PROFILES OF VITAMIN D AMONG PATIENTS WITH RHEUMATOID ARTHRITIS AT THE KENYATTA NATIONAL HOSPITAL

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DECLARATION

I solemnly confirm that this dissertation is my original work and to the best of my knowledge has not been submitted elsewhere for examination

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DEDICATION

I lovingly dedicate this work to my husband, Dr. Norman R. Demba, and my two sons Kyle Emmanuel Demba and Milan Lloyd Demba. Special dedication goes to my sister, Dr. Emily Aradi, to whom I owe the gift of persistence and courage.

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I convey my heartfelt gratitude to the many people who made this proposal a success. First, gratitude to my supervisors: Prof. Joshua Kayima, Prof. Omondi Oyoo, and Prof.Erustus Amayo for constantly guiding me throughout the research process.

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To my sons Kyle Emmanuel Demba and Milan Lloyd Demba, you always make me want to do better in my work.

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My classmates in the Master of Medicine Internal Medicine class of 2016 have been a wonderful source of solace, company, encouragement and correction. May God bless you.

Above all, I owe my existence and success to God Almighty, to whom all honor and glory goes.

LIST OF ABBREVIATIONS

1,25-VD……1,25 dihydroxycholecalciferol

7 DHC……..7 dehydrocholestrol

24-OHase ……24 – hydroxylase

25-VD …….25 hydroxycholecaliferol

ACR…….American college of Rheumatology

ADCC……..Antibody dependent cell-mediated cytotoxicity

ANOVA……...Analysis of variance

Anti-CCP…….Anti- cyclic citrullinated peptide

APCs……Antigen presenting cells

CD……Cluster of differentiation

CRP……..C- reactive protein

CY P450……...Cytochrome P450

DAS-28……Disease activity score -28

DC……Dendritic cell

ESR……..Erythrocyte sedimentation rate

EULAR……European league against rheumatism

GIT……...Gastrointestinal tract

HLA…….Human leukocyte antigen

HPA axis……..Hypothalamic-pitiutary-adrenal axis

IL-1……..Interlukin-1

IL-2……..Interlukin-2

IL-6……..Interlukin-6

IL-10……Interlukin-10

IL-12……. ..Interlukin-12

IL-17……Interlukin-17

KNH……Kenyatta National Hospital

M-HAQ……...Modified-health assessment questionnaire

MHC……Major histocompatibility complex

RA……Rheumatoid arthritis

RF……. ...Rheumatoid factor

SLE……...Systemic lupus erythematosus

SPSS……..Statistical package for social sciences

S-28……. .Swollen joints -28

T-28……...Tender joints -28

TLR……Toll-like receptor

UVB……...Ultraviolet B

VDR……...Vitamin D receptor

VEGF……Vascular-endothelial derived growth factor

.

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ABSTRACT

Background: Rheumatoid arthritis (RA) is an autoimmune, chronic debilitating condition of undetermined cause. It affects numerous extra- articular organ systems. Vitamin D is a steroid hormone synthesized in the skin by the action of ultraviolet B (UVB) irradiation. Active vitamin D is important in the inhibition of T cell proliferation and downregulation of key inflammatory cytokines responsible for the pathogenesis of RA. There is growing evidence demonstrating the association between vitamin D insufficiency and higher incidence of RA as well as increased severity of disease and increased functional disability in RA patients.

Aim: The purpose of this study was to determine serum vitamin D levels among patients with rheumatoid arthritis at the Kenyatta National Hospital (KNH) and its association with disease activity and functional disability.

Methods: This was a descriptive cross-sectional survey involving subjects with RA at the Kenyatta National Hospital. Consecutive sampling technique to recruit patients with rheumatoid arthritis. Ten 10 (mls) of blood was taken from the recruited subjects to determine serum vitamin D levels. Every participant had their demographics, clinical history and disease duration documented. Clinical disease activity index (CDAI) was used to assess activity of disease and severity. It comprised of number of tender joint out of 28 joints (T-28), number of swollen joints out of 28 (S-28) global health assessment score by both the physician and the patient. Disability level was established using the standard modified health assessment questionnaire (MHAQ). Data analyzed correlated their association with serum vitamin D levels. SPSS version 21 was used to analyze the data collected and this entailed descriptive statistics, chi-square, ANOVA and students'-test to compare and correlate vitamin D levels with age, duration of disease, CDAI score and modified HAQ score in RA.

Results: Eighty-one patients mean of 48.7 (SD 13.9), median 48.0(IQR 40.0-59.0) were evaluated. The female to male ratio was 4:1. The average 25-VD concentration was 34.9ng/ml (SD11.6). Thirty-five participants (43.2%) had insufficient vitamin D levels (<30ng/ml), whereas forty-six study participants (56.8%) had sufficiency of Vitamin D. Majority of the patients 54(67.5%) had low disease activity. Fourteen subjects 17.5 % had high disease activity and while 2.5% were on remission. Functional disability was assessed using the modified health assessment questionnaire. Thirty-eight participants (46.5%) demonstrated no disability, 33.8% had mild disability while 9% had severe disability. Correlation between vitamin levels with age, duration of disease, CDAI and HAQ did not attain statistical significance.

Conclusion: Vitamin D insufficiency is high in subjects with rheumatoid arthritis without correlation with age, duration of disease, functional disability and disease activity.

CHAPTER ONE

1.0 INTRODUCTION

Rheumatoid arthritis (RA) is a chronic debilitating autoimmune disorder whose origin not fully understood [1]. It is believed to be due to the breakdown of self-tolerance, and B and T lymphocytes are key in the occurrence of the disease [2]. Importance of T and B lymphocytes is further proved by the introduction of biologic disease- modifying antirheumatic drugs (DMARDS) in the mitigation of RA, which target both T and B lymphocytes and their cytokine profile [3].

Vitamin D is a hormone existing in the skin as an inactive molecule, pre-vitamin D, which is metabolized in the skin by action of ultraviolet irradiation [4]. Its major role is in the metabolism of calcium, by regulating its absorption from the gastrointestinal system [5]. Vitamin D has multiple extra skeletal roles potentiated through its receptor, the vitamin D receptor (VDR). This receptor has been demonstrated in nearly all tissues [6]. Vitamin D is an immunomodulator, a property key in the regulation of B and T cells [7]. Vitamin D deficiency is associated with the occurrence of autoimmune conditions. Various studies have demonstrated increased prevalence of autoimmune conditions in the African-American population due to their relatively lower serum vitamin D compared to Caucasians. [8].

Multiple studies have found serum vitamin D to be considerably lower in rheumatoid arthritis patients compared to controls, and these levels have an inverse relationship with the activity and severity of disease in RA [9].

1.1 STUDY JUSTIFICATION

Rheumatoid arthritis reduces life expectancy and leads to significant disability in affected subjects [15]. Various studies have indicated direct connection between the insufficiency of vitamin D and the occurrence of various autoimmune disorders due to its role in immunomodulation [29]. The deficiency of 25-VD is causes the occurrence, increased disease activity and a more aggressive disease in RA. Among the black population, various studies have shown vitamin D insufficiency even in healthy subjects due to increased melanin concentration impairing vitamin D absorption. These studies have however found considerably reduced levels of vitamin D in RA individuals among black populace compared to those without the disease [8].

In Africa, the studies were in countries with relatively fair skinned individuals in Egypt [37] and Libya, thus there is a gap in the knowledge of this condition in the sub- Saharan region with fairly darker individuals.

In studies where vitamin D was included as part of wholesome RA management, it was noted that there was substantial improvement in disease severity in the subjects [35, 40]. In Kenya, and sub-Saharan Africa, the burden of vitamin D insufficiency in RA individuals is unknown.

This study will serve as a platform for other studies on the inclusion of vitamin D in the management of RA and impact on the severity of disease in our set-up, aiming at possibly combining vitamin D and DMARDS in the overall RA management.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. PREVALENCE OF RHEUMATOID ARTHRITIS

The global prevalence of rheumatoid arthritis is estimated to be 1%. It affects females more than males at a ratio of 3:1[10]. The occurrence rises with advancing age and the gender differences reduce in the older age groups. In Africa, the burden of RA is uncertain due to few studies done. The first described case of RA in Africa according to literature was in 1956 [11]. From the limited studies done of a few select African countries, the prevalence of RA in Africa is estimated at 0.36% [12]. Although it is not a representation of the continent, the crude prevalence is in line with the global prevalence of 1%.

In a study done in South Africa in an urban setting of a predominantly black population,the prevalence of RA combined was estimated to be 2.6% in men, 3.7% in women, and 3.3% in all subjects above fifteen years. [13]

In a study done in 2007,60 patients with RA had been seen in KNH within a period of six months[57]compared to an earlier study in 1979 where 76 patients were seen over a period of 18 months [14]. This rise in number of cases may be a reflection improved referral system or increased urbanization in Kenya.

2.2 PATHOPHYSIOLOGY OF RHEUMATOID ARTHRITIS

Rheumatoid arthritis is an autoimmune, inflammatory disorder of undetermined origin.It primarily involves the joints of hands and feet but it can virtually affect all the joints in the body. Extra-articular manifestations are also common in RA and are associated with significant morbidity and mortality. These manifestations are known to reduce life expectancy averagely by

3-7 years. [15] Being an autoimmune condition, it affects primarily the areas with extensive nervous systems or those relying on small vessel beds**.**

Rheumatoid arthritis is believed to result due to a breakdown in self-tolerance. The exact cause of the disease remains unknown [1].An inter-play of environmental and genetic influences is known to trigger the development of RA. Various viruses have been thought to be the initial trigger; with the viral protein mimicking the human self-proteins thus up regulating the immune system which then attacks the self-antigens [15]. Microorganisms like the Epstein–Barr virus and cytomegalovirus (CMV), together with there are known to be potential triggers for RA pathogenesis and the mechanism is postulated to be molecular mimicry [16].

Following an initial trigger, pro inflammatory cells are up regulated. Interleukin-17(IL-17) is particularly important in development of synovitis. Other cytokines; Interleukin -1(IL-1) and (Interleukin-6) IL-6 are also produced leading to increased synovial inflammation [17].T cell activation results in increased cytokine production as well as release of secondary mediators of inflammation, including histamine, nitric oxide andleukotrienes resulting in extensive joint swelling [17].

Inflammation leads to synovial membrane damage. Mononuclear cells are activated locally within the synovium leading to further synovitis. Further inflammation leads to proliferation of endothelial cells around the blood vessels thus forming new blood vessels (angiogenesis). This is under the influence of vascular-endothelial derived growth factor (VEGF). These vessels are leaky and they contribute to further joint swelling [19].

Pannus, which is a fibro vascular tissue, results from the penetration of proliferating synovial cells, as well as fibroblastic and endothelial cells into the cartilage leading to erosive damage to the joint. The development of a pannus is under the influence of IL-1 which leads to proliferation

of inflammatory cells into the synovium. This process is facilitated by cellular adhesion molecules of the integrin superfamily which lead to endothelial cells binding to laminin, fibronectin and collagen matrix thus leading to invasion into the cartilage [19].

Extra- articular effects such as osteoporosis, anemia of chronic illness and cardiovascular disease occur due to the up-regulation of inflammatory cytokines. This HPA abnormality is an important contributor to RA pathogenesis which explains the fatigue and depression in these patients [20]. In individuals with a genetic preponderance to RA, there is a 60 % chance of getting the disease if a first degree relative has it. The most important environmental trigger in genetically susceptible individuals is smoking [21].

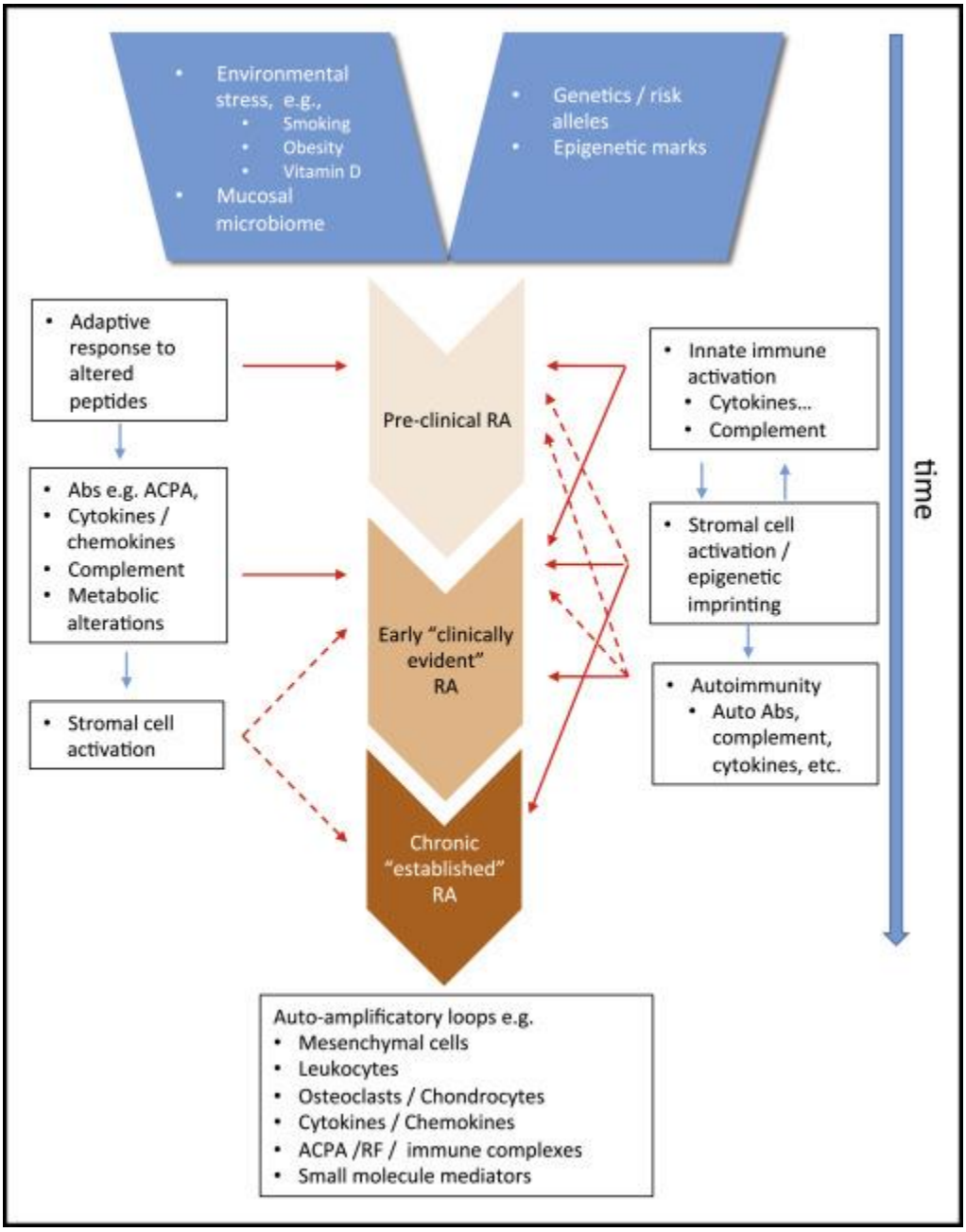


Figure 2.1: The steps involved in the development of rheumatoid ArthritisAdapted from immunopathogenesis of rheumatoid arthritis volume 46, issue 2 83-196 [58].

2.4 VITAMIN D METABOLISM AND ACTIONS

Vitamin D is a hormone produced through the action of UVB irradiation on the skin, from its inactive form, 7-dehydrocholestrol (7- DHC).7- DHCis converted into vitamin D3 using ultra violet rays, a reaction known as non-enzymatic photoisomerization. It then undergoes the first and second hydroxylation processes to produce 1, 25 hydroxycholecalciferol (1, 25 VD) the active vitamin D. These steps take place in the liver and kidney respectively. 24-hydroxylase is an enzyme in the liver which inactivates excessive 1.25-VD[22]. The most studied role of vitamin D is the regulation of calcium in the body by increasing its absorption from the GIT and bone remodeling. Current studies have demonstrated other functions of vitamin D such as immunomodulation and cell differentiation, potentiated through its receptor, the vitamin D receptor (VDR). This receptor is found in nearly all tissues [6]. Sources of vitamin D from the diet include eggs, fish, liver, milk and butter. The action of ultraviolet rays on plants and fungi produces vitamin D2 (ergocalciferol). This compound binds less to vitamin D binding protein and it is also eliminated faster from circulation.

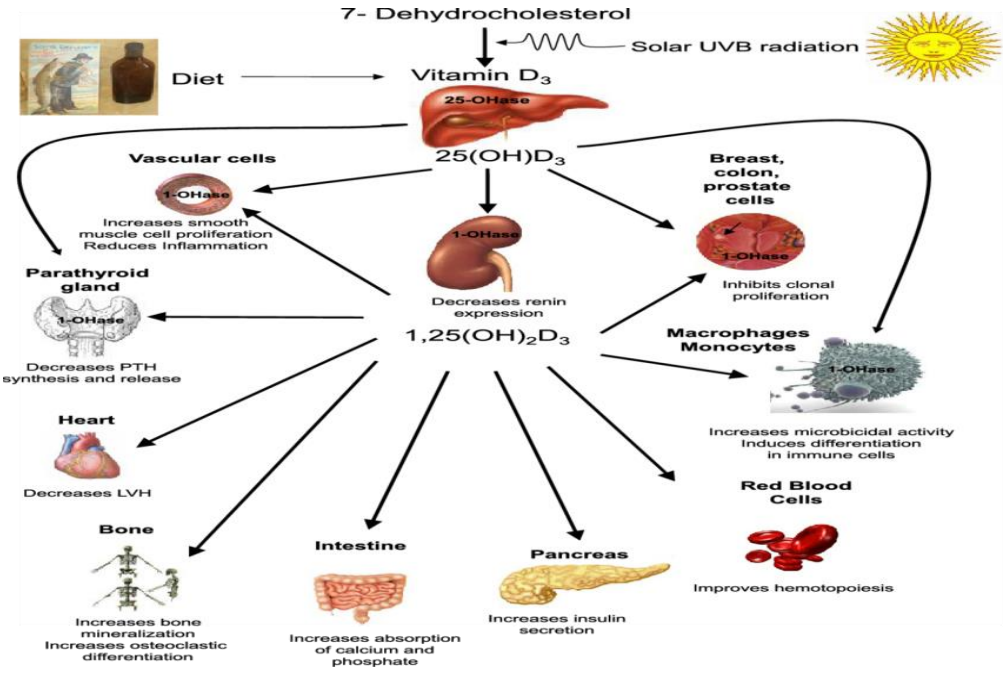


Figure 2.2: Metabolism of Vitamin D: Adapted from American journal of physiology 2009 publication Vol.297[60].

2.5 EFFECTS OF VITAMIN D ON THE IMMUNE SYSTEM

Active vitamin D is known to up-regulate the functions of the innate immune system. It induces the actions of antigen presenting cells such as the dendritic cells and macrophages. Vitamin D enhances phagocytosis in the cells of the immune system, as well as enabling chemataxis of opsonized immune cells. [23]. Dendritic cells (DCs) which are antigen presenting cells (APCs), are the main cells targeted by vitamin D as an immunomodulator. The APCs present antigens including self- antigens in the case of autoimmune conditions to T and B cells. This leads to an up-regulation of pro-inflammatory cytokines and co-stimulatory molecules [24]. Recent literature demonstrates that active vitamin D alters the structure and function of DCs causing them to be tolerogenic and immature. These changes prevent the T-cells from being cytotoxic and induce them to become immunoregulatory cells [25, 26].

Immature DC express reduced human leukocyte antigen class 11(HLA class 11) levels and also demonstrate reduced levels of CD40, CD86 and CD80 which are co-stimulatory molecules. The resultant is a downregulation of inflammatory cytokines such as interleukin-12(IL12), which is highly immunogenic

VDR is found in both B and T cells [30]. In the dormant states of these immune cells, VDR expression is reduced. Once activated during an immune response, the expression of VDR is up-regulated. More than 500 vitamin D genes are regulated during this activation leading to down-regulation of the immune responses [31, 32].

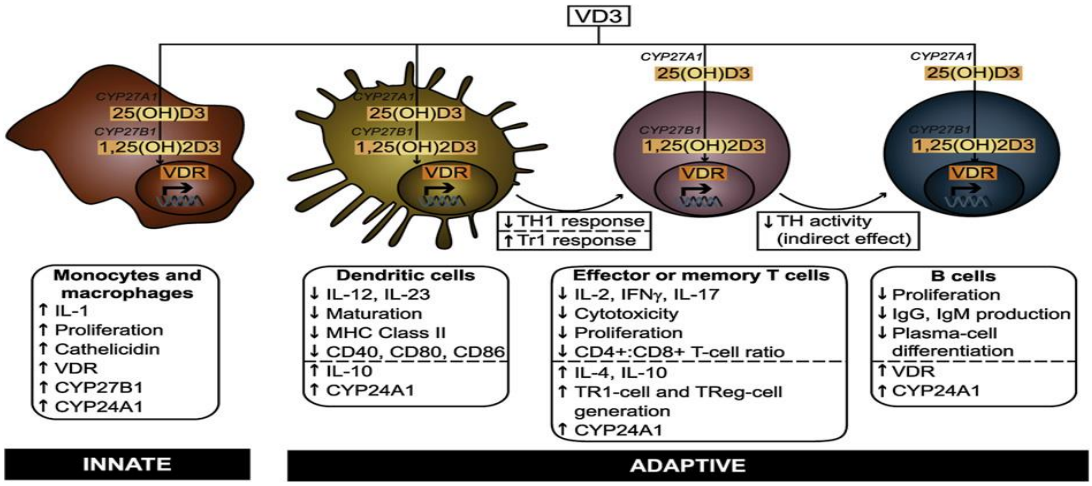


Figure 2.3: vitamin D activity on the immunity: adapted from Natures Reviews immunology 2008 Sep [61].

2.6 CORRELATION BETWEEN VITAMIN D AND RHEUMATOID ARTHRITIS

Active vitamin D induces immunologic tolerance thus, key in the mitigation of RA which primarily results due to breakdown of self- tolerance [26].

A study done in Iowa demonstrated that a greater intake vs. lower intake of vitamin D is shown to have substantial reduction in the risk of RA[35].A study in Amsterdam in 2010 demonstrated

that lower vitamin D levels correlate negatively to activity of disease in RA [36] and was in agreement with an Egyptian study.

In rheumatoid arthritis, boneresorption and damage occurs due to activation of osteoclasts. A study has shown the significance of the mTOR pathway since it enhances osteoclasts survival. The same study demonstrated Vitamin-D inhibiting the mTOR pathway thus significant benefits have been achieved in combining anti-rheumatic drugs (DMARDS) with vitamin D as a wholesome approach to rheumatoid arthritis management [39]. It was noted that Individuals with the greatest vitamin intake have 24.2% reduced risk of rheumatoid arthritis development compared to individuals with the least intake [40].

2.8 RESEARCH QUESTION

What is the burden of vitamin D insufficiency among subjects with rheumatoid arthritis at the Kenyatta National Hospital?

2.9 OBJECTIVES

2.9.1 BROAD OBJECTIVE

To determine serum vitamin D levels among patients with rheumatoid arthritis in KNH and to associate it with age, disease activity and functional disability.

2.9.2 PRIMARY OJECTIVE

To determine the levels of serum 25 hydroxycholecalciferol in patients with rheumatoid arthritis in KNH.

2.9.3 SECONDARY OBJECTIVE

To correlate serum 25 hydroxycholecalciferol levels with age, CDAI score, modified HAQ score and duration of disease in patients with RA.

CHAPTER THREE

METHODOLOGY

3.2 STUDY SITE

The study took place at the Kenyatta National Hospital, rheumatology clinic. The rheumatology clinic runs once a week and about 15- 20 patients are seen every week. It is manned by consultants and residents

3.3 STUDY POPULATION

These were patients above eighteen years, clinically diagnosed with RA sat the rheumatology clinic in Kenyatta National Hospital. They were required to fill a well informed consent form prior to the commencement of the study.

3.4 STUDY VARIABLE

3.4.1 DEPENDENT VARIABLES

Serum 25-hydroxycholecalciferol levels. Vitamin D profile was determined by serum measurement of 25-VD levels. Serum 25-VD estimation is the optimal test to determine body

stores as its metabolite 1-25-VD is rapidly utilized in target organs. Subjects' vitamin D status was classified as follows;

Table 3.1: Vitamin D normal laboratory reference ranges [56]

Vitamin D status	**25-VD (ng/ml)**
Severe deficiency	<10
Moderate deficiency	10-<30
Normal	30-100
Toxicity	>100

For univariate and multivariate analysis cutoff of 20ng/ml was used as literature has demonstrated that at levels above this, one begins to appreciate the immunomodulatory properties of vitamin D [42]. Vitamin D levels were stratified as follows:

Table3. 2: Serum vitamin D correlation status

Deficient < 20
Sufficient ≥20

3.4.2 INDEPENDENT VARIABLES

- **Age**
- **Gender**
- **CDAI score**: This is a measure to determine disease activity and severity. Itwas determined using 28 tender joints, 28 swollen joints and global disease assessment by both physician and patient (Appendix 3)

- **Modified HAQ**: This is a standardized tool used to determine functional disability in rheumatoid arthritis patients.
- **Duration of disease** from time of diagnosis: This was stratified at five-year intervals.

3.5 SAMPLE SIZE DETERMINATION

This present study will use the correction formula for finite population[62] to determine the required minimum sample size

$$n^1 = \frac{NZ^2P(1-P)}{d^2(N-1) + Z^2 P(1-P)}$$

Where;

n^1= sample size with finite correction

N=Population size (=102 RA patients in the rheumatology clinic)

Z=Z statistic for a confidence interval =1.96

P= expected proportion (in proportion of one)

d= precision (in proportion of one) =0.05

In a study by Chiu *et al* (49), the prevalence of Vitamin D deficiency was 50%.

$$n = 102 \text{ X } 1.96^2 X50(0.5)/0.05^2 X101 + 1.96^2 X50X0.5$$

$$n = 81$$

3.6.1 INCLUSION CRITERIA

i) Patients above 18 years.

ii) Confirmed to have RA

iii) Willing to participate and signed an informed consent

3.6.2 EXCLUSION CRITERIA

i) Patients taking multivitamin supplements or any form of vitamin D supplementation.

ii) Pregnant and lactating mothers

3.7 SCREENING AND RECRUITMENT

The principal investigator/assistant went to the rheumatology clinic and identified files of those patients documented to have RA as per the ACR/EULAR criteria were invited to join this study. Only the patients who gave consent were recruited into the study. Baseline demographic and medical history were collected from these patients using standardized questionnaires. The questionnaires captured data on current management for RA and duration of disease since diagnosis with RA.

3.8 ASSAY OF VITAMIN D LEVELS IN SERUM

Ten (10) mls of venous blood were drawn from the antecubital vein of the study subjects and collected in a free (red) vacutainer bottle, and then taken to Lancet Kenya laboratories at the end of the days' collection for estimation of serum 25-VD concentration. It was extracted from serum using acetronitryl with 0.4% acetic acid. This was injected into a chromatographic column, Polaris C18-A 3µ 150 x 2.0mm.

HPLC uses isocratic mode with eluent (MeCN: 0.4% Acetic acid) as the mobile phase. The flow rate was 0.3mls/min and oven temperature 30 degrees Celsius, and detector will be UV-V at wavelength of 280nm. The amount of vitamin D3 was determined by matching the retention of pure standard and a calibration code.

HPLC was the preferred method of assaying for vitamin D in our study because it is more accurate compared to immunoassays which do not distinguish vitamin D2 and D3. Further HPLC

is faster and less dependent on operator errors and is currently the best method of determining vitamin levels in serum [53].

3.9 CLINICAL DISEASE ACTIVITY INDEX (CDAI)

CDAI is a validated tool used to assess the activity of disease in rheumatoid arthritis. It comprises of a summation of five variables, patient assessment of condition and physician assessment of the disease. This will be used to determine disease activity in this stud

3.11 QUALITY ASSURANCE (QA)

Standard operating procedures (SOPs) of Lancet Kenya Laboratories were used for specimen collection, preparation and storage to minimize pre-analytical errors. To ensure quality is maintained, the laboratory tests were carried out in Lancet Kenya laboratories. Machines used were properly calibrated using standard calibration methods and materials. Lancet Kenya laboratory carries out internal and external quality control.

3.12 DATA MANAGEMENT AND ANALYSIS

Each study questionnaire was assigned with a unique study serial number to prevent duplication of data collection. A computer that is protected with a pass word was used to enter the data collected in Microsoft access. The data entered in Microsoft access was integrated into the software Statistical findings where p value of less than 0.05 were considered significant. The findings of this study were presented using tables and graphs.

3.13 ETHICAL CONSIDERATION

Before commencing, permission to carry out this study was sought from the University of Nairobi's Department of Clinical Medicine and Therapeutics, as well as the KNH/UoN Ethics Review Committee. The KNH/UoN Ethics Review Committee reference number for this study was P812/12/2018. Subjects who gave informed consent were recruited into the study. No

patient was coerced into participating. There was no discrimination against any individual who declined to participate. All information collected was treated as confidential. Only blood samples intended for study were drawn and thereafter discarded after analysis

CHAPTER FOUR

RESULTS

Between February and April 2019 94 study subjects were selected. 9 patients who were on multivitamins, pregnant or lactating did not participate Eighty-five patients underwent phlebotomy and 4 of the samples were eliminated due to inadequate or clotted blood samples, leaving eighty-one patients to be analyzed.

STUDY FLOW CHART

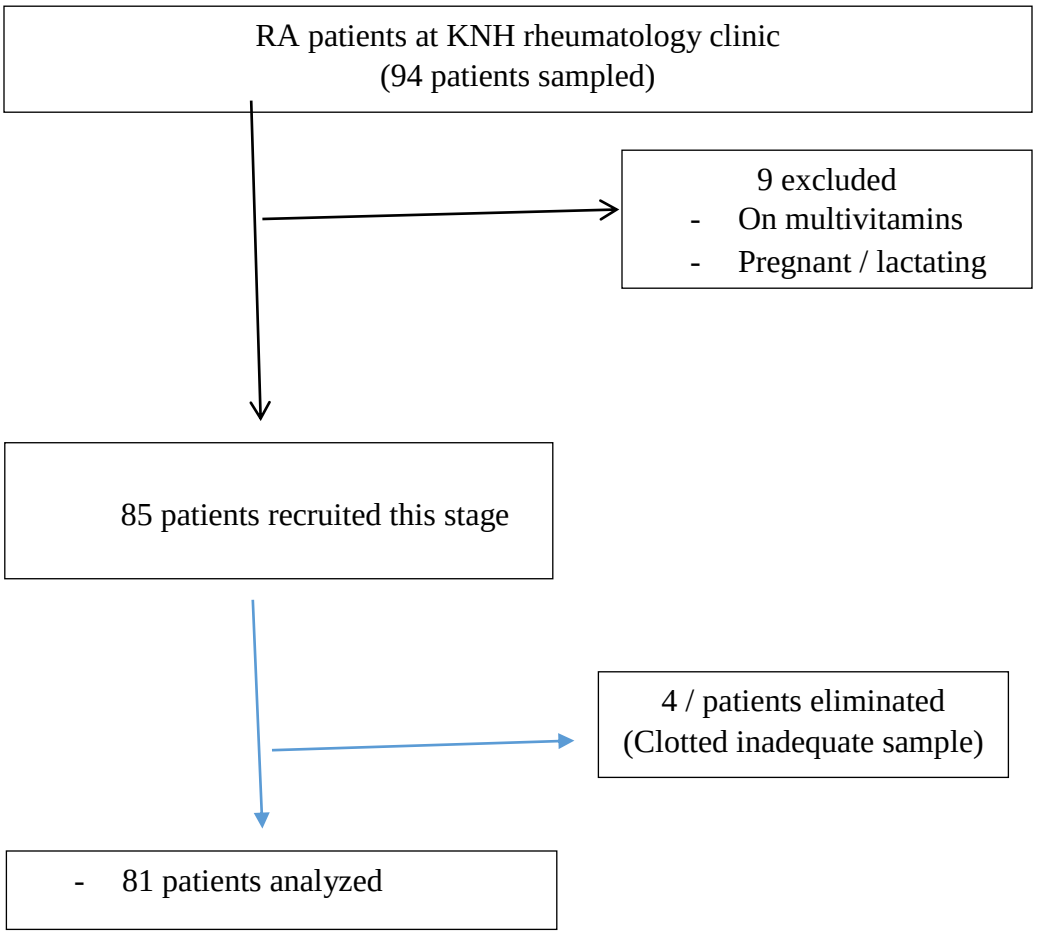


Figure 4.1: Study flow chart of patients with rheumatoid arthritis

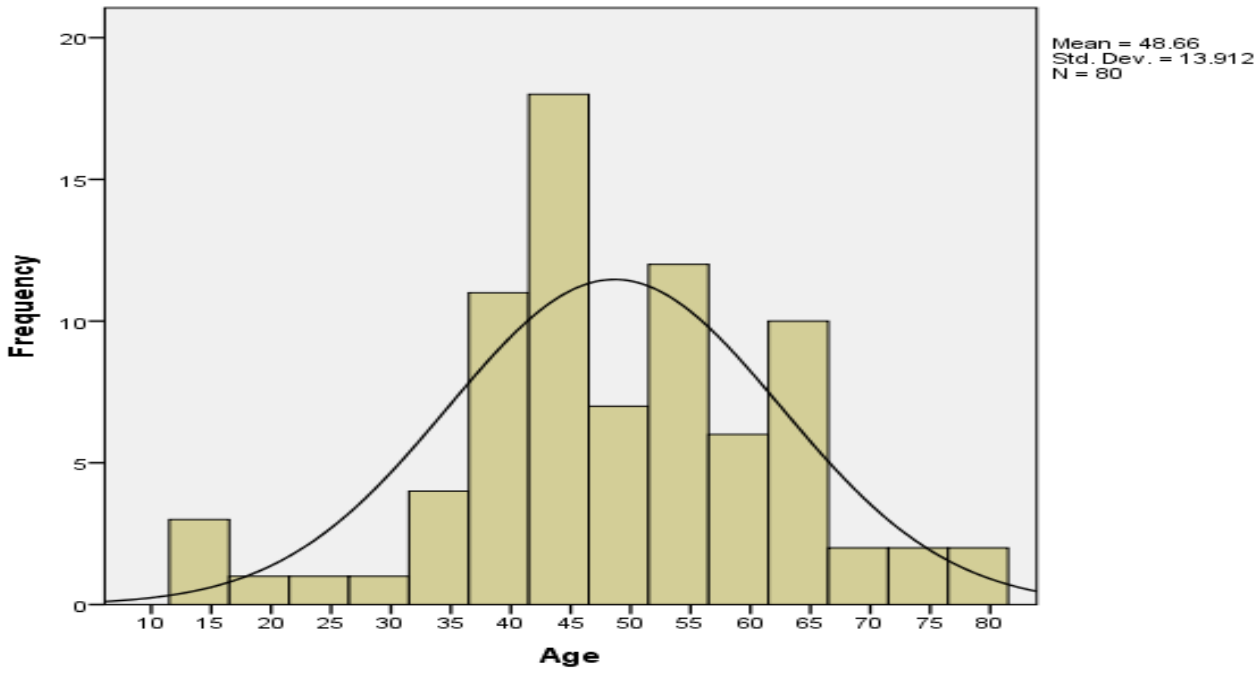
4.1 SOCIO-DEMOGRAPHIC CHARACTERISTICS OF STUDY PARTICIPANTS

The study participants were relatively young (median 48). Sixty-five of the participants (81.3%) were female while 15(18.8%) were male.. Majority of the patients were married 70% and 55% had received secondary education (Table 4.1)

TABLE 4.1: SOCIO-DEMOGRAPHIC CHARACTERISTICS OF THE STUDY PARTICIPANTS (n= 81).

Characteristic	Frequency (%) n=81	95% CI
Age		
Mean (SD)	48.7 (13.9)	
Median (IQR)	48.0 (40.0-59.0)	
Min-Max	14-78	
Age		
<30	5 (6.3)	(2.3-14.6)
30-45	33 (40.0)	(29.4-51.6)
46-60	27 (33.8)	(23.8-45.3)
Above 60	16 (20.0)	(12.2-30.7)
Sex		
Male	16 (18.8)	(11.2-29.4)
Female	65 (81.3)	(70.7-88.8)
Marital status		
Single	18 (22.5)	(14.2-33.5)
Married	55 (70.0)	(58.6-79.5)
Widowed	7 (7.5)	(3.1-16.2)
Level of education		
None	4 (5.0)	(1.6-12.9)
Primary	10 (11.3)	(5.6-20.8)
Secondary	44 (55.0)	(43.5-66.0)
Tertiary	23 (28.7)	(19.5-40.1)

Figure 4.1: Age distribution of the study participants- Histogram



4.2 CLINICAL CHARACTERISTICS OF STUDY PARTICIPANTS

Forty-two patients (52.2%) had less than five years since the diagnosis of RA. 32 patients (39.5%) were on (DMARDS) only, while 39 patients (48.1%) were on a DMARD plus steroids. None of the subjects was on a biologic agent.

TABLE 4.2: CLINICAL CHARACTERISTICS OF THE STUDY POPULATION

Characteristic	Frequency (%) N=80	95% CI
Duration since diagnosis of RA		
0-5	43 (52.5)	(41.1-63.7)
5-10	29 (36.3)	(26.0-47.8)
10-15	5 (6.3)	(2.3-14.6)
>20	4 (5.0)	(1.6-12.9)
Current RA medications		
DMARDS	32 (39.5)	(43.5-66.0)
DMARDS plus Steroids	39 (48.1)	(33.9-56.5)
DMARD + Other immunosuppressants	10 (12.3)	(4.3-27.2)
Biologics	0 (0)	

4.3 LABORATORY CHARACTERISTICS OF STUDY PARTICIPANTS

For descriptive and comparison purposes, the interpretation of the serum 25-VD concentration was based on internationally accepted reference ranges as outlined in the methodology section. Serum 25-VD cut-off was set at 30ng/ml. For purposes of correlation vitamin D deficiency cut point was set at 20ng/ml. Literature has demonstrated that at levels above this, one begins to appreciate the immunomodulatoryproperties of vitamin D [42].

Serum 25-VD concentrations ranged from 19.08 to 57.56. The mean (SD) serum 25-VD concentration in this study population was 34.9 ng/ml with a median of 33.6 (11.2) ng/ml and an inter-quartile range of 24.0 - 44.2ng/ml (Table 4.3). Thirty-five participants (43.2%) had insufficient.

TABLE 4.3: LABORATORY CHARACTERISTICS OF THE STUDY POPULATION

Characteristic	Frequency (%) N=80	95% CI
Serum Vitamin D		
Mean (SD)	34.9 (11.6)	
Median (IQR)	33.6 (24.0-44.2)	
Min-Max	16.0-64.4	
Serum Vitamin D Stratum 1		
>100 (toxicity)	0	
30 -100ng/ml (Normal levels)	46 (56.8)	(47.2-69.5)
10.-29.9 (moderate deficiency)	35 (43.2)	(21.6-42.7)
< 10ng/ml (severe deficiency)	0	
Serum Vitamin D Stratum 2		
≥ 20	72 (90.0)	(80.7-95.3)
< 20ng/ml	8 (10.0)	(4.7-19.3)

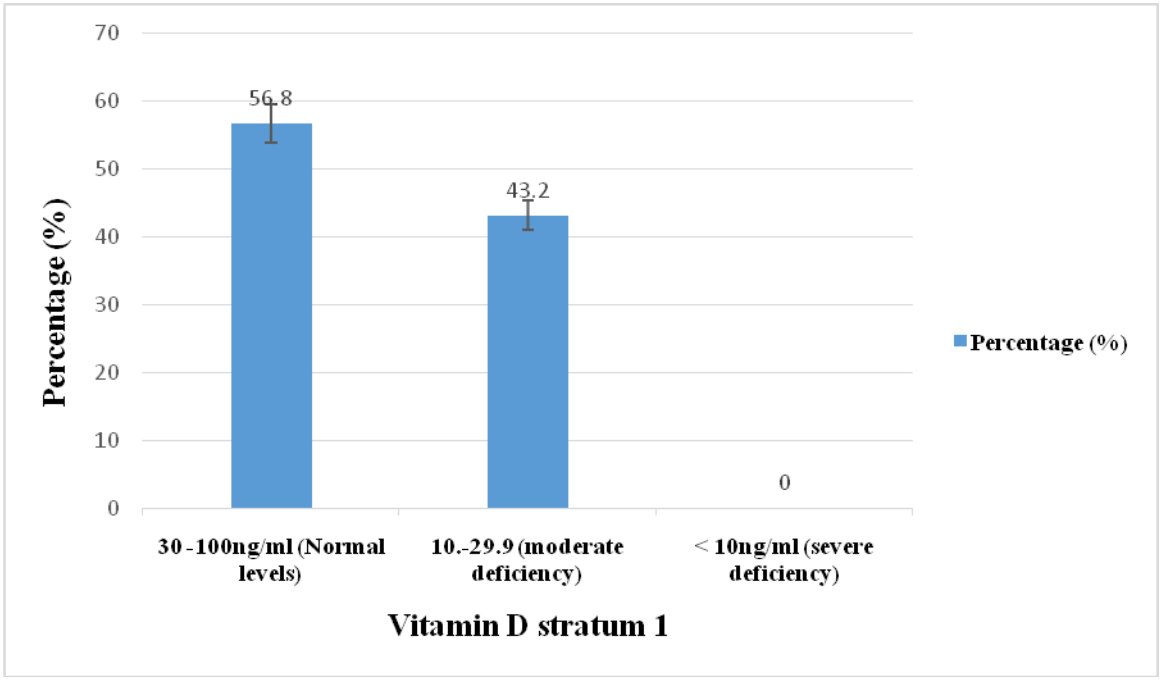


Figure 4.2: Laboratory characteristics of Vitamin D stratum 1 population

4.4 DISEASE ACTIVITY IN THE STUDY POPULATION

Majority 54(67.5%) had low disease activity, while 10 (12.5) had moderate disease activity. Fourteen subjects 17.5 % had high disease activity and while 2 patients 2.5% were on remission.

TABLE 4.4: CDAI Score in patients with RA

Characteristic	Frequency (%) N=80	95% CI
Mean (SD)	14.3 (17.1)	
Median (IQR)	7 (5.0-14.5)	
Min-Max	2-74	
DAS Score		
Remission <0-2.8	2 (2.5)	(0.6-8.6)
Low activity 2.9-10	54 (67.5)	(56.0-77.3)
Moderate activity 10.1-22.0	10 (12.5)	(6.5-22.2)
High activity 22.1-76	14 (17.5)	(10.2-27.9)

4.5 FUNCTIONAL DISABILITY IN THE STUDY POPULATION

Thirty-eight participants (46.5%) demonstrated no disability, twenty-seven(33.8%) had mild disability, six participants (7.5%) had moderate disability, while 9% had severe disability.

TABLE 4.5: HAQ in patients with RA

Characteristic	Frequency (%) N=80	95% CI
No disability 0	38 (46.5)	(36.3-58.9)
Mild disability 1	27 (33.8)	(23.8-45.3)
Moderate disability >1 <2	6 (7.5)	(3.1-16.2)
Severe disability >2	9 (11.3)	(5.6-20.8)

4.6: ASSOCIATIONS BETWEEN VARIABLES.

Univariate analysis was then performed to explore associations between serum 25-VDlevels and various patient and disease characteristics of interest. These characteristics included patients age, duration of disease since diagnosis, disease activity and functional disability. Patients below the age of 45 years were three times more likely to have vitamin D deficiency compared to those above 45 years, and though this is clinically significant. patients with less than five years since the diagnosis of RA had a 1.6 chance of having vitamin D insufficiency compared to those with more than five years' duration since the diagnosis of RA.

TABLE 4.6: ASSOCIATIONS BETWEEN SERUM VIT D WITH AGE, DURATION SINCE DIAGNOSIS, CDAI AND HAQ

	Serum Vitamin D			
	≤ 20	**> 20**	**OR (95% CI)**	**p-value**
Age				
<30	0 (0.0)	5 (100.0)	-	0.441
30-45	6 (18.8)	26 (81.3)	5.3 (1 -28.18)	0.033
46-60	1 (3.7)	26 (96.3)	0.3 (0.03 -2.57)	0.180
Above 60	1 (6.3)	15 (93.8)	0.5 (0.06 -4.38)	0.576
Duration since diagnosis of RA				
0-5	5 (11.9)	37 (88.1)	1.6 (0.36 -7.2)	0.550
5-10	2 (6.9)	27 (93.1)	0.6 (0.11 -3.19)	0.485
10-15	1 (20.0)	4 (80.0)	2.4 (0.23 -24.55)	0.441
>20	0 (0.0)	4 (100.0)	-	0.494
CDAI Score				
Remission <0-2.8	0 (0.0)	2 (100.0)	-	0.633
Low activity 2.9-10	5 (9.3)	49 (90.7)	0.8 (0.18 -3.64)	0.750
Moderate activity 10.1-22.0	0 (0.0)	10 (100.0)	-	0.260
High activity 22.1-76	3 (21.4)	11 (78.6)	3.3 (0.69 -15.84)	0.117
HAQ				
No disability 0	1 (2.6)	37 (97.4)	0.1 (0.01 -0.85)	0.037
Mild disability 1	4 (14.8)	23 (85.2)	2.1 (0.48 -9.15)	0.306
Moderate disability >1 <2	1 (16.7)	5 (83.3)	1.9 (0.19 -18.65)	0.571
Severe disability >2	2 (22.2)	7 (77.8)	3.1 (0.52 -18.39)	0.195

CHAPTER FIVE

DISCUSSION

Association between vitamin D levels and disease activity and functional disability did not show consistent effect since it was not powered to do so. Most of the study participants had no disability (46.5%), with 27% having mild disability. Disease activity among patients in this study also revealed that a greater number had low activity (67.5%). These findings may explain why correlation of these variables with serum vitamin D levels did not show statistical significance though our study was not designed to show causal relationships. Studies from elsewhere have shown an inverse relationship between serum 25D levels and disease activity in RA patients. The same studies have also revealed increased functional disability among patients with low vitamin D levels. In a Chinese study involving 130 RA patients and 80 controls without RA, it was determined that serum 25-VD levels were markedly reduced in RA subjects compared to control groups [43.12 nmol/L (S. D 15.95) *vs* 57.93nmol/L (S. D 15.95), *p* 0.01]. The study also demonstrated that subjects with lower vitamin D levels had a more aggressive and severe disease. Functional disability scores were also higher in patients with low vitamin D levels [34]. A study done in Iowa demonstrated that a greater intake vs. lower intake of vitamin D is shown to have substantial reduction in the risk of RA [35]. A study in Amsterdam in 2010 demonstrated that lower vitamin D levels correlate negatively to activity of disease in RA [36] and was in agreement with an Egyptian study that showed that patients with lower serum vitamin D levels had higher DAS-28 scores and higher HAQ scores [37].

In a group of predominantly older men with RA in the US, higher prevalence occurred with anti-CCP positivity and non-whites. Patients with lower levels had a higher DAS score [38].

In our study, all the 81 patients (100%) were on DMARDS which are the cornerstone drugs in the management of RA. These drugs have been shown to mitigate RA manifestations . The use of DMARDS in the subjects might be the explanation to reduced functional disability and reduced disease activity in the patients. Also, proper follow up and management in a tertiary institution with specialist doctors may be the explanation for better disease control in the patients.

Indeed, this is the first study looking at profile of serum vitamin D levels among rheumatoid arthritis patients at Kenyatta National Hospital. In addition, the vitamin D assay technique that was employed is rapid, accurate, precise, well validated [53] and comparable to the gold standard [54, 55].

The American association of Clinical Chemistry in July 2012 rated the performance of this method as excellent to world class. Comparator studies utilized a similar method.

5

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.0 CONCLUSION

There was a n increased incidence of vitamin D insufficiency in the study populace. It is prudent to identify RA disease mitigation factors such as vitamin D levels. We did not establish correlation between disease activity and functional disability with vitamin D levels.

6.1 RECOMMENDATIONS

The study recommends further studies to explore:

1. Vitamin D profile – case-control studies for the same variables

2. Interventional studies analyzing the role of vitamin D supplementation on rheumatoid arthritis

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APPENDICES

APPENDIX I: INFORMED CONSENT (ENGLISH)

STUDY TITLE: PROFILE OF VITAMIN D AMONG PATIENTS WITH RHEUMATOID ARTHRITIS AT THE KENYATTA NATIONAL HOSPITAL.

Study number:

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Purpose and Benefits

We wish to conduct a research on patients attending the rheumatology outpatient clinic at Kenyatta National Hospital, to identify those with rheumatoid arthritis who have deficiency of Vitamin D compared to the ones without rheumatoid arthritis. This study aims to determine the vitamin D levels among rheumatoid arthritis patients and draw possible comparison with their severity of disease. We will include patients aged 18 years and above.

If you are found to have deficiency or vitamin D, we shall give nutritional advice and prescribe the relevant supplements for your benefit.

Procedures

This study will be conducted through a questionnaire administered with the assistance of a trained study assistant. We will also document your age, gender, marital status, level of education and medical history. 10mls of venous blood will be drawn from a peripheral vein in

your forearm using an aseptic technique. This quantity of blood will be used to determine Vitamin D levels as well as ESR.

Safeguarding Privacy

The interviewer signed a pledge to keep all information about you secure. Your name will be removed from all records involved in the study. A number will be assigned to the survey questionnaire instead. Only project staff will have access to the study data. We will not use your name when we report results of the study.

Benefits

Your taking part in this study will help us determine how prevalent vitamin D deficiency is among patients with rheumatoid arthritis, and help advance knowledge on possible future interventions that might benefit these patients, such as supplementation of vitamin D as part of their overall management.

There are no direct benefits to you. There will be little or no discomfort while drawing blood. However, the overall impact for the patient s with RA may be great because new data on vitamin D deficiency will help us know the prevalence of deficiency, and form a basis for future research on whether supplementation may reduce the severity of disease in rheumatoid arthritis.

Risks

There are no known risks to you as a person taking this survey. You will suffer a littlediscomfort due to drawing of blood from your forearm during the procedure. We will do all possible to

ensure that we minimize the discomfort. Should there be any muchpain or discomfort that requires medical attention, or any complication, we undertake to provide the necessary care free of charge.

If you have any questions about this research, you may call Dr. Sylviah M. Aradi on 0723102535. Kenyatta National Hospital/University of Nairobi- Ethical Review Committee, Email: k.research@knh.or.ke P.O BOX 20723-00202, Nairobi Kenya; Telephone number: +2542726300-

Participant's Rights: Your participation in this study is voluntary and if you decline to participate, you will not be denied any services that are normally available to you.

Right to withdrawal: You have a right to withdraw from this study at any point and there will be no victimization. Your participation to the study is voluntary and you are entitled to pull out of the study any time.

Confidentiality: We will make every effort to protect your identity. You will not be identified in any report or publication of this study or its results.

CONSENT CERTIFICATE

Respondent Agreement

The Study has been explained to me. I consent to participate. I have had a chance for my questions to be answered. I know that I may refuse to participate or to stop the interview at any time without any loss of health care benefits that I am otherwise receiving. I understand that if I have questions about this study or my rights in taking it, I may contact Dr.Sylviah M. Aradi on 0723102535. Further, I understand that the information recorded by the investigator will be confidential

Respondent Signature_____ Date_____

Interviewer Signature_____ Date_____

Contacts of the investigator

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FOMU YA IDHINI

UTANGULIZI

Mimi niDkt. Sylviah M. Aradi, kutoka Chuo Kikuu cha Nairobi. KwasasanasomeauzamilikatikaTibayaNdani. Kama sehemuyamasomoyanguyauzamifu, nahitajikakufanyamradiwautafiti.

NinafanyauchunguzikuhusuviwangovyachembechembeyaVitamini D katikawagonjwawaviungo, katikaHospitaliKuuya Kenyatta.

Lengokuuyautafiti- Lengo la utafitihuunikuamuaiwapowagonjwawaviungowana viwangopungufuvya vitamin D. Hiinisababuupungufuhuuwawezakutatizatibayaonakuharakishamadharayaugonjwahuo.

Taratibuzitakazohusishwa - Lazimakukubalikushirikikatikautafitisisikuulizamaswali

machahekulingananautafitiprofoma.

Ndipotutakuwakufanyamtihaniwakimwiliambayoinahusishauchunguziwauzitowamwili, urefunaupanawakiuno.

Tutatoadamumililitananekwautaratibunabilamajerahailikufanyautafitiwaviwangovyavitamini D.

UkipatikananaviwangodunivyaVitamini D, tutakuelezajinsiyakutumiavyakulanamadawakutibushidahiyo.

Hakiyakokamamshirikikatikautafitihuu- Ushirikiwakokatikautafitihuuniwa kujitolea. Hataukichaguakushiriki au ukataekushirikihaitaathirimatibabuyako.Unauhuruwakujiondoakatikautafitihuuwakatiwowote. Unauhuruwakuulizamaswalikablayakutiasahihikatikafomuyaidhininawakatiwautafiti. Maswalayoteyatahifadhiwakwasiriwakatiwote.

Hakiyakujiondoa;

wewekamamshirikiunahakiyakujiondoakwenyeutafitiwakatiwowotebilayakudunishwa

Hasarazaushiriki - Hakunahasarayoyoteutakayopitia au kupata.Unawezashuhudia uchungukidogowakatiwakutoadamu, lakinihakunauwezekanowamadharayoyote. Ikiwanauchungumwingi, tutagharamiadawayamaumivu.

Manufaayakushiriki- Mwishonimwautafitihuu, nitawasilishamatokeoyautafitikatikaidarayaTibayaNdanikatika Chuo Kikuu cha Nairobi.Habarizozotemuhimu zitakazotokananautafitinaambazozitafanyatibakuwa bora, wagonjwawatafahamishwailihatuamwafakaichukuliwe.

Siri- Habarizotezitakazokusanywawakatiwautafitizitahifadhiwakwasiri. Ni watafiti pekeendiowanaowezakufikiahabarizakibinafsi.

Habarizitakazokusanywazitaandikwanakuainishwabilakutajawashiriki.

Ikiwaunaswalilolotewakatiwautafiti, unawezakuwasilianawafuatao: DKT. SylviahAradi, chuokikuu cha nairobi, Simuyamkono 0723102535. Idarayamafundishoyaudaktarinamatibabuyamagonjwa,

CHETI CHA IDHINI

Kablasijakuhusishakatikautafitiwangu,

naombautiesahihikatikafomuyaidhiniiliyopohapochini.Fomuhiiyaidhinihaitahusishwanamajibuya ko.

Kauliyaridhaa: Nimesomahabarihapojuunanimepatamajibuyamaswaliyoyote

SAHIHI……. TAREHE……

JINA …….

MKUU WA UCHUNGUZI

SAHIHI ……TAREHE……

JINA ……

Ahadiyamhusika

Ninakiriyakwambanimeelezwanakufafanuliwamuktadhawautafitihuu, nanimeelewayakwambanihiariyangukuhusika. Pia, nimeelewayakwambanawezakujiondoakwenyeutafitihuuwakatiwowotebilakuhatarishamatibabuyangu.Aidha, nimeelewayakwambamaswaliyotenitakayozuakuhusiananautafitihuuyatajibiwanamtafitimkuu, DktSylviahAradikupitiasimuyarununu) 0723102535, au baruapepe sylviaharadi.sa@gmail.com.

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APPENDIX II: DATA COLLECTION TOOL

STUDY TITLE: **PROFILES OF VITAMIN D AMONG PATIENTS WITH RHEUMATOID ARTHRITIS AT THE KENYATTA NATIONAL HOSPITAL**

1. Socio-demographic information

1. Study ID number_____

2. Age (years)_____

3. Gender: Male Female (Tick one)

4. Marital status: Single Married Separated/divorced Widowed (Tick one)

5. Education level: None Primary Secondary Tertiary (Tick one)

2. Past/current medical History

i). Current rheumatoid arthritis medications

a) DMARDS

b) DMARDS plus steroids

c) Biologic agents

d) None

ii).Duration of RA since diagnosis

- 0-5 years
- 5-10 years
- 10-15 years
- Above 20 years

3. Laboratory parameters

1) Vitamin D levels (ng/dl) _____

Category of vitamin D status

- _30ng/dl (sufficiency)
- 20.1-29.9ng/dl (insufficiency)
- <20ng/dl (deficiency)

4. CDAI score

- Remission 0-2.8
- Low activity 2.9-10
- Moderate activity 10.1-22.0

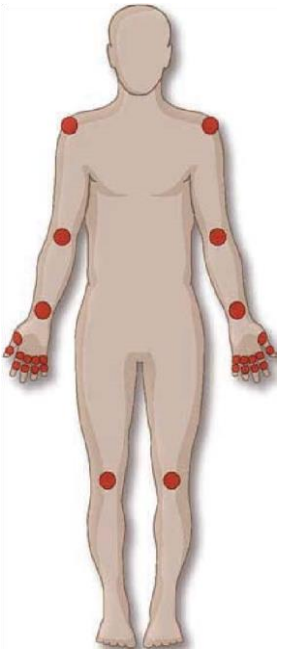
- High activity 22.1-76

5. Modified health assessment questionnaire

- No disability 0
- Mild disability 1
- Moderate disability >1 < 2
- Severe disability >2

APPENDIX III: CDAI SCORE

Joint	Left		Right	
	Tender	Swollen	Tender	Swollen
Shoulder				
Elbow				
Wrist				
MCP 1				
MCP 2				
MCP 3				
MCP 4				
MCP 5				
PIP 1				
PIP 2				
PIP 3				
PIP 4				



PIP 5				
Knee				
Total	Tender:		Swollen:	

Patient Global Assessment of Disease Activity

Considering all the ways your arthritis affects you, rate how well you are doing on the following scale:

Very □□□□□□□□□□□□□□□□□□□□□ Very
Well 0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10 Poor

Provider Global Assessment of Disease Activity

Very □□□□□□□□□□□
Well 0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 □ 5.5 □ 6.0 □ 6.5 □ 7.0 □ 7.5 □ 8.0 □ 8.5 □ 9.0 □ 9.5 □ Very 10 Poor

CDAI scoring

Variable	Range	Value
Tender joint score	(0-28)	
Swollen joint score	(0-28)	
Patient global score	(0-10)	
Provider global score	(0-10)	
Add the above values to calculate the CDAI score	**(0-76)**	

CDAI Score Interpretation	
0.0 – 2.8	Remission
2.9 – 10.0	Low Activity
10.1 – 22.0	Moderate Activity
22.1 – 76.0	High Activity

APPENDIX IV A: MODIFIED HEALTH ASSESMENT QUESTIONAIRRE (ENGLISH)

Please check (✕) the **one** best answer for your abilities**.**

At this moment, are you able to:	Without Any Difficulty (0)	With some difficulty (1)	With much difficulty (2)	Unable to do (3)
1. Dress yourself, including tying Shoelaces and doing buttons?	❑	❑	❑	❑
2. Get in and out of bed?	❑	❑	❑	❑
3. Lift a full cup or glass to your mouth?	❑	❑	❑	❑
4. Walk outdoors on flat ground?	❑	❑	❑	❑
5. Wash and dry your entire body?	❑	❑	❑	❑
6. Bend down to pick up clothing from the floor? …	❑	❑	❑	❑
7. Turn faucets on and off?	❑	❑	❑	❑
8. Get in and out of a car?	❑	❑	❑	❑

APPENDIX IVB: MODIFIED HEALTH ASSESMENT QUESTIONAIRRE

(KISWAHILI)

Wekaalamaya (✕) kwajibulakosahihi**.**

Kwawakatihuuunawezakufanya majukumuhaya:	Bila upungufu (0)	Kwa upungufu kadri (1)	Upungufu Zaidi (2)	Siwezi (3)
1. Kujivishanguo, pamojanakufungakambazaviatu navitaki?	❑	❑	❑	❑
2. Kupandanakutokakitandani?	❑	❑	❑	❑
3. Kuinuakikombehadimdomoni?	❑	❑	❑	❑
4. Kutembeamahalipasiponamwiunuko?	❑	❑	❑	❑
5. Kuoganakupanguzamwiliwote?	❑	❑	❑	❑
6. Kuinamanakuokotanguosakafuni? ……	❑	❑	❑	❑
7. Kufunguanakufungamifereji?	❑	❑	❑	❑
8. Kuingianakutokakwenyegari?	❑	❑	❑	❑

Scoring

The disability index is the mean of the eight items. If more than 2 items are blank, do not score the index.

INTERPRETATION =MEAN OF THE 8 ITEMS

DISABILITY	**HAQ SCORE**
No disability	0
Mild disability	1
Moderate disability	>1 < 2
Severe disability	>2