Baetselier et al. 2016). Sigma diagnostic method provides a systematic approach to verifying reference intervals by assessing the precision, accuracy, and overall performance of test methods. Reference range mean, sample mean and the percentage deviations

were established based on the formula as provided by Baetselier et al, (2016). The calculated percentages deviations for the iron parameters were used to determine whether the established reference range was different from the manufacturers provided reference intervals as indicated in the table 3.1 below.

3.10.1 Reference range tolerance limit verification calcualtion formular.

$$\textbf{Reference Range Mean} = \left\{ \left(\frac{\text{Upper Limit - Lower Limit}}{2} \right) + \text{lower limit} \right\}$$

$$\textbf{Sample mean: } = \frac{\text{Sum of the sample values}}{N}$$

(N= Total counted entities)

$$\textbf{\%Deviation} = \left\{ \left(\frac{\text{Selected Sample Mean}}{\text{Reference Range Mean}} \text{ x100} \right) - 100 \right\}$$

Table 3 1:Tolerance limit Table For clinical chemistry parameters

Analyte	% Deviation limit	Analyte	% Deviation limit
Alanine aminotransferase	+/− 14%	Creatinine	+/− 10%
Albumin	+/− 8%	Glucose	+/− 8%
Alkaline Phosphatase	+/− 20%	Total Iron	+/− 20%
Amylase	+/− 20%	LDL / LDH	+/− 14%
Aspartate aminotransferase	+/− 14%	LD-1	+/− 20%
Total Bilirubin	+/− 14%	Magnesium	+/− 16%
Calcium	+/− 9%	Total Protein	+/− 8%
Chloride	+/− 4%	Triglycerides	+/− 16%
Total Cholesterol	NA	Blood Urea	+/− 6%
Cholesterol, HDL	+/− 20%	Uric Acid	+/− 12%
Creatine Kinase	+/− 20%	All Other Analytes	+/− 20%
Creatinine Kinase-MB	+/− 20%		

Table courtesy of African journal of laboratory medicine (Baetselier *et al.* 2016).

3.11 Data analysis

Data from the study was input into MS-Excel® 2013 by Microsoft Corporation, crosschecked against the printed copies, and analyzed with Statistical Package for Social Sciences (IBM SPSS) version 23.0. The normality test was computed using the Kolmogorov-Smirnoff (KS) for sample sizes above 50 to determine whether the data is parametric or non-parametric. The data was non-parametric. Descriptives statistics for the continuous variables was established and recorded in terms of median and median range. Mann-Whitney U test and Kruskal wallis for inferential statistics to compare the differences between the various groups, chi square test was used for categorical data across the various social demographic groups, with $p < 0.05$ considered a significant difference. The data are presented in the form of tables.

3.11 Ethical considerations

Permission to conduct the study was obtained from the MMUST Institutional Ethical Review Committee (IREC) (Appendix IX), and authorization to carry out the research was sought from the institutional ethical committee of Moi Teaching & Referral Hospital/Moi University (MTRH/MU-IREC) (Appendix X) and the National Commission for Science, Technology, and Innovation (NACOSTI) (Appendix XI). The confidentiality of the participant's information was paramount in this study. This was ensured through the use of unique coded numbers and not participants' names. The data collected from the study was password-protected, and only authorized individuals were allowed to access the data. Before this study, all healthcare establishments and the research team were informed about the ethical issues. Written or oral consent was obtained from participants (documented on the information sheets), as mandated by the research ethics. All procedures that were taken in this study ensured the safety of the

participants. No direct handouts were provided to the participants for them to take part in the study. However, the study established reference intervals that will facilitate the accurate diagnosis of iron-related disorders. This will also provide the clinician with proper medical decision points for patients and an overall outcome of better patient care and management.

CHAPTER FOUR

RESULTS

4.1 Social-demographic and anthropometric measures of study participants

A total of 290 healthy adults participated in the study, this consisted of 157 males and 133 females. The males accounted for 54.1%, where else the females accounted for 45.9% of the total participants. The males were aged between 18 and 64 years, with a median age of 35 years; the females were aged between 18 and 63 years, with a median age of 29 years.

The participants were grouped into three age groups, as shown on Table 4.1, namely, early adulthood (18–34 years), early middle age (35–44 years), and late middle age (45–64 years). Those aged 18–34 had the highest number of participants, accounting for 112 (38.6%) participants. Males in this group were 62 (21.4%), and females were 50 (17.2%). The age group between 35 and 44 years had 90 participants, who accounted for 31.0% of the total participants. In this group, the males were 47 (16.2%), and the females were 43 (14.8%). The late middle age group (45–64 years) had 88 participants who contributed to 30.4% of the total participants; the males were 48 (16.6%), and the females were 40 (13.8%).

The study participants' ages were similar in all three age groups ($P = 0.902$); a comparison of the statistical differences in the study participants' heights between genders and across the age groups was also conducted. The gender comparison showed a statistically significant difference between the genders with a $p < 0.0001$. Male and female height comparisons over the three age groups were similar overall, with a P value of 0.955 for males and a P value of 0.808 for females, respectively.

On the comparison of weight and BMI on gender and as well across the age groups and among the sexes was done. On significant differences between males and females, it was found for weight, a p value of <0.0001 and for BMI had a p value of <0.0001

The comparison of weight and BMI between genders was done, and a p value of <0.0001 and P~<0.0001 for weight and BMI were realized, respectively. This showed that there were statistically significant differences between the males and females. In the comparison of weight and BMI across the three age groups per gender, it was found that there were no significant differences. The weight and BMI values were (P = 0.633) and (P = 0.892) for males, respectively; for females, the values were (P = 0.357) and (P = 0.239) for the weight and BMI, respectively.

BMI categorization on the basis of underweight, normal, and overweight was done, and this showed that there were no significant variations in their distribution among the study participants in the three age groups (P = 0.193). The majority of the participants among the groups were normal, amounting to (219/290) 75.5%, with those who were underweight (7/290) accounting for 2.4% and those who were overweight (64/290) accounting for BMI (22.1%).

On the social lives of the participants, comparisons were made across the three age groups. On education, it was noted that 159/290 (54.8%) had attained secondary education or above, 113/290 (39.0%) had only primary education, and those who never attended school accounted for 18/290 (6.2%). Education was comparable across the three age groups, with a P value of 0.874. On the marital status, the majority of the participants were married, from the three age groups, accounting for a total of 214/290 (73.7%); those single accounted for 16.9%; and those separated or divorced accounted for 9.4%. The marital status was different across the groups (P = <0.0001). The group

analysis revealed a significant difference among the singles, with the age group between 18 and 34 years accounting for 65.3% in that category when compared to the other two groups. The rest of the categories (married and divorced) were evenly distributed among the groups.

On religion, protestants were the majority, with 73.7% of the total study participants; Catholics had 17%; and Muslims accounted for 9.3%. The distribution among the age groups was comparable (P = 0.914). The majority of the participants were found to be self-employed, accounting for 64.3%; those with formal employment accounted for 24.2%; and those who had no occupation accounted for 11.5%. The group distribution on occupation showed a significant difference, with a P value of 0.0001. The early adulthood group had the majority of those with no occupation, accounting for 70.6% of the total in that categorization compared to the other groups.

Table 4 1: Demographic and anthropometric characteristics of all the study participants per age group

Characteristic **Age group. n (%)**		**Early Adulthood 18-34 years n=112/290 (38.6)**	**Early mid Adulthood 35-44 years n=90/290 (31.0)**	**Late mid Adulthood 45-64 years n=88/290 (30.4)**	***Totals***	***Age gps (P)***	***Gender (P)***
Gender	Male. n (%)	62/157 (21.4)	47/157 (16.2)	48/157 (16.6)	**157(54.1%)**	0.902	-
	Female. n (%)	50/133 (17.2)	43/133 (14.8)	40/133 (13.8)	**133(45.9%)**		
Height. cm	Male. H (%)	173.4 (33.3)	174.2 (33.4)	173.8 (33.3)	-	0.955	**<0.000**
	Female. H (%)	164.5 (33.4)	164.2 (33.3)	163.8 (33.3)	-	0.808	**1***
Weight, Kg	Male. W (%)	67.3 (32.4)	69.2 (33.3)	71.2 (34.3)		0.633	**<0.000**
	Female. W (%)	56.5 (31.4)	61.2 (34.0)	62.4 (34.6)	-	0.357	**1***
BMI, Kg/m^2	Male. B (%)	21.5 (28.1)	24.8(35.0)	24.6(36.9)	-	0.892	**<0.000**
	Female. B (%)	22.4 (31.4)	24.2 (33.9)	24.8 (34.7)	-	0.239	**1***
			BMI category, n (%)				
Underweight (<18.5kg/m^2)		4 (1.4)	2 (0.7)	1 (0.3)	**7(2.4%)**		
Normal (18.5 – 24.9 kg/m^2		90 (31.0)	68 (23.4)	61 (21.0)	**219(75.4%)**	0.193	-
Overweight (>25.0 kg/m^2)		18 (6.2)	20(7.0)	26(9.0)	**64(22.2%)**		
			Education level, n (%)				
None		8 (2.8)	4 (1.4)	6 (2.1)	**18(6.3%)**		
Primary		41 (14.1)	35 (12.1)	37 (12.7)	**113(38.9%)**	0.874	-
Secondary & above		63 (21.7)	51 (17.6)	45 (15.5)	**159(54.8%)**		
			Marital status, n (%)				
Single		32 (11.0)	9 (3.1)	8 (2.8)	**49(16.9%)**		
Married		74 (25.5)	71 (24.5)	69 (23.7)	**214(73.7%)**	**0.001***	**-**
Separated		6 (2.1)	10 (3.7)	11 (3.7)	**27(9.4%)**		
			Religion, n (%)				
Catholic		19 (6.6)	15 (5.2)	15 (5.2)	**49(17%)**		
Protestant		81 (27.8)	66 (22.8)	67 (23.1)	**214(73.7%)**	0.914	-
Muslim		12 (4.1)	9 (3.1)	6 (2.1)	**27(9.3%)**		
			Occupation, n (%)				
None		24 (8.1)	3 (1.0)	7 (2.4)	**34(11.5%)**		
Self		63 (21.8)	63 (21.8)	60 (20.7)	**186(64.3%)**	**0.001***	**-**
Formal		25 (8.6)	24 (8.4)	21 (7.2)	**70(24.2%)**		

Data shown are median values for continuous variables and numbers (n) and proportion (%) of study subjects for categorical variables. Data analysis was conducted using chi-square tests for categorical data and Kruskal wallis test for continuous data. Significance was set at a *P* value <0.05 and significant *P*-values **bolded***

4.2: Demographic and anthropometric characteristics of all the study participants per Location.

Participants for the study were recruited across the entire county, representing the six subcounties of Uasin Gishu County. The number of participants, recruited per each subcounty depended on the population size of the subcounty, with the more populated counties having a higher number of the participants. Turbo subcounty had a total of 57 participants who accounted for 19.6%, Soy had 54 participants (18.6%), Kapseret 48 (16.6%), Moiben 45 (15.5%), Kesses 44 (15.2%), and Ainabkoi had 42 participants accounting for 14.5%. Table 4.2 summarizes the social-demographic and anthropometric measures of study participants per each subcounty. The study also carried out social demographic and anthropometric characterization of the study population based on the participants location origins (subcounties). The distribution of both males and females was comparable among the groups ($P = 0.998$), and the heights of both males and females were comparable across all the locations, with a value of ($P = 1.000$) for males and ($P = 0.992$) for females. The weight and BMI from all six locations showed no significant differences, with ($P = 0.799$) and ($P = 0.173$) for weight, males and females, respectively. The BMI for males was ($P = 0.149$) and for females was ($P = 0.134$), respectively.

On BMI categorization on the basis of underweight, normal, and overweight, an even distribution was noted across all the locations ($P = 0.863$). On the social life aspect, all parameters were comparable across the locations. Education had a P value of 0.438, marital status ($P = 0.693$), religion ($P = 0.0557$), and occupation had a P value of 0.533.

The data corrected from the different subcounties showed that there was no any association between the location of origin and the various parameters such as the BMI, height, weight, education levels, marital status, religion and occupation as shown in table 4.2

Table 4 2: Demographic and anthropometric characteristics of all the study participants per Sub counties

Characteristic n (%)		Turbo 57 (19.6)	Soy 54 (18.6)	Kapseret 48 (16.6)	Moiben 45(15.5)	Kesses 44(15.2)	Ainabkoi 42(14.5)	*P*
Male. n (%)		32(11.0)	30(10.3)	26(9.0)	24(8.3)	23(7.9)	22(7.6)	0.998
Female. n (%)		25(8.6)	24(8.3)	22(7.6)	21(7.2)	21(7.2)	20(6.9)	
Height. cm	**Male**	172.4(16.5)	173.9 (16.7)	174.5 (16.7)	173.8 (16.7)	174.3 (16.7)	173.9 (16.7)	1.000
	Female	164.3 (16.7)	163.8 (16.6)	163.7 (16.6)	164.2 (16.7)	164.4 (16.7)	164.6 (16.7)	0.922
Weight, Kg	**Male**	69.3 (16.7)	68.8(16.6)	69.5 (16.7)	68.9 (16.6)	69.7 (16.8)	69.2 (16.6)	0.799
	Female	59.5(16.5)	60.3 (16.7)	59.4 (16.5)	59.8 (16.6)	61.2 (17.0)	60.1 (16.7)	0.173
BMI Kg/m^2	**Male**	22.5 (15.7)	23.9 (16.7)	24.3 (17.0)	23.6 (16.5)	24.8 (17.3)	24.1 (16.8)	0.149
	Female	24.4 (17.1)	22.8 (16.0)	23.8 (16.7)	24.6 (17.2)	22.9 (16.0)	24.3 (17.0)	0.134
BMI category, n (%)								
Underweight 18.5kg/m^2		1 (0.3)	1 (0.3)	2 (0.7)	0 (0.0)	1 (0.3)	2 (0.7)	
Normal (18.5-24.9 kg/m^2		41 (14.1)	39 (13.4)	36 (12.4)	38 (13.1)	33 (11.4)	32 (11.0)	0.863
Overweight (>25.0 kg/m^2)		15 (5.2)	14 (4.8)	10 (3.4)	7 (2.4)	10 (3.4)	8 (2.8)	
Education level, n (%)								
None		4 (1.4)	1 (0.3)	4 (1.4)	5 (1.7)	2 (0.7)	1 (0.3)	
Primary		20 (6.9)	21 (7.2)	17 (5.9)	20 (6.9)	14 (4.8)	21 (7.2)	0.438
Secondary & above		33 (11.4)	32 (11.0)	27 (9.3)	20 (6.9)	28 (9.7)	20 (6.9)	
Marital status, n (%)								
Single		11 (3.8)	7 (2.4)	10 (3.4)	5 (1.7)	10 (3.4)	6 (2.1)	
Married		38 (13.1)	41 (14.1)	32 (11.0)	37 (12.8)	32 (11.0)	34 (11.7)	0.693
Separated		8 (2.8)	6 (2.1)	6 (2.1)	3 (1.0)	2 (0.7)	2 (0.7)	
Religion, n (%)								
Catholic		13 (4.5)	6 (2.1)	10 (3.4)	8 (2.8)	8 (2.8)	4 (1.4)	
Protestant		41 (14.1)	44 (15.2)	33 (11.4)	33 (11.4)	29 (10.0)	34 (11.7)	0.557
Muslim		3 (1.0)	4 (1.4)	5 (1.7)	4 (1.4)	7 (2.4)	4 (1.4)	
Occupation, n (%)								
None		4 (1.4)	6 (2.1)	4 (1.4)	6 (2.1)	7 (2.4)	7 (2.4)	
Self		36 (12.4)	34 (11.7)	31 (10.7)	31 (10.7)	30 (10.3)	23 (7.9)	0.533
Formal		17 (5.9)	14 (4.8)	13 (4.5)	8 (2.8)	7 (2.4)	12 (4.1)	

Data shown are median values for continuous variables and numbers (n) and proportion (%) of study subjects for categorical variables. Data analysis was conducted using chi-square tests for categorical data and Kruskal wallis test for continuous data.

4.3: The establishment of normal serum ferritin reference interval values for adult males and females.

The establishment of the normal serum ferritin reference intervals were done using the non parametric method. The method was used after a normality test was done using the Kolmogorov-Smirnov, showed that the data was not normally distributed. At a confidence interval of 95%, the reference values were created, using the 2.5th and 97.5 percentiles. The 2.5^{th} and 97.5^{th} percentiles denotes the lower limits (LL) and upper limits (UL), respectively. The Mann-Whitney U test for independent samples (a non-parametric test) was used to compare the medians for the two sexes statistically. Statistics were deemed different if $P \leq 0.05$. The established normal ferritin reference intervals were as follows: males: 28.30–425.60 ng/mL; females: 11.15–155.87 ng/mL; and combined males and females: 13.89–415.62 ng/mL. On comparison of the gender statistical differences, a *p* value of <0.0001 was realized. Verification of the established normal ferritin reference intervals and for the reference intervals that are provided by the manufacturer's company (Cobas) was done based on the sigma diagnostic method per age group.

The tolerance limits and deviation percentages results were as follows: - Males: -18.81, females: -16.05, and for the established combined male and female (M/F): -38.60. The results were deemed to be acceptable if the tolerance limit was ≤ +/-20%, as shown in Table 4.3.

Table 4 3: Established normal Ferritin RIs for adults aged 18-64 years, Uasin Gishu County, Kenya.

	Gender	N	Mean	Median	Percentiles 2.5	Percentiles 97.5	Refence intervals	Tolerance limit (% Dev.)
Ferritin (ng/mL)	Male	157	184.20	164.40	28.30	425.60	28.30 - 425.60	-18.81
	Female	133	70.11	64.28	11.15	155.87	11.15 - 155.87	-16.05
	M/F	290	131.87	116.43	13.89	415.62	13.89-415.62	**-38.60***

Comparison of the established ferritin RIs for male and female

	Gender	N	Mean	Median	Refence value	Z- score	*p*-value
Ferritin (ng/mL)	Male	157	184.20	164.40	28.30 - 425.43	-10.214	**<0.0001***
	Female	133	70.11	64.28	11.15 - 155.87		

Verification of the established Ferritin reference intervals **and manufacturer's (Cobas) per age group**

	Gender	N	Mean	Median	Established RIs (ng/mL)	Cobas RIs (ng/mL)	Cobas RIs Tolerance limit (%)	UG RIs Tolerance limit (%)
18–34 years								
	Male	62	184.38	164.95	28.30-425.43	30.00-400	-14.24	-18.72
	Female	50	68.08	53.06	11.15 - 155.87	15.00-150	-17.48	-18.54
	M/F	112	132.44	127.64	13.89-415.62	-	-	**38.33***
35-44 years								
	Male	47	184.86	168.25	28.30 – 425.43	30.00-400	-14.02	-18.52
	Female	43	71.21	64.85	11.15 – 155.87	15.00-150	-13.68	-14.73
	M/F	90	130.57	97.36	13.89-415.62	-	-	**-39.20***
45-64 years								
	Male	47	183.31	158.45	28.30 – 425.43	30.00-400	-14.74	-19.20
	Female	43	71.53	66.00	11.15 – 155.87	15.00-150	13.30	-14.35
	M/F	88	132.50	117.10	13.89-415.62	-	-	**-38.30***

*ng/mL=Nanograms per milliliter, M/F=Male & Female. *p≤0.05, RIs= Reference interval, .*
Bolded* - Tolerance limit >+/-20

4.4: Verification of the established Ferritin reference intervals and manufacturer's reference intervals (Cobas) per location

Table 4.4 shows the verification of the established normal ferritin reference intervals and for those provided by the manufacturer (Cobas). These were validated using the Sigma diagnostic

technique based on the research participants' subcounties of origins. This was aimed at determining the acceptability of the reference intervals for use in the various sub-counties. The results were as follows: The established ferritin reference intervals for Uasin Gishu county, the results showed that the male and female individual reference intervals were within the acceptable tolerance limits; however, the combined male and female (M/F) reference intervals exceeded the permissible limits. The results obtained from the manufacturer's reference intervals indicated that they were all within the acceptable tolerance limits of < +/-20%,.

Table 4 4: Verification of the established Ferritin RIs and manufacturer's RIs (Cobas) per location

Location	Gender	N	Mean	Median	Established RIs (ng/mL)	Cobas RIs (ng/mL)	Cobas RIs Tolerance limit (%)	UG RIs Tolerance limit (%)
Turbo	Male	32	184.87	152.95	28.30 – 425.43	30.00-400	-14.01	-18.51
	Female	25	71.33	65.33	11.15 – 155.87	15.00-150	-13.54	-15.78
	M/F	57	135.07	118.32	13.89-415.62	-	-	**-37.11***
Soy	Male	30	184.69	168.98	28.30 - 425.43	30.00-400	-14.10	-18.59
	Female	24	70.72	65.05	11.15 - 155.87	15.00-150	-14.28	-15.32
	M/F	54	134.04	136.18	13.89-415.62	-	-	**-37.58***
Kapseret	Male	26	183.09	148.15	28.30 – 425.43	30.00-400	-14.84	-19.29
	Female	22	69.67	62.51	11.15 – 155.87	15.00-150	-15.55	-16.45
	M/F	48	131.10	107.70	13.89-415.62	-	-	**-38.95***
Moiben	Male	24	185.23	153.15	28.30 – 425.43	30.00-400	-13.85	-18.35
	Female	21	70.72	64.51	11.15 – 155.87	15.00-150	-14.28	-15.32
	M/F	45	131.79	95.79	13.89-415.62	-	-	**-38.63***
Kesses	Male	23	183.45	174.50	28.30 - 425.43	30.00-400	-14.67	-19.14
	Female	21	69.18	61.52	11.15 - 155.87	15.00-150	-16.14	-17.16
	M/F	44	128.91	98.77	13.89-415.62	-	-	**39.97***
Ainabkoi	Male	22	183.52	172.75	28.30 – 425.43	30.00-400	-14.64	-19.10
	Female	20	68.65	61.47	11.15 – 155.87	15.00-150	-16.79	-17.79
	M/F	42	128.82	123.57	13.89-415.62	-	-	**-40.02***

ng/mL=Nanograms per milliliter, M/F=Male & Female., RIs= Reference interval. ***Bolded* - Tolerance limits > +/-20***

4.5: Established normal Iron reference intervals for adults aged 18-64 years, Uasin Gishu County, Kenya.

The creation of iron reference intervals followed the CLSI/IFCC criteria. The non-parametric technique was adopted, with the 2.5th and 97.5th percentiles serving as the lower (LL) and upper limits (UL), respectively. The established normal iron reference interval values are as follows: Males: 8.27–32.02 umol/L; females: 5.21–25.45 umol/L; combined male–female (M/F): 5.40–29.52 umol/L. A statistical difference between genders was found with a p-value of less than 0.0001. The sigma diagnostic technique was used to verify the established Uasin Gishu reference intervals for entire study groups per sex and per age groups as well. The provided reference intervals by the reagent manufacturer (Cobas) were s well verified based on the age group, all aimed at ensuring that since single reference intervals are provided for the entire adult population per sex, a verification could test their potency in all the age groups. The established Uasin Gishu reference intervals the verification results for tolerance limits for males, female, and the combined male and female (M/F) were within the acceptable limits as shown on Table 4.5. On the categorization based on the three age groups, the results showed that established Uasin Gishu reference intervals were all within the acceptable tolerance limits as shown in table 4.5.

The verification of the provided Cobas supplied reference intervals showed that the reference intervals for males were within the acceptable tolerance limit of -/+20 hence deemed suitable for use. However, the provided reference intervals for the females were NOT within the acceptable tolerance limits as they were beyond -/+20 for the three age groups hence can be deemed unsuitable for use for the Uasin Gishu County as shown in table 4.5 below.

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Table 4 5: Established normal Iron reference intervals for adults aged 18-64 years, Uasin Gishu County, Kenya.

					Percentiles			Tolerance limit (%
	Gender	**N**	**Mean**	**Median**	**2.5**	**97.5**	**Refence intervals**	**Dev.)**
	Male	157	16.26	15.22	8.27	32.02	8.27 - 32.02	-19.29
Iron	Female	133	12.54	11.63	5.21	25.45	5.21 - 25.45	-18.20
(umol/L)	M/F	290	14.55	13.83	5.40	29.52	5.40-29.52	-16.67
Comparison of the established Iron RIs for male and female								
	Gender	**N**	**Mean**	**Median**	**Refence value**		**Z- score**	**Sig. (*p*-value)**
Iron	Male	157	16.26	15.22	8.27 - 32.02		-5.527	**<0.0001***
(umol/L)	Female	133	12.54	11.63	5.21 - 25.45			
Verification of the established Iron RIs and manufacturer's RIs (Cobas) per age group								
					Established RIs	**Cobas RIs**	**Cobas RIs Tolerance limit**	**Established RIs Tolerance limit**
	Gender	**N**	**Mean**	**Median**	**(umol/L)**	**(umol/L)**	**(%)**	**(%)**
18–34	Male	62	16.23	15.19	8.27 - 32.02	10.6 -28.3	-16.56	-19.43
years	Female	50	12.81	12.14	5.21 - 25.45	6.6 - 26.0	**-21.41***	-16.44
	M/F	112	14.70	14.08	5.40 - 29.52	-	-	-15.80
35-44	Male	47	16.15	15.22	8.27 - 32.02	10.6 -28.3	-16.97	-19.88
years	Female	43	11.88	12.31	5.21 - 25.45	6.6 - 26.0	**-27.12***	-19.70
	M/F	90	14.31	13.60	5.40 - 29.52	-	-	-18.40
45-64	Male	48	16.16	15.30	8.27 - 32.02	10.6 -28.3	-16.92	-19.78
years	Female	40	12.43	10.83	5.21 - 25.45	6.6 - 26.0	**-23.74***	-18.92
	M/F	88	14.46	13.19	5.40 - 29.52	-	-	-17.18

*umol/L - micromole per litre; N- number of samples analyzed; Sig.- significance; M/F=Male & Female. ***p≤0.05**; RIs – Reference interval, ***bolded -Tolerance limits >+/-20%***

4.6: Verification of the established Iron RIs and manufacturer's RIs (Cobas) per Location

The established Uasin Gishu and the manufacturer's (Cobas) were verified using the sigma diagnostic method, based on the subcounty of origin of the participants. The results observed from the study showed that all the Uasin Gishu established reference interval tolerance limits were within the acceptable tolerance limits of -/+20 in all the locations for both male and females. The manufacturer's provided reference intervals were suitable for males as they were within the acceptable tolerance limits of -/+20. However, the provided manufacturer reference

intervals for the females were outside the acceptable tolerance limits of -/+20 in all locations. These results indicates that these reference intervals are not suitable for the Uasin Gishu female population as shown in Table 4.6.

Table 4 6: Verification of the established Iron RIs and manufacturer's RIs (Cobas) per Location

Location	Gender	N	Mean	Median	Established RIs (umol/L)	Cobas RIs (umol/L)	Cobas RIs Tolerance limit (%)	Established RIs Tolerance limit (%)
Turbo	Male	32	16.15	15.39	8.27 - 32.02	10.6 -28.3	-16.97	-19.83
	Female	25	12.63	12.54	5.21 - 25.45	6.6 - 26.0	**-22.52***	-17.61
	M/F	57	14.61	13.50	5.40 - 29.52	-	-	-16.32
Soy	Male	30	16.17	15.34	8.27 - 32.02	10.6 -28.3	-16.86	-19.73
	Female	24	12.34	11.83	5.21 - 25.45	6.6 - 26.0	**-24.29***	-19.50
	M/F	54	14.47	14.16	5.40 - 29.52	-	-	-17.12
Kapseret	Male	26	16.14	15.08	8.27 - 32.02	10.6 -28.3	-17.02	-19.88
	Female	22	12.38	11.34	5.21 - 25.45	6.6 - 26.0	**-24.05***	-19.24
	M/F	48	14.31	14.04	5.40 - 29.52	-	-	-18.04
Moiben	Male	24	16.79	15.70	8.27 - 32.02	10.6 -28.3	-13.68	-16.65
	Female	21	12.43	1231	5.21 - 25.45	6.6 - 26.0	**-23.74***	-18.92
	M/F	45	14.62	13.19	5.40 - 29.52	-	-	-16.27
Kesses	Male	23	16.16	13.38	8.27 - 32.02	10.6 -28.3	-16.92	-19.78
	Female	21	12.73	12.45	5.21 - 25.45	6.6 - 26.0	**-21.90***	-18.92
	M/F	44	14.32	13.30	5.40 - 29.52	-	-	-17.98
Ainabkoi	Male	22	16.17	14.66	8.27 - 32.02	10.6 -28.3	-16.86	-19.73
	Female	20	12.70	11.72	5.21 - 25.45	6.6 - 26.0	**-22.09***	-17.16
	M/F	42	14.52	13.41	5.40 - 29.52	-	-	-16.84

umol/L - micromole per litre; N- number of samples analyzed; M/F=Male & Female; RIs – Reference interval, ***bolded -Tolerance limits above** ≤ +/-20%*

4.7 Established normal Transferrin and UIBC RIs for adults aged 18-64 years

Transferrin and unsaturated iron binding capacity (UIBC) reference intervals were established based on the IFCC/CLSI guidelines. The following values for the reference intervals were established for Transferrin: 2.13–4.01 g/L and 2.08–4.04 g/L for males and females, respectively; combined males and females: 2.11–4.02 g/L. For UIBC, the established reference

intervals were: 20.50–64.08 umol/L and 20.07–64.21 umol/L for males and females, respectively, and 20.33–64.05 umol/L for combined males and females. As shown in Table 4.7, a comparison between genders for the two analytes was done; transferrin had a *P*~0.829 and UIBC had a *P*~1.00. These *P* values were an indication that there were no significant statistical differences between genders for the two analytes as shown in Table 4.7 below.

Table 4 7: Established normal Transferrin and UIBC RIs for adults aged 18-64 years, Uasin Gishu County, Kenya.

					Percentiles			Tolerance limit
	Sex	**N**	**Mean**	**Median**	**2.5**	**97.5**	**Refence intervals**	**(% Dev.)**
	M	157	2.83	2.78	2.13	4.01	2.13 - 4.01	-7.82
Transferrin	F	133	2.82	2.75	2.08	4.04	2.08 - 4.04	-7.84
(g/L)	M/F	290	2.83	2.76	2.11	4.02	2.11 - 4.02	-7.67
	M	157	39.61	40.20	20.50	64.08	20.50 - 64.08	-6.34
UIBC	F	133	44.29	40.3	20.07	64.21	20.07 - 64.21	5.10
(umol/L)	F/M	290	41.76	40.25	20.33	64.05	20.33 - 64.05	6.09

Comparison of the established Transferrin and UIBC RIs for male and female

	Sex	**N**	**Mean**	**Median**	**Refence value**	**Z- score**	***p*-value**
Transferrin	M	157	2.83	2.78	2.13 - 4.01		
(g/L)	F	133	2.82	2.75	2.08 - 4.04	-0.113	0.829
UIBC	M	157	39.61	40.20	20.50 - 64.08		1.00
(umol/L)	F	133	44.29	40.3	20.07 - 64.21	-4.435	

*umol/L - micromole per litre; g/L – grams/Litre, N- number of samples analyzed; Sig.- significance; M/F=Male & Female. *p≤0.05; RIs – Reference interval, *Tolerance limit= ≤ +/-20%.*

4.8 Verification of the established Transferrin and UIBC RIs & manufacturer's RIs per age groups

The established Uasin Gishu reference intervals and the manufacturer's (Cobas) provided reference intervals were verified using the sigma diagnostic method, based on age groups. This was done to establish the viability of both the established Uasin Gishu County reference intervals and those provided by the reagent manufacturer using the sigma diagnostic verification method. As indicated in Table 4.8, the results showed that both the established

Uasin Gishu reference intervals and those provided by the reagent manufacturer were all within the acceptable tolerance limits for the two parameters with Tolerance limits of ≤ +/-20%, as shown in Table 4.8.

Table 4 8: Verification of the established Transferrin and UIBC RIs & manufacturer's RIs per age groups

		Sex	N	Mean	Median	UG RIs	Cobas RIs	Cobas RIs Tolerance limit	UG RIs Tolerance limit
18–34 years.	**Tran** (g/L)	M	62	2.90	2.80	2.13 - 4.01	2.0 -3.6	3.57	-5.54
		F	50	2.89	2.75	2.08 - 4.04	2.0 -3.6	3.21	-5.86
		M/F	112	2.89	2.76	2.11 - 4.02	2.0 -3.6	3.21	-5.71
	UIB (umol/L)	M	62	39.23	40.40	20.50- 64.08	20-62.0	-4.32	-7.23
		F	50	42.05	40.20	20.07- 64.21	20-62.0	2.56	-0.21
		M/F	112	40.49	40.25	20.33-64.05	20-62.0	-1.2	-4.03
35-44 years.	**Tran** (g/L)	M	47	2.75	2.70	2.13 - 4.01	2.0 -3.6	-1.79	-10.42
		F	43	2.78	2.76	2.08 - 4.04	2.0 -3.6	-0.71	-9.12
		M/F	90	2.77	2.76	2.11 - 4.02	2.0 -3.6	-1.07	-9.62
	UIBC (umol/L)	M	47	40.26	40.80	20.50-64.08	20-62.0	-1.80	-4.80
		F	43	44.47	39.90	20.07 - 64.21	20-62.0	8.46	5.53
		M/F	90	42.28	40.75	20.33 - 64.05	20-62.0	3.12	0.21
45-64 years.	**Tran** (g/L)	M	48	2.82	2.80	2.13 - 4.01	2.0 -3.6	0.71	-8.14
		F	40	2.78	2.71	2.08 - 4.04	2.0 -3.6	-0.71	-9.45
		M/F	88	2.80	2.77	2.11 - 4.02	2.0 -3.6	0.0	-8.65
	UIBC (umol/L)	M	48	39.07	39.65	20.50 - 64.08	20-62.0	-4.70	-6.67
		F	40	46.89	45.60	20.07 - 64.21	20-62.0	14.37	11.27
		M/F	88	42.84	40.00	20.33 - 64.05	20-62.0	4.49	1.54

*Tran=transferrin, UIBC=unsaturated iron binding capacity, M=male, F=female, M/F=male & female, RIs= reference intervals, UG=Uasin Gishu, *Tolerance limit= ≤ +/-20%*

4.9 Verification of the established Transferrin and UIBC reference intervalss & manufacturer's RIs per location

Based on the sigma diagnostic method, the established transferrin and unsaturated iron binding capacity reference intervals and the provided manufacturer's (Cobas) reference interval were verified as per the study participants subcounties of origin. The observed tolerance limits for both the established and provided reference intervals were within the permissible range of ≤ +/- 20% in all the locations in both males and females as shown in Table 4.9. This was an indication that both the manufacturer's and the established reference intervals can be applied in safely for the entire Uasin Gishu population.

Table 4.9: Verification of the established Transferrin and UIBC RIs & manufacturer's RIs per location

		Sex	N	Mean	Median	UG RIs	Cobas RIs	Cobas RIs Tolerance limit (%)	UG RIs Tolerance limit (%)
Turbo	**Tran**	M	32	2.83	2.87	2.13 - 4.01	2.0 -3.6	1.07	-7.82
		F	25	2.84	2.87	2.08 - 4.04	2.0 -3.6	1.43	-7.19
		M/F	57	2.83	2.87	2.11 - 4.02	2.0 -3.6	1.07	-7.67
	UIBC	M	32	40.70	40.75	20.50 - 64.08	20-62.0	-073	-3.76
		F	25	48.02	49.70	20.07 - 64.21	20-62.0	17.12	13.55
		M/F	57	43.91	42.70	20.33 - 64.05	20-62.0	7.10	4.08
Soy	**Tran**	M	30	2.82	2.78	2.13 - 4.01	2.0 -3.6	0.71	-8.14
		F	24	2.92	2.90	2.08 - 4.04	2.0 -3.6	4.29	-4.89
		M/F	54	2.87	2.85	2.11 - 4.02	2.0 -3.6	2.5	-6.36
	UIBC	M	30	39.53	40.35	20.50 - 64.08	20-62.0	-3.58	-6.53
		F	24	44.32	40.35	20.07 - 64.21	20-62.0	8.10	4.80
		M/F	54	41.66	42.70	20.33 - 64.05	20-62.0	1.61	-1.26
Kapseret	**Tran**	M	26	2.78	2.69	2.13 - 4.01	2.0 -3.6	-0.71	-9.45
		F	22	2.72	2.68	2.08 - 4.04	2.0 -3.6	-2.86	-11.40
		M/F	48	2.75	2.68	2.11 - 4.02	2.0 -3.6	-1.79	-10.27
	UIBC	M	26	39.10	39.00	20.50 - 64.08	20-62.0	-4.63	-7.54
		F	22	46.15	39.95	20.07 - 64.21	20-62.0	12.56	9.13
		M/F	48	42.33	39.65	20.33 - 64.05	20-62.0	3.24	0.33
Moiben	**Tran**	M	24	2.74	2.65	2.13 - 4.01	2.0 -3.6	-2.14	-10.75
		F	21	2.78	2.70	2.08 - 4.04	2.0 -3.6	-0.71	-9.45
		M/F	45	2.76	2.68	2.11 - 4.02	2.0 -3.6	-1.43	-11.58
	UIBC	M	24	38.57	39.40	20.50 - 64.08	20-62.0	-5.93	-8.80
		F	21	43.13	40.2	20.07 - 64.21	20-62.0	5.20	1.99
		M/F	45	40.93	39.80	20.33 - 64.05	20-62.0	-0.17	-2.99
Kesses	**Tran**	M	23	2.87	2.94	2.13 - 4.01	2.0 -3.6	2.50	-6.51
		F	21	2.50	2.67	2.08 - 4.04	2.0 -3.6	-10.70	-8.79
		M/F	44	2.84	2.73	2.11 - 4.02	2.0 -3.6	1.43	-7.3
	UIBC	M	23	39.18	39.96	20.50 - 64.08	20-62.0	-4.44	-7.35
		F	21	43.51	44.60	20.07 - 64.21	20-62.0	6.12	2.88
		M/F	44	41.02	39.96	20.33 - 64.05	20-62.0	0.05	-2.77
Ainabkoi	**Tran**	M	22	2.94	2.84	2.13 - 4.01	2.0 -3.6	5.00	-4.23
		F	20	2.85	2.71	2.08 - 4.04	2.0 -3.6	1.79	-6.19
		M/F	42	2.90	2.78	2.11 - 4.02	2.0 -3.6	3.57	-5.38
	UIBC	M	22	40.28	41.15	20.50 - 64.08	20-62.0	-1.76	-4.75
		F	20	39.59	37.90	20.07 - 64.21	20-62.0	-3.44	-6.38
		M/F	42	39.95	40.40	20.33 - 64.05	20-62.0	-2.56	-5.31

*N – number of samples analyzed, % Dev. - Percentage deviation, M=male, F=female, M/F=male & female, RIs= reference intervals, UG=Uasin Gishu, *Tolerance limit= ≤ +/-20%*

CHAPTER FIVE

5.0 DISCUSSION

Iron profile tests play an important role in the diagnosis and therapy monitoring of iron-related disorders in patients. To interpret iron-panel laboratory tests, it's important to understand the typical reference ranges for a certain area or population. This study was set to establish and verify normal reference intervals for routinely analyzed iron profile tests that are population-based, based on the healthy population of Uasin Gishu County. The iron profile parameters that were considered for this study were ferritin, iron, transferrin, and unsaturated iron binding capacity (UIBC).

5.1 Demographic and anthropometric characteristics of iron profile study participants.

The study participant recruitment enrolled 290 healthy adults, consisting of 157 males, who accounted for 54.14%, and 133 females, representing 45.87%. The number of females was lower than that of males, which is thought to have statistical significance. The low turn up of females in this study is in agreement with other studies that use the same criteria such as Borai A. *et al* (2023). The lower number of female participants was due to specific exclusion criteria, such as oral contraceptive use, pregnancy, menstruation, and recent lactation, which affect iron and hemoglobin levels (Shkodzik, 2020; Nwadike, 2021).

The study found that the male study participants had more height and weight when compared to the females, which showed statistically significant differences between the two genders. Height and weight levels are thought to have an indirect impact on iron and

ferritin levels in individuals. This can be attributed to the fact that tall heights and heavy weights in males have a number of physiological and lifestyle variables that may favor iron and ferritin levels. In general, men have more muscular mass and blood volume than women as a result of their weight and height. These factors favor men, who often have higher iron needs, resulting in higher ferritin and iron levels. As muscle mass increases, so does the body's need for iron, potentially leading to lower blood iron levels due to greater use and redistribution. This scenario is in agreement with a study done by Chen Z. *et al. (*2022) on the association between iron status and muscle mass in adults. Males as well are associated with more physical activities and sports; these activities can increase muscle oxygen needs, increasing the overall iron and ferritin levels in taller people, especially in men. In conclusion, we can say that the differences in the iron profile reference intervals between males and females can be directly associated with weight and height.

The study also found that there was a significantly statistical differences in the BMI between the genders, females had more of their study participants with high levels of BMI and majorly contributed to those classified as overweight 9BMI .25.0). Obesity is thought to have a complicated connection with iron profile levels, frequently resulting in altered iron metabolism and probable reduced iron levels. This can be associated with persistent, low-grade inflammation. Adipose tissue secretes pro-inflammatory cytokines such as interleukin-6 (IL-6), which stimulates the synthesis of hepcidin, a major regulator of iron metabolism. Elevated hepcidin levels inhibit the ferroportin channels in the gut, hence decreasing iron absorption. Hepcidin's high levels also enhance iron sequestration in macrophages and liver cells, making it less accessible for erythropoiesis. These scenarios

of reduced iron levels in females owing to obesity are consistent with other similar studies on obesity as a developing emergency for iron deficiency conducted by Aigner, E. *et al.* (2014) and Alshwaiyat, N. *et al.* (2021)

5.2 Established normal Ferritin RIs for adults aged 18-64 years, Uasin Gishu County, Kenya.

The established reference intervals for ferritin were: 28.41–425.60 ng/mL for males, 11.15–155.87 ng/mL for females, and 13.89–415.62 ng/mL for combined males and females. The established results were similar to results from other researchers, such as Ichihara *et al*. (2018), and Omuse *et al.* (2020), with slight variations on both lower limit and upper limits. This slight variation can be attributed to factors that affect reference intervals in different populations such as diet, altitude, race, age, and genetics. The established reference intervals were verified using the Sigma Diagnostics method to test their validity and applicability to the Uasin Gishu county population. Using the sigma diagnostic method, the study also verified the manufacturer's (Cobas) ferritin reference intervals currently in use. This technique was utilized to assess their viability and applicability as compared to the established Uasin Gishu reference intervals. A gender comparison was also done for the established Uasin Gishu reference intervals to establish whether there were any statistical differences between the genders using the Mann-Whitney U test.

On verification of the established ferritin reference intervals, it was noted that individual males and females were within the permissible tolerance ranges, which are +/-20; however,

the combined established male and female reference intervals were outside the permissible tolerance limits, with -38.60. This eventuality highlights the necessity of distinct ferritin reference intervals for males and females due to physiological differences. This situation underscores the necessity of employing distinct ferritin reference intervals for males and females separately. This scenario aligns with the recommendations of the reagent manufacturer (Cobas), which supplies separate ferritin reference intervals for males and females. This is also in agreement with other researchers, such as Omuse *et al.* (2020), who recommend separate male and female reference intervals for ferritin.

Results from the comparison of gender-specific ferritin reference intervals using the Mann-Whitney U test yielded a *P* value of <0.0001, an indication that there was a statistically significant difference between males and females. This scenario is in agreement with other studies done by other researchers, such as Ichihara *et al*. (2018), Omuse *et al.* (2020), and Uchechukwu *et al*. (2021), Ferritin, being an iron-storing protein, is profoundly influenced by several physiological factors. One key factor is the natural blood loss experienced by women of reproductive age during menstruation, often leading to lower iron and ferritin levels compared to males. Women may lose up to 1 mg of iron daily during their menstrual cycle, contributing significantly to this disparity (Weyand A. *et al* 2023). Additionally, body size and muscle mass also play a role. Males typically have a larger body mass and muscle mass, which results in higher storage capacity for iron and ferritin. Research by Harrison-Findik (2010) highlights that larger muscles store more iron and ferritin. Genetic makeup further contributes to these differences. Androgens in men have a direct stimulatory effect on the bone marrow, working with erythropoietin to promote its

production in the kidney. Conversely, estrogen has an inhibitory effect on the bone marrow in women, affecting their iron requirement and absorption (Murphy 2014). Recognizing and accounting for these differences is crucial for accurate interpretation of test results and effective clinical care. Different patterns of iron accumulation exist according to age, sex, and race.

The established ferritin reference ranges were further verified based on two categories, namely the age groups and the study participants' location of origin. The age category included three age groups, and the location was divided into six groups, as shown in Tables 4.3 and 4.4. This was done to determine the suitability of the established ferritin reference intervals in the various categories. The purpose of this was to ensure that all age groups and locations were adequately covered and that the established reference intervals could be safely applied to them all. The three age groups were 18–34 years, 35–44 years, and 45–64 years. The location categorization is comprised of the six subcounties of Uasin Gishu County, namely: Turbo, Kapseret, Moiben, Kesses, Ainabkoi, and Soy. The study results from the verifications showed that the individual male and female ferritin reference intervals were all suitable for use in all age groups; however, the established combined male and female reference intervals were all outside the permissible tolerance limits, with -38.33 for the age group, -39.20 for those aged 35–44 years, and -38.30 for those aged 45–64 years. Under the location categorization, all individual male and female established ferritin reference intervals were within the permissible tolerance limits and could be safely applied in all the locations; however, the established combined male and female reference intervals were outside the permissible ranges for all the locations. Turbo had (-37.11), Soy

had (-37.58), Kapseret had (-38.95), Moiben had (-38.63), Kesses had (-39.97), and Ainabkoi had (-40.02). Based on these observed tolerance limit deviations, it was concluded that the established individual male and female ferritin reference intervals were suitable for utilization across all age groups and in all locations per sex. However, it was also noted that the combined male and female reference intervals exceeded permissible tolerance limits across all locations, rendering them unsuitable for application for the Uasin Gishu population.

The results of this study show that the combined male and female ferritin reference interval is not suitable for application for either of the sexes. This might be due to the significant physiological and genetic differences between males and females, which were also evidently highlighted by the statistically significant differences between the two genders.

This approach of verifying individual sex reference intervals and combining male and female reference intervals acknowledges and addresses the inherent differences between males and females, leading to better diagnostic accuracy and improved patient treatment outcomes.

To assess the suitability of the provided manufacturer's (Cobas) ferritin reference intervals currently in use, validation using the Sigma diagnostics method was done for all categories. This validation was meant to test the suitability of the provided ferritin reference intervals for the Uasin Gishu population. As per the results, it was observed that all the obtained values fell within the permissible tolerance limits for all categories. These results indicated that the provided Cobas ferritin reference intervals are suitable for use with the population

of Uasin Gishu County. This scenario might occur due to various reasons that favor the two different populations, such as sharing similar demographics (age, sex, ethnicity, and health status), the use of similar standardized assay methods, and the use of similar technologies and equipment.

5.3 Established normal iron RIs for adults aged 18-64 years, Uasin Gishu County, Kenya.

The establishment of iron reference intervals for the healthy population aged 18–64 years, for both males and females, was conducted in accordance with CLSI guidelines. The reference intervals obtained were as follows: for males, 8.27–32.02 µmol/L; for females, 5.21–25.45 µmol/L; and for the combined male and female group, 5.40–29.52 µmol/L, as detailed in Table 4.5. The established iron reference intervals were then verified using the sigma diagnostic method to assess their suitability for use in Uasin Gishu County, considering various categories such as three age groups and six groups based on the participants' location of origin, as shown in Tables 4.5 and 4.6. For the reference intervals to be considered suitable, they had to fall within the allowable percentage deviation tolerance limits of ±20%. The study results on verification under age groups and location categories showed that the established iron reference intervals were all within the permissible tolerance limits, an indication that they were all suitable for use by the adult population in Uasin Gishu County.

Using the Mann-Whitney U test for gender-specific reference interval differences was conducted; this gave a *P* value of <0.0001. This indicated a statistically significant

difference between males and females. The study results on gender statistically significant difference, was in line with other similar studies done by researchers such as Bakan *et al* (2016), Borai A. *et al* (2016), Shah S. *et al* (2018), and Bawua A. *et al* (2020). This variation between gender, can be attributed to various physiological factors, such as the natural blood loss during the menstrual cycle in women of reproductive age, which leads to lower iron levels in them. Women can lose up to 1 mg of iron daily during menstruation, and since over 70% of the body's iron reserves are in circulation, this loss significantly impacts iron levels. Additionally, the difference in body size and muscle mass between males and females plays a role; males typically have a larger body mass, resulting in more storage for iron and higher levels of both iron and ferritin. Harrison-Findik (2010) noted that men's larger muscles store more iron and ferritin. Iron levels are influenced by genetic makeup and hormones, with androgens in men directly stimulating the bone marrow and promoting erythropoietin production in the kidney. Conversely, estrogen in women has an inhibitory effect on the bone marrow, affecting iron requirements and absorption (Murphy 2014). These gender differences align with similar studies on iron and ferritin reference intervals by researchers like Ichihara *et al.* (2018) and Uchechukwu *et al.* (2021). Given these statistically significant gender differences, it is recommended to use separate male and female iron reference intervals rather than a combined reference interval for a more accurate diagnosis.

This study also evaluated the suitability of the manufacturer's (Cobas) provided iron reference intervals, traditionally used in Uasin Gishu County (males: 10.6-28.3 µmol/L, females: 6.6-26.0 µmol/L). Using the sigma method, the tolerance limits were calculated

for all the categories. The yielded results indicated that only the tolerance limit values from males were within the permissible ranges for all the categories; however, the tolerance limits for females were all outside the permissible limits. This was an indication that the provided iron reference intervals were only suitable for use in males, as shown in Tables 4.5 and 4.6. The obtained female tolerance limit values were as follows: those aged 18–34 had -21.41; those aged 35–44 had -27.92; and those aged 45–64 had -23.74. Under location categorization, the tolerance limits achieved for females were as follows: for Turbo, -24.21; for Soy, -22.52; for Kapseret, -24.05; for Moiben, -23.76; for Kesses, -21.90; and for Ainabkoi, -22.05. These results indicated that the manufacturer's provided iron reference intervals for females are unsuitable for use with the adult female population of the Uasin Gishu population. The discrepancies between the manufacturer-provided reference intervals and those established for Uasin Gishu can be attributed to several factors.

The observed discrepancies between the Uasin Gishu established female iron reference and those provided by the manufacturer’s can be attributed to geographic differences that can also influence other factors such as diet, altitude, genetics, race, health status, and exposure environments. This phenomenon can also be attributed to the fact that the sample sizes used to establish the reference intervals in the two populations varied, hence contributing to statistical differences. Lastly, the manufacturers' reference intervals are typically constructed based on controlled settings adhering to strict quality control requirements. In contrast, since this study was based on the local population in

uncontrolled settings, this difference in methodology could have further contributed to the discrepancies observed between the two sets of reference intervals.

5.4 Established normal establishment of transferrin and unsaturated iron binding capacity (UIBC) RIs for adults aged 18-64 years, Uasin Gishu County, Kenya.

The establishment of transferrin and unsaturated iron binding capacity (UIBC) reference intervals for the healthy population aged 18–64 years, for both males and females, was conducted in accordance with CLSI guidelines. The reference intervals obtained for transferrin were as follows: for males, 2.13–4.01 g/L; for females, 2.08–4.04 g/L; and for combined males and females, 2.11–4.02 g/L. For unsaturated iron binding capacity (UIBC), it was 20.50–64.08 umol/L, 20.07–64.21 umol/L, and 20.33–64.05 umol/L for males, females, and combined males and females, respectively. The established reference intervals were then verified using the sigma method to test their suitability for use in the various categories and groups. Comparisons for gender-specific reference interval differences for each analyte were done using the Mann-Whitney U tests.

On the verification of the established reference intervals for both transferrin and unsaturated iron binding capacity (UIBC) under the age group and location categories, the results realized were within the acceptable tolerance limits of $\pm \leq 20\%$, as shown in Tables 4.7, 4.8, and 4.9. These results indicated that the established transferrin and unsaturated iron binding capacity reference intervals were suitable and could be safely used for all age groups and in all the locations within Uasin Gishu County. It was also realized that the combined male and female reference intervals for the two analytes can be safely employed

for both sexes. This scenario is in tandem with the reagent manufacturer, Cobas, which supplies shared reference intervals for both males and females for each analyte (transferrin and UIBC).

Gender-specific reference interval differences were determined using the Mann-Whitney U tests for each analyte, and a *P* value of 0.829 was realized for transferrin and a *P* value of 1.00 for UIBC. These values were greater than 0.05, which indicated that there were no statistically significant differences between the genders for both transferrin and UIBC. This scenario is in agreement with similar studies done by other researchers, such as Borai A. *et al.* (2016), Bawua A. *et al.* (2020), and Omuse *et al.* (2020). The balance depicted by transferrin can be attributed to the fact that, from a biological perspective, transferrin's' basic function remains constant across the sexes. Transferrin is an iron transporter, and this elementary physiological function does not significantly differ between sexes. This occurrence can be attributed to the fact that transferrin, as a transport protein for iron in the blood, is not hormonally dependent, and its levels can fluctuate according to individual physiological demands rather than sex differences. Transferrin reference intervals depend on population demography, age, genetics, and the general health condition of individuals (Rieger *et al.*, 2016). The established unsaturated iron binding capacity reference interval showed no statistically significant differences between the genders. The phenomenon can be attributed to the fact that the UIBC's purpose is to measure transferrin's ability to transport iron. The fact that transferrin and unsaturated iron binding capacity reference intervals show no statistically significant differences between genders is thought to be the

reason for the reagent manufacturer providing a shared male and female reference interval for each of the two analytes.

The manufacturer's provided transferrin and UIBC reference intervals were validated, and the Sigma diagnostic procedure was used to verify the stated transferrin and UIBC reference intervals from the manufacturer (Cobas). The provided manufacturer's transferrin and UIBC reference intervals are 2.0–3.6 g/L and 20–62.0 umol/L, respectively. The two analytes were verified based on their age groups and origin regions. The study findings revealed that the manufacturer's specified reference ranges for the two analytes were appropriate for usage in Uasin Gishu County. This eventuality can be attributed to the fact that transferrin, an iron transport protein, is tightly controlled by biological mechanisms that are generally consistent across groups and are not hormone- or diet-dependent. This may also be due to both using the same process to construct the reference intervals.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1: Conclusion

The study established sex-specific reference intervals for routinely used iron profiles for the adult population of Uasin Gishu County aged 18–64 years. The reference intervals were validated using the Sigma diagnostic method to check whether they met the required tolerance limits.

On establishing ferritin reference intervals for the healthy population aged 18–64 years in Uasin Gishu County, it was noted that the established individual reference intervals for males and females met the permissible tolerance limits and were suitable for use with the Uasin Gishu population. However, the tolerance limit obtained from the established combined male and female ferritin reference intervals was found to be outside the permissible tolerance limits. This means that the established combined male and female reference intervals are not suitable for use and cannot be applied to both sexes. This scenario is an indication that individual ferritin reference intervals should be used for males and females separately.

On establishing iron reference intervals for the healthy population aged 18–64 years for males and females, results obtained on the percentage deviations showed that they met the permissible ranges, which means that the established iron reference intervals are suitable for use by the Uasin Gishu population. However, the iron reference intervals also showed a statistically significant difference between genders, this is an indication that males and

females cannot share combined reference intervals hence, separate iron reference intervals should be used for males and females, which is in agreement with the reagent manufacturer (Cobas), who supplies separate iron reference intervals for males and females. The research also revealed the provided iron reference intervals for females by the reagent manufacturer were outside the permissible tolerance ranges, which necessitates the importance of every laboratory to locally establish their own reference intervals or verify reference intervals from foreign sources.

The established transferrin and unsaturated iron binding capacity (UIBC) reference intervals were found to have no gender statistically significant differences. The combined male and female reference intervals on verification for the two analytes were found to be suitable for both sexes and can be safely applied for both males and females.

The manufacturer provided reference intervals on verification using the Sigma diagnostic method, and it was noted that some of the provided reference intervals were not suitable for use by the Uasin Gishu population. The parameters that met the required tolerance limit, are deemed suitable for use. However, since there are differences in both the lower limits (LL) and upper limits (UL) when compared with the established Uasin Gishu county reference intervals, it's imperative to address the noteworthy varied range between the lower and upper limits of these reference intervals on the basis of the clinical significance that supersedes statistical significance (Sharma 2021). The implication of such varied interval values is far-reaching, particularly pertaining to the role of reference intervals in clinical applications, and can be classified as clinically meaningful differences (CMD) (Page 2014). The overreliance on manufacturer-provided reference intervals or entirely on

statistical *p-value* analysis may inadvertently misclassify numerous healthy cases, resulting in the eventual mismanagement of these individuals' health (Nahm 2017). These results will provide improved iron profile test results evaluation and interpretation, providing clinicians with better decision-making reference points that will result in better overall quality healthcare offered to the population served.

6.2: Recommendation

The findings of this study on ferritin reference intervals show that the reference intervals are sex-specific, and their interval values vary significantly with those of the reagent manufacturer. It is therefore recommended that locally established reference intervals be used, since they better define the local population's state of health. The use of foreign reference intervals should be done with caution, and each laboratory should either define its own ferritin reference intervals or rigorously validate those provided by other populations.

Iron reference intervals are sex-specific for the local adult population; the use of separate male and female reference intervals is recommended. The application of foreign reference intervals should be subjected to verification before use, as evident discrepancies were depicted in this study following the validation of the provided manufacturer's iron reference intervals.

Transferrin and UIBC reference intervals are not sex-specific; the use of common references for both sexes is acceptable. The use of reference intervals for these analytes

from foreign sources must be subjected to verification before their application to ensure accuracy in diagnosis.

6.2.1 Recommendations for action

Other laboratories should establish their own local population reference intervals for iron profile and any other biochemical parameters, as per the methodologies they are using, and not entirely rely on the reference intervals supplied by the manufacturers.

6.2.2 Recommendations for further research

This study recommends that similar iron profile reference intervals be devised for the pediatric population and the elderly (>64 years), as the current established reference intervals are for people aged 18 to 64 years and may not serve the mentioned populations accurately.

6.3: limitations

The study participants' honesty in accurately filling out the questionnaires with the right information on their lifestyles or any other underlying conditions that could not be examined in the laboratory and could have a direct impact on iron profile levels.

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APPENDICES

Appendix 1: Research Consent form

Topic: Laboratory Reference Intervals for selected Iron profile parameters in adult Population of Uasin Gishu County, Kenya.

Consent explanation: My name is Willington Kasimu (Mobile phone; 0723748171) a Master's student in the Department of Medical Laboratory Sciences, Masinde Muliro University of science and technology (MMUST). PO Box 90-50100 Kakamega, Kenya; Phone: +254 (0) 702597360/1.

I am conducting a study on the establishment of local reference intervals for the iron profile. The information in this form will help you make an informed decision on whether or not to participate in this study. Please read through carefully and feel free to ask any questions about the study. I will read it out to those who are not able to read.

Description: The interpretation of clinical chemistry laboratory results is based on comparison to reference quantitative intervals known as normal ranges.

Purpose: This study is aimed at establishing local quantitative reference intervals for the iron profile that will guide the accurate interpretation of the clinical chemistry results.

Benefits: There will be no tokens or direct benefits to the participants. However, the study will establish reference intervals which will facilitate accurate diagnosis of iron related

disorders. This will also facilitate the clinician with proper medical decision points for patients and an overall outcome of better patient care and management.

Risks & discomforts: One potential risk of being in the study is the loss of privacy. However, the samples will only be used for the intended purpose of this study and any personal information collected will be handled with high confidentiality. The procedure of blood sample collection has no major side effects as only **4** milliliters (MLs) of blood will be required, but might cause slight discomfort.

Procedure: If you accept to participate in this study, you have to read and understand the contents of this document, then you will be provided with a participant's questionnaire to complete before sample collection. The sample will be collected after which, we will test your Heamoglobin level and as well screen for HIV, Syphilis, and Hepatitis B and C.

Sample collection

This study will involve blood collection Venous blood will be collected from the antecubital fossa veins and at least 4mls will be collected. This will only be done once for the entire study.

Voluntarism: Enrolment in the study is of free will.

Subject's rights: As a volunteer to this study as a participant, your role will be the sample donation which will be used for the purpose of the study. As this is a voluntarism activity your right to consent or fail to consent is respected. As well, if you accept to take part in the study, you have the right to withdraw your consent or discontinue participating at any time without penalty. Your privacy will be maintained in all published and written data emanating from the study.

If you have questions about your rights as a study participant or are dissatisfied at any time with any aspect of this study, you may contact - anonymously, if you wish – MTRH/MU-IREC (Moi Teaching & Referral Hospital building, 2nd floor. Door No. 219, P.O. Box. 3-30100 Eldoret, Kenya, Office line: +254787723677. Email: irecoffice@gmail.com or irec@mtrh.go.ke.

I have read this form or had it read to me in a language that I understand. I have discussed the information with the study staff. My questions have been well answered. My decision on whether or not to take part in the study is voluntary. If I decide to join the study I may withdraw at any time.

By signing this form, I give my consent to be a participant in this study.

Participant Name: _____. Sign/Thumbprint: _____. Date: _____

Staff Conducting Study Name: _____. Sign. _____ Date: _____

Consent form: Kiswahili version

Mada: Maingiliano ya Marejeleo ya Maabara kwa vigezo vya wasifu vya madini ya Iron vilivyochaguliwa katika Idadi ya watu wazima ya Kaunti ya Uasin Gishu, Kenya.

Maelezo ya idhini: Jina langu ni Willington M. Kasimu (Simu ya rununu; 0723748171) mwanafunzi wa Shahada ya MSc. katika Idara ya Sayansi ya Maabara ya Matibabu, Chuo Kikuu cha Sayansi na teknolojia cha Masinde Muliro (MMUST). Saduku la Posta: 90-50100 Kakamega, Kenya; Simu: + 254 (0) 702597360/1.

Ninafanya utafiti juu ya uanzishwaji wa vipindi vya kumbukumbu vya ndani kwa wasifu wa madini ya iron. Habari katika fomu hii itakusaidia kufanya uamuzi wenye habari juu ya kushiriki au kutoshiriki katika utafiti huu. Tafadhali soma kwa uangalifu na jisikie huru kuuliza maswali yoyote juu ya utafiti. Nitasoma kwa wale ambao hawawezi kusoma.

Maelezo: Tafsiri ya matokeo ya maabara ya kemia ya kliniki ni msingi wa kulinganisha na vipindi vya kumbukumbu vya idadi inayojulikana kama safu za kawaida.

Kusudi: Utafiti huu unakusudia kuanzisha vipindi vya kumbukumbu vya kiwango cha ndani kwa wasifu wa madini ya iron, ambao utaongoza tafsiri sahihi ya matokeo ya kemia ya kliniki.

Faida: Hakutakuwa na ishara au faida za moja kwa moja kwa washiriki. Walakini, utafiti huo utaanzisha safu za kumbukumbu ambazo zitawezesha utambuzi sahihi wa shida zinazohusiana na chuma. Hii pia itawezesha kliniki na vidokezo sahihi vya uamuzi wa matibabu kwa wagonjwa na matokeo ya jumla ya utunzaji bora wa wagonjwa na usimamizi.

Hatari na usumbufu: Hatari moja inayowezekana ya kuwa katika utafiti ni upotezaji wa faragha. Walakini, sampuli zitatumika tu kwa madhumuni yaliyokusudiwa ya utafiti huu

na habari yoyote ya kibinafsi iliyokusanywa itashughulikiwa kwa usiri mkubwa. Utaratibu wa ukusanyaji wa sampuli ya damu hauna athari kubwa kama tu mililita 4 (ml) ya damu itahitajika, lakini inaweza kusababisha usumbufu mdogo.

Utaratibu: Ikiwa unakubali kushiriki katika utafiti huu, lazima usome na uelewe yaliyomo kwenye hati hii, basi utapewa dodoso la mshiriki kukamilisha kabla ya ukusanyaji wa sampuli. Sampuli hiyo itakusanywa baada ya hapo, tutajaribu kiwango chako cha Heamoglobin na pia skrini ya VVU, Syphilis, na Hepatitis B na C.

Ukusanyaji wa sampuli ya damu

Utafiti huu utahusisha ukusanyaji wa damu Damu ya Venous itakusanywa kutoka kwa mishipa ya fossa ya antecubital na angalau 4mls zitakusanywa. Hii itafanywa mara moja tu kwa utafiti wote.

Kujitolea: Uandikishaji katika utafiti ni wa hiari ya bure.

Haki za mada: Kama kujitolea kwa utafiti huu kama mshiriki, jukumu lako litakuwa mchango wa mfano ambao utatumika kwa madhumuni ya utafiti. Kwa kuwa hii ni shughuli ya hiari haki yako ya kukubali au kushindwa kukubali inaheshimiwa. Vile vile, ikiwa unakubali kushiriki katika utafiti, una haki ya kuondoa idhini yako au kuacha kushiriki wakati wowote bila adhabu. Usiri wako utadumishwa katika data zote zilizochapishwa na zilizoandikwa kutoka kwa utafiti.

Ikiwa una maswali juu ya haki zako kama mshiriki wa utafiti au haujaridhika wakati wowote na kipengele chochote cha utafiti huu, unaweza kuwasiliana - bila majina, ikiwa unataka – MTRH/MU-IREC (Moi Kufundisha na Jengo la Hospitali ya Marejeleo, sakafu ya 2. Mlango Na. 219, Sanduku la posta. 3-30100 Eldoret, Kenya, Simu ya rununu: + 254787723677. Barua pepe: irecoffice@gmail.com au irec@mtrh.go.ke.

Nimesoma fomu hii au ilisomwa kwa lugha ambayo ninaelewa. Nimejadili habari hiyo na wafanyikazi wa masomo. Maswali yangu yamejibiwa vizuri. Uamuzi wangu juu ya kama au kushiriki katika utafiti ni wa hiari. Ikiwa nitaamua kujiunga na utafiti naweza kujiondoa wakati wowote.

Kwa kusaini fomu hii, ninatoa idhini yangu kuwa mshiriki.

Jina la Mshiriki:_____. Ishara/Thumbprint: _____. Tarehe: _____

Wafanyikazi Wanaofanya Jina la Utafiti: _____. Saini: _____ Tarehe: _____

Appendix 11 Participants Questionnaire

PARTICIPANTS NUMBER: _____STUDY CASE NUMBER: _____

UNIQUE NUMBER: _____PLACE OF BIRTH: _____

GENDER: __DATE OF BIRTH: ___/___/__(DD/MM/YY). NATIONALITY: _____

PERIOD OF STAY IN UASIN GISHU_____MONTHS/YEARS

Whether suffered from any of the following Ailments	**YES**	**NO**	**Whether having any of the following**	**YES**	**NO**
High blood pressure			Lactation		
Diabetes mellitus			Use of oral contraceptives		
Renal disease			Pregnancy/LMP:		
Tuberculosis			Menses		
Any allergy			On any form of medication		
Epilepsy			Drug abuse		
Malaria			Cigarette smoking		
HIV/AIDS			Alcohol consumption		

Syphilis			Recent surgery/hospitalization		
Heart disease			Frequent blood donor		
Had surgery					
Hepatitis B and C					
Stomach ulcers					
Jaundice					

Appendix III: Laboratory request form

LABORATORY REFERENCE INTERVALS FOR IRON PROFILE FOR ADULT STUDY

Participant's Details:

Unique Code……...

Age…….

Gender M……...F……

IP/OP No……

Lab No……...

Name…….

Sign……

Date……

Date of sample collection……
Time of sample collection……

Phlebotomist **……**

Sample Type; Tick appropriately

Blood ☐

Participants Vitals

Temperature …….

Weight: ……Height:……BMI:…..….

BP/Pulse……..

Relevant clinical information
……..……

Investigation Required

IRON ☐ TRANSFERRIN ☐

FERRITIN ☐ UIBC ☐

NOTE:

THIS REQUEST FORM IS ONLY INTENDED FOR THIS STUDY ALONE AND SHOULD NOT BE USED AS A SUBSTITUTE FOR THE FACILITY REQUEST FORM

Appendix IV: Map of the study area

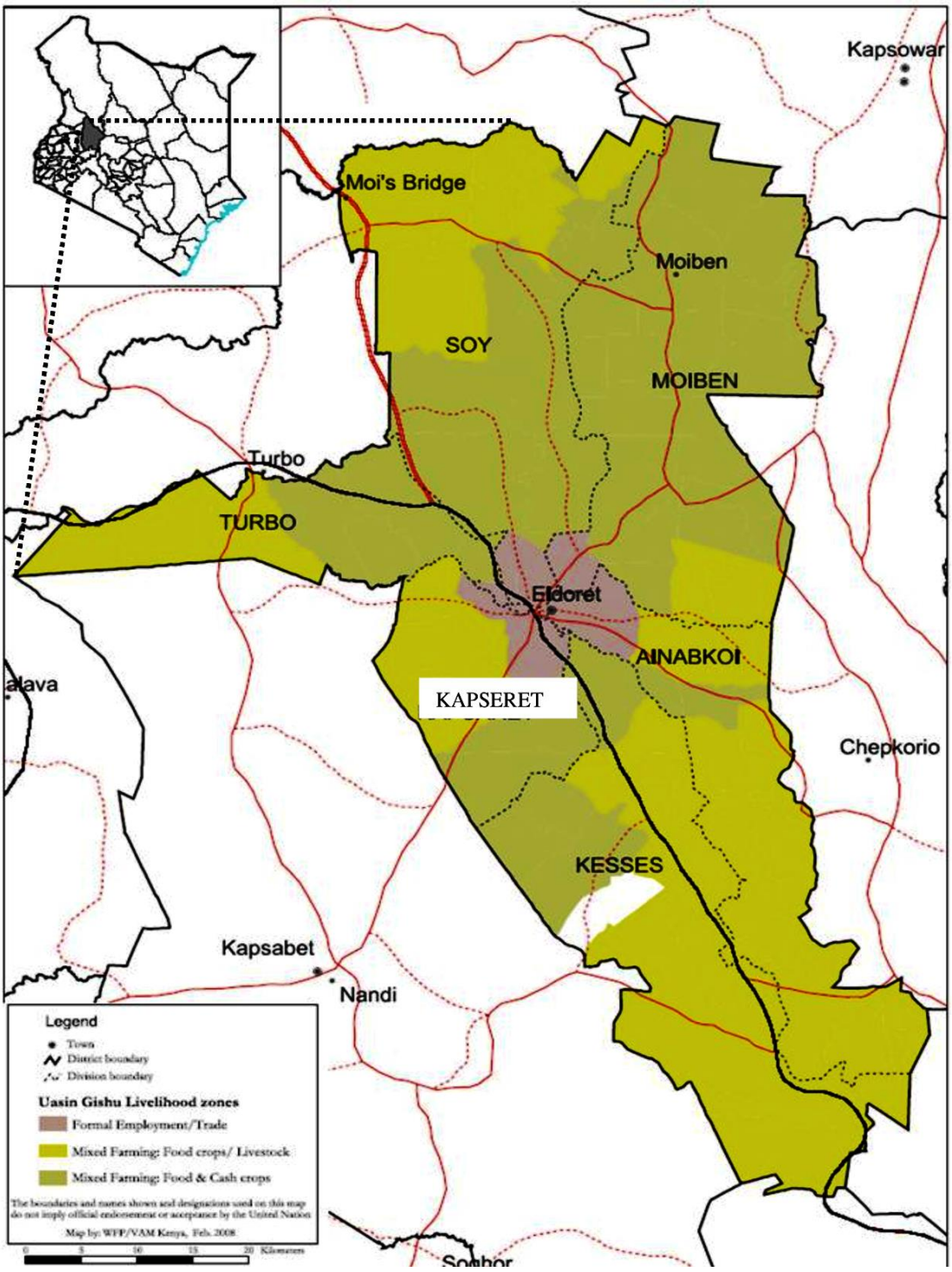


Figure 3.1 Map of Uasin Gishu county

Appendix V: IREC approval

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY
Tel: 056-31375
Fax: 056-30153
E-mail: ierc@mmust.ac.ke
Website: www.mmust.ac.ke
P. O. Box 190,
50100.
Kakamega,
KENYA

Institutional Scientific and Ethics Review Committee (ISERC)

REF: MMU/COR: 403012 Vol 6 (01)

Date: April 14th, 2023

To: Mr. Kasimu Willington Mutunga.

Dear Mr.,

RE: LABORATORY REFERENCE INTERVALS FOR SELECTED IRON PROFILE PARAMETERS IN ADULT POPULATION OF UASIN GISHU COUNTY, KENYA.

This is to inform you that the ***Masinde Muliro University of Science and Technology Institutional Scientific and Ethics Review Committee (MMUST-ISERC)*** has reviewed and approved your above research proposal. Your application approval number is **MMUST/IERC/142/2023.** The approval covers for the period ***March 14th, 2023 to March 14th, 2024.***

This approval is subject to compliance with the following requirements;

i. Only approved documents including informed consents, study instruments, MTA will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***MMUST-ISERC.***
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***MMUST-ISERC*** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***MMUST-ISERC*** within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to ***MMUST-ISERC.***

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed

Yours Sincerely,

Prof. Gordon Nguka (PhD)
Chairperson, Institutional Scientific and Ethics Review Committee

Copy to:
- The Secretary, National Bio-Ethics Committee
- Vice Chancellor
- DVC (PR&I)

Appendix VI: DPS approval

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY (MMUST)

Tel: 056-30870
Fax: 056-30153
E-mail: directordps@mmust.ac.ke
Website: www.mmust.ac.ke

P.O Box 190
Kakamega – 50100
Kenya

Directorate of Postgraduate Studies

Ref: MMU/COR: 509099 **Date:** 13th March 2023

Willington Mutunga Kasimu
HML/G/01-55333/2020,
P.O. Box 190-50100,
KAKAMEGA.

Dear Mr. Kasimu,

RE: APPROVAL OF PROPOSAL

I am pleased to inform you that the Directorate of Postgraduate Studies has considered and approved your Masters Proposal entitled: "***Laboratory Reference Intervals for Selected Iron Profile Parameters in Adult Population of Uasin Gishu County, Kenya"*** and appointed the following as supervisors:

1. Dr. Godfrey M. Gitonga - MMUST
2. Dr. Ezra Kombo Osoro - MMUST

You are required to submit through your supervisor(s) progress reports every three months to the Director Postgraduate Studies. Such reports should be copied to the following: Chairman, School of Public Health, Biomedical Sciences and Technology Graduate Studies Committee and Chairman, Medical Laboratory Sciences Department. Kindly adhere to research ethics consideration in conducting research

It is the policy and regulations of the University that you observe a deadline of three years from the date of registration to complete your Master's thesis. Do not hesitate to consult this office in case of any problem encountered in the course of your work.

We wish you the best in your research and hope the study will make original contribution to knowledge.

Yours sincerely,

MASINDE MULIRO UNIVERSITY
OF SCIENCE AND TECHNOLOGY
DIRECTORATE OF POSTGRADUATE STUDIES
P.O. Box 190 - 50100 KAKAMEGA (K)
Date:..... Sign:.....

Prof. Stephen O. Odebero, PhD, FIEEP
DIRECTOR, DIRECTORATE OF POSTGRADUATE STUDIES

Appendix VII: MU/MTRH IREC

MTRH/MU-INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471//2/3

MOI UNIVERSITY
COLLEGE OF HEALTH SCIENCES
P.O. BOX 4606
ELDORET
Tel: 33471/2/3

Reference: IREC/501/2023
Approval Number: 0004487

27th July, 2023

Kasimu Willington Mutunga,
Masinde Muliro University of Science & Technology,
Department of Medical Laboratory Sciences,
P.O. Box 90-30100,
KAKAMEGA-KENYA.

Dear Mr. Mutunga,

LABORATORY REFERENCE INTERVALS FOR SELECTED IRON PROFILE PARAMETERS IN ADULT POPULATION OF UASIN GISHU COUNTY, KENYA

This is to inform you that ***MTRH/MU-IREC*** has reviewed and approved the above referenced research proposal. Your application approval number is ***FAN: 0004487.*** The approval period is **27th July, 2023- 26th July, 2024.**
This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, Material Transfer Agreements (MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***MTRH/MU-IREC***.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***MTRH/MU-IREC*** within 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***MTRH/MU-IREC*** within 72 hours.
v. Clearance for export of biological specimens must be obtained from ***MOH at the recommendation of NACOSTI*** for each batch of shipment.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to ***MTRH/ MU-IREC.***

Prior to commencing your study; you will be required to obtain a research license from the National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and other relevant clearances from study sites including a written approval from the CEO-MTRH which is mandatory for studies to be undertaken within the jurisdiction of Moi Teaching & Referral Hospital (MTRH) and its satellites sites.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
27 JUL 2023
APPROVED
P. O. Box 4606 • 30100 ELDORET

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc	CEO	-	MTRH	Dean	-	SOP	Dean	-	SOM
	Principal	-	CHS	Dean	-	SON	Dean	-	SOD

Appendix VIII: NACOSTI research license

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **710110**

Date of Issue: **01/May/2023**

RESEARCH LICENSE

This is to Certify that Mr.. WILLINGTON MUTUNGA KASIMU of Masinde Muliro University of Science and Technology, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Uasin-Gishu on the topic: LABORATORY REFERENCE INTERVALS FOR SELECTED IRON PROFILE PARAMETERS IN ADULT POPULATION OF UASIN GISHU COUNTY, KENYA for the period ending : 01/May/2024.

License No: **NACOSTI/P/23/25705**

710110

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

See overleaf for conditions

THE SCIENCE, TECHNOLOGY AND INNOVATION ACT, 2013 (Rev. 2014)
Legal Notice No. 108: The Science, Technology and Innovation (Research Licensing) Regulations, 2014

The National Commission for Science, Technology and Innovation, hereafter referred to as the Commission, was the established under th Science, Technology and Innovation Act 2013 (Revised 2014) herein after referred to as the Act. The objective of the Commission shall be to regulate and assure quality in the science, technology and innovation sector and advise the Government in matters related thereto.

CONDITIONS OF THE RESEARCH LICENSE

1. The License is granted subject to provisions of the Constitution of Kenya, the Science, Technology and Innovation Act, and other relevant laws, policies and regulations. Accordingly, the licensee shall adhere to such procedures, standards, code of ethics and guidelines as may be prescribed by regulations made under the Act, or prescribed by provisions of International treaties of which Ken is a signatory to
2. The research and its related activities as well as outcomes shall be beneficial to the country and shall not in any way;
   i. Endanger national security
   ii. Adversely affect the lives of Kenyans
   iii. Be in contravention of Kenya's international obligations including Biological Weapons Convention (BWC), Comprehensive Nuclear-Test-Ban Treaty Organization (CTBTO), Chemical, Biological, Radiological and Nuclear (CBRN).
   iv. Result in exploitation of intellectual property rights of communities in Kenya
   v. Adversely affect the environment
   vi. Adversely affect the rights of communities
   vii. Endanger public safety and national cohesion
   viii. Plagiarize someone else's work
3. The License is valid for the proposed research, location and specified period.
4. The license any rights thereunder are non-transferable
5. The Commission reserves the right to cancel the research at any time during the research period if in the opinion of the Commission t research is not implemented in conformity with the provisions of the Act or any other written law

Appendix IX: Quality control report for the iron profile analytes

Before running the samples for the analyte levels, quality assurance was ensured, the required calibration was done, and quality control was run as well using the manufacturer's supplied and recommended pathological and normal controls and documented as indicated in Table 4.10 below.

Table 4 10:The quality control (QC) report

Analyte (Unit)	QC type	Assigned QC report				Study QC Report			
		Range	Mean	SD	%CV	Mean	SD	%CV	Results
Ferritin	PNU	85-165	125	1.0	0.8	132.00	2.21	1.67	134.20
	PPU	153-459	306	2	0.6	301.20	3.23	1.07	308.10
Iron	PCC 1	15.7-22.9	19.3	1.2	6.22	19.58	1.74	8.79	18.93
	PCC 2	36.2-52.4	44.3	2.7	6.09	44.19	2.9	6.56	43.44
Transferrin	PCC 1	1.52-2.18	1.85	0.11	5.95	1.92	0.15	7.81	1.89
	PCC 2	2.69-3.89	3.29	0.20	6.08	3.36	0.23	6.84	3.53
UIBC	PCC 1	27.5-41.9	34.7	2.40	6.92	38.27	2.35	6.14	33.60
	PCC 2	35.7-54.9	45.3	3.20	7.06	45.23	3.49	7.71	46.01

*PCC1: PreciControl ClinChem Multi Normal:
*PCC2: PreciControl ClinChem Multi Pathological
*PNU: Precinorm protein,
*PPN: Precipath protein.

Appendix X: Normality Test

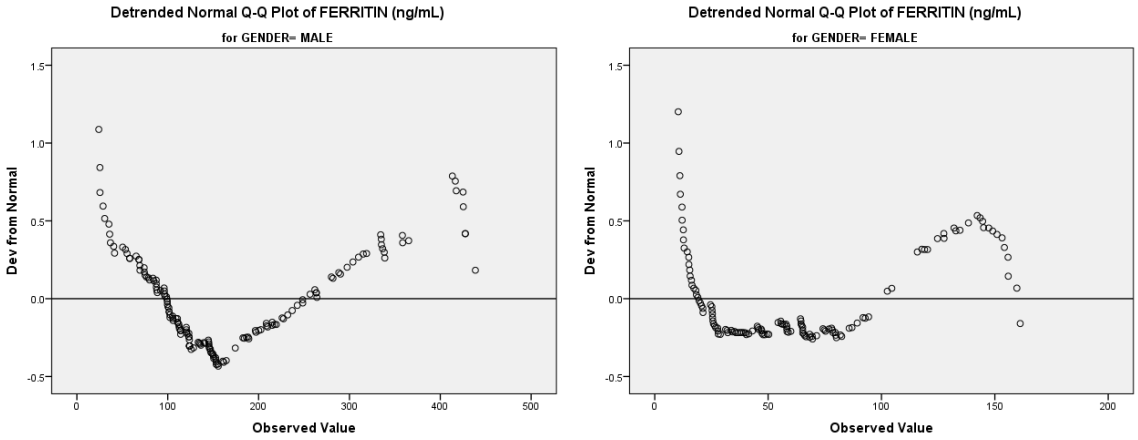
Table 4.11

	Kolmogorov-Smirnov				
	Statistic	**df**	**Sig.**	**skewness**	**kurtosis**
FERRITIN (ng/mL)	.144	290	< 0.0001	1.422	1.780
IRON (umol/L)	.086	290	< 0.0001	0.796	0.551
TRANSFERRIN (g/L)	.087	290	< 0.0001	0.798	0.785
UIBC (umol/L)	.083	290	< 0.0001	0.196	-0.347

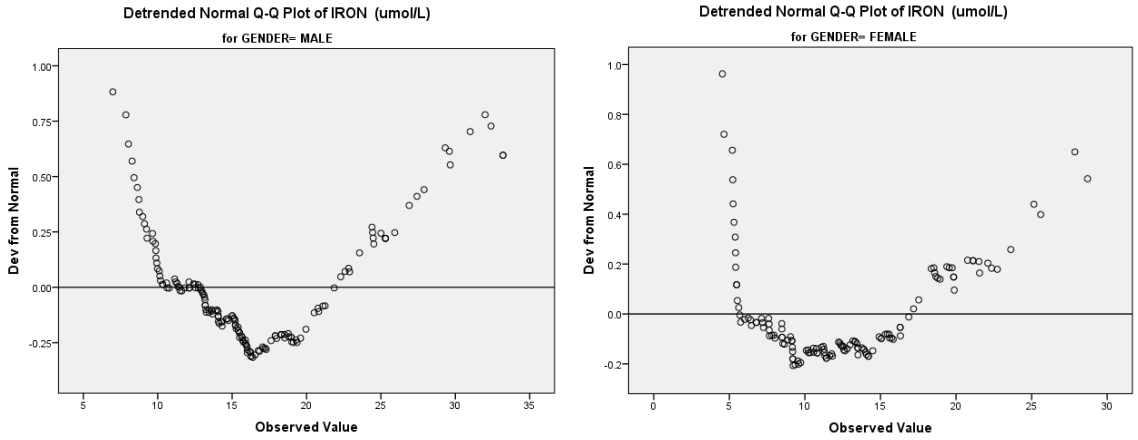
df: Degrees of freedom, **sig.:** Significance

* p-value of <0.05, (reject the null hypothesis that the data is normally distributed).

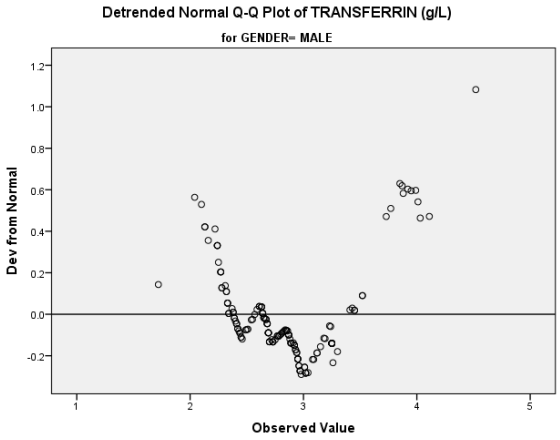
Appendix XI: Detrended normal q-q plot ferritin male & female

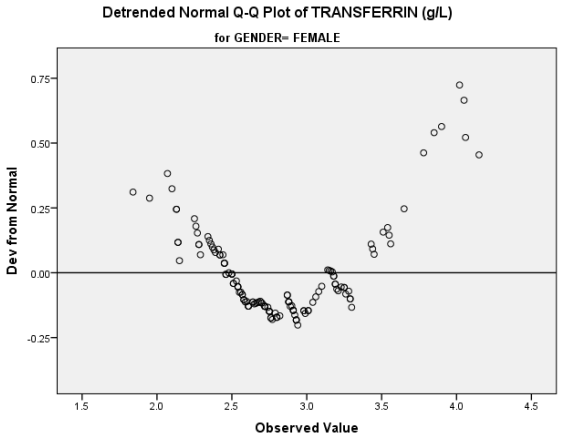


Appendix XIII: Detrended normal q-q plot iron male& female



Appendix XIV: Detrended normality q-q plot transferrin male & female





Appendix XV: Detrended normality q-q plot UIBC male & female