CORRELATION BETWEEN RETICULOCYTE HEMOGLOBIN EQUIVALENT AND SERUM MARKERS OF IRON STATUS OF END STAGE RENAL DISEASE PATIENTS ON HEMODIALYSIS IN KENYATTA NATIONAL HOSPITAL

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DECEMBER, 2023

STUDENT'S DECLARATION

I do hereby declare that this study is my original work and has not been presented for the award of any degree at any other institution or university. Where I have used another person's work, I have acknowledged and referenced.

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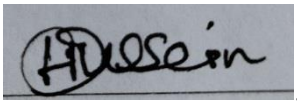
Signed: ……  …… Date: …08/12/2023……

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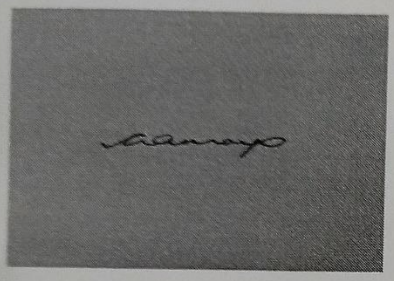

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ABBREVIATIONS AND ACRONYMS

CBC – Complete Blood Count

CKD – Chronic Kidney Disease

EDTA – Ethylene Diamine Tetra-acetic Acid

eGFR – Estimated Glomerular Filtration Rate

EPO – Erythropoietin

ESA – Erythropoiesis Stimulating Agent

ESRD – End Stage Renal Disease

Hb – Hemoglobin

HD – Hemodialysis

HIV – Human Immunodeficiency Virus

ID – Iron Deficiency

IDA – Iron Deficiency Anemia

IV – Intravenous

KDIGO – Kidney Disease Improving Global Outcomes

KNH – Kenyatta National Hospital

MCH – Mean Corpuscular Hemoglobin

MCHC – Mean Corpuscular Hemoglobin Concentration

MCV – Mean Corpuscular Volume

PCV - Packed Cell Volume

PLT - Platelet

RBC – Red Blood Cell

RDW – Red cell Distribution Width

Ret He – Reticulocyte Hemoglobin Equivalent

rHuEPO – Recombinant Human Erythropoietin

SPSS – Statistical Package for the Social Sciences

TIBC – Total Iron Binding Capacity

TSAT – Transferrin Saturation

UIBC – Unsaturated Iron Binding Capacity

WBC – White Blood Cell

DEFINITION OF TERMS

Chronic Kidney Disease (CKD) - Kidney damage or a decreased glomerular filtration rate (GFR) of less than 60 mL/min/1.73 m^2 for at least 3 months

End Stage Renal Disease (ESRD) – Kidney failure with an estimated glomerular filtration (eGFR) rate less than 15 mL per minute per 1.73 m^2 body surface area, or those requiring dialysis irrespective of glomerular filtration rate (GFR)

Hemodialysis - A medical procedure to remove fluid and waste products from the blood and to correct electrolyte imbalances. This is accomplished using a machine and a filter / dialyzer, also referred to as an "artificial kidney." Hemodialysis is used to treat both acute (temporary) and chronic (permanent) kidney failure.

Reticulocyte Hemoglobin Equivalent (Ret He) – A measure of the quantity of Hb in immature red blood cells (reticulocytes). It has been noted to be a useful indicator of iron deficiency and iron deficiency anemia.

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ABSTRACT

Background: Anemia is an almost universal complication of end stage renal disease (ESRD). Iron deficiency contributes significantly towards anemia in ESRD, having a local prevalence of 35%. Iron status is traditionally assessed by ferritin and transferrin saturation (TSAT). Studies show biological, analytical and intra-patient variability in these tests. Reticulocyte hemoglobin equivalent (Ret He), a new parameter in the complete blood count (CBC), measures hemoglobin content in reticulocytes and can be used to determine iron status in ESRD. Its measurement is cheap, fast and convenient, making its use ideal in our local setting. There is no local data to provide guidelines for its use in Kenya.

Objective: This study aims to investigate the correlation between Ret He and serum markers of iron status of ESRD patients on hemodialysis (HD) in Kenyatta National Hospital (KNH).

Methodology: This study is a cross-sectional descriptive study. Using consecutive sampling, ninety ESRD patients undergoing HD in KNH were recruited. Blood was collected for measuring CBC, Ret He, iron, ferritin and TSAT. Patient interviews and files were utilized to obtain relevant demographic and medical information.

Data analysis: Data analysis was carried out using the Statistical Package for Social Sciences. The data summary was also, reported as histograms, frequency tables, line graphs, bar graphs, pie charts, and written reports.

Significance of the study: The expected outcome is a correlation between Ret He and serum markers of iron status that can inform the use of Ret He as an alternative test for iron status assessment, thus improving anemia treatment, of ESRD patients undergoing HD.

1.0 CHAPTER ONE: INTRODUCTION

1.1 BACKGROUND

Traditionally, iron status in chronic kidney disease (CKD) has been evaluated by two serum markers, transferrin saturation (TSAT) and ferritin (1). Despite their widespread use, and despite their use being recommended in a number of guidelines, many studies have demonstrated shortcomings in these tests (2) (3) (4) (5).

Several newer markers of iron status are available, including reticulocyte hemoglobin equivalent (Ret He) and percentage hypochromic red cells (PHRC) (6). These markers have been found to be reliable, with good specificity and sensitivity, for assessing iron status in CKD (7) (8) (9).

One of the questions raised by the Kidney Disease Improving Global Outcomes (KDIGO) guideline, which guides physicians regarding iron deficiency anemia (IDA) treatment in CKD, is on the optimal laboratory tests physicians could utilize to direct their decisions on appropriate iron therapy application in CKD. The guideline has, despite its recommendation for use of TSAT and ferritin, raised concerns regarding their appropriateness and suitability in IDA treatment in CKD. It does mention that, among other iron status tests, use of Ret He and PHRC may be considered as alternatives to ferritin and TSAT (10).

Ferritin is a plasma protein which binds iron for transport in blood. Ferritin is thus an indirect measure of body iron stores. Reduced ferritin is an indicator for iron deficiency (ID). Being an acute phase protein, ferritin is raised in pro-inflammatory states, and this limits its use for iron status evaluation in CKD, as CKD is an inflammatory condition (2).

Ret He measures the quantity of hemoglobin (Hb) in reticulocytes, young erythrocytes that develop to mature red blood cells (RBCs) in 24 to 48 hours. The quantity of Hb in reticulocytes reflects the quantity of iron available to the bone marrow for Hb synthesis a few days before the production of new erythrocytes. Ret He thus reflects the amount of iron available for erythrocyte production in a clinically significant timeframe, making it an early and appropriate indicator of the body’s iron status (4).

2.0 CHAPTER TWO: LITERATURE REVIEW

2.1 ANEMIA IN CKD

Anemia, a frequent complication in all CKD stages, is an almost global complication in ESRD (11). Anemia of CKD has various etiologies, the main ones being decreased erythropoietin (EPO) production with increasing worsening of kidney function, and changes in iron metabolism resulting in a change in the body's iron status. A new occurrence or advancement of pre-existing anemia could indicate an issue resulting in loss of blood or hindering bone marrow synthesis of RBCs. The most frequent correctable etiology of long-standing or exacerbating anemia in CKD is IDA, except for anemia principally attributable to CKD (10). Reversal of anemia is known to not only reduce morbidity and mortality, but also to improve quality of patients' lives by slowing progression of CKD (12).

In a local study at KNH renal unit (2011), Gitari established an anemia prevalence of 98% amongst ESRD patients undergoing hemodialysis (HD), with a mean Hb of 7.7 ± 1.9 g/dL. Almost 35% of patients had IDA. More than a third (35%) had absolute ID, while slightly greater than 1 in 4 (26%) had functional ID. Half (50%) of all patients were on iron sucrose, while a similar number (53%) were on EPO. He thus established that, despite therapeutic measures, anemia, ID and IDA were prevalent in ESRD patients undergoing HD (13).

In monitoring of anemia in CKD patients not using an erythropoiesis stimulating agent (ESA), the KDIGO guideline recommends that patients on HD have their Hb concentration measured when clinically indicated, and at least monthly, and, an initial workup for anemia to include the following: retic count with CBC, TSAT, ferritin, folic acid and vitamin B12 (10).

2.2 CLASSIFICATION OF IRON DEFICIENCY

2.2.1 Absolute iron deficiency

This is characterized by TSAT < 20% and ferritin < 200ng/mL for CKD patients undergoing HD, and TSAT < 20% and ferritin < 100ng/mL for those not undergoing HD (1). Absolute ID is a reflection of both reduced circulating iron and depleted body iron stores in the presence of normal iron homeostasis and erythropoiesis (4). Approximately 1 in 5 CKD patients starting HD tend to have absolute ID.

2.2.2 Functional iron deficiency

This is described by TSAT < 20% and ferritin > 100ng/mL for all CKD patients (1). Functional ID is usually a result of inflammatory states in the body such as due to infection (14). Inflammation results in production of cytokines that induce iron sequestration by macrophages, reduce production of endogenous EPO and decrease responsiveness of bone marrow to EPO stimulation for RBC production (15). Hepcidin overproduction is also believed to be present in pro-inflammatory states, thus contributing to functional ID (16) (17) (18). The result is inadequate and slow release of iron from body iron stores to allow for Hb synthesis.

2.3 STAGES OF IRON DEFICIENCY

2.3.1 Early stage

Also known as the pre-latent stage, this stage marks the depletion of body iron stores. Laboratory investigations that are useful in this stage (and their expected findings) include:

- Stainable bone marrow iron (reduced or absent)
- Serum ferritin (decreased)
- Reticulocyte hemoglobin equivalent (Ret He) (reduced)

The gold standard for diagnosis of iron deficiency is stainable bone marrow iron. However, this test is highly invasive and is thus not performed routinely.

2.3.2 Intermediate stage

This stage is also known as the latent stage and is characterized by reduced circulating iron availability and erythropoiesis which is iron deficient.

Laboratory tests utilized in this stage (and their expected findings) include:

- Serum iron (reduced)
- TIBC (elevated)
- TSAT (decreased)
- Serum transferrin (elevated)
- Free erythrocyte protoporphyrin (FEP) (raised)

- Zinc protoporphyrin (ZPP) (elevated)
- Soluble transferrin receptors (sTfR) (increased)

2.3.3 Late stage

At this stage, iron deficiency anemia develops.

Laboratory investigations of importance in this stage (and their expected findings) include:

- Hemoglobin concentration (Hb) (reduced)
- Red cell indices including MCV and MCH (both are low)
- Red cell distribution width (RDW) (increased)

2.4 EVALUATION OF IRON STATUS

2.4.1 Importance of iron status evaluation in CKD

The KDIGO guideline recommends iron status evaluation once in 3 months in the course of ESA treatment. Other recommendations include testing iron status for surveilling response to a dose of intravenous (IV) iron, following a bleeding episode, while starting or raising ESA dose, and during other instances in which body iron stores could become exhausted. Evaluation of iron status will also be needed in investigating those who have reduced responsiveness to ESA therapy (10).

2.4.2 Iron status evaluation using ferritin and TSAT

The highly utilized markers in evaluating iron status are ferritin and TSAT. Whereas ferritin is used to evaluate body iron storage, TSAT is utilized to assess availability of iron for ongoing RBC production. TSAT and ferritin are utilized concurrently in diagnosing ID, evaluating iron status, and determining responsiveness to EPO administration and iron therapy.

Ferritin, an acute phase protein, is synthesized by the liver and is affected by inflammation. Therefore, it should be analyzed cautiously in CKD, particularly when occult inflammation is suspected (2). Ferritin also displays biological variability, as its levels are lower in women compared to its levels in men (6).

TSAT is less influenced by inflammation, but is not readily available and is costly for a majority of patients on HD who are already burdened with the cost of HD and medication for

the underlying cause of their CKD. TSAT is calculated from measurement of iron, which also has an inherent biological limitation of diurnal variability that can affect TSAT calculation (19).

2.4.3 Caution in interpretation of iron status test results using ferritin and TSAT

A greatly reduced ferritin (≤ 29 ng/ml) points to ID (20). Other than in this instance, ferritin and TSAT demonstrate reduced specificity and sensitivity in CKD for estimating body iron stores and determining responsiveness to EPO administration and iron therapy (21). Significant intra-patient variation independent of variations in body iron stores additionally limits their use.

In those having an inflammatory disorder, ferritin should be analyzed cautiously, as it might not accurately estimate body iron stores or determine a response to EPO administration and iron treatment comparably with when there is no inflammation. C-reactive protein (CRP) measurement could indicate a hidden inflammatory condition which could lead to a raised ferritin and poor response to ESA (22).

2.4.4 Iron status evaluation using Ret He

Ret He, introduced by Sysmex, measures Hb concentration of reticulocytes. During erythropoiesis, reticulocytes exit bone marrow and enter the bloodstream to develop into RBCs within a couple of days. As reticulocytes are actively using iron for Hb synthesis and remain in circulation for just 24 to 48 hours before maturing into RBCs, their Hb concentration closely mirrors the quantity of iron that was available to the bone marrow for erythrocyte production within a few days of its measurement (4). Ret He thus provides real time information regarding iron availability for Hb production. Thus, Ret He would be expected to fall within hours to days from the onset of the development of ID.

Studies have demonstrated greatly lower analytical and biological variations in Ret He compared to TSAT and ferritin (23). Ret He is neither affected by inflammation nor malignancy. Ret He informs on early Hb changes and could be utilized for surveilling timely Hb improvement to iron therapy and ESA administration (24).

A low Ret He can occur in other microcytic anemias such as thalassemia, while a normal Ret He may occur in IDA coexisting with megaloblastic anemia (25).

A limitation of the mainstream use of Ret He for iron status assessment is the lack of a definitive optimal cutoff value to define ID, with various studies quoting cutoff values between 25pg and 33pg. Due to rapid maturation of reticulocytes, measurement bias may occur if there is time delay in analysis after sample collection (6). This limitation can be overcome with timely analysis of the sample within 6 hours of its collection.

2.5 SENSITIVITY AND SPECIFICITY OF RET HE FOR IRON STATUS ASSESSMENT

Ucar et al (2018) in a study on 217 patients diagnosed with anemia, demonstrated that a Ret He cutoff of 25pg indicated ID with 49% specificity and 90% sensitivity in IDA. (26).

Eckhardt et al (2011) in a study on 44 CKD patients on HD and fixed-dose EPO who received 400 mg of IV iron, found Ret He at a cutoff of 29.5pg had good specificity and sensitivity (95% and 72%) for diagnosing ID (8).

Davidkova et al (2015) in a study on pediatric patients on dialysis, found that the utility of Ret He for diagnosing ID (cutoff 29pg, 75% specificity, 90% sensitivity) was good (7).

Dalimunthe et al (2016) in a study on 72 patients on HD found that Ret He is a good parameter for ID diagnosis and a salient marker for IV iron therapy response (cutoff 32pg, 62% specificity, 82% sensitivity) (27).

Wirawan et al (2017) demonstrated in a study on 106 CKD patients on HD that at a Ret He cutoff of 29pg, the specificity and sensitivity for evaluating response to iron therapy in CKD patients on HD were 94% and 95.5% (28).

Brugnara et al (2006) using 1500 blood specimens of dialysis patients, compared Ret He to conventional markers of ID (Hb <11 g/dl, ferritin <100 ng/ml, TSAT <20%, iron <40 ug/dl), and found that at a Ret He cutoff of 27pg, ID could be diagnosed with 83% specificity and 93% sensitivity (29).

Chinudomwong et al (2020) in a retrospective study of 953 blood test reports, identified that at a Ret He cutoff of 30pg, IDA can be diagnosed with a 97% specificity, 96% sensitivity, 99.6% NPV and 80% PPV (30).

Kim et al (2007) in a study on 140 HD patients on rHuEPO and IV iron treatment, determined that Ret He at a cutoff of 32pg is suitable for the evaluation of ID (84% specificity, 96% sensitivity) (31).

Miwa et al (2009) studied 217 HD patients of whom those diagnosed with ID were put on iron supplementation, and found that at a Ret He cutoff of 33pg, 65% specificity and 74% sensitivity were attained, making Ret He a dependable marker of ID (32).

Toki et al (2017) investigated 211 blood samples from patients, and found that Ret He allows an ID diagnosis with good precision, and thus Ret He could be a reliable parameter for assessing ID (cut-off 28pg, 68% sensitivity, 91% specificity) (33).

Urrechaga et al (2016) studied 40 HD patients on EPO treatment, and found that Ret He is a valid marker of iron status of HD patients (cutoff 31pg, 87% specificity, 79% sensitivity) (34).

PROBLEM STATEMENT

Anemia is a frequent complication of CKD and a nearly global complication of ESRD. Anemia is an adverse indicator of CKD progression and an independent risk factor for mortality and morbidity in ESRD. ID contributes significantly to anemia in ESRD, with a local prevalence of 35% (13), necessitating the need for regular follow-up and initiation of EPO administration and iron supplementation when required.

The iron status of patients with IDA in CKD has been traditionally assessed by two serum markers, ferritin and TSAT. Despite their widespread use, and despite their use being recommended in guidelines, studies have demonstrated biological, analytical and intra-patient variability in these tests. These biochemical tests are expensive and not always readily available, take long to perform and require taking a different blood sample than that used to diagnose anemia.

STUDY JUSTIFICATION

This study aimed at determining the utility of an alternative test, Ret He, for assessing iron status of ESRD patients undergoing HD in KNH, with a view to improving the standard of care by providing the rationale to use an affordable, readily available, quickly measured and convenient marker of iron status that can enable timely intervention of EPO administration and iron therapy to be instituted, thus improving patient outcomes.

RESEARCH QUESTION

Is there any correlation between Ret He and serum markers of iron status of ESRD patients on HD in KNH?

STUDY OBJECTIVES

Broad Objective

To investigate the correlation between Ret He and serum markers of iron status of ESRD patients on HD in KNH

Specific Objectives

i) To determine anemia prevalence, using Hb concentration, of ESRD patients on HD in KNH

ii) To assess the Ret He concentration of ESRD patients on HD in KNH

iii) To evaluate the iron status, using TSAT and ferritin, of ESRD patients on HD in KNH

iv) To correlate the Ret He with ferritin and TSAT of ESRD patients on HD in KNH

3.0 CHAPTER THREE: METHODOLOGY

3.1 STUDY DESIGN

A prospective cross-sectional descriptive study.

3.2 STUDY SITES

KNH is a leading teaching and referral hospital in the entire Eastern Africa, located in Nairobi, Kenya.

- KNH renal unit

  Its renal unit consists of 120 beds for regular HD. Patients with hepatitis B and HIV are dialyzed separately. The unit operates every day of the week.

- KNH hematology laboratory

  KNH hematology lab is equipped with the latest hematology analyzers to carry out a wide range of hematologic tests for cellular hematology as well as hemostasis and thrombosis. The laboratory utilizes the Sysmex platform. Blood transfusion tests and services are provided from a different lab.

- KNH biochemistry laboratory

  KNH biochemistry lab is well equipped to perform a wide range of biochemistry tests including those utilizing immunoassays. Its two main analyzers are the Abbott and Mindray platforms.

3.3 STUDY DURATION

July 2022 to December 2022

3.4 STUDY POPULATION

Patients from the age of 18 years with ESRD on HD for 3 months or more.

3.5 STUDY ELIGIBILITY CRITERIA

3.5.1 Inclusion criteria

- Adults from age 18 years with ESRD on HD for 3 or more months who give written informed consent

3.5.2 Exclusion criteria

- Patients with known liver disease, malignancy or hemoglobinopathies

3.6 CASE DEFINITIONS

a) ESRD

Patients with an eGFR of <15ml/min/1.73m^2

b) Iron status definitions (1)

i) Normal iron status

Ferritin ≥ 200ng/ml

TSAT ≥ 20%

ii) Functional iron deficiency

Ferritin > 100ng/ml

TSAT < 20%

iii) Absolute iron deficiency

Ferritin < 200ng/ml

TSAT < 20%

iv) Iron overload

Ferritin > 500ng/ml

TSAT > 50%

Serum iron ≥ 30 µmol/l

c) Anemia definitions (35)

i) Hemoglobin < 13.0g/dl for males

ii) Hemoglobin < 12.0g/dl for females

iii) Iron deficiency anemia

Hemoglobin < 13.0g/dl for males and < 12.0g/dl for females

Ferritin < 200ng/ml

TSAT < 20%

3.7 SAMPLE SIZE

Calculated using Fisher's formula:

$$N = \frac{Z^2 P(1-P)}{d^2} = \mathbf{350}$$

Where:

N is the required sample size

Z is the confidence interval at 95% (1.96)

P is the local prevalence of ID in ESRD, 35% (13)

d is the margin of error at 5% (0.05)

The number of patients on dialysis at KNH renal unit is a fixed (finite) population of 120 patients. Due to the limited number of dialysis machines and the time it takes to dialyze each patient (4 hours), the renal unit cannot accommodate more than 120 patients per week. Each patient requires hemodialysis twice a week, and, with their condition being end-stage, their need for dialysis is long-term / lifelong.

This population varies only slightly in a year, and usually less than 10 new patients are added per year, and only when a patient passes away or transfers to another dialysis facility, thereby maintaining a population size of 120. Thus, for a 4-month duration of data collection, it is assumed that the population will vary little, and it will not be possible to achieve a sample size of 350 during the 4 months.

Thus, adjustment of the sample size for a finite population using Cochran's formula:

$$n = \frac{n_0}{1+\frac{(n_0-1)}{N}} = \mathbf{90}$$

Where:

n_0 is the Fisher's sample size calculation (350)

N is the finite population size (120)

n is the new, adjusted sample size

3.8 SAMPLING METHOD

Patients who fit the inclusion criteria were sampled via consecutive sampling until 90 study participants are recruited.

3.9 STUDY FEASIBILITY

KNH renal unit has a fixed population of 120 dialysis patients in any given week, each of whom is dialyzed twice per week. These patients are on regular twice-weekly maintenance hemodialysis which is long-term / life-long. Some have been on dialysis for more than 1 year (13). The patient population only occasionally changes, usually due to a patient passing away or moving to another dialysis facility, at a rate of less than 2 new patients per month.

One to two study participants were recruited per day for each day of the week. At least 30 participants were recruited per month, allowing 90 participants to be recruited over at least 3 months, and at most 4 months.

3.10 SCREENING AND RECRUITMENT

3.10.1 Research assistant

A renal-trained KNH staff nurse working within the renal unit was recruited as a research assistant to the principal investigator. Since the nurse was part of trained pre-existing staff at the renal unit, she was already knowledgeable about the workings of the renal unit, and also provided familiarity to the recruited participants, as well as had authority to access participant's medical records. The assistant's roles and responsibilities were to assist the principal investigator in recruiting patients by notifying them about the study and asking them to provide written consent. Once written consent was obtained, the assistant was to fill in the questionnaire and draw blood for laboratory testing. The assistant did not play a role in any other part of the study.

3.10.2 Recruitment procedure

The principal investigator and/or study assistant were positioned in KNH renal unit's client registration station, and screened the clients' files. Clients diagnosed with ESRD and meeting the inclusion criteria were notified about the study, then asked to complete a consent form.

After completing a consent form, a unique study number were assigned and recorded, in addition to the client's hospital number and full name, so as to avoid repeat recruitment in future sessions.

3.11 STUDY VARIABLES

3.11.1 Dependent variables

- CBC parameters including total white cell count, Hb concentration and platelets
- Reticulocyte parameters including reticulocyte count and Ret He
- Serum markers of iron status including ferritin, iron, UIBC, TIBC and TSAT

3.11.2 Independent variables

- Age
- Sex
- Cause(s) of CKD

3.11.3 Qualitative variables

- Age of patients
- Sex of patients
- Cause(s) of CKD

3.11.4 Quantitative variables

- CBC parameters including total white cell count, Hb concentration and platelets
- Reticulocyte parameters including reticulocyte count and Ret He
- Serum markers of iron status including ferritin, iron, UIBC, TIBC and TSAT

3.12 CLINICAL METHODS

The principal investigator and/or study assistant, after obtaining consent, administered the study questionnaires (Appendix I: Data collection tool) in a quiet room within KNH renal unit to gather information from enrolled study participants. Demographics including sex and age were documented. Data on when HD was commenced were obtained through interview. Information on etiology of CKD were recorded from the study participant's file.

3.13 PARTICIPANT SELECTION AND RECRUITMENT

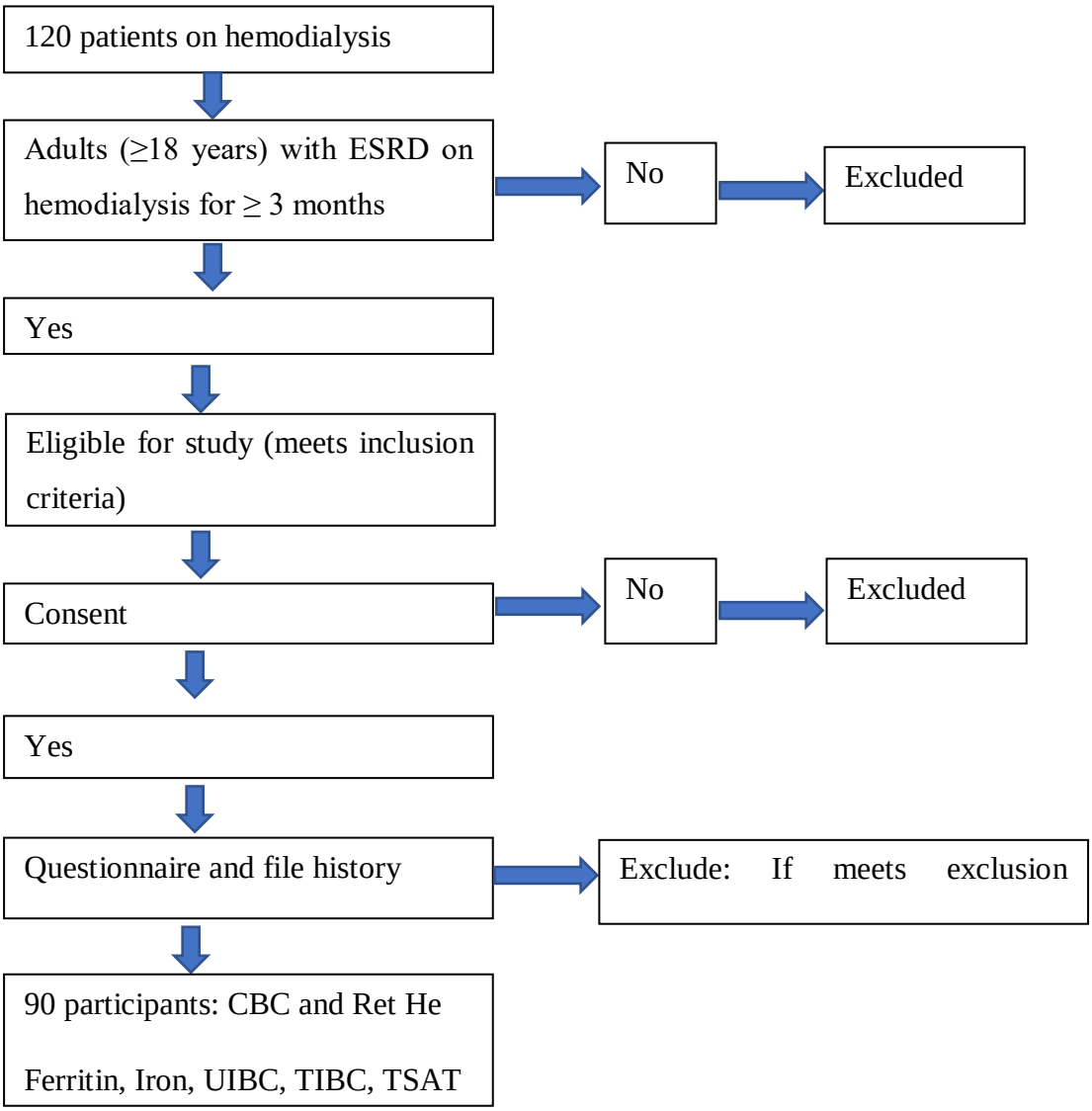

Figure 1: Flow chart depicting participant selection and recruitment.

3.14 LABORATORY METHODS

Prior to commencement of HD, 6mls of blood were removed from an antecubital vein at the study participant's bedside via the standard venipuncture method.

4mls of blood were placed in a plain sterile vacutainer for measurement of ferritin, iron and UIBC. The vacutainer were transported to KNH biochemistry laboratory for centrifugation, and serum separated into two micro vials and refrigerated to – 20^{o} centigrade.

Serum ferritin, serum iron and UIBC were measured using the Mindray BS-2000M analyzer. TIBC and TSAT were calculated using measured iron and UIBC.

TIBC = Serum iron + UIBC

TSAT = Serum iron x 100

TIBC

2mls of blood was put in an Ethylene Diamine Tetra Acetic acid (EDTA) sterile vacutainer for measurement of Ret He and CBC. The vacutainer were taken to KNH hematology laboratory for immediate analysis. Ret He and CBC were assayed with the Sysmex XN-1000 instrument.

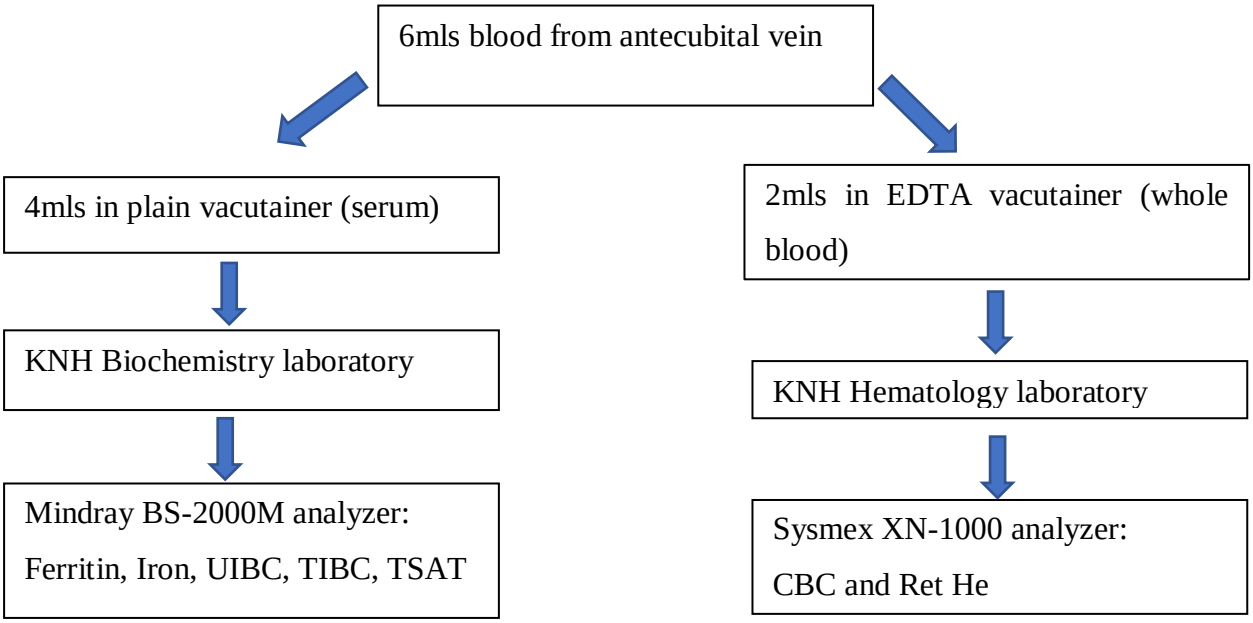

Figure 2: Flow chart depicting laboratory procedures.

3.15 MATERIALS AND REAGENTS

3.15.1 Materials

- Phlebotomy – alcohol swabs, tourniquet, 10cc syringes, needles G21 and G23, cotton swabs, micropore
- Vacutainers – red top plain, purple top EDTA
- Serum micro vials

3.15.2 Reagents

- Iron (Fe) Kit (Colorimetric Assay)
- Ferritin (FER) Kit (Particle-enhanced Immunoturbidimetric Assay Method)
- Unsaturated Iron Binding Capacity (UIBC) Kit (Colorimetric Method)

3.16 QUALITY ASSURANCE

The approved methods of blood sample collection were followed, including use of suitable vacutainers and correct venipuncture area cleansing technique.

Correct sample labeling and storage were carried out.

All laboratory tests were verified before they are performed on study participant specimens.

Standard operating procedures were complied with in every part of the study.

KNH hematology and biochemistry laboratories' internal quality control and external quality assurance procedures were carried out.

All COVID-19 prevention regulations and protocols were adhered to.

3.17 DATA MANAGEMENT

All study participants' samples and records were coded using unique identifiers to de-identify the study participants.

All study participants' hard copy questionnaires and laboratory test results were securely and safely stored in a fire-proof locked safe.

All study participants' soft copy file records were password protected and only the principal investigator and his supervisors were allowed to access them.

Two data backups were created and securely and safely stored for all hard copy and soft copy study participants' records.

3.18 DATA STATISTICAL ANALYSIS

Qualitative data (participants' age, sex, and cause(s) of CKD) was collected primarily via participant interviews, and verified via participants' medical records/files. This data was recorded in the study questionnaire. Quantitative data (participants' laboratory tests results) were obtained after laboratory analysis of participants' blood samples, and was recorded in the data collection tool (Appendix I: Data collection tool).

All sets of participants' data were entered in a predesigned Microsoft Excel spreadsheet. This data underwent statistical analysis using SPSS, following its verification and cleaning.

Quantitative (continuous) variables were reported using counts, medians, means, variances, standard deviations and ranges, while qualitative (categorical) variables were described using percentages or proportions (Section 3.22: Dummy tables).

Prevalence of anemia was calculated using frequencies.

Sensitivity and specificity of Ret He were established using TSAT and ferritin as the standard iron status indicators.

A Ret He receiver operating characteristic (ROC) curve was developed for determining the best Ret He cutoff for iron deficiency diagnosis.

Associations between study participants' Ret He, TSAT and ferritin were analyzed using Pearson correlation.

Tests of significance was done using chi square for age and sex.

Associations were considered statistically significant if p value is ≤ 0.05.

Data summary was reported as descriptive statistics and graphically displayed as frequency tables, histograms, bar graphs, line graphs and pie charts.

3.19 DATA DISSEMINATION

After analysis of the data, the findings of the study will be disseminated in seminars, conferences and medical meetings. For publishing in a peer-reviewed medical journal, a manuscript shall be developed.

3.20 ETHICAL CONSIDERATIONS

This study was carried out with permission from Department of Human Pathology, Faculty of Health Sciences as well as UoN/KNH Ethics and Research Committee (ERC).

The study adhered to the prescribed codes of conduct for researchers by the ERC.

Persons qualified to take part in this study were enrolled when they provide written informed consent as per this defined procedure:

a) They were informed the reason for this study

b) They were notified that this study involves local research

c) They were guaranteed that enrollment is not compulsory, and if they refuse to take part, medical attention was still provided

d) They were explained the study methods including laboratory tests to be performed

e) They were notified that they may exit the study at any stage without penalty

f) They were informed of the medical benefits as well as any physical and psychological harms

g) They were provided with their laboratory test reports, which was also availed to their attending physicians

h) They were informed that expenses pertaining to laboratory tests were paid for by the principal investigator

i) They were assured that study information was securely and safely stored and full confidentiality kept

Clinical procedures were carried out with adherence to COVID-19 prevention measures.

3.21 STUDY LIMITATIONS

The gold standard for evaluating iron status, bone marrow iron stain, was not performed in the study participants.

C-Reactive Protein (CRP) level was not measured to determine the presence and extent of inflammation in the study participants.

4.0 CHAPTER FOUR: RESULTS

4.1 STUDY PARTICIPANTS' DEMOGRAPHICS

The table below shows that 42 (46.7%) of the study participants were aged between 50– 69, 29 (32.2%) between 30– 49, 11 (12.2%) between 18– 29 and only 8 (8.9%) whose age was 70 and above. On the other hand, there were more males 51 (56.7%) than females 39 (43.3%) involved in the study.

Table 1: Study participants' demographics N=90

Variable	Categories	Frequency (Percent)
Age	18 – 29	11 (12.2)
	30 – 49	29 (32.2)
	50 – 69	42 (46.7)
	70 and above	8 (8.9)
Sex	Male	51 (56.7)
	Female	39 (43.3)

4.2 CAUSES OF CKD AND DURATION OF DIALYSIS IN STUDY PARTICIPANTS

The table below shows that among the causes of CKD in study participants, 53 (58.9%) had only hypertension, 34 (37.8%) had both diabetes mellitus and hypertension, and only 2 (2.2%) who had only diabetes mellitus, while 1 (1.1%) was found to suffer from chronic glomerulonephritis.

Table 2: Cause(s) of CKD and duration of hemodialysis N=90

Variable	Categories	Frequency (Percent)
Cause(s) of CKD	Hypertension	53 (58.9)
	Diabetes Mellitus	2 (2.2)
	HTN + DM	34 (37.8)
	Urinary tract obstruction	0

	Vesicoureteral reflux Chronic glomerulonephritis Nephrotic syndrome Autoimmune diseases Congenital abnormalities Other _____	0 1 (1.1) 0 0 0
Duration of dialysis	< 6 months 6 – 12 months 13 – 24 months > 24 months	46 (51.2) 22 (24.4) 13 (14.4) 9 (10)

A simple majority of study participants 46 (51.2%) had been on hemodialysis for less than 6 months, while only 9 (10%) had been on hemodialysis for longer than 2 years.

4.3 LABORATORY PARAMETERS FOR STUDY SUBJECTS

The table below shows the summary of the hematological and biochemical laboratory parameters for the study subjects. The hemoglobin mean level was 9.83 g/dl (+/- 1.91 g/dl SD) ranging from 6.2 to 13.7 g/dl. The Ret He mean was 31.74% (+/- 3.95% SD) ranging from 22.4% to 39.3%. From the iron status parameters, ferritin had a mean of 525.04 ng/ml (+/- 411.51 ng/ml SD) with a range of 11 to 1300 ng/ml, while TSAT had a mean of 30.08% (+/-17.92% SD), ranging from 6.47% to 92.58%.

Table 3: Summary of laboratory parameters N=90

VARIABLE	MEAN +/- SD	MINIMUM	MAXIMUM
Total WBC	6.78 +/- 1.99	3.57	13.48
RBC	3.59 +/- 0.68	2.05	5.21
Hemoglobin	9.83 +/- 1.91	6.2	13.7
PCV	29.48 +/- 6.01	17.1	41.6

MCV	83.73 +/- 6.60	63.0	96.0
MCH	28.27 +/- 2.28	21.2	32.3
MCHC	33.38 +/- 1.21	30.6	36.5
RDW	15.20 +/- 1.74	12.8	20.6
PLT	289.18 +/- 92.29	110	833
Ret He	31.74 +/- 3.95	22.4	39.3
Retic count	1.72 +/- 0.80	0.63	4.84
Serum ferritin	525.04 +/- 411.51	11	1300
Serum iron	10.32 +/- 5.91	2.2	38.3
UIBC	27.74 +/- 13.19	2.5	59.8
TIBC	38.06 +/-11.57	6.6	71.5
TSAT	30.08 +/-17.92	6.47	92.58

4.4 COMPLETE BLOOD COUNT AND RETICULOCYTE PARAMETERS

The table below shows the distribution of the hematological parameters of the respondents. A majority of patients (80, 88.9%) had low hemoglobin levels.

Table 4: Distribution of complete blood count and reticulocyte parameters N=90

Variable	Low: Number (%)	Normal: Number (%)	High: Number (%)
Total WBC	5 (5.6)	82 (91.1)	3 (3.3)
RBC	64 (71.1)	26 (28.9)	0
Hemoglobin	80 (88.9)	10 (11.1)	0
RDW	0	67 (74.4)	23 (25.6)
PLT	1 (1.1)	87 (96.7)	2 (2.2)
Retic count	0	78 (86.7)	12 (13.3)

4.5 SERUM MARKERS OF IRON STATUS

The table below shows the distribution of the biochemical parameters of the respondents.

Table 5: Distribution of serum markers of iron status N=90

VARIABLE	Low: Number (%)	Normal: Number (%)	High: Number (%)
Serum ferritin	8 (8.9)	23 (25.5)	59 (65.6)
Serum iron	33 (36.7)	55 (61.1)	2 (2.2)
UIBC	30 (33.3)	60 (66.7)	0
TIBC	61 (67.8)	29 (32.2)	0
TSAT	31 (34.4)	47 (52.3)	12 (13.3)

4.6 PREVALENCE OF ANAEMIA USING HAEMOGLOBIN LEVELS

The table below shows the proportion of patients who had anemia with hemoglobin cut offs based on sex. Using these parameters, 80 (88.8%) participants were anemic. Of these, 45 (50%) were moderately anemic, while 4 (4.4%) had severe anemia. Only 10 participants (11.2%) had a normal hemoglobin level.

Table 6: Prevalence of anemia using hemoglobin N=90

Variable		Category	Frequency (Percent)
Hemoglobin	Normal	(≥ 12 F; ≥ 13 M)	10 (11.2)
	Mild	10 – < 12 F; or < 13 M	31 (34.4)
	Moderate	7 – < 10	45 (50)
	Severe	< 7	4 (4.4)

As shown in the figure below, the study participants observed haemoglobin level distribution with a number of points, one at hemoglobin of 8.0 – 8.5g/dl, the other at 10.0 – 10.5g/dl and another at 12.5 – 13.0g/dl.

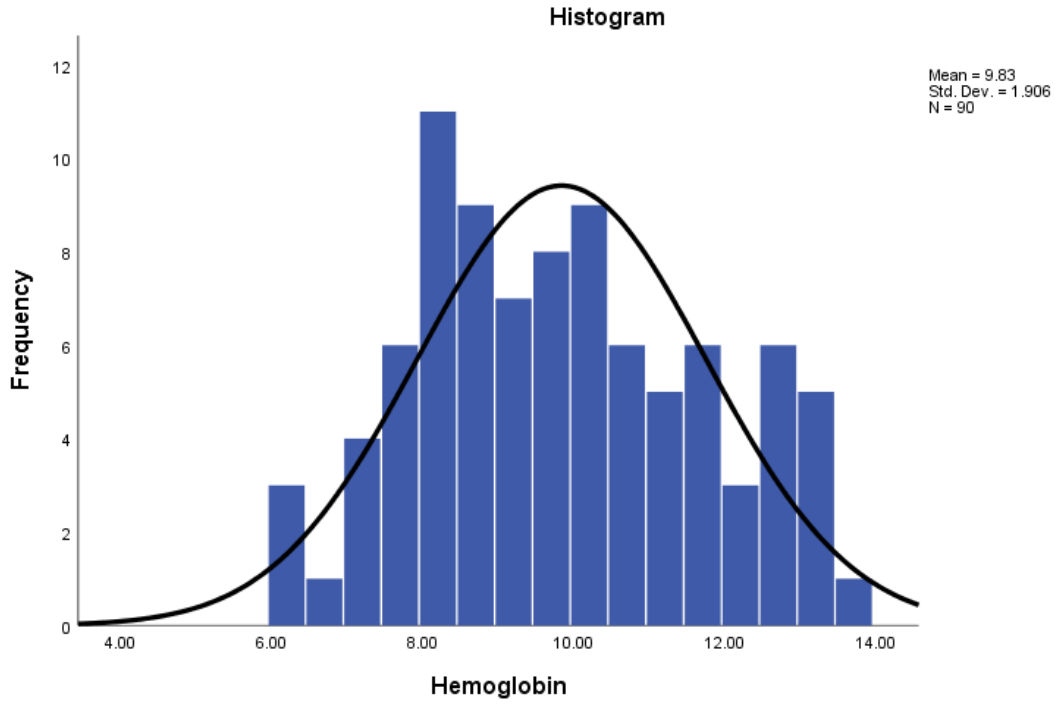

Figure 3: Hemoglobin Distribution

4.7 EVALUATION OF IRON STATUS USING TSAT AND FERRITIN

The evaluation of iron status was done and analysis revealed that 18 (20.0%) of study participants had absolute iron deficiency (Ferritin < 200ng/ml & TSAT < 20%) while 13 (14.4%) had functional iron deficiency (Ferritin > 100ng/ml & TSAT < 20%). 47 (52.2%) had normal iron status (Ferritin ≥ 200ng/ml & TSAT ≥ 20%), while 12 (13.3%) had iron overload (Ferritin > 500ng/ml, TSAT > 50% & Serum iron ≥ 30 µmol/l).

Table 7: Distribution of iron status N=90

STATUS	N =	Percentage %
Normal	47	52.3
Iron overload	12	13.3
Absolute iron deficiency	18	20.0
Functional iron deficiency	13	14.4

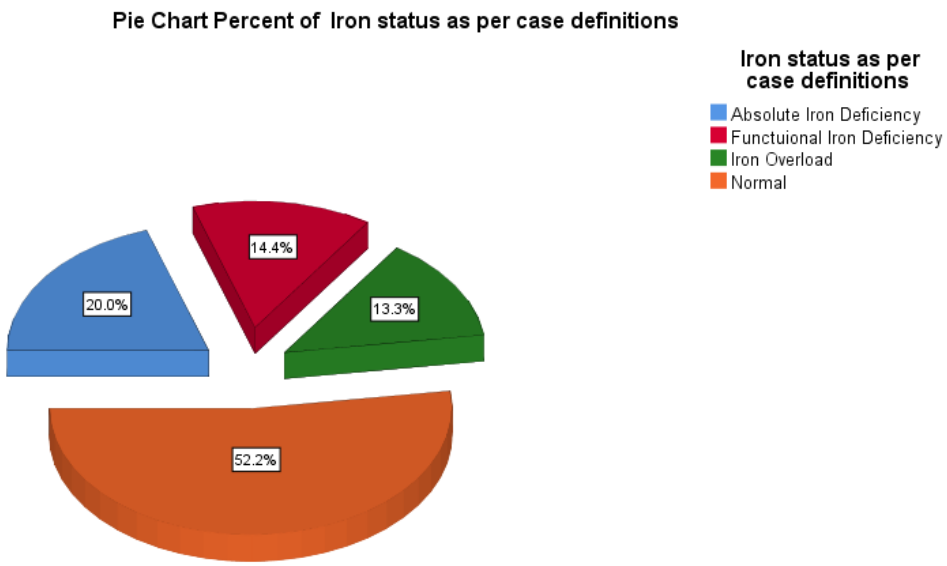

Figure 4: Iron Status as per case definitions

4.8 RETICULOCYTE HEMOGLOBIN EQUIVALENT (RET HE) CONCENTRATION ASSESSMENT

The table below shows that 64 (71.1%) of study participants had their reticulocyte hemoglobin equivalent above 30%, while only 8 (8.9%) had their Ret HE below 25%.

Table 8: Distribution of Ret HE N=90

Variable	Category	Frequency (Percent)
Ret HE	20 – < 25	8 (8.9)
	25 – < 30	18 (20.0)
	30 – < 35	46 (51.1)
	35 – < 40	18 (20.0)

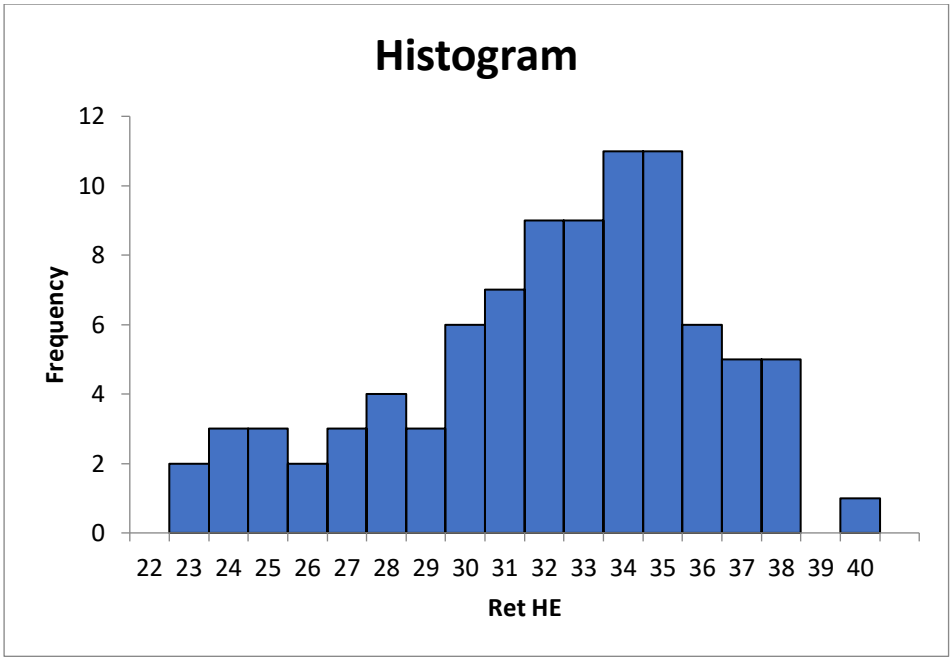

Figure 5: Reticulocyte hemoglobin equivalent (RET HE) distribution

4.9 CORRELATION OF RET HE AND SERUM IRON STATUS MARKERS

The results from the table below shows that there is a moderate positive correlation between Ret HE and serum ferritin at .492. Also, there is a moderate positive correlation between Ret HE and TSAT at .467. On the other hand, the correlation is statistically significant at the 0.01 level with a p value (.000).

Table 9: Correlation of Ret HE and serum iron status markers N=90

Correlations				
		Ret HE	Serum Ferritin	TSAT (calculated)
Ret HE	Pearson Correlation	1	.492	.467
	Sig. (2-tailed)		.000	.000
	N	90	90	90
Serum Ferritin	Pearson Correlation	.492	1	.648
	Sig. (2-tailed)	.000		.000
	N	90	90	90
TSAT	Pearson	.467	.648	1

(calculated)	Correlation			
	Sig. (2-tailed)	.000	.000	
	N	90	90	90

4.10 CUT-OFF LEVEL FOR DIAGNOSIS OF IRON DEFICIENCY USING RET HE

Receiver operating characteristic (ROC) analysis was carried out to evaluate the ability to detect iron deficiency using Ret-HE value, according to the Cut-off level for diagnosis of iron deficiency data from these cases were analyzed to determine the cutoff level of Ret-HE

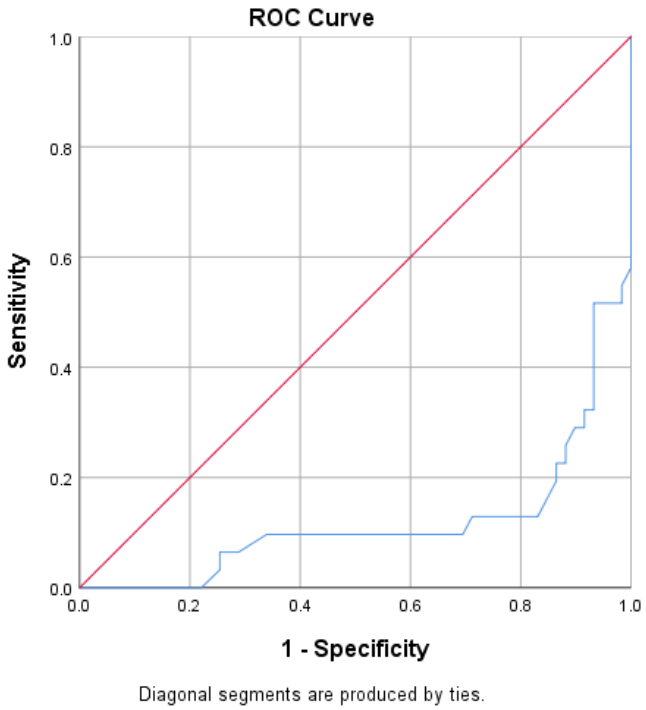

Figure 6: Receiver Operating Characteristic (ROC) curve

Table 10: Area under the curve

Test Result Variable(s): RET HE

			Asymptotic 95% Confidence Interval	
Area	Std. Error[a]	Asymptotic Sig.[b]	Lower Bound	Upper Bound
.119	.042	.000	.036	.201

The test result variable(s): RET HE has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

The analysis is statistically significant at p value .000 which is (< .05).

Table 11: Coordinates of the curve

Test Result Variable(s): RET HE

Positive if Greater Than or Equal To[a]	Sensitivity	1 - Specificity
21.400	1.000	1.000
22.600	.968	1.000
22.950	.935	1.000
23.500	.903	1.000
24.150	.839	1.000
24.500	.806	1.000
24.650	.774	1.000
25.000	.742	1.000
25.550	.710	1.000
25.950	.677	1.000
26.150	.645	1.000
26.500	.613	1.000
26.950	.581	1.000
27.200	.548	.983
27.650	.516	.983
28.350	.516	.966
28.750	.516	.949
28.850	.516	.932
29.050	.484	.932
29.250	.419	.932
29.400	.387	.932
29.550	.355	.932

29.750	.323	.932
30.050	.323	.915
30.350	.290	.915
30.550	.290	.898
30.650	.258	.881
30.750	.226	.881
30.850	.226	.864
31.000	.194	.864
31.200	.161	.847
31.350	.129	.831
31.450	.129	.814
31.650	.129	.797
31.850	.129	.763
32.000	.129	.746
32.200	.129	.729
32.500	.129	.712
32.800	.097	.695
32.950	.097	.644
33.050	.097	.610
33.150	.097	.576
33.300	.097	.542
33.500	.097	.508
33.750	.097	.475
33.950	.097	.441
34.150	.097	.424
34.350	.097	.390
34.450	.097	.373
34.550	.097	.356

34.700	.097	.339
34.850	.065	.288
34.950	.065	.271
35.050	.065	.254
35.250	.032	.254
35.450	.000	.220
35.700	.000	.203
36.150	.000	.186
36.450	.000	.169
36.550	.000	.136
36.650	.000	.119
36.950	.000	.102
37.300	.000	.085
37.450	.000	.068
37.600	.000	.051
37.850	.000	.034
38.650	.000	.017
40.300	.000	.000

The test result variable(s): RET HE has at least one tie between the positive actual state group and the negative actual state group.

When the cutoff level of Ret-HE was set at a higher sensitivity and specificity, the maximal cutoff level was 28.9, with sensitivity 51.6% and specificity 93.2%.

5.0 CHAPTER FIVE: DISCUSSION

This study set out to correlate serum iron status parameters (ferritin and TSAT) with Ret HE in patients with ESRD on hemodialysis. There were 90 study participants involved. This is the first study in the region to investigate the correlation of Ret HE with traditional serum iron status markers (ferritin and TSAT) and to attempt to establish a Ret HE cut off that can be used to diagnose iron deficiency as both an early and relatively inexpensive marker.

The study findings showed there was a higher proportion of males involved in the study where 51 (56.7%) were males and females were 39 (43.3%). This is comparable to Gitari's findings with a sample size of 165, with males being at 60.6%, and females at 39.4% (2). Similarly, Maina in her study at KNH on prevalence and correlates of anemia in CKD also had a higher proportion of males at 56.6%. It has also been documented that more men than women receive kidney replacement therapy, potentially related to underlying biology and faster progression of CKD in men, hence more males in ESRD requiring hemodialysis (4).

The study also observed that 50 (55.6%) of the study participants were aged above 50 years, whereas 40 (44.4%) were below 50 years of age, with a mean age of 50.3 years and a range of 18 – 76 years. This is comparable to Gitari's findings where 59.4% of respondents were above the age of 50, with a mean age of 45 years and a range of 14 – 80 years (2). This reflects the pathogenesis of CKD as a chronic process that takes several years to develop from the time of diagnosis of the underlying cause, such as diabetes mellitus or hypertension, to the eventual progression to chronic renal failure, thus presenting more in the middle aged and elderly population.

Amongst the causes of CKD, almost all study participants (89, 98.9%) had either hypertension or diabetes mellitus or both as the underlying cause of their CKD. Only 1 (1.1%) study participant was found to suffer from chronic glomerulonephritis. This finding reflects the high burden of lifestyle diseases not only to the causation of chronic kidney disease but also to the need for dialysis services in our region.

With regards to the duration of dialysis, 46 (51.2%) of study participants had been on dialysis for less than 6 months, while only 9 (10%) had been on dialysis for longer than 24 months. Gitari had the same finding, where a majority (43%) of respondents had been on dialysis for less than 6 months. This could reflect either mortality related to ESRD or its complications, or it could also be as a result of patients transferring to other dialysis facilities for continuation of maintenance dialysis.

The mean hemoglobin level was 9.83g/dl (+/- 1.91 SD) ranging from 6.2 to 13.7 g/dl. Gitari found a mean hemoglobin level of 7.9 g/dl (+/- 1.9 SD) with a range of 3.8 to 13.2g/dl (2). The higher mean hemoglobin and narrower range found in this study could reflect increased treatment measures instituted for the correction of previously diagnosed anemia. Anemia (Hb <13g/dl for males, <12g/dl for females) was observed in 80 (88.9%) of participants, which is comparable to Gitari's findings of a prevalence of anemia of 98.2% in his study population. The high prevalence of anemia is in keeping with other global studies that have demonstrated almost universal occurrence of anemia in patients in ESRD. Among the anemic patients, those with moderate anemia were 45 (50%) while those with mild anemia were 31 (34.4%). Only 4 (4.4%) of study participants had severe anemia. This could reflect stronger interventions taken in anemic patients to reverse anemia so as to avert complications related to severe anemia such as heart failure and increased mortality.

Absolute iron deficiency was found in 18 (20.0%) of the study subjects, while 13 (14.4%) had functional iron deficiency. Of the 90 study participants, 47 (52.2%) had normal iron status. Only 12 (13.3%) had iron overload. Gitari found normal iron status in 36.4% of his study participants, while only 2.4% had iron overload. The higher prevalence of iron overload in this study (13.3%) could reflect more aggressive measures for correcting iron deficiency or a possibility of anemic patients being on iron supplements despite not suffering from iron deficiency. Iron deficiency anemia was found in 31 (34.4%) of the study participants, which is the same finding as Gitari (34.5%) in his study. This underscores the high occurrence of iron deficiency and its contribution to anemia in the setting of CKD, and highlights the need for correctly identifying iron deficient patients and instituting appropriate interventions to avert IDA associated morbidity and mortality.

Correlation of reticulocyte hemoglobin equivalent and serum iron status markers demonstrated a moderate positive correlation, which was also statistically significant. Also, the performance of Ret HE, when compared to traditional parameters for iron deficiency anemia diagnosis is observed to be excellent (7).

With 52% sensitivity and 93% specificity, the cutoff value of Ret HE for the diagnosis of iron deficiency in ESRD patients on HD at KNH renal unit was found to be 28.9pg. The analysis was also statistically significant. Several studies done worldwide have shown a similar cut-off, with values ranging from 27pg to 32pg. Wirawan et al (2017) found that at a cutoff of 29pg, with 94% specificity and 95.5% sensitivity, Ret HE was suitable for evaluating IDA in

CKD patients on HD (28). Similarly, Toki et al (2017) demonstrated that Ret HE could be used to diagnose ID with 68% sensitivity and 91% specificity, at a cut off of 28pg. A meta-analysis done by Kilic et al (2022) on the effect of Ret HE on the diagnosis of IDA showed that various cutoff values of Ret HE were obtained ranging from 28 to 29pg, with the mean cut off value being 28.2pg.

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

This study arrived at various conclusions as outlined below:

1. The prevalence of anemia amongst HD patients with ESRD was 88.9%, with a majority having a moderate degree of anemia.

2. The Ret He concentration of ESRD patients on HD in KNH had a mean of 32.05pg.

3. The proportion of iron deficiency contributing to anemia in ESRD patients on HD remains unchanged since a previous study. Iron overload was found in 13.3% of ESRD patients undergoing HD.

4. There was positive correlation between Ret He with ferritin and TSAT of ESRD patients on HD in KNH. Reticulocyte hemoglobin equivalent may therefore be considered a useful indicator of iron deficiency in HD patients with ESRD. At a cut off of 28.9pg, Ret HE had a sensitivity of 52% and specificity of 93% . This cut-off may therefore be used to determine the presence of iron deficiency in ESRD patients undergoing HD in our setting.

6.1.1 Limitations of the study

1. Iron deficiency was defined using serum ferritin, which is subject to large biological variability. However, it is the most common method still used to determine iron status.

2. This was a single centre study with a relatively small sample size of 90 patients which may limit the generalizability of the study findings.

6.2 RECOMMENDATIONS

1. Ret HE should be used as an affordable marker of iron status amongst HD patient with ESRD. Prevalence of anemia and iron deficiency remains high in this population, thus there is a need assess their hemoglobin levels regularly. Ret He can provide information on iron status at the same time.

2. Obtain more data to validate the cut-off value of Ret He to identify iron deficiency

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APPENDICES

CORRELATION BETWEEN RET HE AND SERUM FERRITIN / TSAT OF ESRD PATIENTS ON HD IN KNH

Appendix I: Data collection tool

A. DEMOGRAPHIC DATA

1. Date _____
2. Study number _____
3. Hospital number _____
4. Name _____
5. Age _____
6. Sex Male / Female

B. MEDICAL DATA

1. Cause(s) of chronic kidney disease _____

   _____

   _____

2. Duration of hemodialysis < 3 months / ≥ 3 months
3. Other medical conditions:
   a) Hemoglobinopathy _____
   b) Malignancy _____
   c) Liver disease _____
   d) Infection _____

C. LABORATORY RESULTS

CBC – PARAMETER	VALUE
Total WBC	
RBC	
Hemoglobin	
PCV	
MCV	
MCH	
MCHC	
RDW	
PLT	
Reticulocyte count	
Ret He	

SERUM – PARAMETER	VALUE
Serum ferritin	
Serum iron	
UIBC	
TIBC (calculated)	
TSAT (calculated)	
Iron status as per case definitions	

Appendix II: Informed consent English version

PARTICIPANT INFORMATION AND CONSENT FORM FOR ENROLLMENT IN THE STUDY

This Informed Consent form is for adult patients with end stage renal disease undergoing hemodialysis at KNH renal unit. It were administered to eligible patients. We are requesting you to participate in this research whose title is "CORRELATION BETWEEN RETICULOCYTE HEMOGLOBIN EQUIVALENT AND SERUM MARKERS OF IRON STATUS OF END STAGE RENAL DISEASE PATIENTS ON HEMODIALYSIS IN KENYATTA NATIONAL HOSPITAL"

Principal Investigator: Dr Husein Janoowalla

Institution: Department of Human Pathology, Faculty of Health Sciences, University of Nairobi.

Contact: 0721233767

This Informed Consent Form has three parts:

a) Information Sheet (informs you in a brief overview about the research with you).

b) Certificate of Consent (for you to sign if you agree to take part).

c) Statement by the researcher/person taking consent.

A copy of the informed consent form were provided to you.

Part I: Information Sheet

Introduction

My name is Dr Husein Janoowalla, a postgraduate student in the Department of Human Pathology, Faculty of Health Sciences, University of Nairobi. I am carrying out research that involves testing your blood to tell us if you have low hemoglobin (anemia), and if you do, if

it is due to lack of iron in your body. The research will compare one blood test (reticulocyte hemoglobin equivalent) with other blood tests to determine the relationship of the first test with the other tests and to investigate if it is sufficient enough to tell us that the cause of your anemia is due to lack of iron in your body.

Purpose of the Research

I will provide you with information about the study and invite you to be a participant in this study. There may be some words that you don't comprehend. Please ask me to explain as we go through the information, and I will explain. After receiving the information concerning the study, you are encouraged to seek clarification in case of any doubt.

Type of Research Intervention

This research will involve the use of questionnaires and patient files with your doctor's permission. You were consenting for blood collection via a needle inserted into a vein in your right/left arm and the pReceiver operating characteristic (ROC)edure will take up to 5 minutes. No major risk is associated with the pReceiver operating characteristic (ROC)edure; in case of any discomfort the needle were removed, and we will keep you comfortable. Laboratory tests were done at Kenyatta National Hospital at my expense.

Voluntary participation/right to refuse or withdraw

You are free to participate or not. Whether you choose to participate or not, all the services you receive at this hospital will continue and nothing will change. If you decide against participating, you were offered the treatment that is routinely provided in this hospital for your condition. You have a choice to refuse or withdraw your participation at any point in the study.

Confidentiality

The information obtained in this study were treated confidentially and only be available to the principal investigator and the study team. Your name will not be used. Any personal information will have a number on it instead of your name. We will not be sharing the identity of those participating in this research. Your laboratory test results were relayed to your doctor for your further treatment.

Study Procedure

After agreeing and consenting to participate in the study, 6ml of blood were collected via a needle inserted into a vein in your right/left arm and the pReceiver operating characteristic (ROC)edure will take up to 5 minutes. No major risk is associated with the pReceiver operating characteristic (ROC)edure; in case of any discomfort the needle were removed, and we will keep you comfortable. I will ask you a few questions and also take some information from your file including your age, gender, cause(s) of your chronic kidney disease and any other relevant medical condition you may be having.

Sharing the Results

The knowledge obtained from this study were shared with the policymakers in KNH and doctors through publications and conferences. Confidential information will not be shared.

Your laboratory test results were channeled to your attending doctor.

Benefits

The benefits of joining the study include:

- Contribution to the advancement of the treatment of other patients with the same condition as yours
- Free full blood count and serum markers of iron status whose results were used for your treatment

Risks

There were no major risk involved in enlisting for this study. Minimal pain may be experienced during the sample collection pReceiver operating characteristic (ROC)ess. There may be some bleeding and formation of a swelling at the site of needle insertion. Care were taken to ensure this does not happen, but if it does, then medical attention were provided.

Cost and Compensation

There were no cost incurred for participating in this study, nor is there any compensation offered.

This research has been reviewed and approved by the UoN/KNH Ethics and Research Committee, which is a committee whose task is to make sure that research participants are protected from harm.

Who to contact?

If you wish to ask any questions later, you may contact:

Principal Investigator:

Dr Husein Janoowalla

Department of Human Pathology,

Faculty of Health Sciences,

University of Nairobi.

0721233767

University of Nairobi Supervisors:

Prof. Angela Amayo

Associate Professor, Clinical Chemistry Thematic Unit

Department of Human Pathology

Faculty of Health Sciences

University of Nairobi

Prof. Jessie Githanga

Associate Professor, Hematology and Blood Transfusion Unit

Department of Human Pathology

Faculty of Health Sciences

University of Nairobi

Secretary, KNH/UoN Ethics and Review Committee:

Contact telephone numbers 2726300 ext. 44102, email: uonknh_erc@uonbi.ac.ke

Part II: Certificate of Consent

I have read and understood the above information/the above information has been read out and explained to me. I have had the opportunity to ask questions and the questions that I have asked have been answered satisfactorily. I voluntarily agree and consent to participate in this research.

Name of participant _____

Signature of participant _____

Date _____

If non-literate:

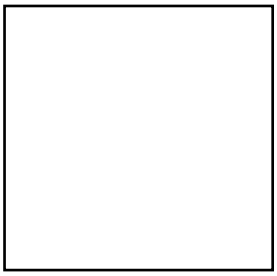
I have witnessed the reading and explanation of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I can confirm that the individual has given consent voluntarily.

Name of witness_____

Signature of witness _____

Date _____

Thumb print of participant



Part III: Statement by the Researcher

I have read out the consent form to the participant and made sure that the participant understands the following:

A decision to refuse to participate or withdraw from the study will not in any way compromise the care of treatment. All information given were handled with confidentiality.

The results of this study may be published to facilitate research and improve clinical guidelines. I can confirm that the participant is allowed to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the approval has been given voluntarily.

A copy of the Informed Consent Form has been provided to the participant.

Name of researcher/person taking consent _____

Signature of researcher/person taking consent_____

Date_____

Appendix III: Informed consent Swahili version

Fomu ya Makubaliano ya Kujiunga na Utafiti

Fomu ya makubaliano

Nimeelezwa utafiti huu kwa kina. Nakubali kushiriki utafiti huu kwa hiari yangu. Nimepata wakati wa kuuliza maswali na nimeelewa kuwa iwapo nina maswali zaidi, ninaweza kumwuliza mtafiti mkuu au watafiti waliotajwa hapa juu.

Jina la mshiriki_____

Sahihi ya mshiriki _____

Tarehe_____

Kwa wasioweza kusoma na kuandika

Nimeshuhudia usomaji na maelezo ya utafiti huu kwa mshiriki. Mshiriki amepewa nafasi ya kuuliza maswali. Nathibitisha kuwa mshiriki alipea ruhusa ya kushiriki bila ya kulazimishwa.

Jina la shahidi_____ Alama ya kidole cha mshiriki

Sahihi la shahidi_____

Tarehe _____

Ujumbe kutoka kwa mtafiti

Nimemsomea mshiriki ujumbe kiwango ninavyoweza na kuhakikisha kuwa mshiriki amefahamu yafuatayo:

Kutoshiriki au kujitoa kwenye utafiti huu hautadhuru kupata kwake kwa matibabu. Ujumbe kuhusu majibu yake yatahifadhiwa kwa siri.

Matokeo ya utafiti huu yanaweza chapishwa ili kuwezesha kuzuia na kutibu upungufu wa damu kwa wagonjwa wenye ugonjwa sugu wa figo.

Ninathibitisha kuwa mshiriki alipewa nafasi ya kuuliza maswali na yote yalijibiwa vilivyo.

Ninahakikisha kuwa mshiriki alitoa ruhusa bila ya kulazimishwa.

Mshiriki amepewa nakala ya hii fomu ya makubaliano.

Jina la mtafiti _____

Sahihi ya mtafiti _____

Tarehe_____