PREVALENCE AND PROFILES OF RHEUMATOID ARTHRITIS BIOMARKERS AMONG TYPE II DIABETES MELLITUS PATIENTS AT MOI TEACHING AND REFERRAL HOSPITAL, ELDORET.

By

DORCUS A. ADHIAMBO

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN MEDICAL BIOCHEMISTRY, DEPARTMENT OF BIOCHEMISTRY AND CLINICAL CHEMISTRY, SOM, CHS, MOI UNIVERSITY.

DECLARATION

Declaration by the Candidate:

This thesis is my original work to the best of my knowledge and has not been presented for a degree in any other university or institution. No part of this thesis may be reproduced without the prior written permission of the author and/or Moi University.

Dorcus A. Adhiambo..... Date.....

(CANDIDATE)

Declaration by Supervisors

This thesis has been submitted for examination with approval of University supervisors**.**

Dr. Caleb I. Nyamwange ……Date……..

Department of Biochemistry and Clinical Chemistry,

SOM, CHS, Moi University, Eldoret, Kenya

Prof. Hezron S. Nyandieka……Date ……..

Department of Biochemistry and Clinical chemistry,

SOM, CHS, Moi University, Eldoret, Kenya.

DEDICATION

I dedicate the accomplishment of this thesis to my lovely family who has been of great support during the entire period. Above all I thank the almighty God for his providence.

ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic, progressive autoimmune disease of unknown cause. It is characterized by persistent inflammation that primarily affects the peripheral joints. It usually starts as an insidious symmetrical arthritis and has an unpredictable and variable course, although pain and disability can be minimized if the condition is recognized early and treated promptly and appropriately. In Kenya, there are minimum data regarding the prevalence of RA biomarkers in type II diabetic patients as a focus to reduce Non–communicable disease related morbidity and mortality.

Objective: To determine the prevalence and profiles of early RA biomarkers among type II diabetes mellitus patients attending Clinic at Moi Teaching and Referral Hospital (MTRH).

Methods: This was a quantitative cross sectional study conducted in Diabetic Clinic Chandaria MTRH for four months from May to August 2018. One hundred and eighty (180) parsons aged 18 years and above were recruited at the phlebotomy section to participate in the study using convenient sampling technique. A structured questionnaire was used to collect socio-demographic data. Venous blood samples were obtained for the measurement of Rheumatoid Factor, Anti- Cyclic Citrullinated proteins and Tenascin-C in the Laboratory using agglutination tests and Enzyme Linked Immunosorbent Assays (ELISA). Data was analyzed using EPI INFO for measures of central tendencies mean, median, standard deviation. Measures of associations Odds ratio, cross tabulation and logistic regression outcome were calculated at 95% confidence interval to correlate relationship of type II diabetes and Rheumatoid Arthritis.

Results: The mean age of the participants' was 55.2 years (SD 15.4) and the modal age was 58 years with the age range between 22 and 89 years. Females comprised of 67% of the participants. The mean years the participants have lived with T2DM was 8.4 (SD 6.2). Prevalence of biomarkers (RF, Anti-CCP and TNC) among T2DM was 10%, 17.8% and 96.7% respectively. Among the participants 7 (3.9%) had elevated early biomarker hence a prevalence of 3.9% of RA among people living with T2DM. The mean elevated serum level of Anti–CCP was 21.9 U/ml with females (11.7%) males (6.1%). Comparing positivity for RA, Anti-CCP/RF-ve 76.1 % (137), Anti-CCP/ RF +ve 3.9 %(7), Anti-CCP +ve/ RF-ve 13.9% (25) and Anti-CCP-ve/ RF +ve 6.1 %(11). Likely hood of RF negative to be Anti-CCP positive OR=3.5(P=0.0184).Majority had elevated TNC (96/7%) with mean of 577pg/ml.

Conclusion: The proportion of participants living with T2DM and RF antibodies was 10%. Prevalence of RA among type II diabetes patients was 3.9% with higher occurrence at the age of 71-.80 years. Females were more likely to have RA antibodies than males. The proportions with elevated Anti- CCP biomarker were 17.8% hence more specific to RA. Majority had elevated levels of antibodies to Tenascin C a pro-inflammatory marker hence not associated to Rheumatoid Arthritis.

Recommendation: Routine screening for early biomarkers of RA in People living with type II diabetes

Glossary; *Rheumatoid Arthritis, Rheumatoid factor, Biomarkers, Screening, Phlebotomy***.**

TABLE OF CONTENTS

DECLARATION---------- ii

DEDICATION---------- iii

ABSTRACT----------iv

TABLE OF CONTENTS----------v

LIST OF TABLES----------viii

LIST OF FIGURES----------ix

ACKNOWLEDGMENTS----------x

ABBREVIATIONS----------xi

DEFINITION OF TERMS ----------xiii

CHAPTER ONE ----------1

1.0 INTRODUCTION ----------1

1.1 Background Information----------1

1.1.1 Diagnosis of Rheumatoid Arthritis----------2

1.1.2 Diabetes and rheumatoid arthritis----------4

1.2 Problem Statement ----------9

1.3 Justification----------10

1.4 Research Questions: ----------10

1.5 Objectives----------11

1.5.1 Broad Objective of the study ----------11

1.5.2 Specific Objectives ----------11

CHAPTER TWO----------12

2.0 LITERATURE REVIEW----------12

2.1Introduction ----------12

2.1.1Aetiology of Rheumatoid Arthritis----------13

2.1.2 Pathogenesis of Rheumatoid Arthritis ----------14

2.1.3 Methods of Rheumatoid Arthritis diagnosis----------16

2.2 Rheumatoid Arthritis Biomarkers---17

2.3 Rheumatoid arthritis (RA) and Diabetes Mellitus (DM).---18

CHAPTER THREE---21

3.0 METHODOLOGY---21

3.1 Study Area and Population. ---21

3.2 Study Design ---21

3.3 Sampling ---21

3.4 Sample Size---22

3.5 Eligibility Criteria ---22

3.5.1 Inclusion Criteria ---22

3.5.2 Exclusion Criteria---23

3.6 Recruitment of Study Participants ---23

3.7 Data Collection Procedures ---23

3.7.1Questionnaire---23

3.7.2Collection of Blood Samples---23

3.8 Laboratory Analysis of the Samples for Biomarkers---24

3.8.1screening for Rheumatoid Factor (RF) ---24

3.8.2 The Human Tenascin-C (TNC). ---26

3.9Data management plan---29

3.9.1Data collection, entry and storage ---29

3.9.2 Statistical Analysis and presentation---29

3.10 Ethical consideration---30

CHAPTER FOUR ---31

4.0 RESULTS AND ANALYSIS ---31

4.1 Socio- demographic characteristics of the participants. ---31

4.2 Occurrences of Rheumatoid Factor amongst participants. ---32

4.2.1Rheumatoid Factor occurrence and Gender of the participants.---33

4.2.2 Rheumatoid Factor occurrence and age of participants ---------- 34
4.3 Serum levels of Rheumatoid Arthritis biomarkers in participants. ---------- 35
4.3.1Inflammatory marker Tenascin-C ---------- 35
4.4 Evaluation of serum Anti-CCP marker levels in the participants. ---------- 37
4.5: Comparison of positivity for rheumatoid arthritis biomarkers. ---------- 41
CHAPTER FIVE ---------- 42
5.0 DISCUSSION ---------- 42
5.1 Introduction ---------- 42
5.2 Occurrence of Rheumatoid Factor among the diabetic participants. ---------- 43
5.3 Serum levels of Rheumatoid Arthritis markers ---------- 46
5.3.1Tenascin C antibodies evaluated in the participants. ---------- 46
5.3.2Anti-citrullinated peptide/protein antibodies (Anti-CCP): ---------- 47
5.3.3 Comparison of RF and anti-CCP positivity ---------- 48
CHAPTER SIX ---------- 49
6.0 CONCLUSION AND RECOMMENDATION ---------- 49
6.1Conclusion ---------- 49
6.2 Recommendation ---------- 51
6.3 Limitations ---------- 52
6.4 The Study strength ---------- 52
REFERENCES ---------- 53
APPENDICES ---------- 63
Appendix 1: Consent to Participate in the Research Study: English version. --- 63
Appendix 2 : Questionnaire ---------- 65
Appendix 3:IREC Approval Letter. ---------- 66
Appendix 4: Hospital Approval (MTRH) ---------- 67
Appendix 5: Laboratory Request Form ---------- 68

LIST OF TABLES

Table 4.1.1: Rheumatoid Factor and Blood glucose levels of the participants.....33

Table 4.1.3: Showing logistic Regression of age groups and Rheumatoid Factor. 34

Table 4.2.2: Showing Tenascin-C status in relation to gender of the participants 35

Table 4.2.3: Frequency of inflammatory marker (Tenascin-C) in relation to age groups of the participants.....36

Table 4.3.1: Anti-CCP status and gender of the participants.....37

Table 4.3.2: Showing frequencies of Anti-CCP marker levels in age groups of participants.....38

Table 4.4.1: Comparison of status of RF and Anti-CCP antibodies as RA biomarker39

Table 4.4.2: Comparison of RF and Tenascin -C antibodies status among participants 40

LIST OF FIGURES

Figure 1: Interpretation of RF results.....25

Figure 2: protocol of TNC Laboratory analysis.....28

Figure 3: Age distribution of participants enrolled in the study (n=180) 31

Figure 4: The occurrence of Rheumatoid Factor among the participants.....32

Figure 5: Showing the distribution of RF in different age groups. 34

Figure 6: Showing Comparative positivity of Rheumatoid Arthritis markers among the participants (n=180).....41

ACKNOWLEDGMENTS

The success of this work was as a result of a collaborative effort of a number of people without whom it would never been a success.

First I want to thank God Almighty, whose abundant grace has seen me through from the beginning to the end of this journey.

I also wish to recognize the wise counsel and able guidance of my University supervisors: Dr. Caleb Nyamwange and Prof. Hezron Nyandieka who walked with me through the research journey and helped me out of the labyrinth into the successful completion of this work; thank you so much for patiently showing me the way.

I would like to gratefully and sincerely\ thank the Ampath Reference laboratory fraternity for helping me with data collection, Storage and testing of all my samples.

Lastly I thank the entire staff of Medical Biochemistry department, my colleagues, friends who provided an enabling environment for the successful completion of this work. My prayer for you all- may God bless you mightily.

I remain solely responsible for any short comings in this work.

ABBREVIATIONS

ACCP	Anti-cyclic citrullinated peptide
ACPAs	Antibodies against citrullinated peptides
ACR	American College of Rheumatology
ADA	American diabetes Association
ADA	American diabetes association
AFR	Sub-Saharan Africa
AHA	American Heart Association
AMR	American Region
ARA	American Rheumatism association
CC	Carbonyl Content
CDC	Center for Disease control
CHD	Coronary heart disease
CRP	C- reactive protein
CTD	connective tissue disorders
DCCT	Diabetes control and complication trial assay.
DM	Diabetes mellitus
DMI	Diabetes Management information
ELISA	Enzyme linked immunosorbent assay
EMR	Eastern Mediterranean Region
EUR	European Region
FPG	Fasting plasma glucose
GDM	Gestational diabetes mellitus
HbAIc	Glycated/ glycosylated hemoglobin
HLA	Human Leukocyte Antigen

IDF	International diabetes federation
IFG	Impaired fasting glucose
IREC	Institutional research and ethics committee
LMIC	Low and Middle income countries
MTRH	Moi Teaching and Referral Hospital
NGSP	National glycohemoglobin standardization program
OGTT	Oral glucose tolerance test
RA	Rheumatoid Arthritis
RF	Rheumatoid factor
SEAR	Southeastern Asia Region
SPSS	Statistical Package for social sciences
STR	soft-tissue rheumatism
TNC	Tenascin – C Antibodies.
WHO	World health organization
WPR	West Pacific Region

DEFINITION OF TERMS

Diabetes mellitus: A chronic metabolic disorder that occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces

Arthritis: Joint inflammation, conditions that affects the joints, the tissue that surround the joints and other connective tissues (the swelling and tenderness of one or more joints)

Biomarkers: A naturally occurring molecule, gene or characteristic by which a pathological or physiological processes, diseases can be identified.

Citrullination: The posttranslational conversion of peptidylcitrulline to peptidylarginine by the enzyme PAD (peptidyl arginine deaminase)

Deimination: The conversion of an imine group; especially the post-translational modification of the amino acid arginine in a protein into the amino acid citrulline.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Rheumatoid arthritis (RA) is a chronic progressive autoimmune disease of unknown cause which predominantly is characterized by persistent inflammation of the intra- and peri-articular synovial membranes with a symmetric polyarticular distribution. The condition particularly involves the small joints of hands and feet at the peripheral joints as reported by Gibofsky, (2012). Concomitant symptoms of systemic inflammation with fatigue and malaise are frequently observed, and at times low-grade fever can be experienced. Rheumatoid Arthritis is a known frequent inflammatory disorder that may affect many tissues and organs but principally it affects the synovial joints. Despite advancements in treatments, patients continue to have higher mortality and morbidity prematurely than the general population due to auto antibodies, (Nielen *et al., 2*004).Onset of rheumatoid arthritis can occur at any age, but peaks between 30 and 50 years where disability is common and significant(Roy *et al*, 2016). Some of the risk factors predisposing an individual to Rheumatoid Arthritis have been assumed to include genetic susceptibility as evident in familial clustering and monozygotic twin studies (Georgiadis *et al.,* (2006). About 50 percent of other documented risks are related to genetic factors; older age, a family history of the disease, and female gender which have been associated with increased risk of RA even though the gender difference is less prominent in older patients (Wasserman A., 2011). Early menarche at 10 years of age or younger and with very irregular menstrual periods have increased the RA risks (Karlson *et al*., 2004). However a study done by Guthrie and colleagues established that pregnancy more often than not can reduce remission of rheumatoid arthritis when they analyzed

women parity as an immunologic tolerance with regard to long-lasting impact on RA onset or remission. The reduction on risk of RA correlated to the period or duration women had taken since the last child birth in which the study recorded a reduction among women whose last birth occurred 1-5 years previously but progressively increase as the duration increased i.e. for those 5-15 years since last birth had higher risk of developing RA (Guthrie *et al*., 2010). Furthermore, the risk of RA in women also decreases if breastfeeding is practiced for longer period of time of at least 24 months but no effect has been reported from the use of oral contraceptive or supplements like vitamin E as means of preventing precipitation of rheumatoid arthritis s precipitation especially in females (Karlson *et al.,* 2008).The Cardiovascular risk factors other than age, gender, family history and smoking are diabetes, hypertension, dyslipidaemia, obesity and sedentary lifestyle has also been noted in RA patients to pose higher burden leading to morbidity as well as mortality as reported by John *et al*, 2009. Other studies have suggested that myocardial Rheumatoid Arthritis affects between up to 1% of adult population worldwide and can start at any age where the peak onset age has been hypothesized to be between 30 and 55 years (Roy *et al.*, 2015).

1.1.1 Diagnosis of Rheumatoid Arthritis

Typical presentation of patients with Rheumatoid arthritis present with pain and stiffness in multiple joints. The wrists, proximal inter-phalangeal joints, and meta-carpophalangeal joints are most commonly affected. Morning stiffness that can last for more than one hour suggests inflammatory reactions. Boggy swelling due to synovitis may be visible, or subtle synovial thickening may be palpable on joint examination. Patients may also present with more indolent arthralgias before the onset

of clinically apparent joint swelling. Systemic symptoms of fatigue, weight loss, and low-grade fever may occur with active disease.

In 2010, the American College of Rheumatology and European League against Rheumatism collaborated to create new classification criteria for RA as an effort to diagnose RA earlier in patients who may not meet the 1987 American College of Rheumatology classification criteria. (Wasserman A., 2011). These 2010 criteria do not include presence of rheumatoid nodules or radiographic erosive changes, both of which are less likely to occur in early Rheumatoid Arthritis. The diagnosis of RA is made via the stipulated criteria by thorough assessment of patient symptoms, physical examination and additional investigations in accordance with the 2010 American College of Rheumatology's classification criteria which include, morning stiffness in and around the joints for 1 hour, arthritis of 3 joint areas, arthritis of hand or wrist joints symmetrical arthritis, rheumatic nodules, serum rheumatoid factor (RF) and radio graphical changes (Aletaha *et al*., 2010). Autoimmune diseases such as RA are often characterized by the presence of auto-antibodies. Rheumatoid factor as antibody specific for the Fc portion of human IgG has been historically considered a marker for RA and was one of the diagnostic criteria for RA that was established by the American College of Rheumatology (ACR) as was evaluated by (Banal *et al*., 2009). The specificity of the RF test has been documented to be relatively poor with some percentage of false positivity in the general population, RF is found in many patients with other diseases of infectious or autoimmune origin such as hepatitis C, and in healthy older persons. Consequently, a search for better diagnostic markers, especially those with improved specificity for RA, ensued. Although Rheumatoid Factor has a little diagnostic utility; it has been retained for several years as a core diagnostic tool in practice because of its prognostic capacity due to lack of alternative rapid test.

Citrullination (deimination) of proteins is a chemical reaction which occurs when inflammatory cells release enzymes in the local tissues. Citrullination of synovial antigens, especially fibrin, during synovial inflammation probably allows the induction of anti-cyclic citrullinated peptide (anti-CCP) antibody in RA patients through an antigen conducted activation of B cells (Kayshap et al., 2015). The Anti-citrullinated protein antibody is more specific for RA and may play a role in disease pathogenesis. The greater sensitivity and specificity of anti-CCP than IgM/ IgG RF and its probable predictability of erosive disease in Rheumatoid Arthritis or the eventual precipitation of undifferentiated arthritis into RA makes these antibodies potentially important surrogate biomarkers for the diagnosis and prognosis in Rheumatoid Arthritis (Lee *et al*, .2006)

The evolution of laboratory assays used in the diagnosis of RA has documented Anti-Citrullinated peptide antibodies (ACCP) detection to be of higher level of diagnostic accuracy to RA with about 88- 99% specificity (Aggarwal, *et al.,* 2009). The updated RA Classification Criteria of 2010 has since included Anti-CCP detection as one of the important assays for the diagnosing the disease (Aletaha *et al*., 2010). Other tests that may be done include: complete blood count, CRP, erythrocyte sedimentation rate (ESR), joint ultrasound or magnetic resonance imaging (MRI), joint x-rays and synovial fluid analysis.

1.1.2 Diabetes and rheumatoid arthritis

Diabetes is classified into four classes, mainly Type 1 diabetes (results from β-cell destruction in the pancreas, usually leading to absolute insulin deficiency), Type 2 diabetes which results from a progressive insulin secretors defect on the background of insulin resistance, Gestational diabetes mellitus (GDM) diabetes that is diagnosed during pregnancy and other secondary types of diabetes due to genetic defects in β-

cell function, insulin action, diseases of the exocrine pancreas (such as cystic fibrosis), and drug- or chemical-induced diabetes. The WHO describes diabetes as a growing epidemic threatening to overwhelm health services and undermine economies, especially in the developing countries. Further, diabetes affects more than 250 million people worldwide, and is expected to affect over 380 million by 2025. Type 2 diabetes constitutes about 85% of the diabetic cases, followed by type 1 at 10% then the secondary and gestational types account for about 5 % (Cheng *et al.,* 2012). USA has the highest prevalence of diabetes among all developed countries with 11 per cent of American adults suffering from the disease followed by Singapore, Maltain second and third place respectively. An estimation of 14.2 million adults aged 20–79 years has diabetes in the sub-Saharan Africa (SSA) region, representing a regional prevalence of 2.1–6.7 %. Sub-Saharan Africa has the highest proportion of undiagnosed cases of diabetes; over two-thirds of people with diabetes are unaware of their status, majority of these people with diabetes live in cities or urban areas, even though the population in the region is predominantly rural. With increasing urbanization and population ageing, diabetes will pose a greater threat to Africa as reported by International Diabetes Federation (IDF, 2015) in Belgium. Epidemiological surveys conducted by the Nairobi-based Diabetic Management and Information Centre (DMI) gave the estimated prevalence of diabetes mellitus in Kenya as 3% by 2003 and that it was above 6% in 2007. In some rural parts of Kenya such as Nyeri county in central Kenya and Kilifi county in the Kenyan coast the prevalence is as high as 11.6% and above 20% respectively among the richer families(Chege 2010).The World Health Organization estimates that the prevalence of diabetes in Kenya at 3.3% (WHO, 2009) and predicts a rise to 4.5% by 2025 (Chege, 2010). Due to this burden of diabetes and its complications there is need to assess for

RA in people living with diabetes because diabetes and RA are co morbid and in most cases they are rarely screened for Rheumatoid Arthritis in Kenya. Generally in the population two thirds of Diabetes mellitus still remains undiagnosed while a third is lately diagnosed. Diabetes Mellitus type II comprises 90% of all the diabetes cases, followed by type 1diabetes mellitus at 10% and the secondary or gestational type of diabetes account for about 5% (WHO 2008). These patients spend three quarters of their time seeking treatment or are immobilized because of inability to move from one point to another due to joint pains. Data from CDC 2008 indicated that more than half of people living with diabetes mellitus also suffer from Arthritis in general; however, the association between diabetes mellitus and Rheumatoid arthritis which is a common type of arthritis has not been well established as demonstrated by Center for Disease Control in US, 2008.

The diagnostic criteria for Diabetes Mellitus involve standardized, quality assured laboratory methods to be applied when venous plasma glucose and HbA1c are measured. The current gold standard for diagnosing diabetes is measurement of glucose in venous plasma. This measurement can be accurate only if glycolysis is inhibited in the blood sample as soon as the sample is drawn from the body. This can be achieved in two ways. Either the blood tube is stored on ice and the blood is centrifuged within 30 minutes, or glycolysis in the tube is effectively inhibited by appropriate additives (citrate plus fluoride; fluoride by itself is not sufficient). The glucose levels stated in the practice guidelines apply to venous plasma (Kerner *et al*., 2014). This diagnostic criteria that was earlier developed by ADA (2013) is based on one of the four abnormalities or findings as follows: HbA1c ≥6.5%., Fasting plasma glucose ≥126 mg/dl (7.0 mol/l), random elevated blood glucose with symptoms, oral glucose tolerance test (OGTT) plasma glucose ≥200 mg/dl (11.1 mol/l), and in a

patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dl (11.1 mol/l) is indicative of diabetes.

Biomarkers are integral to disease stratification since they have the potential to transform the management of Rheumatoid Arthritis by enabling; early diagnosis, assessment and prediction of disease severity, selection of therapy, and monitoring of response to therapy (Maecker *et al.,* 2012).The traditional RA biomarker rheumatoid factor (RF) is highly sensitive to most autoimmune diseases and not specific to RA (Venrooij WJ, 2002). However, the diagnostic sensitivity of RF and anti-CCP antibody positivity in early synovitis has been reported to range between 40% and 71% respectively (Nielen, 2004). The following RA biomarkers (CCP and TNC) can be used for early diagnosis of RA because of their sensitivity and specificity.

Citrullination or deimination is the conversion of the amino acid arginine in a protein into the amino acid citrulline. Citrulline is not one of the 20 standard amino acids encoded by DNA in the genetic code. Instead, it is the result of a post-translational modification in local tissues. Citrullination of synovial antigens, especially fibrin, during synovial inflammation probably allows the induction of anti- cyclic citrullinated peptide (Anti-CCP) antibody in RA patients through an antigen conducted activation of B cells. Capacity to form antibodies to citrullinated peptides and not citrullination of peptides *per se* seems to be unique to RA; the exact significance of antibodies to these peptides, however, remains uncertain. Greater sensitivity and specificity than IgM RF and probable predictability of erosive disease in RA or the eventual development of undifferentiated arthritis into RA makes Anti-CCP antibodies potentially important surrogate markers for the diagnosis and prognosis in RA.

During Citrullination (deimination) of proteins, a chemical reaction occurs when inflammatory cells release enzymes in local tissues. Anti-CCP antibodies are potentially important surrogate markers for the diagnosis and prognosis of RA (Lee W *et al*, 2006). The antibodies against cyclic citrullinated proteins /peptides (A-CCP) have been found to predict development and severity of RA (Van der Helm-van, 2005). Importantly, anti-CCP antibodies are present early in disease (Rantapaa-Dahlqvist S, 2003). As a diagnostic tool for RA, CCP test has a sensitivity of 68%, with a high specificity of 97–98% (Suwannalai *et al*., 2012). This specificity of the anti-CCP helps in distinguishing RA from other autoimmune diseases, such as systemic lupus erythromatosus (SLE) associated with erosive arthritis (Mediwake et al., 2001). Tenascin-C a pro inflammatory matrix glycoprotein molecule that is absent from healthy joints but highly expressed in the joints of patients with RA (Udalova *et al*., 2011), have been found to be elevated in the cartilage, synovium and synovial fluid of patients with RA (Goh *et al*.,2010). It is an immunodominant peptide in citrullinated Tenascin-C (cTNC5) antibodies detected in around half of the patients with inflammation associated with RA years before the disease onset. (Schwenzer *et al.,* 2016), it stimulates the production of pro-inflammatory cytokines in human macrophages and synovial fibroblasts through activation of toll- like receptors-4.

1.2 Problem Statement

Rheumatoid Arthritis is an inflammatory disorder that affects many tissues and organs but principally the joints. Despite advances in treatments, RA patients continue to have higher mortality and morbidity rates than the general population. The condition is affecting between 0.5-1 percent of adult population worldwide. Its incidence, prevalence and trend are difficult to define and predict. Worldwide, 2/3 of Diabetes Mellitus still remains undiagnosed while 1/3 is diagnosed late however by 2007 IDF estimated 246 million people to be living with the diabetes. In 2009 Diabetes Atlas publication, the International Diabetes Federation, reported the global burden of diabetes in 2010 at 285 million and projected an increase to 438 million by 2030, if no interventions are put in place (IDF Atlas, 2009).This rise in diabetes can be associated with demographic and social changes such as globalization, urbanization, aging population and adoption of unhealthy lifestyles. These impacts could be felt on socioeconomic well-being of humanity since patients with diabetes do not only have diabetes-related co-morbidity but also have non diabetes-related co morbidities, like musculoskeletal diseases such as rheumatoid Arthritis which causes dysfunction of bone and joints (Struijs et al., 2006). Diabetes Mellitus type II comprises 90% of all the diabetes cases and the fact that there are no/few reports in Kenya, indicating the prevalence of RA in DM calls for screening of this condition in diabetes patients, since the only available information regarding the same was conducted at KNH in 1979 on RA patient. Rheumatoid arthritis is not widely recognized as co-morbid condition of diabetes which can pose potential difficulties and complications in the management of the two conditions. The study therefore aims at establishing early biomarkers of RA.

1.3 Justification

Since the proportion of people living with diabetes and Rheumatoid Arthritis is not well established. This study was therefore undertaken to determine the predictive biomarkers of Rheumatoid Arthritis in people living with diabetes to extrapolate those who might be at risk of developing arthritis to curb the burden of the disease complications beforehand. In Kenya, there are no reports which have evaluated the occurrence of Rheumatoid Arthritis in patients living with type II diabetes mellitus and vice versa. Therefore by considering the difficulties always encountered during prevention and management of the two conditions, this study was considered to be of importance. The resulting data will give care providers and patients themselves a better understanding on how to manage both conditions using an interdisciplinary approach since the occurrence is an "egg - chick phenomenon". This study will present a platform to explore biomarkers especially Anti-CCP marker as a routine diagnostic test in patients with T2DM due to increasing prevalence of type II diabetes mellitus.

1.4 Research Questions:

1. What is the prevalence of Rheumatoid Factor (RF) among patients with type II diabetes mellitus?
2. What is the occurrence and levels of RA Biomarkers (Anti –CCP and TNC) in T2DM patients?

1.5 Objectives

1.5.1 Broad Objective of the study

The general objective of this study was to determine the prevalence and profiles of RA biomarkers among type II diabetes mellitus patients attending Clinic at Moi Teaching and Referral Hospital (MTRH).

1.5.2 Specific Objectives

1. To determine the prevalence of Rheumatoid Factor (RF) among type II diabetes mellitus patients.
2. To determine serum levels and occurrence of Anti-CCP and anti-TNC markers in T2DM patients.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1Introduction

Rheumatoid Arthritis (RA) is a severe, progressive, co-morbid systemic inflammatory disease of unknown etiology. Onset of rheumatoid arthritis can occur at any age, but its peaks have been documented to be between 30 and 50 years where disability is common and significant. Since timely intervention with new and effective treatments can alter the course of the disease, reduce functional impairment, and lengthen life, better biomarkers for diagnosis and prognosis are needed to identify these patients at an early stage in order to fine-tune therapeutic options to the individual patient (Kayshap *et al*., 2015). The chronic systemic inflammatory disorder may affect many tissues and organs but principally affects the small joints (Gibofsky, 2012). The prevalence of RA in the western world is 1–2% (Alamanos *et al*., 2006). Its believed that Rheumatoid Arthritis (RA) affects between 0.5 to 1% of the adult population worldwide (Roy *et al.,* 2015). The RA prevalence meta–analysis conducted by Rudan, Sidhu *et al.,* 2015 on low and middle income countries (LIMC) following the WHO regional divisions of the world, gave different estimates of Rheumatoid Arthritis with different prevalence rates. The regions included Southeast Asia (SEAR) 0.40%, Eastern Mediterranean Region (EMR) 0.37%, European Region (EUR) 0.62%, American Regions (AMR) 1.25% and finally Western Pacific Regions (WPR) 0.42% respectively. However, this analysis could not be performed for Sub – Sahara Africa (AFR) due to limited data that could not reveal the exact proportions of people living with RA. The African prevalence estimates of rheumatoid arthritis in urban settings, per country, varied from 0.13% in Algeria , 0.6% in the Democratic Republic of Congo to a maximum meta-analysis overall prevalence of 2.5% in South Africa

(an analysis done on 3 studies). Nevertheless the overall prevalence could not be reported because of significant heterogeneity between the studies conducted in Nigeria which documented on prevalence of RA where they concluded that the exact prevalence has not been well established (Usenbo, *et al*., 2015). The incidence and prevalence and trends of RA are difficult to define and predict (Davis, *et al*., 2014). RA can start at any age, but the peak age of onset is between 30 and 55 years (Roy,*et al*., 2015). Although some patients have mild self-limited disease, many experience joint destruction, severe physical disability and multiple co-morbidities such as long-term damage of the joints, dysfunction, and failure of different organs like the eyes, kidneys, nerves, heart, and blood vessels (Plenge *et al.*, 2007). Mortality rates are twice as high in patients with RA compared to the general population and this gap appears to be widening. (Wolfe *et al.*, 1994).The incidence of RA seems to increase with age whereby it has been confirmed to be more in women than men (Santos-Altamirano *et al.*, 2015).

2.1.1Aetiology of Rheumatoid Arthritis

Rheumatoid arthritis is a disease involving autoimmune reactions, though it is yet unclear what initiates the autoimmune response (Klareskog, *et al*., 2006). Several risk factors such as genetic, environmental and hormonal are thought to interact at the onset of the disease triggering aetiopathogenesis of RA (van der Woude *et al*, 2010). Studies of twins have revealed that heredity contributes approximately 60% to the risk of developing RA (MacGregor *et al.*, 2000). The human leukocyte antigen (HLA) class II surface molecule, HLA-DRB1 shared epitope (SE), has been shown to account for up to 40% of the genetic influence towards the development of RA (Gregersen *et al.*, 1987), though other studies have suggested that the overall contribution of HLA shared epitope may have been over-estimated and that the

contribution of risk from shared epitope is confined mainly to patients sero-positive for antibodies against citrullinated peptides (ACPAs) (van der Woude *et al*, 2010).Prolonged and heavy smoking increases the risk of RA(Costenbader *et al*, 2006), which is thought to facilitate protein citrullination in the lungs, and subsequently precipitating an autoimmune response that is more pronounced in individuals carrying the HLA-DRSE genes.The interaction between HLA SE and smoking in ACPA-positive RA patients induces post-translational modification of arginine into citrulline which results in significant alterations in the structure and function of various protein antibodies related to the RA disease such as rheumatoid factors (RFs) and ACPAs. A specific combination of these antibodies have positive prediction in the progress of RA almost 100% (Rantapää- Dahlqvist *et al.*, 2003).There is an over representation of female sex in RA and this is thought to be due to life events such as menarche, pregnancy, lactation and usage of oral contraceptives hence suggesting a role of hormonal factors in the etiology of RA (Berglin *et al.*,2010).

2.1.2 Pathogenesis of Rheumatoid Arthritis

In an established RA disease, the lining of the synovial cavity is characterized by a thickening of synoviocytes, there is a marked increase in the volume of the sub-linear layers, edema, and an infiltration of mononuclear cells which are both diffusely spread and aggregated in lymphoid follicles. Hypotheses regarding the initiation of the inflammatory process exist: an “outside-in” and an “inside-out” hypothesis which explains that the inflammation could start in the synovial tissue from which the inflammatory process spreads into adjacent tissues and bone marrow; alternatively it could originate from an immunological process in the bone marrow with the synovial membrane as the final (Schett and Firestein, 2010).The inflammatory cells, if in

inappropriate large amount, destroy body tissue. The synovial fluid accumulates and the joints swell in time and thicken into a pannus (abnormal tissue). Over time the pannus erodes the joint's cartilage and, possibly, scar tissue will be formed, connecting bone ends. Later, this scar tissue can ossify wherewith the joints get immobilized and deformed. The surrounding structures of the inflammatory joints, as tendon sheaths, bursas and origins of muscles are often involved in supporting joint deformations as well. There are several reasons why there could be an association between these two conditions (RA and DM): Both are autoimmune diseases with systemic inflammation as the characteristic feature (Dall *et al*., 2012), and the conditions are linked to cardiovascular diseases (Stamatelopoulus *et al.,* 2009) as a consequence of sedentary lifestyle, predisposing one to the development of insulin resistance and Diabetes (Sattar *et al.,* 2003).Diabetes is a broadly recognized health issue and, as part of a health strategy to improve disease management, it is important to consider all related health issues and to investigate their relationships (ADA, 2013).This is necessitated by the fact that rheumatoid arthritis is not widely recognized as co-morbid condition in diabetes whereby it's not considered part of the potential cause of difficulties in the management of diabetes. Several co-morbidities/complications such as long-term damage, dysfunction, and failure of different organs like the eyes, kidneys, nerves, heart, and blood vessels (ADA, 2013) are known to exist with both diabetes and RA. Rheumatic and musculoskeletal diseases have fourth highest global impact on disability-adjusted life years and are the second leading cause of disability as measured by the number of years lived with disability (Mody., 2017).Further, data from CDC 2008 indicated that more than half of people living with diabetes also suffer from arthritis, however, the association between Type II diabetes Mellitus and Rheumatoid arthritis, has not been

demonstrated (CDC, 2008). In Kenya, the proportion of people living with RA and diabetes is not known since no studies have been carried out on the same. Therefore there is need for this study to determine early biochemical markers of RA in patients living with type II diabetes mellitus so as to ascertain those who might be at risk of developing arthritis later in life. This information can be applied during management of both conditions before full precipitation of RA.

2.1.3 Methods of Rheumatoid Arthritis diagnosis

RA follows a variable course which is indeterminate with regards to joint injury and functional outcomes thus making its diagnosis challenging and difficult. The American College of Rheumatology (ACR) /European League against Rheumatism (EULAR) classification of RA (Aletaha *et al.*,2010),a criterion that was formulated in 1956 by a committee of American Rheumatism association (ARA) and have undergone several revisions to enable easy diagnosis (Arnett *et al.*, 1988). There is need for an aggressive form of testing panel for confirmation and identification rheumatoid arthritis from other inflammatory conditions.(Nielsen *et al*, 2012).The main biomarker used for the diagnosis of rheumatoid arthritis (RA) is rheumatoid factors (RF) and an antibody directed against the Fc portion of immunoglobulin G (IgG) in the body. RF IgM latex is the commonly measured parameter in clinical practice as the first line diagnostic test (Rantapää Dahlqvist *et al*., 2003). Other tests like antibodies to citrullinated peptides are usually measured by enzyme-linked immunosorbent assays (ELISAs) using CCPs as antigen. Anti-CCP antibodies have a similar sensitivity to RF for RA but have a much higher specificity of between 95 to 98 percent (Schellekens *et al*., 2000). Some tests like anti-mutated citrullinated vimentin gives similar results to anti-CCP hence can as well be used as an alternative (van Venrooij and Pruijn 2000). It has been suggested that diagnostic

accuracy of both two markers; anti-CCP antibody and IgM–RF maximizes specificity and sensitivity as compared to one test done alone. The presence of anti-CCP antibody is associated with development of rheumatoid arthritis and greater radiographic progression, while RF is a major predictor of bone damage (Zeng *et al*, 2003).This study aimed at assessing whether the Anti-CCP antibody or IgM RF were the reasonable testing strategy which could capture missing potentially treatable diabetic patients with pre existing antibodies that may lead to development of rheumatoid Arthritis.

2.2 Rheumatoid Arthritis Biomarkers

The traditional RA biomarker rheumatoid factor (RF), are highly sensitive to most autoimmune diseases and not specific to RA and may be present in patients with other diseases, such as hepatitis C, and in healthy older persons. (Van Venrooij *et al*., 2002). However, the diagnostic sensitivity of RF and anti-CCP antibody positivity in early synovitis has been reported to range between 40% and 71% respectively (Nielen *et al*., 2004) therefore the following RA biomarkers can be used for early diagnosis of RA because of their sensitivity and specificity. The antibodies against cyclic citrullinated proteins /peptides (A-CCP) have been found to be more specific for RA and may play a role in disease pathogenesis by its ability to predict the development and severity of RA (Van der Helm-van *et al*., 2005).Importantly, anti-CCP2 antibodies are present early in disease (Rantapaa-Dahlqvist., 2003). Citrullination (deimination) of proteins is a chemical reaction which occurs when inflammatory cells release enzymes in local tissues. Citrullination of synovial antigens, especially fibrin, during synovial inflammation probably allows the induction of anti-cyclic citrullinated peptide (Anti-CCP) antibody in RA patients through an antigen conducted activation of B cells. Capacity to form antibodies to

citrullinated peptides and not citrullination of peptides *per se* seems to be unique to RA; the exact significance of antibodies to these peptides, however, remains uncertain. Greater sensitivity and specificity than IgM RF and probable predictability of erosive disease in RA or the eventual development of undifferentiated arthritis into RA makes Anti-CCP antibodies potentially important surrogate markers for the diagnosis and prognosis in RA. As a diagnostic tool for RA, CCP test (anti-CCP) has a sensitivity of 68%, with a high specificity of 97–98% which helps in differentiating RA from other autoimmune diseases such as systemic lupus erythromatosus (Parawee *et al*., 2011). Further, Anti-CCP2 assays are also helpful in various clinical settings, including early diagnosis of Rheumatoid Arthritis, diagnosing the RF-negative RA and differentiating other forms of RF-positive arthritis which could be infection related like hepatitis C virus-related joint involvement. (Pallazi *et al.*,2012). Secondly, Tenascin-C which is a pro-inflammatory matrix or glycoprotein has also been considered to be possible biomarker in most of the inflammatory conditions. It's always absent from healthy joints but highly expressed in the joints of patients with RA (Udolova *et al*., 2011) where its level have been noticed to be elevated in the cartilage and synovial fluids. (Goh *et al.,* 2010). This immuno dominant peptide in citrullinated Tenascin- C antibodies can be detected in a round half of the RA patients' years before the disease onset.

2.3 Rheumatoid arthritis (RA) and Diabetes Mellitus (DM).

Diabetes mellitus (DM) is a hereditary, chronic and endocrine-metabolic disorder characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action or both (Jafari-Koshki *et al*., 2015). Accumulation of glucose in the serum could have different effects on health and quality of life. Long-term damage and dysfunctional organs are its consequences. The risk of cardiovascular and renal

disorders is 2 times and 17 times greater in these patients. One-third of new cases of kidney disorders arise in diabetic patients. (Sadeghi *et al.,* 2012). Mortality rates in diabetic patients are 1.5-2.5% greater compared to healthy people; their survival is 10-15 years shorter than the general population, and the costs of a diabetic patient is 3.8 times higher than other patients (Jafari-Koshki *et al*., 2015).As a health issue, diabetes lowers the quality of life of the patients.

It has been demonstrated that type 2 diabetes mellitus is a well-known risk factor for the development of cardiovascular disease, cerebrovascular disease and peripheral vascular diseases (VinodMahato, *et al*., 2011). Some of the most common complications of diabetes are related to the heart, blood vessels, eyes, kidneys, and nerves leading to their damage or inability to function properly (Stamatelopoulos, *et al*., 2009).People with diagnosed diabetes are nearly twice as likely to have arthritis as a complication, indicating a diabetes-arthritis connection since what starts off as a hormonal problem can progress into joint problems and cardiovascular problems (John, *et al*., 2009). Sometimes diabetes causes musculoskeletal changes that lead to symptoms such as joint pain and stiffness; swelling; nodules under the skin, particularly in the fingers; tight, thickened skin; trigger finger; carpal tunnel syndrome; painful shoulder, severely affected feet after having diabetes for several years, joint damage (Alkaabi *et a*l., 2003) .Literature also reported that RA patients' were 50 % more likely to develop type 2 diabetes as compared with non-RA. Attaining sedentary lifestyle because of RA pain further increases the risk for type 2 diabetes mellitus (Zhou *et al*., 2012).

RA and Diabetes are both disorders with unknown causality and similar risk factors; the association between these two conditions has not been clearly defined (Doran.,

2007). In another study conducted on the association of the two conditions there was no conclusion whether there is an increased prevalence of Diabetes in patients with RA or an increased prevalence of RA in patients with diabetes. One of the most relevant studies in this area was performed by Simard and Mittleman in 2007 which showed that there was no association between RA and Diabetes Mellitus persons who were 60 year old. (Simard and Mittleman 2007).Further, there is no available data reflecting on how the prevalence of Cardiovascular disease is affected by the co-morbidity of Diabetes Mellitus and Rheumatoid Arthritis as mentioned by Tentolouris *et al.* (2018) when they evaluated the presence of concomitant RA in patients with Diabetes Mellitus and followed them for a minimum of 10 years in which they concluded in their findings that there was low prevalence in the proportion of RA in people living with type II diabetes.This therefore poses the need for the determination of predictive biochemical markers of Rheumatoid Arthritis in patients with diabetes mellitus so as to ascertain those who might be at risk of developing arthritis early enough.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Area and Population.

The study was conducted at Moi Teaching and Referral Hospital (MTRH) which is a Referral hospital serving the north-rift and western regions of Kenya. It's located in Uasin Gishu County where both urban and rural patients are seen. It is the second largest referral hospital in Kenya where different chronic and communicable diseases are taken care of. The hospital has 800 bed capacity, receives patients from western Kenya, parts of Eastern Uganda and the Southern Sudan. Diabetes clinic operates three days in every week due to high number of patients turn out.

The study targeted all confirmed Type II diabetes mellitus patients aged ≥18 years old who have lived with the condition for duration more than one year and presented themselves at the Diabetes Clinics MTRH located at Chandaria Cancer and chronic disease Center between May – August 2018.

3.2 Study Design

It was a descriptive cross-sectional study.

3.3 Sampling

Sampling was done by convenient sampling of all type II diabetes mellitus patients presenting at the diabetic clinic on the appointed clinic dates during the period of the study and fulfilled the eligibility criteria.

3.4 Sample Size

The sample size for the participants was calculated using Fischer's formula as follows: (Mugenda 1999),

$$n = \frac{Z^2 \alpha * Pq}{d^2}$$

Where, n = sample size

$Z^2\alpha$=Confidence interval level

P=Prevalence rate no of the conditions

q = 1- p

d = degree of accuracy

Prevalence of diabetes in Kenya according to Diabetes Management Information (DMI) survey report of 2012 is 10.7% in urban set up was used to calculate the minimum sample size as follows:

$N=1.96^2*0.107(0.893)/0.05^2$

= 164

NB: The DMI established 10.7% prevalence of diabetes used to calculate the sample size since it's the population under investigation in the entire study**.**

3.5 Eligibility Criteria

3.5.1 Inclusion Criteria

- All patients who were confirmed type II diabetes mellitus for a period of > 1 year and above.
- All the patients with Type II diabetes mellitus who were 18 years and above.

3.5.2 Exclusion Criteria

- Patients who were seriously ill to talk and sign the participants consent form.
- Patients with Type II diabetes mellitus for period less than one year after diagnosis.

3.6 Recruitment of Study Participants

The study participants were conveniently selected immediately they were from the doctor's consultation room during clinic dates at the phlebotomy station for privacy. Participants were then taken through the consent form and after consenting an interview administered using the structured questionnaire. Venous blood sample was then collected by a qualified phlebotomist in plain red top tubes for screening and determination of the Rheumatoid arthritis biomarkers in the laboratory.

3.7 Data Collection Procedures

3.7.1Questionnaire

The questionnaire administered by the investigator determined demographic and socioeconomic variables such as medical history concerning the duration of which patients have lived with type II diabetes and when diagnosed , the type of drug(s) the patient is currently using for the management, smoking, any other illnesses and alcohol intake or drug abuse. (See appendix II).

3.7.2Collection of Blood Samples

Five millilitres (5mls) of venous blood sample was collected from each participant and put in a plain red top tube and allowed to clot. The blood was then centrifuged at 3,000 revolutions per minute (rpm) for 10 minutes at room temperature to separate serum from blood elements. The serum was then aliquoted into two cryovials (tubes) one tube for real time analysis and screening of Rheumatoid Factor antibodies and

another tube stored in a deep freezer at -70^{0}c (Ampath Bio-repository department) well labelled with unique participants identifiers for confidentiality. The frozen sample was later used for other RA biomarkers analysis (Anti-CCP and Anti-Tenascin- C) using Elisa technique.

3.8 Laboratory Analysis of the Samples for Biomarkers

3.8.1screening for Rheumatoid Factor (RF)

Rheumatoid factors (RF) are antibodies directed against antigenic sites in the Fc fragment of human and animal IgG. Their frequent occurrence in rheumatoid arthritis makes them useful for diagnosis and monitoring of the disease. Rheumatoid Factor (RF) was determined based on the ability of rheumatoid arthritis sera to agglutinate sensitized sheep red cells, as was observed by Waaler and Rose. The reagent consisting of biologically inert latex beads coated with human gamma globulin as was described by Singer and Plotz. The Rheumatoid Factor kit is based on the principle of an immunological reaction between human IgG bound to biologically inert latex particles and rheumatoid factors in the test specimen. When serum containing rheumatoid factors is mixed with the latex reagent, a visible agglutination/aggregates occurs which is visualized in a well lighted place. The RF latex reagent sensitivity has been adjusted to detect a minimum of 8 IU/ml of rheumatoid factors according with the WHO International Standard without previous sample dilution. The major advantage of this method is rapid performance (3 minute reaction time) and lack of heterophile antibody interference.

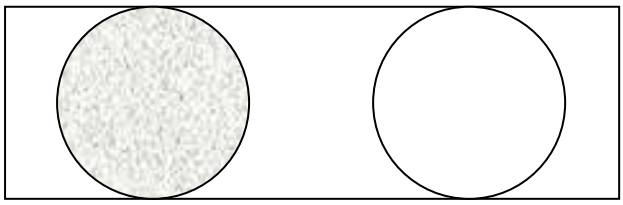
Materials and Components used for the determination of RF included the following: RF Latex Reagent: A suspension of uniform polystyrene particles coated with IgG (human) in glycine buffer, pH 8.2, **RF Positive Control Serum**: A stabilized, pre-diluted human serum containing at least 8 IU/ml of RF,. **RF Negative**

Control Serum: A stabilized, pre-diluted human serum containing less than 8 IU/ml of RF, Reaction Slide/tile, Pipette/Stir Sticks and Timer.

Protocol (Qualitative Rheumatoid Factor Test)

All the reagents and specimens were brought to room temperature before use, One drop (50 µl) of the RF Positive Control and Negative Control was placed on respective field of the reaction slide. Using pipettes which were provided in the kit, one drop of the undiluted specimens was then put on successive fields, Retaining Pipette/Stir Sticks for mixing. The RF Latex Reagent was then gently re-suspended and a drop of (50 µl) added to each test field after which the mixture spread using the stirrer over entire test field. The slide was then rotated using a mechanical rotor at 80-100 revolution per minute for duration of 2 minutes then results read immediately under direct light. Presence of agglutination of the latex particle was then observed as shown in (figure 1) below. Agglutination indicates RF concentration of equal or more than 8 IU/ml in the serum. Interpretation of the results was done as follows: **Negative Result:** A negative reaction is indicated by a uniform milky suspension with no agglutination observed with the RF Negative Control.

Positive Result: A positive reaction is indicated by any observable agglutination in the reaction mixture. The specimen reaction should be compared to the RF Negative and Positive Controls.



Positive Negative

Figure 1: Interpretation of RF results

3.8.2 The Human Tenascin-C (TNC).

Tenascin-C (TNC) is a glycoprotein that in humans is encoded by the TNC gene. It is expressed in the extracellular matrix of various tissues during development, disease and injury. Tenascin-C is the founding member of the gene family (Tenascin-C). In the embryo it is made by migrating cells like the neural crest; it is also abundant in developing tendons, bone and cartilage. TN-C clearly plays a role in cell signaling as evidenced by its ability to be induced during events such as trauma or inflammation. Also, TN-C is important in regulating cell proliferation and migration, especially during developmental differentiation and wound healing.

Test Principle:

Enzyme-Linked Immunosorbent Assay (ELISA) kits (DL-TNC-HU) used sandwich method were the micro titer plate provided in the kit was pre-coated with an antibody specific to TNC. Standards or samples were then added to the appropriate micro titer plate wells with a biotin-conjugated antibody preparation specific to TNC. Next Avidin conjugated to horseradish peroxidase (HRP) was then added to each micro plate well and incubated. After TMB substrate solution was added, only those wells that contain TNC, biotin-conjugated antibody and enzyme-conjugated Avidin exhibited a change in color. The enzyme-substrate reaction was terminated by the addition of sulphuric acid as stop solution and the color change measured spectrometrically at a wavelength of 450nm ±10nm. The concentration of TNC in the samples was then determined by comparing the optical densities (OD) of the samples to the standard curve. The ODs were then converted to concentrations for easy interpretation of the results. Any value above 58.7pg/ml was regarded as positive according to the manufacturer's manual.

Equipment, Chemicals and materials used for the determination of TNC Elisa.

- Pre-coated, ready to use 96 well strip plate.
- Plate sealer for 96 wells.
- The standard concentration (2000pg/ml, 1000pg.ml,500pg/ml,250pg/ml,62.5pg/ml, 31.1pg/ml.)
- Diluents buffer
- Distilled Water.
- TMB substrate
- Wash buffer (30 x concentrate)
- Stop solution.
- 1000mls Measuring Cylinder
- Multi-channel Pipette and disposable tips.
- Eppendorf tubes for diluting samples
- Elisa Micro-plate reader at 450 ± 10nm filter.
- Absorbent paper for blotting the micro titer plate
- Container for wash solution.

Protocol Summary

Prepared all reagents, samples, and standards as instructed

Added 100 µL standards or samples to appropriate wells incubate at 37°C for 2 hours.

Discard plate content. Do not wash. Add 100 µL biotinylated Antibody in to all wells

Incubate at 37°C for 1 hour.

Wash each well three times with 300 µL 0.01M PBS (or TBS)

Add 100 µL detection working solution Incubate at 37°C for 30 minutes

Wash each well five times with 300 µL 0.01M PBS (or TBS)

Add 90 µl of prepared TMB Incubate at 37°C in dark for 15-25 minutes

Add 50 µL Stop Solution and read OD at 450 nm within 30 minutes.

Figure 2: protocol of TNC Laboratory analysis.

Anti-CCP Testing

Secondly, Anti- CCP analysis was also analyzed based on the IMTEC – CCP Antibodies which is an indirect ELISA for the quantitative measurement of IgG class auto -antibodies which involved immobilization of cyclic citrullinated peptides (CCP) to the surface of micro-titer plate and subsequent binding of anti-CCP antibodies from human serum or plasma. The bound anti CCP antibodies are detected with a horseradish peroxidase (HRP) labeled antibody that is directed against human IgG. When substrate added a color developed whose intensity is proportional to the concentration of the anti-CCP antibodies in the serum. The results were then interpreted according to the manufacturers instruction manual which indicated positives /elevated values as > 25u/ml and normal levels as < 25u/ml.

3.9Data management plan

3.9.1Data collection, entry and storage

The data obtained from the structured questionnaire and biochemical analysis were cleaned, coded and stored in Ms Excel, Optical densities interpolated to concentration by Graph pad prism version7.Software and then the whole data set analyzed using the EPI INFO software and the soft copy of these data were stored in an external hard disc as a backup of the information.

3.9.2 Statistical Analysis and presentation

The results analyzed using EPI INFO for measures of central tendencies like mean, median, standard deviation. Measures of associations like Odds ratio, cross tabulation and logistic regression option with p-value of <0.05 applied when analyzing the data collected to correlate relationship of diabetes and Rheumatoid Arthritis.

3.10 Ethical consideration

Ethical clearance obtained when a proposal was submitted to Institution Research and Ethics Committee (IREC), a committee of Moi University and Moi Teaching and Referral Hospital that deals with ethical issues on human subjects, for review and approval. (Ref. IREC/2017/149 and ELD/MTRH/R&P/10/2/V.2/2010).Written consent was then obtained from willing diabetes participants ages >18 years old. Patient's privacy was taken into consideration by allocating unique identifiers for the participants. The results from the RF screening communicated to the participants in real time.

The data generated from the study shall be published without any participants identities to the hospital management for policy implementation and abstract sent for publication in a selected scientific journal.

CHAPTER FOUR

4.0 RESULTS AND ANALYSIS

4.1 Socio- demographic characteristics of the participants.

The study recruited a total of 180 diabetic participants between the months of May to August 2018. About 43% of the participants were aged 41-60 years, 36% were between ages 61and 80 years and 21% were either below 40 years or more than 81 years of age. Approximately 67% (121/180) of participants enrolled in the study were females while males constituted 33%.(59/180).The mean age of the participants was 55.2 years with standard deviation of ± 15.4 years, the modal age was 58 years with the age range of between 22 and 89 years. The duration in years that participants have lived with diabetes was 8.4± 6.2 years while on medication.

The mean glucose levels of the participants were 10.5 (SD ± 5.7) mmol/l and a mode of 6.3 mmol/l. The participants with uncontrolled glucose levels of more than 10mmol/l were 45.6% (82/180) while those with controlled glucose levels were 54.4 %.(98/180).

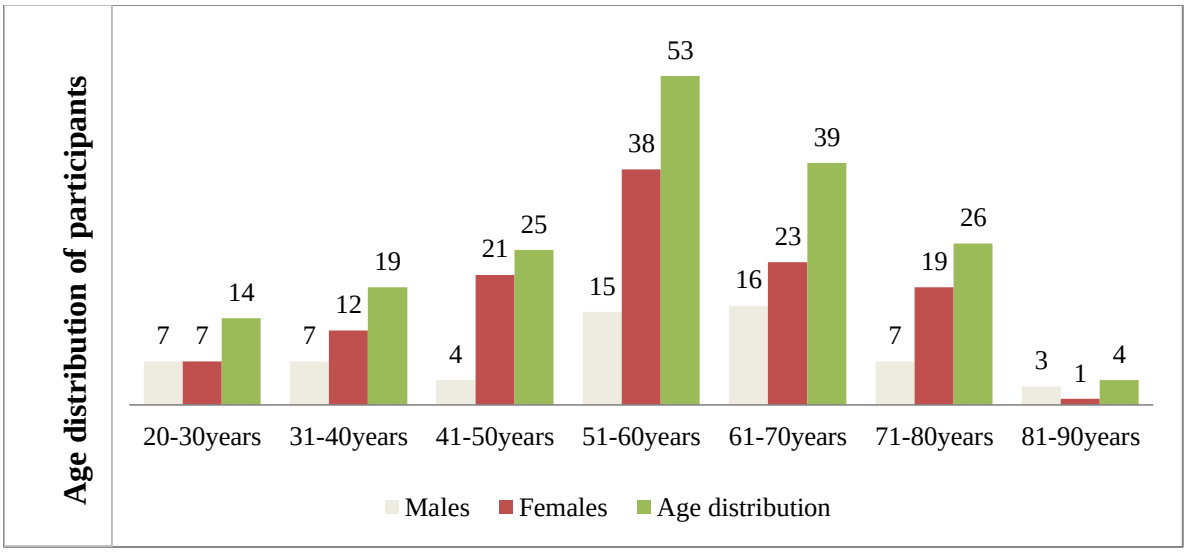


Figure 3: Age distribution of participants enrolled in the study (n=180)

4.2 Occurrences of Rheumatoid Factor amongst participants.

The overall RF positivity proportion found in the participants was 10 %(18/180). However when the participants were segregated by their glucose levels, occurrence of RF was more likely to be pronounced in hyperglycemic participants than in the group with controlled glucose levels. On logistic regression analysis, it showed no statistical significant difference between glucose levels and RF occurrences (p= 0.4517).

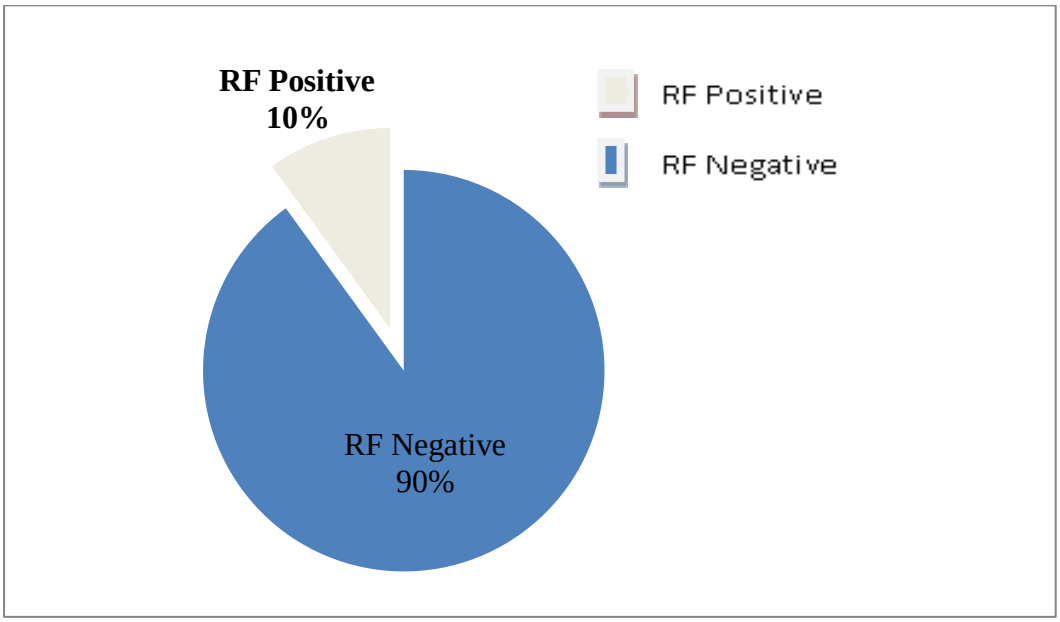


Figure 4: The occurrence of Rheumatoid Factor among the participants.

Table 4.1.1: Rheumatoid Factor and Blood glucose levels of the participants.

GLUCOSE LEVELS / RF STATUS	Controlled glucose levels(<11.1mm0/l)	Uncontrolled glucose (>11.1mmol/l)	Total
Negative	89(49.4%)	73(40.6%)	162(90%)
Positive	9(5.0%)	9(5.0%)	18(10%)
Total	**n=98(**54.4%)	**n=82(**45.6%)	100%

The proportion of the participants who had controlled blood glucose levels were 54.4 %(98/180)of which 5.0% (9/180) were RF positive while those with uncontrolled glucose levels were 45.6% (82/180) with a positivity of 10.9% (9/180) for RF factor. This showed no statistically significant difference between those with controlled and uncontrolled glucose levels.

4.2.1Rheumatoid Factor occurrence and Gender of the participants.

Females were one hundred and twenty one 67.2 %(121/180) while 32.8 %(59/180) were males. Among the 121 females 12.4 %(15/121) were RF positive while among the males 5.1 %(3/59) were positive. There was no statistical significant difference in the occurrence of Rheumatoid Factor and gender of the participants, though females were 2.6 times likely to have rheumatoid factor antibodies as compared to male participants (OR=2.64, CI=0.7335-9.51,P =0.137).

4.2.2 Rheumatoid Factor occurrence and age of participants

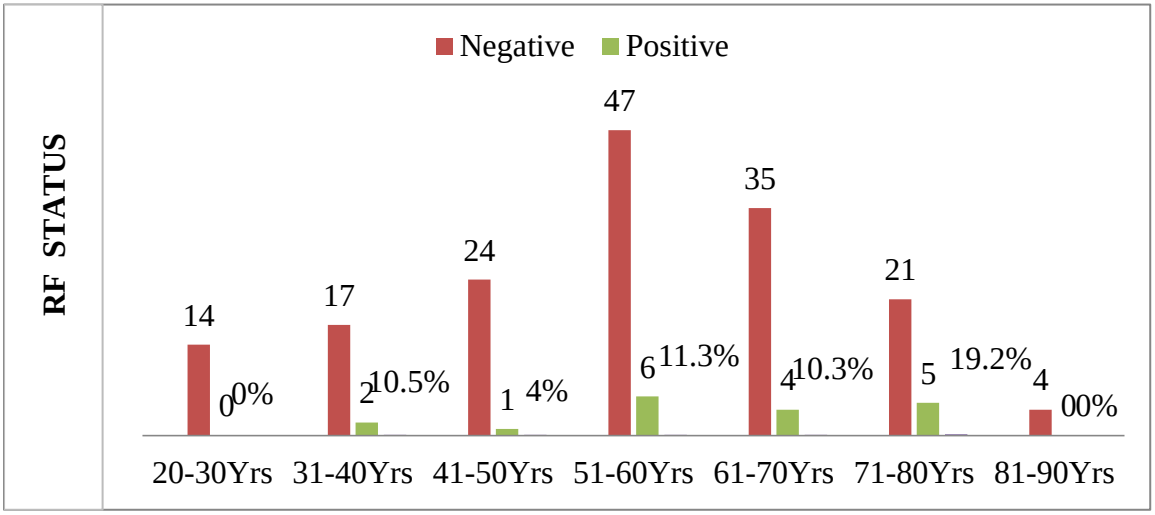


Figure 5: Showing the distribution of RF in different age groups.

The above figure shows the occurrence of RF among participants in relation to age. The most affected age group is 71-80 years of which 19.2 %(5/26) were positive for Rheumatoid Factor. Those below 30 years and above 81 years old did not have RF antibodies. On logistic regression to compare the age groups and RF occurrences, there was no statistically significant difference between age of the participants and the occurrences of Rheumatoid Factor (OR =1.3100, CI=0.9218- 1.8618, P= 0.1321).

Table 4.1.3: Showing logistic Regression of age groups and Rheumatoid Factor.

Term	Odds Ratio	95% C.I.	P-Value
Age (41-50)/ 31-40	*0.3547*	*0.0297-4.2290*	*0.4124*
Age (51-60)/ 31-40	*1.0851*	*0.1995-5.9026*	*0.9247*
Age (61-70)/ 31-40	*0.9714*	*0.1616-5.8390*	*0.9747*
Age (71-80) /31-40	*2.0239*	*0.3482-11.7646*	*0.4324*

The age groups 31-40 years was used as a baseline during logistic regression where there was no statistically significant difference between the age groups, although those aged 71-80 years had two times higher chances of having rheumatoid factor antibodies as compared to the younger age group of 31-40 years as shown in the table above (Table 4.1.3)

4.3 Serum levels of Rheumatoid Arthritis biomarkers in participants.

4.3.1Inflammatory marker Tenascin-C

The mean levels of TNC pro inflammatory marker in serum among the participants was 577pg/ml with standard deviation of 541pg/ml. The participants with controlled glucose 2.1 % (2/98) had normal levels of TNC and 97.9% elevated levels. The participants with uncontrolled glucose levels 4.9 %(4/82) had normal TNC levels while 95.1% had elevated levels. However there was no statistically significant difference between glucose levels and Tenascin C status (P=0.3057).

Table 4.2.2: Showing Tenascin-C status in relation to gender of the participants

GENDER	**TNC STATUS**	
	Normal	**Elevated**
MALE n =*59(32.8%)*	**1(**0.6%)	**58(32.2**%)
FEMALE *n=121(67.2%)*	**5(**2.8%)	**116(**64.4%)
TOTAL n =100%	**6(3.3%)**	**174(96.7%)**

About 96.7% (174/180) were TNC positive while 3.3 %(6/180) were negative. Among the males, 0.5 %(1/180) had normal TNC levels and 32.2% (58/180) had elevated TNC levels while in the Females 2.8% (5/180) had normal TNC levels with 95.9 %(116/121) having elevates TNC levels as shown in table 4.2.2 above. However there was no statistical significant difference between Tenascin C levels and gender of the participants. (P=0.4079, OR=0.4000, CI=0.0457-3.5035).

Table 4.2.3: Frequency of inflammatory marker (Tenascin-C) in relation to age groups of the participants

TNC Levels	Age groups						
	20-30yrs N=14	**31-40yrs N=19**	**41-50yrs N=25**	**51-60yrs n=53**	**61-70yrs n=39**	**71-80yrs n=26**	**81-90yrs n=4**
Mean levels (pg/ml)	*466.3*	*687.2*	*560.0*	*738.0*	*701.0*	*515.0*	*1123.0*
Normal Levels	1(7.1%)	1(5.3%	1(4.0%)0	1(1.9	0(0.0%)	2(7.7%)	0(0.0%)
Elevated levels	13(92.8%)	18(94.7%)	24(96.0%)	52(98.1%)	39(100%)	24(92.3%)	4(100%)

The Table above shows that among the 180 participants recruited in the study ,the age 51-60years old constituting 29.9% (53/180).The highest proportion of participants with elevated levels of Tenascin- C were ages 60-79yrs and 81-90 years (100%) while the least affected age group was 20-30 years with (92.8%)% (4/180) having elevated levels of TNC.. On logistic regression, there was no significant statistical difference between Tenascin C and age groups of the participants. (OR=1.1551, CI=0.6798 – 1.9629, P=0.5939).

The statistical analysis of the means age categories of the participants by linear regression showed significant difference in the means (p=0.03268).

4.4 Evaluation of serum Anti-CCP marker levels in the participants.

Anti-CCP was evaluated as a predictive marker that is highly specific of rheumatoid arthritis. Among the respondents about 17.8% (32/180) had elevated levels of Anti-CCP while 82.2 % (148/180) had normal Anti-CCP levels (Table 4.3.1). The mean serum level of Anti- CCP among the participants was 7.640 U/ml with a standard deviation of 11.8 U/ml. The mean for those participants with elevated serum levels of Anti–CCP was 21.9 U/ml, while those with normal serum levels of Anti- CCP were 4.5 U/ml in relation to the normal levels according to manufacturers' manual which indicated positives /elevated values as > 25u/ml.

Table 4.3.1: Anti-CCP status and gender of the participants

SEX	**Anti- CCP Status**	
	Normal	**Elevated**
Male n=*59(32.8%)*	48(26.6%)	11(6.1%)
Female n=*121(67.2%)*	100(67.6%)	21(11.7%)
Total *(100%)*	148(82.2%)	32(17.8%)

The mean levels of Anti-CCP antibodies in females were 8.1u/ml and in men was 6.8u/ml. A total of one hundred and forty eight participants 82.2% had normal levels of Anti-CCP and thirty two had elevated levels of Anti-CCP 17.8%. Among the participants with elevated levels of Anti-CCP, females constituted 11.7 %(21/180) and males 6.1 %(11/180).The status of Anti-CCP showed no statistical significant difference with the gender of the participants (P=0.8303, OR=0.9156, CI=0.4088 – 2.0508).

Table 4.3.2: Showing frequencies of Anti-CCP marker levels in age groups of participants.

Age Groups(N)	Anti-CCP Levels			Logistic regression analysis		
	Elevated Levels freq. (%)	Normal Levels	Mean Levels (U/ml)	Odds ratio	95% C.I.	P-Value
31-40Yrs=19	5(26.3)	14	*13.5*	*2.1429*	*0.3500-13.1207*	*0.4097*
41-50Yrs=25	6(24.0)	19	*6.1*	*1.8947*	*0.3273-10.9683*	*0.4756*
51-60Yrs=53	6(11.3)	47	*7.1*	*0.7660*	*0.1370-4.2830*	*0.7614*
61-70Yrs=39	7(17.9)	32	*6.6*	*1.3125*	*0.2384-7.2262*	*0.7547*
71-80Yrs=26	5(19.2)	21	*7.0*	*1.4286*	*0.2393-8.5276*	*0.6956*
81-90Yrs =4	1(25.0)	3	*11.2*	*2.0000*	*0.1326-30.1624*	*0.6166*

The Table (4.3.2) above shows that the age group with the highest levels of Anti-CCP specific biomarker was the age group 31- 40 years with 26.3%(5/19), while the least 51-60 years 11.3% (6/53). However, overall logistic regression of Anti-CCP status for the age categories of the respondents showed no significant statistical association between age groups and status of Anti-CCP (P=0.8321).On the other hand, the logistic regression analysis comparing the age groups either did not show any statistically significant difference between the groups as shown in the above (Table 4.3.2). However, those in the age groups 31-40yrs, 41-50yrs and 81-90yrs.Were 2.1, 1.9 and 2 times more likely to have raised levels of Anti- CCP respectively as compared to the baseline age group 20-30 years.

Table 4.4.1: Comparison of status of RF and Anti-CCP antibodies as RA biomarker

RF STATUS	Anti-CCP Status		
	Normal levels/negative n=148	**Elevated levels/positive n=**32	**Total participants n=**180
Negative	137(76.1%)	25(13.9%)	162(90%)
Positive	11(6.1%)	7(3.9%)	18(10%)
Total Anti-CCP	82.2%	17.8%	100%

The above table (4.4.1) shows that the positivity/status for RF was 10.0% while that of Anti-CCP was 17.8%. The participants who were both Anti-CCP negative and RF negative were 76.1 % (137), Anti-CCP positive and RF positive were 3.9 %(7), Anti-CCP positive and RF negative were 13.9 %(25) and finally the respondents with Anti-CCP negative and RF positive was 6.1 %(11).When logistic regression analysis was done; there was a significant statistical difference between RF and Anti-CCP status, at the same time, the participants who were RF negative showed 3.5 times more likely to be positive for Anti-CCP antibodies (OR=3.4884, CI=1.2342- 9.8598, P=0.0184).

Table 4.4.2: Comparison of RF and Tenascin -C antibodies status among participants

	TNC STATUS		
RF STATUS	**Negative** **n=**6	**Positive** **n=**174	**Total** **n=**180
Negative	6 (3.3%)	156 (86.7%)	162(90%)
Positive	0	18(10%)	18(10%)
Total %	(3.3%)	(96.7%)	(100%)

The above table (4.4.2) shows that all the participants positive for RF were also positive for Tenascin-C antibodies 96.7%(174), the participants who were TNC positive and RF negative were 86.7%(156), TNC negative as well as RF negative were 3.3%(6) and TNC negative with RF positive was none.. However on logistic regression analysis, there was no significant statistical difference seen between RF and TNC antibodies (P= 0.40628).

4.5: Comparison of positivity for rheumatoid arthritis biomarkers.

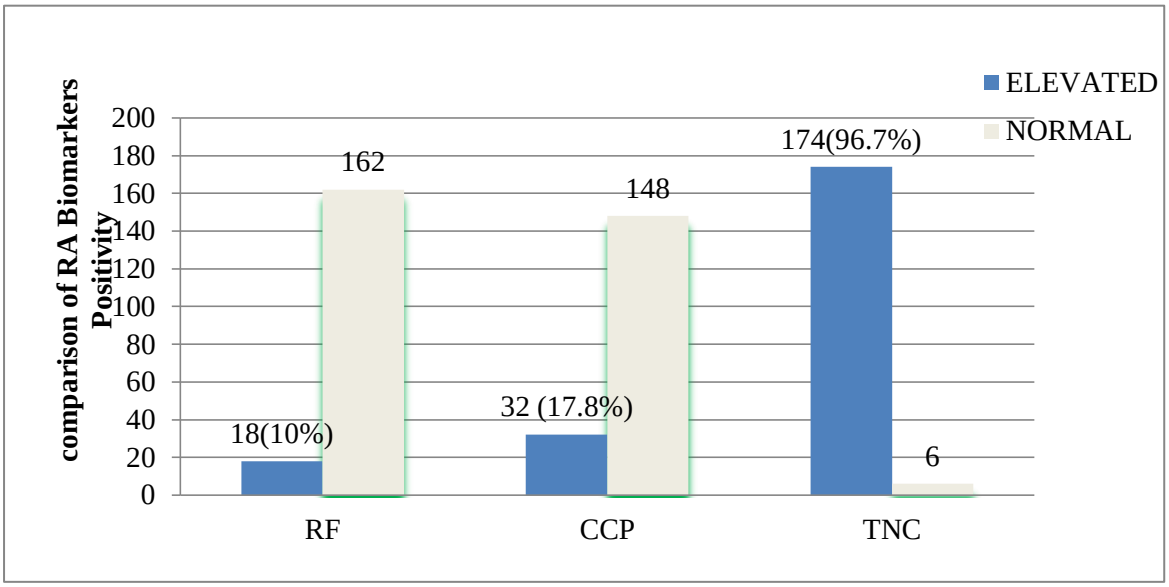


Figure 6: Showing Comparative positivity of Rheumatoid Arthritis markers among the participants (n=180)

Figure 6 above shows the comparative evaluation of RF, TNC and Anti-CCP antibodies positivity in general. TNC antibodies were elevated in all participants with positive Rheumatoid Factor (RF). However, Anti-CCP and RF antibodies were present in thirty two and eighteen participants (17.8% and 10.0%) respectively. All the three markers were simultaneously present in 3.9% (7/180) participants.

Among the RF negative participants, positivity for Anti-CCP antibodies increased to15.4% (25/162) upwards from 10.0 %(18) RF positive. Logistic regression analysis showed that there was statistically significant difference between RF and Anti-CCP levels, and that participants who were RF negative were about 3.5 times likely to be positive for Anti-CCP antibodies.(OR=3.4884, CI=1.2342- 9.8598, P=0.0184).

CHAPTER FIVE

5.0 DISCUSSION

5.1 Introduction

For decades, the diagnosis of RA has been primarily based on clinical manifestations due to lack of reliable alternative diagnostic tests. Approximately one third of the RA patients do not fulfill the ACR classification criteria, which makes the diagnosis of this disease difficult in the early stages (Kayshap *et al*., 2015). Adding to the problem is the fact that substantial irreversible joint damage occurs within the first 2 years by the time the diagnosis can be confirmed by radiological or Laboratory testing (Serdaroğlu *et al.*, 2008). This prompted this cross-sectional study which was designed to investigate the biomarkers of rheumatoid arthritis among adults above 18 years of age living with type II diabetes mellitus at Moi Teaching and Referral Hospital. The study documented the duration that the participants had lived with diabetes to be $\geq$ 8.4 years with their glucose levels either controlled or uncontrolled according to WHO reference normal levels of 11.1mmol/l.

This study also investigated other laboratory tests that can support in the prognostic management of Rheumatoid Arthritis as a co-morbid condition to type II diabetes mellitus. These assays done in this study included: detection of predictive Auto-antibodies such as the levels of pro-inflammatory circulating Tenascin-C glycoprotein, Anti-CCP which is the confirmatory biomarker to rheumatoid arthritis and Rheumatoid Factor a screening test.

The major findings of the current study discussed therefore are inclusive of demographic characteristics like age and gender, Occurrence of Rheumatoid Factor and serum levels/status of rheumatoid arthritis biomarkers (TNC and Anti-CCP).

5.2 Occurrence of Rheumatoid Factor among the diabetic participants.

This study established that the proportion of Rheumatoid Factor (RF) among the type II diabetes mellitus adults patients greater than 18 years enrolled in the study was 10% (18/180) of which females 83.3 %(15) and males were 16.7 %(3). RF has been widely used in practice as a serological marker for the diagnosis of rheumatoid arthritis. Even though ACR criteria included RF testing, its specificity is limited. To be precise, this showed that all the participants who had RF also experienced inflammations from undifferentiated conditions RF inclusive since RF can be detected in patients with other rheumatic diseases, infectious diseases, in 5% of healthy individuals and in 10-20% of persons aged above 65 years. (Farid *et al*., 2013).Current findings from this study are similar to a survey conducted by Silveira *et al.*, 2007 on out patients in Porto Alegre, Brazil that yielded a pre-test probability of RF of 17%. Their proportion included other connective tissue disorders (CTD), spondylo-arthropathies (SA), soft-tissue rheumatism including fibromyalgia and osteoarthritis (STR), non-rheumatologic autoimmune disorders (NRA), cancers (CA), and other non-classified diseases that tested positive for RF. However, these results differed to those by Raj S *et al*., (2014) in Kottayam, Kerala, India which reported an RA proportion of (24.4%) 47/192 among diabetes type 2 patients. Further, the current results contrasted a report of a study conducted by Centre for disease control (CDC) in US which showed high the prevalence of arthritis among adults diagnosed with diabetes to be approximately 52.0 % (CDC. 2008) as compared to the general worldwide RA prevalence of 1 -2% as reported by (Rudan., *et al* 2015). Again, another high proportion of RF prevalence was shown by a study in Atlanta Georgia on adults with Diabetes mellitus which found around 48.1% RF sero-positivity. Cheung *et al*., (2012). However, a low occurrence in RF was found in an investigation on

Danish population at Copenhagen University Hospital which revealed 4.3% sero-positive RF in a proportion of non-rheumatoid arthritis populations as reported by Nielsen *et al.,* (2012). Further, low proportions of 2.2% diabetes co morbid RA was reported by Bartels et al., 2012 while monitoring patients with or without RA in a US based Medicare study. Therefore these differences could be related to the fact that Rheumatoid factor (RF) can be found in many undifferentiated forms of diseases other than rheumatoid arthritis, variation in medical practices and care by the participants as well as variability of prevalence in environmental and genetic risk factors.

The mean age of the participants in this study was 55.2 years with the youngest being 20 years and the oldest 89 years. The results of the current study indicated that participants with diabetes and RA were older than 31 years with the majority in between 50 and 80 years at 83.3%. This is in agreement with a study done by Lee *et al*., 2003 in Boston USA which found the occurrence of RF to be high among those in the ages of 40 to 70 years. It also concurred with the report done by Bizzaro *et al*., (2013) in Italy who found the mean age to be 52 (SD =16) years and majority were in 40 to 70 years age category. Another study conducted by Kashyap *et al*., (2015) in new Delhi, India also found that most of the patients belonged to 21‑ 50 years age group (70.59%).Similarly, study by Symmons, (2002) on incidence and prevalence of RA in UK showed that RA was rare in both men and women before the age of 35 but a peak prevalence was found in the 55- to 64-yr age group for both genders.

However, another study by Elshafie *et al*., 2016 comparing Sudanese and the Swedish populations, found the majority of the respondent's age for RA to be 41–45year in females and 46–50 years in males though these ages seemed younger than the age found in this study hence contrasted with the findings of the current study. It is well

known that the risk of developing arthritis increases with age just as is the risk of type 2 diabetes mellitus increases with age especially after age of 45 years; probably because people tend to exercise less, decreasing muscle mass, weight gain as they age and increased parity among women.

This study established the **gender** proportions of female participants as 67.2 %(121/180) while that of men to be 32.8 %(59/180). It documented that females were more affected by RF than males. The positivity among the females to males was 12.4%(15/121) and 5.1%(3/59) respectively giving a ratio of 2:1 and that females had about 2.6 more chances of having RF as compared to males. This result is comparable with a study in India with a ratio of 1:3 Kashyap *et al.,* (2015), a worldwide ratio of 1:2.5 as by Lee *et al.,* (2001), and a study by Elshafie, *et al*.,(2016) on Sudanese and Swedish populations 1:3.Further, it also agreed with a study done in Northeast of European patients with RA: Finland 1:2.5, Denmark 1:3, and the Netherlands 1:2 respectively by Sokka *et* al.,(2009).However, the ratios contrasted with a study done by Ouédraogo *et al*., (2011) in Burkina Faso who reported male to female ratio of 1:4, Egypt 1:6 by Abdel-Nasser *et al* (2008), Cameroon1:19 by Singwe-Ngandeu *et al.,* (2010), and Senegal 1:10 by Lekpa *et al.,* (2012). The higher occurrence of RF in females could be associated hormonal changes during lactation and pregnancy, therapy encountered during menopause and lifestyle changes. (Intriago *et al.,* 2019).

5.3 Serum levels of Rheumatoid Arthritis markers

The study analyzed possible associations of both the positivity and serum concentrations of RF, TNC anti-CCP antibodies as RA biomarkers. These markers could be handy as to disease stratification and could lead to the transformation in the management of RA in diabetics including but not limited to early diagnosis, identifying the patients at risk in their early stages, assessment, prediction of disease severity, selection of therapy, and monitoring of response to therapy Maecker *et al.*, (2012**)**.

5.3.1Tenascin C antibodies evaluated in the participants.

This study determined the serum levels of circulating TNC antibodies which is a pro-inflammatory glycoprotein. The overall mean level of TNC found in the study population was577pg/ml. About 96.7% of the participant had elevated levels showing that most of the participants had pro-inflammatory reactions. Among the males about 98.3% (58/59) and 95.9% (116/121) females had elevated levels respectively. However, logistic regression showed no significant statistical difference of TNC between the gender (OR=0.4000, CI=0.0457-3.5035, P=0.4079). The results of this study are consistent with a study in United Kingdom which found higher circulating levels of Tenascin-C (TNC) with a mean of 118.2ng/ml, p= < 0.0001 and 82.5%positivity (Page *et al.,* 2012). Further, another study in Birmingham, UK in early arthritis patients reported Anti-TNC levels higher with a mean of 193.1±449.8 pg/ml with a positivity of 81.3% (Raza *et al*., 2016).The elevated TNC levels in diabetes patients could be as a result of a wide range of inflammatory conditions, all of which are associated with chronic arthritis or not since pro inflammatory mediators can interact with one another and the magnitude of interaction increases as the level of pro inflammatory mediators increase. Chung *et al*., (2019)

It has been shown that Raised serum TNC is not directly associated with RA. Raised serum levels of TNC could be manifested in diseases with unknown arthritic or rheumatic origin which can be cryptogenic pneumonia, cardiovascular diseases such as myocardial infarction and dilated cardiomyopathy Terasaki *et al*., (2007). These results suggested that high serum TNC levels recorded could have been due to tissue dysfunction, metabolic syndrome, immune dysfunction, and neuronal problems Michaud *et al., (*2013).

5.3.2Anti-citrullinated peptide/protein antibodies (Anti-CCP):

The study established that Antibodies to citrullinated proteins in the serum samples of one hundred and eighty participants recruited and analyzed to be 17.8% (n=32) positive. It also indicated that the mean serum concentration among participants with elevated antibodies to CCP was 21.9 (SD± 23u/ml) higher than normal mean levels of 4.5(SD=2.2u/ml). These results are comparable to a study in Amsterdam, Netherlands by Ursum *et al*., (2010) which showed higher positivity of Anti-CCP 56% than IgM-RF 47% at the baseline of their study. These findings are also consistent to a study conducted among the undifferentiated arthritis (UA) in Italy by Bizzaro *et al*.,(2013) report which depicted positivity of RF to Anti-CCP as 41.4% and 41.8% respectively with a higher levels in serum.

However these current results, contrasted a study done in Taiwan among the patients which reported high Anti-CCP concentration of 198.2 (SD=175.7) U/ml with 10.4 - 40.0 I U/ml, among normal group with $p< 0.001$. Chang *et al*., (2016).The variations in the concentrations of Anti-CCP levels from studies could possibly be due to validation in methods of testing, populations of study as either confirmed rheumatoid arthritis or polyarthritis with or without other co-morbidities.

5.3.3 Comparison of RF and anti-CCP positivity

The results of this current study showed that those who were both Anti-CCP and RF negative were 92.6%(137), Anti-CCP positive and RF positive were 21.9%(7), Anti-CCP positive and RF negative were 78.1%(25) and finally the respondents with Anti-CCP negative and RF positive was 7.4%(11) with significant statistical association (P=0.0184 O,R=3.4884, CI=1.2342- 9.8598).Those RF negative participants showed about 3.5 times likely to be positive for Anti-CCP antibodies. These results are in agreement with the study in Hungary where significant correlation between RF and Anti-CCP showed P < 0.01) by Kapitany *et al., (*2008) with Anti-CCP antibodies with 56% both RF and Anti-CCP positive. However, it is also comparable to study conducted by Kashyap *et al.*, 2015 which reported 5.9% and 14.7% RF and Anti-CCP positivity respectively. In agreement with other studies, but unlike popular perception, RF had a good sensitivity and specificity, giving useful positive and negative predictive values. However, anti-CCP had equal sensitivity and much better specificity and was, thus, even more useful. Combining anti-CCP and RF results using the cutoffs recommended by the manufacturers yielded higher diagnostic power than either by itself, both for the double positive and double-negative groups.

Unfortunately, a study in Ontario Best Practices Research Initiative (OBRI) showed low proportions compared to the study. It reported 27% (Anti-CCP negative/ RF negative), 6.2 %(Anti-CCP positive /RF negative), 12.1% (Anti-CCP negative /RF positive) and 54.7% (Anti-CCP positive / RF positive) with a statistical significant difference p=0.02 Pope *et al.,* (2018).The reason for this discrepancy may be due to differences in patient selection and whether the patients had received treatment or not. Again most of the literature is extracted from a defined RA population unlike this current study with population of diabetic participants with or without RA.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1Conclusion

The proportion of participants T2DM with RF antibodies in the study was 10%. The most affected age group with higher occurrence of Rheumatoid Factor was 71-.80 years. It is well known that the risk of developing arthritis increases with age just as the risk of type 2 diabetes mellitus increases with age especially after age of 45 years when people tend to lead laxity life style, probably because people tend to exercise less, decreasing muscle mass, weight gain as they age and increased parity among women.

The females were more likely to have RF antibodies than males in the study. This higher occurrence of RF in females could be associated with hormonal changes during lactation and pregnancy or therapy encountered during menopause as well as lifestyle changes which can be associated to urbanization.

Most of the participants regardless of gender had their glucose levels controlled however; those with uncontrolled glucose levels had higher proportion of RF positives occurrences. This showed that coexistence of the two conditions leads to difficulty in the management of glucose levels.

On the other hand, when the predictive biomarkers were analyzed, most of the participants had raised levels of antibodies to Tenascin C which is a pro-inflammatory marker hence was not directly associated to Rheumatoid Arthritis. It came out clearly as a general pro inflammatory marker which can be found in almost all chronic metabolic diseases inclusive of diabetes. The Increased levels in TNC antibodies could have resulted from a wide range of inflammatory diseases causing the immune

reactions such as cardiovascular diseases, cryptogenic pneumonia, Tumors or other autoimmune diseases which are undifferentiated.

This study showed that anti-CCP antibodies were more sensitive and specific diagnostic marker of RA than the other parameters analyzed for the diagnosis of RA during the study. The proportions that were positive to Anti CCP were 17.8% this was higher than 10% RF positive rated. The variations in the concentrations of Anti-CCP levels from studies could possibly be due to validation in methods of testing, populations of study as either confirmed rheumatoid arthritis or polyarthritis with or without other co-morbidities. Another difference could have emerged from the different treatment regimens given to the participants. In summary, combined detection of the three parameters can be beneficial when confirming a diagnosis of RA among the people living with diabetes mellitus.

6.2 Recommendation

Patients with RA showed considerable variability in disease activity making it difficult to predict the onset of the disease. Further research needs to be done on Tenascin-C specifically in terms of genetical composition to clearly bring out the exact part of the domain that does elicit its pro- inflammatory reactions since it has several domain binding receptors. Therefore the diagnostic and prognostic genetically established biomarkers will have the potential to benefit patients, by enabling precise and timely diagnosis of conditions and selection of the most appropriate therapy.

Other biomarkers need to be redefined further in the general population to attain sensitivity and specificity sufficient for the accurate identification of individuals who will develop Rheumatoid arthritis early enough, to facilitate implementation of appropriate intervention for better management since the population studied in this current research was small and specific(T2DM).

In the event that the patient benefits from the same, stratified medicine through clinical trials and drug developers also can have a share by enriching cohorts in clinical trials with patients likely to respond to therapeutic, prognostic biomarkers hence a reduction on the trial size required to determine therapeutic efficacy.

Replication and validation of biomarkers in prospective clinical trials will be of paramount importance, as it will proof their impact on management of disease and utilization of health-care resources. From this research we anticipate that a paramount clinical trial and enabling technologies based on biomarkers will increasingly take a leading role in the practice of medicine especially in the north rift region of Kenya.

6.3 Limitations

- Investigations and analysis on various therapies/drugs given for the management of patients with type II diabetes mellitus could not be done due to inability of some patients to remember the names of their drugs.
- The cross-sectional design restricted the analysis of an relationship between the two diseases hence it was impossible address disease incidence either temporarily, or causality since the prevalence of both RA and diabetes depends upon disease incidence and duration.

6.4 The Study strength

The inclusion of participants from various geographical areas is of benefit to the study for the generation of more information since the data collected contained both urban and rural setup.

REFERENCES

Abdel-Nasser, A. M., Mahmoud, M. H., El Mansoury, T. M., & Osman, A. M. (2008). Anti-CCP2 is an adjunct to, not a surrogate for, rheumatoid factor in the diagnosis of rheumatoid arthritis: diagnostic utility of anti-CCP2 antibodies in Egyptian patients with rheumatoid arthritis. *Scandinavian journal of rheumatology*, *37*(5), 329-336.

Aggarwal, R., Liao, K., Nair, R., Ringold, S., & Costenbader, K. H. (2009). Anti-citrullinated peptide antibody (ACPA) assays and their role in the diagnosis of rheumatoid arthritis. *Arthritis and rheumatism*, *61*(11), 1472.

Alamanos, Y., & Drosos, A. A. (2005). Epidemiology of adult rheumatoid arthritis. *Autoimmunity reviews*, *4*(3), 130-136.

Aletaha, D., Neogi, T., Silman, A. J., Funovits, J., Felson, D. T., Bingham III, C. O., ... & Combe, B. (2010). 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis & rheumatism, 62(9), 2569-2581.

Alkaabi, J. K., Ho, M., Levison, R., Pullar, T., & Belch, J. J. F. (2003). Rheumatoid arthritis and macrovascular disease. *Rheumatology*, *42*(2), 292-297.

American Diabetes Association. (2013). Standards of medical care in diabetes—2013. *Diabetes care*, *36*(Supplement 1), S11-S66.

American Diabetes Association. (2014). Standards of medical care in diabetes--2014. *Diabetes care*, *37*, S14-S80.

Arnett, F. C., Edworthy, S. M., Bloch, D. A., Mcshane, D. J., Fries, J. F., Cooper, N. S., ... & Hunder, G. G. (1988). The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology*, *31*(3), 315-324.

Atlas, D. (2015). International diabetes federation. *IDF Diabetes Atlas, 7th edn. Brussels, Belgium: International Diabetes Federation*.

Banal, F., Dougados, M., Combescure, C., & Gossec, L. (2009). Sensitivity and specificity of the American College of Rheumatology 1987 criteria for the diagnosis of rheumatoid arthritis according to disease duration: a systematic literature review and meta-analysis. *Annals of the rheumatic diseases*, *68*(7), 1184-1191.

Bartels, C. M., Saucier, J. M., Thorpe, C. T., Kind, A. J., Pandhi, N., Hansen, K. E., & Smith, M. A. (2012). Monitoring diabetes in patients with and without rheumatoid arthritis: a Medicare study. *Arthritis research & therapy*, *14*(4), 1-9.

Berglin, E., Kokkonen, H., Einarsdottir, E., Ågren, Å., & Rantapää Dahlqvist, S. (2010). Influence of female hormonal factors, in relation to autoantibodies and genetic markers, on the development of rheumatoid arthritis in northern Sweden: a case–control study. *Scandinavian journal of rheumatology*, *39*(6), 454-460.

Bizzaro, N., Bartoloni, E., Morozzi, G., Manganelli, S., Riccieri, V., Sabatini, P., ... & Gerli, R. (2013). Anti-cyclic citrullinated peptide antibody titer predicts time to rheumatoid arthritis onset in patients with undifferentiated arthritis: results from a 2-year prospective study. *Arthritis research & therapy*, *15*(1), 1-9.

Centers for Disease Control and Prevention (CDC. (2008). Arthritis as a potential barrier to physical activity among adults with diabetes--United States, 2005 and 2007. *MMWR. Morbidity and mortality weekly report*, *57*(18), 486-489.

Chege, M. P. (2010). Risk factors for type 2 diabetes mellitus among patients attending a rural Kenyan hospital. *African journal of primary health care & family medicine*, *2*(1).

Cheng, Y. J., Imperatore, G., Caspersen, C. J., Gregg, E. W., Albright, A. L., & Helmick, C. G. (2012). Prevalence of diagnosed arthritis and arthritis-attributable activity limitation among adults with and without diagnosed diabetes: United States, 2008–2010. *Diabetes Care*, *35*(8), 1686-1691.

Cheung, B. M., & Li, C. (2012). Diabetes and hypertension: is there a common metabolic pathway?. *Current atherosclerosis reports*, *14*(2), 160-166.

Chung, H. Y., Kim, D. H., Lee, E. K., Chung, K. W., Chung, S., Lee, B., ... & Yu, B. P. (2019). Redefining chronic inflammation in aging and age-related diseases: proposal of the senoinflammation concept. *Aging and disease*, *10*(2), 367.

Costenbader, K. H., Feskanich, D., Mandl, L. A., & Karlson, E. W. (2006). Smoking intensity, duration, and cessation, and the risk of rheumatoid arthritis in women. *The American journal of medicine*, *119*(6), 503-e1.

Dall, T. M., Yang, W., Halder, P., Pang, B., Massoudi, M., Wintfeld, N., ... & Hogan, P. F. (2014). The economic burden of elevated blood glucose levels in 2012: diagnosed and undiagnosed diabetes, gestational diabetes mellitus, and prediabetes. *Diabetes care*, *37*(12), 3172-3179.

Davis, L. A., Whitfield, E., Cannon, G. W., Wolff, R. K., Johnson, D. S., Reimold, A. M., ... & Caplan, L. (2014). Association of rheumatoid arthritis susceptibility gene with lipid profiles in patients with rheumatoid arthritis. *Rheumatology*, *53*(6), 1014-1021.

de Vries, R. (2011). Genetics of rheumatoid arthritis: time for a change!. *Current opinion in rheumatology*, *23*(3), 227-232.

Del Rincon, I. D. (2003). Arthritis Rheum 2001; Watson DJ. *Journal of Rheumatology*.

Dobiasˇova, M. (2004). Atherogenic index of plasma [log (triglycerides/HDL-cholesterol)]: theoretical and practical implications.

Doran, M. (2007). Rheumatoid arthritis and diabetes mellitus: evidence for an association?. *The Journal of rheumatology*, *34*(3), 460-462.

Elshafie, A. I., Elkhalifa, A. D., Elbagir, S., Aledrissy, M. I., Elagib, E. M., Nur, M. A., ... & Rönnelid, J. (2016). Active rheumatoid arthritis in Central Africa: a comparative study between Sudan and Sweden. *The Journal of rheumatology*, *43*(10), 1777-1786.

Farid, S. S., Azizi, G., & Mirshafiey, A. (2013). Anti-citrullinated protein antibodies and their clinical utility in rheumatoid arthritis. *International journal of rheumatic diseases*, *16*(4), 379-386.

Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical chemistry*, *18*(6), 499-502.

Georgiadis, A. N., Papavasiliou, E. C., Lourida, E. S., Alamanos, Y., Kostara, C., Tselepis, A. D., & Drosos, A. A. (2006). Atherogenic lipid profile is a feature characteristic of patients with early rheumatoid arthritis: effect of early treatment–a prospective, controlled study. *Arthritis research & therapy*, *8*(3), 1-7.

Gibofsky, A. (2012). Overview of epidemiology, pathophysiology, and diagnosis of rheumatoid arthritis. *The American journal of managed care*, *18*(13 Suppl), S295-302.

Goh, F. G., Piccinini, A. M., Krausgruber, T., Udalova, I. A., & Midwood, K. S. (2010). Transcriptional regulation of the endogenous danger signal tenascin-C: a novel autocrine loop in inflammation. *The Journal of Immunology*, *184*(5), 2655-2662.

Gregersen, P. K., Silver, J., & Winchester, R. J. (1987). The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology*, *30*(11), 1205-1213.

Guthrie, K. A., Dugowson, C. E., Voigt, L. F., Koepsell, T. D., & Nelson, J. L. (2010). Does pregnancy provide vaccine-like protection against rheumatoid arthritis?. *Arthritis & Rheumatism*, *62*(7), 1842-1848.

Han, C., Robinson, D. W., Hackett, M. V., Paramore, L. C., Fraeman, K. H., & Bala, M. V. (2006). Cardiovascular disease and risk factors in patients with rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis. *The Journal of rheumatology*, *33*(11), 2167-2172.

Ikewuchi, C. C. (2009). Alteration of plasma lipid profiles and atherogenic indices by Stachytarpheta jamaicensis L.(Vahl). *Biokemistri*, *21*(2).

Ikewuchi, C. C. (2009). Alteration of plasma lipid profiles and atherogenic indices by Stachytarpheta jamaicensis L.(Vahl). *Biokemistri*, *21*(2).

Intriago, M., Maldonado, G., Cárdenas, J., & Ríos, C. (2019). Clinical characteristics in patients with rheumatoid arthritis: differences between genders. *The Scientific World Journal*, *2019*.

John, H., Kitas, G., Toms, T., & Goodson, N. (2009). Cardiovascular co-morbidity in early rheumatoid arthritis. *Best Practice & Research Clinical Rheumatology*, *23*(1), 71-82.

Kaduka, L. U., Kombe, Y., Kenya, E., Kuria, E., Bore, J. K., Bukania, Z. N., & Mwangi, M. (2012). Prevalence of metabolic syndrome among an urban population in Kenya. *Diabetes care*, *35*(4), 887-893.

Kapitány, A., Szabó, Z., Lakos, G., Aleksza, M., Vegvari, A., Soos, L., ... & Szekanecz, Z. (2008). Associations between serum anti-CCP antibody, rheumatoid factor levels and HLA-DR4 expression in Hungarian patients with rheumatoid arthritis. *The Israel Medical Association journal*, *10*(1), 32.

Karlson, E. W., Mandl, L. A., Hankinson, S. E., & Grodstein, F. (2004). Do breast-feeding and other reproductive factors influence future risk of rheumatoid arthritis?: Results from the Nurses' Health Study. *Arthritis & Rheumatism*, *50*(11), 3458-3467.

Karlson, E. W., Shadick, N. A., Cook, N. R., Buring, J. E., & Lee, I. M. (2008). Vitamin E in the primary prevention of rheumatoid arthritis: the Women's Health Study. Arthritis Care & Research, *59*(11), 1589-1595.

Kashyap, B., Tiwari, U., Garg, A., & Kaur, I. (2015). Diagnostic utility of anti-CCP antibodies and rheumatoid factor as inflammatory biomarkers in comparison with C-reactive protein and TNF-a in rheumatoid arthritis. *Tropical Journal of Medical Research*, *18*(1), 5-5.

Kerner, W., & Brückel, J. (2014). Definition, classification and diagnosis of diabetes mellitus. *Experimental and Clinical Endocrinology & Diabetes*, *122*(07), 384-386.

Klareskog, L., Stolt, P., Lundberg, K., Källberg, H., Bengtsson, C., Grunewald, J., ... & Alfredsson, L. (2006). A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA–DR (shared epitope)–restricted immune reactions to autoantigens modified by citrullination. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology*, *54*(1), 38-46.

Klareskog, L., Widhe, M., Hermansson, M., & Rönnelid, J. (2008). Antibodies to citrullinated proteins in arthritis: pathology and promise. *Current opinion in rheumatology*, *20*(3), 300-305.

Lee, D. M., & Schur, P. H. (2003). Clinical utility of the anti-CCP assay in patients with rheumatic diseases. *Annals of the rheumatic diseases*, *62*(9), 870-874.

Lee, W., & Weisman, M. H. (2006). The predictive power of anti-cyclic citrullinated peptide antibodies: window into understanding gene/environment/immunity interactions. *The Journal of rheumatology*, *33*(7), 1216-1218.

Lekpa, F. K., Ndongo, S., Tiendrebeogo, J., Ndao, A. C., Daher, A., Pouye, A., ... & Diop, T. M. (2012). Rheumatoid arthritis in Senegal: a comparison between patients coming from rural and urban areas, in an urban tertiary health care center in Senegal. *Clinical rheumatology*, *31*(11), 1617-1620.

Liao, K. P., Gunnarsson, M., Källberg, H., Ding, B., Plenge, R. M., Padyukov, L., ... & Alfredsson, L. (2009). A specific association exists between type 1 diabetes and anti-CCP positive rheumatoid arthritis. *Arthritis and rheumatism*, *60*(3), 653.

Lindstrom, T. M., & Robinson, W. H. (2010). Biomarkers for rheumatoid arthritis: making it personal. *Scandinavian Journal of Clinical and Laboratory Investigation*, *70*(sup242), 79-84.

MacGregor, A. J., Snieder, H., Rigby, A. S., Koskenvuo, M., Kaprio, J., Aho, K., & Silman, A. J. (2000). Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology*, *43*(1), 30-37.

Maecker, H. T., Lindstrom, T. M., Robinson, W. H., Utz, P. J., Hale, M., Boyd, S. D., ... & Fathman, C. G. (2012). New tools for classification and monitoring of autoimmune diseases. *Nature Reviews Rheumatology*, *8*(6), 317-328.

Mediwake, R., Isenberg, D. A., Schellekens, G. A., & Van Venrooij, W. J. (2001). Use of anti-citrullinated peptide and anti-RA33 antibodies in distinguishing erosive arthritis in patients with systemic lupus erythematosus and rheumatoid arthritis. *Annals of the rheumatic diseases*, *60*(1), 67-68.

Michaud, M., Balardy, L., Moulis, G., Gaudin, C., Peyrot, C., Vellas, B., ... & Nourhashemi, F. (2013). Proinflammatory cytokines, aging, and age-related diseases. *Journal of the American Medical Directors Association*, *14*(12), 877-882.

Michaud, M., Balardy, L., Moulis, G., Gaudin, C., Peyrot, C., Vellas, B., ... & Nourhashemi, F. (2013). Proinflammatory cytokines, aging, and age-related diseases. *Journal of the American Medical Directors Association*, *14*(12), 877-882.

Mugenda, O. M., & Mugenda, A. G. (1999). *Research methods: Quantitative and qualitative approaches*. Acts press.

Myasoedova, E., Crowson, C. S., Kremers, H. M., Roger, V. L., Fitz-Gibbon, P. D., Therneau, T. M., & Gabriel, S. E. (2011). Lipid paradox in rheumatoid arthritis: the impact of serum lipid measures and systemic inflammation on the risk of cardiovascular disease. *Annals of the rheumatic diseases*, *70*(3), 482-487.

Nielen, M. M., van Schaardenburg, D., Reesink, H. W., Van de Stadt, R. J., van der Horst-Bruinsma, I. E., de Koning, M. H., ... & Dijkmans, B. A. (2004). Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology*, *50*(2), 380-386.

Nielsen, S. F., Bojesen, S. E., Schnohr, P., & Nordestgaard, B. G. (2012). Elevated rheumatoid factor and long term risk of rheumatoid arthritis: a prospective cohort study. *Bmj*, *345*.

Nieves, M. P. (2015). *The Association between Rheumatoid Arthritis and Type 2 Diabetes Mellitus* (Doctoral dissertation, Walden University).

Nishimura, K., Sugiyama, D., Kogata, Y., Tsuji, G., Nakazawa, T., Kawano, S., ... & Kumagai, S. (2007). Meta-analysis: diagnostic accuracy of anti–cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis. *Annals of internal medicine*, *146*(11), 797-808.

Onat, A., Can, G., Kaya, H., & Hergenç, G. (2010). "Atherogenic index of plasma"(log10 triglyceride/high-density lipoprotein− cholesterol) predicts high blood pressure, diabetes, and vascular events. *Journal of clinical lipidology*, *4*(2), 89-98.

Ouédraogo, D. D., Singbo, J., Diallo, O., Sawadogo, S. A., Tiéno, H., & Drabo, Y. J. (2011). Rheumatoid arthritis in Burkina Faso: clinical and serological profiles. *Clinical rheumatology*, *30*(12), 1617-1621.

Page, T. H., Charles, P. J., Piccinini, A. M., Nicolaidou, V., Taylor, P. C., & Midwood, K. S. (2012). Raised circulating tenascin-C in rheumatoid arthritis. *Arthritis research & therapy*, *14*(6), 1-9.

Palazzi, C., Buskila, D., D'Angelo, S., D'Amico, E., & Olivieri, I. (2012). Autoantibodies in patients with chronic hepatitis C virus infection: pitfalls for the diagnosis of rheumatic diseases. *Autoimmunity reviews*, *11*(9), 659-663.

Plenge, R. M., Cotsapas, C., Davies, L., Price, A. L., De Bakker, P. I., Maller, J., ... & Altshuler, D. (2007). Two independent alleles at 6q23 associated with risk of rheumatoid arthritis. *Nature genetics*, *39*(12), 1477-1482.

Pope, J. E., Movahedi, M., & Rampakakis, E. (2019). ACPA and RF as predictors of sustained clinical remission in patients with rheumatoid arthritis: data from the Ontario Best practices Research Initiative (OBRI)(vol 4, e000738, 2018). *RMD OPEN*, *5*(2).

Prete, P. E., & Gurakar-Osborne, A. (1997). The contribution of synovial fluid lipoproteins to the chronic synovitis of rheumatoid arthritis. *Prostaglandins*, *54*(4), 689-698.

Raj, S., Vijayan, R., Rajan, G. V., & Sugathan, R. (2014). Relationship of rheumatoid factor positivity to prevalence of joint manifestations in type 2 diabetes which are unrelated to rheumatoid arthritis. *International Journal of Research in Medical Sciences*, *2*(2), 489.

Rantapää-Dahlqvist, S., De Jong, B. A., Berglin, E., Hallmans, G., Wadell, G., Stenlund, H., ... & van Venrooij, W. J. (2003). Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis & Rheumatism*, *48*(10), 2741-2749.

Rantapää-Dahlqvist, S., De Jong, B. A., Berglin, E., Hallmans, G., Wadell, G., Stenlund, H., ... & van Venrooij, W. J. (2003). Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis & Rheumatism*, *48*(10), 2741-2749.

Raza, K., Schwenzer, A., Juarez, M., Venables, P., Filer, A., Buckley, C. D., & Midwood, K. S. (2016). Detection of antibodies to citrullinated tenascin-C in patients with early synovitis is associated with the development of rheumatoid arthritis. *RMD open*, *2*(2).

Roy, A., Dubey, A., Hijam, D., Arunkumar, N., Prabita, O., Das, S., ... & Devi, T. I. (2016). Study of Serum Lipid Profile and Vitamin E in Rheumatoid Arthritis. *Annals of International Medical and Dental Research*, *1*(3), 221.

Rudan, I., Sidhu, S., Papana, A., Meng, S. J., Xin–Wei, Y., Wang, W., ... & Global Health Epidemiology Reference Group (GHERG. (2015). Prevalence of rheumatoid arthritis in low–and middle–income countries: A systematic review and analysis. *Journal of global health*, *5*(1).

Sadeghi Mazidi A, Kharazmi E, Javanbakht M, Heidari A, Bayati M.(2012)Economics cost of diabetes in type II diabetic patients.

Santos-Altamirano, A., Rodríguez-Falcón, M., Meaney, A., Izaguirre-Gutiérrez, F., Velázquez, H., Rubio-Gayosso, I., ... & Meaney, E. (2015). Atherosclerotic risk in middle-aged women with rheumatoid arthritis. *Revista mexicana de cardiología*, *26*(2), 70-77.

Sattar, N., & McInnes, I. B. (2005). Vascular comorbidity in rheumatoid arthritis: potential mechanisms and solutions. *Current opinion in rheumatology*, *17*(3), 286-292.

Sattar, N., McCarey, D. W., Capell, H., & McInnes, I. B. (2003). Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis. *Circulation*, *108*(24), 2957-2963.

Schellekens, G. A., Visser, H., De Jong, B. A., Van Den Hoogen, F. H., Hazes, J. M., Breedveld, F. C., & Van Venrooij, W. J. (2000). The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology*, *43*(1), 155-163.

Schett, G., & Firestein, G. S. (2010). Mr Outside and Mr Inside: classic and alternative views on the pathogenesis of rheumatoid arthritis. *Annals of the rheumatic diseases*, *69*(5), 787-789.

Schwenzer, A., Jiang, X., Mikuls, T. R., Payne, J. B., Sayles, H. R., Quirke, A. M., ... & Midwood, K. S. (2016). Identification of an immunodominant peptide from citrullinated tenascin-C as a major target for autoantibodies in rheumatoid arthritis. *Annals of the rheumatic diseases*, *75*(10), 1876-1883.

Scott, D. L., & Wolfe, F. (2010). Huizinga TW. *Rheumatoid arthritis. Lancet*, *376*, 1094-108.

Serdaroğlu, M., Çakırbay, H., Değer, O., Cengiz, S., & Kul, S. (2008). The association of anti-CCP antibodies with disease activity in rheumatoid arthritis. *Rheumatology international*, *28*(10), 965.

Silman, A. J., & Pearson, J. E. (2002). Epidemiology and genetics of rheumatoid arthritis. *Arthritis research & therapy*, *4*(3), 1-8.

Silveira, I. G., Burlingame, R. W., von Mühlen, C. A., Bender, A. L., & Staub, H. L. (2007). Anti-CCP antibodies have more diagnostic impact than rheumatoid factor (RF) in a population tested for RF. *Clinical rheumatology*, *26*(11), 1883-1889.

Simard, J. F., & Mittleman, M. A. (2007). Prevalent rheumatoid arthritis and diabetes among NHANES III participants aged 60 and older. *The Journal of rheumatology*, *34*(3), 469-473.

Singer, J. M., & Plotz, C. M. (1958). Slide latex fixation test: a simple screening method for the diagnosis of rheumatoid arthritis. *Journal of the American Medical Association*, *168*(2), 180-181.

Singwe-Ngandeu, M., Finckh, A., Bas, S., Tiercy, J. M., & Gabay, C. (2010). Diagnostic value of anti-cyclic citrullinated peptides and association with HLA-DRB1 shared epitope alleles in African rheumatoid arthritis patients. *Arthritis research & therapy*, *12*(2), 1-7.

Situnayake, R. and G. Kitas (1997)Dyslipidaemia and rheumatoid arthritis. Annals of the rheumatic diseases,56(6),341-342.

Situnayake, R. D., & Kitas, G. (1997). Dyslipidaemia and rheumatoid arthritis. *Annals of the Rheumatic Diseases*, *56*(6), 341-342.

Sokka, T., Toloza, S., Cutolo, M., Kautiainen, H., Makinen, H., Gogus, F., ... & Pincus, T. (2009). Women, men, and rheumatoid arthritis: analyses of disease activity, disease characteristics, and treatments in the QUEST-RA study. *Arthritis research & therapy*, *11*(1), 1-12.

Stamatelopoulos, K. S., Kitas, G. D., Papamichael, C. M., Chryssohoou, E., Kyrkou, K., Georgiopoulos, G., ... & Sfikakis, P. P. (2009). Atherosclerosis in rheumatoid arthritis versus diabetes: a comparative study. *Arteriosclerosis, thrombosis, and vascular biology*, *29*(10), 1702-1708.

Struijs, J. N., Baan, C. A., Schellevis, F. G., Westert, G. P., & Van Den Bos, G. A. (2006). Comorbidity in patients with diabetes mellitus: impact on medical health care utilization. BMC health services research, 6(1), 1-9.

Suwannalai, P., Trouw, L. A., Toes, R. E., & Huizinga, T. W. (2012). Anti-citrullinated protein antibodies (ACPA) in early rheumatoid arthritis. *Modern rheumatology*, *22*(1), 15-20.

Symmons, D. P. (2002). Epidemiology of rheumatoid arthritis: determinants of onset, persistence and outcome. *Best practice & research Clinical rheumatology*, *16*(5), 707-722.

Tentolouris, A., Thanopoulou, A., Tentolouris, N., Eleftheriadou, I., Voulgari, C., Andrianakos, A., & Sfikakis, P. P. (2018). Low prevalence of rheumatoid arthritis among patients with pre-existing type 2 diabetes mellitus. *Annals of translational medicine*, *6*(20).

Terasaki, F., Okamoto, H., Onishi, K., Sato, A., Shimomura, H., Tsukada, B., ... & Kitabatake, A. (2007). Higher serum tenascin-C levels reflect the severity of heart failure, left ventricular dysfunction and remodeling in patients with dilated cardiomyopathy. *Circulation Journal*, *71*(3), 327-330.

Turesson, C., O'fallon, W. M., Crowson, C. S., Gabriel, S. E., & Matteson, E. L. (2003). Extra-articular disease manifestations in rheumatoid arthritis: incidence trends and risk factors over 46 years. *Annals of the rheumatic diseases*, *62*(8), 722-727.

Udalova, I. A., Ruhmann, M., Thomson, S. J., & Midwood, K. S. (2011). Expression and immune function of tenascin-C. *Critical Reviews™ in Immunology*, *31*(2).

Ursum, J., Bos, W. H., van Dillen, N., Dijkmans, B. A., & van Schaardenburg, D. (2010). Levels of anti-citrullinated protein antibodies and IgM rheumatoid factor are not associated with outcome in early arthritis patients: a cohort study. *Arthritis research & therapy*, *12*(1), 1-7.

Usenbo, A., Kramer, V., Young, T., & Musekiwa, A. (2015). Prevalence of arthritis in Africa: a systematic review and meta-analysis. *PloS one*, *10*(8), e0133858.

van der Helm-van, A. H., Verpoort, K. N., Breedveld, F. C., Toes, R. E., & Huizinga, T. W. (2005). Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis. *Arthritis research & therapy*, *7*(5), 1-10.

van der Woude, D., Alemayehu, W. G., Verduijn, W., de Vries, R. R., Houwing-Duistermaat, J. J., Huizinga, T. W., & Toes, R. E. (2010). Gene-environment interaction influences the reactivity of autoantibodies to citrullinated antigens in rheumatoid arthritis. *Nature genetics*, *42*(10), 814-816.

Van Halm, V. P., Peters, M. J. L., Voskuyl, A. E., Boers, M., Lems, W. F., Visser, M., ... & Nurmohamed, M. T. (2009). Rheumatoid arthritis versus diabetes as a risk factor for cardiovascular disease: a cross-sectional study, the CARRE Investigation. *Annals of the rheumatic diseases*, *68*(9), 1395-1400.

Van Venrooij, W. J., Hazes, J. M., & Visser, H. (2002). Anticitrullinated protein/peptide antibody and its role in the diagnosis and prognosis of early rheumatoid arthritis. *Netherlands Journal Medicine, 60*(10), 383-388.

VinodMahato, R., Gyawali, P., Raut, P. P., Regmi, P., Singh, K. P., Pandeya, D. R., & Gyawali, P. (2011). Association between glycaemic control and serum lipid profile in type 2 diabetic patients: Glycated haemoglobin as a dual biomarker.

Wallberg-Jonsson, S., Ohman, M. L., & Dahlqvist, S. R. (1997). Cardiovascular morbidity and mortality in patients with seropositive rheumatoid arthritis in Northern Sweden. *The Journal of rheumatology*, *24*(3), 445-451.

Wasserman, A. (2011). Diagnosis and management of rheumatoid arthritis. *American family physician*, *84*(11), 1245-1252.

Wolfe, F., Freundlich, B., & Straus, W. L. (2003). Increase in cardiovascular and cerebrovascular disease prevalence in rheumatoid arthritis. *The Journal of rheumatology*, *30*(1), 36-40.

Wolfe, F., Mitchell, D. M., Sibley, J. T., Fries, J. F., Bloch, D. A., Williams, C. A., ... & Cathey, M. A. (1994). The mortality of rheumatoid arthritis. *Arthritis & Rheumatism*, *37*(4), 481-494.

Zeng, X., Ai, M., Tian, X., Gan, X., Shi, Y., Song, Q., & Tang, F. (2003). Diagnostic value of anti-cyclic citrullinated Peptide antibody in patients with rheumatoid arthritis. *The Journal of rheumatology*, *30*(7), 1451-1455.

Zhuo, Q., Yang, W., Chen, J., & Wang, Y. (2012). Metabolic syndrome meets osteoarthritis. *Nature Reviews Rheumatology*, *8*(12), 729.

APPENDICES

Appendix 1: Consent to Participate in the Research Study: English version.

Title: Biomarkers of rheumatoid Arthritis among diabetes patients at Moi Teaching and Referral Hospital.

Investigator: Dorcus A. Adhiambo, School of Medicine, Moi University

Institutional Review Board (IRB): Research approved by IREC at Moi University.

Purpose and Background: The purpose of the research is to determine the predictive biomarkers levels in undiagnosed Rheumatoid arthritis patients at Diabetes Clinics in MTRH Eldoret. The data will provide a rough estimate of the condition locally and an insight of the problem in Kenya at large.

Procedure: Nurses at the clinics take the demographical parameters and blood pressure measurements of the participant, and then the blood samples will be collected by the investigator.

Benefit: The research has direct benefit to the participant.

Risk: There are no financial, physical or social risks. Psychological risk maybe minimal

And if the subject feels uncomfortable they may decide not to participate in the study.

Confidentiality: To ensure confidentiality, there will be no names identifying the participants but codes known only by the investigator be used. The data from the study will solely be used for purpose of the study.

Voluntary participation: My participation in the research is voluntary and I am free to withdraw from the study at any time.

Questions: In case of further questions, comments or complaints relating to the study/ if any need arise that want to withdraw from the study, will contact, the investigator

through P.O. Box 4606, school of Medicine or phone number 0726-469-712 or email address dorcusadhiambo3@gmail.com.

Consent

I have read or have been read to the description of the research study. The investigator or his/her representative has explained the study to me and has answered all of the questions I have been asked at this time. I have been told of the potential risks, discomforts and side effects as well as the possible benefits of the study. I freely volunteer to take part in this study.

_____	_____	_____
Name of Participant	Signature of subject/thumbprint (Witness to print if the Subject is unable to write	Date & Time
_____	_____	_____
Name of person Obtaining Consent	Signature of person Obtaining Consent	Date& Time
_____	_____	_____
Printed name of Investigator	Signature of Investigator	Date &Time

Appendix 2 : Questionnaire

Section A. Demographic data

1. Name/ Initials _____Study No_____ Date_____
2. Diabetic clinic No_____
3. Age in years _____
4. Gender Male ☐ Female ☐
5. Body height in Kgs_____ weight in meter/centimeters_____
6. Blood glucose levels_____
7. Blood pressure measurements_____

Section B: Presenting complains / treatment and substance abuse

8. Are you Diabetic? Yes ☐ No ☐
9. For how long have you lived with diabetes_____(years)
10. Are you taking any medicine? Yes ☐ No ☐

    If yes specify_____

11. Other than diabetes, do you experience stiffness/pain /fever/swelling in any of the following body parts? (tick as appropriate)

    My fingers___, my hands/wrists___, my elbows___, my shoulders___, my neck___, my back___, my toes___, my feet/ankles___, my knees___, my hips___.

12. Do you use/drink/smoke any drug/substance in your life?

    Yes ☐ No ☐

13. If yes in Q (12) above, which drug/substance?

    I) Illicit brew (Changaa, Busaa, Muratina) for_____years

    ii) Cigarette ☐ for _____years

    iii) Miraa ☐ for _____years

Appendix 3:IREC Approval Letter.

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

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

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET

Reference: IREC/2017/149
Approval Number: 0002017

22nd January, 2018

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
22 JAN 2018
APPROVED
P. O. Box 4606 - 30100 ELDORET

Dorcus A. Adhiambo,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

Dear Ms. Adhiambo,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee has reviewed your research proposal titled:-

"Biomakers of Rheumatoid Arthritis among Diabetes Patients at Moi Teaching and Referral Hospital".

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 2017*** on 22nd January, 2018. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 21st January, 2019. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

DR. S. NYABERA
DEPUTY-CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

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

Appendix 4: Hospital Approval (MTRH)

An ISO 9001:2015 Certified Hospital

MOI TEACHING AND REFERRAL HOSPITAL

Telephone: (+254)053-2033471/2/3/4
Mobile: 722-201277/0722-208795/0734-600461/0734-683361
Fax: 053-2061749
Email: ceo@mtrh.go.ke/directorsofficemtrh@gmail.com

Nandi Road
P.O. Box 3 – 30100
ELDORET, KENYA

Ref: ELD/MTRH/R&P/10/2/V.2/2010

26th January, 2018

Dorcus A. Adhiambo,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

APPROVAL TO CONDUCT RESEARCH AT MTRH

Upon obtaining approval from the Institutional Research and Ethics Committee (IREC) to conduct your research proposal titled:-

"Biomakers of Rheumatoid Arthritis among Diabetes Patients at Moi Teaching and Referral Hospital".

You are hereby permitted to commence your investigation at Moi Teaching and Referral Hospital.

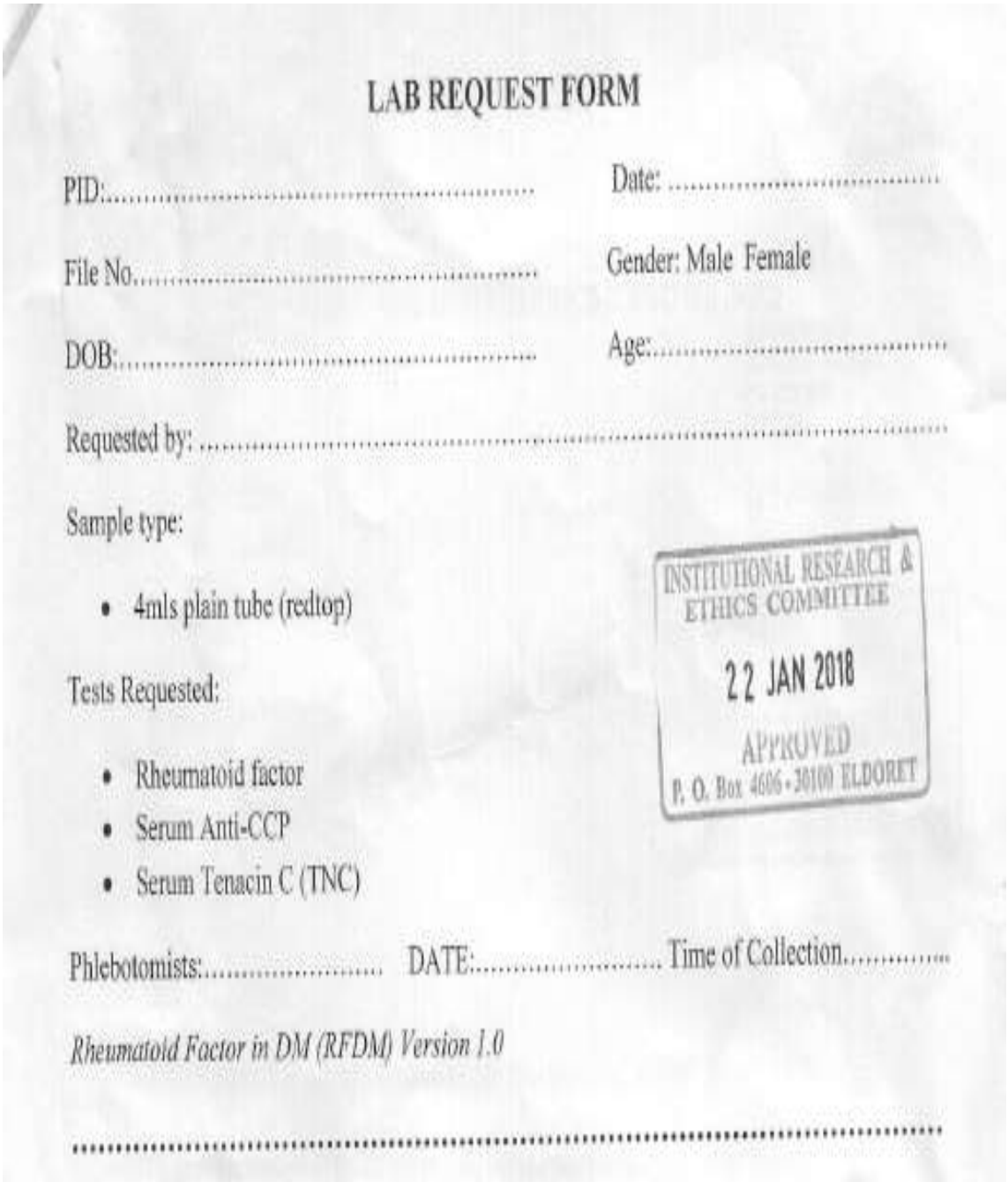
MOI TEACHING AND REFERRAL HOSPITAL
CEO
APPROVED
26 JAN 2018

DR. WILSON K. ARUASA, *MBS*
CHIEF EXECUTIVE OFFICER
MOI TEACHING AND REFERRAL HOSPITAL

cc - DCEO, (CS)
- Director of Nursing Services (DNS)
- HOD, HRISM

All correspondence should be addressed to the Chief Executive Officer
Visit our Website: www.mtrh.go.ke
A WORLD CLASS TEACHING AND REFERRAL HOSPITAL

Appendix 5: Laboratory Request Form



LAB REQUEST FORM

PID:..... Date: .....

File No..... Gender: Male Female

DOB:..... Age:.....

Requested by: .....

Sample type:

- 4mls plain tube (redtop)

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
22 JAN 2018
APPROVED
P. O. Box 4606 - 30100 ELDORET

Tests Requested:

- Rheumatoid factor
- Serum Anti-CCP
- Serum Tenacin C (TNC)

Phlebotomists:..... DATE:..... Time of Collection.....

Rheumatoid Factor in DM (RFDM) Version 1.0

.....