University of Nairobi

CORRELATION BETWEEN VITAMIN D LEVEL AND BONE MINERAL DENSITY IN KENYAN ADULTS AGED 50 YEARS AND ABOVE AT KENYATTA NATIONAL HOSPITAL

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JULY 2020

DECLARATION

DECLARATION

I hereby declare that this thesis is my original work. I also declare that it was developed with the guidance of my supervisors. It has not been submitted to any other university for any purpose.

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CERTIFICATE OF AUTHENTICITY

CERTIFICATE OF AUTHENTICITY

This is to certify that this dissertation is the original work of Dr. Masenge David Nyangau, a Master of Medicine student in Orthopedic Surgery at the University of Nairobi. This research will be carried out at Kenyatta National Teaching and Referral Hospital.

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DEDICATION

I dedicate this dissertation to my parents, my son Jezreel and her mother Razoah, my sister faith, my brother inlaw Kevin and the rest of my family for their love, support, and patience.

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LIST OF ABBREVIATIONS

ALP	Alkaline Phosphatase
BMD	Bone Mineral Density
BMI	Body Mass Index
BUA	Broadband Ultrasound Attenuation
CBD	Central Business District
DPA	Dual Photon Absorptiometry
DEXA	Dual X-ray Absorptiometry
EA	East Africa
GCs	Glucocorticoids
HIV	Human Immunodeficiency Virus
IPAQ	International Physical Activity Questionnaire
ISCD	International Society of Clinical Densitometry
KNH	Kenyatta National Hospital
ERC	Ethics and Research Committee
OPG	Osteoprotegerin
OPAC	Osteoporosis Prevention and Age Control
QUS	Quantitative Ultrasound
QCT	Quantitative Computer Tomography
pDXA	Peripheral Dual X-ray Absorptiometry
RANKL	Receptor Activator Nuclear Kappa Ligand
RANK	Receptor Activator Nuclear Kappa
SoS	Speed of Sound
SPSS	Statistical Package for the Social Sciences
UV-B	Ultraviolet B
VCT	Voluntary Counseling and Testing
WHO	World Health Organization
PI	Principal Investigator
RA	Research Assistant

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ABSTRACT

Background: There is inconsistent evidence in the orthopedic literature on presence of a correlation between vitamin D levels and reduced bone mineral density (BMD) and the relationship is sometimes controversial. Nine percent of Kenya's population is at least 50 years and above. Quantitative ultrasound (QUS) is an appropriate tool for determining BMD profile.

Main Objective: To evaluate the correlation between serum 25-hydroxyvitamin D (25(OH)D) levels and BMD.

Methodology: A cross-sectional study was conducted on 126 patients in Kenyatta national Hospital (KNH). Calcaneal QUS was used to measure BMD. The participants were asked to remove their shoes and stand on one foot on the ultrasound machine. Two measurements were conducted on both feet for all study participants. Descriptive data were presented as proportions and means with their standard deviations (SD) appropriately. Fisher's exact Test or Chi-square Test were used in comparison of categorical variables while Mann-Whitney test or Kruskal-Wallis Anova were used in comparing serum vitamin D levels (continuous variable) in the age categories appropriately. Point biserial correlation coefficient test was used to assess the correlation between vitamin D and BMD. Stepwise linear and stepwise logistic regression were used to adjust for confounders.

Results: The patients' mean age was 61.31± SD of 8.18. Of the 126 patients, 64.3% were males, and 63.7% had a history of bone fracture. The proportion of reduced BMD was 87.3% while 12.7% normal. Vitamin D deficient (<20 ng/mL), insufficient (20–29 ng/mL) and normal (≥ 30 ng/mL) were 10.3%, 31.7% and 57.9%, respectively. There was no evidence that serum vitamin D correlated differently in orthopedic outpatients and inpatients with BMD (*p*-value = 0.189). A statistically significant weak positive bivariate correlation of 0.351 was found between serum vitamin D and BMD. Serum vitamin D was associated/correlated with reduced BMD [adjusted OR: 1.16, 95% confidence interval (CI): 1.08 – 1.25; *p*-value <0.001] compared to normal BMD in adjusted logistic model. In an adjusted linear regression model, patients with reduced BMD had 7.53 (95% CI: 1.29–13.76; *p*-value =0.018) higher serum vitamin D levels than normal BMD. Patients in age category 70 – 79 years disproportionately had reduced BMD than other age categories. The proportions of reduced BMD were statistically significantly different across the age groups (p-value = 0.029).

Conclusion: Serum vitamin D had a weak but positive bivariate correlation with BMD, and in a multivariate analysis, serum vitamin D was strongly associated with reduced BMD. Therefore, vitamin D can be used in predicting reduced BMD. The prevalence of reduced BMD was high in this group of patients. Further prospective clinical research with Dual X-ray Absorptiometry (DEXA) may be needed to reveal why even with high levels of vitamin D, patients still have reduced BMD. The role of diet/vitamin D supplementation needs to be considered in future research.

CHAPTER ONE

1. INTRODUCTION

1.1 Bone Mineralization Density and Osteoporosis

Bone Mineralization Density (BMD) is described as mineral bone matter quantity per centimeter square. It is commonly used as an indicator of the risk of a fracture or development of osteoporosis. A low or decreased bone density indicates a higher probability of the development of osteoporosis or a fracture (2). In addition to BMD, susceptibility to a fracture and bone strength depend on arrangement and trabecular connectivity, biochemical aspects (like strain/stress response, elasticity and failure point), and other factors like bone shape, size, turnover, and architecture (3).

Osteoporosis is the commonest metabolic bone disease globally affecting a population of over 200 million people resulting in psychosocial, physical, and economic effects. Many a time it is undertreated overlooked since it is clinically silent unless it shows a fracture. In Kenya Osteoporosis is not considered a health priority. There is a lack of clinical guidelines for the diagnosis and management of osteoporosis (4).

1.2 Factors Affecting Bone Mineralization Density

Risk factors on osteoporosis may be categorized into 2 broad classes:

i) Non-modifiable risk factors.

ii) Modifiable risk factors.

1.2.1 Modifiable Factors

1.2.1.1 Weight

Study in Canada women aged between 40 and 59 Low weight and BMI predicted osteoporosis and was related to raised fracture risk in younger women. The negative impact of low body weight on the health of bone should be more widely observed (5). Another research conducted to further look into the relation across weight, BMI, and BMD in an Iranian men population. The results indicate that both BMI and weight are related to BMD of hip and vertebrae and obesity and overweight reduced the risk for osteoporosis (21,20).

1.2.1.2 Corticosteroid use

It the commonest type of secondary osteoporosis and the first cause in young people before the age of 50 years. GCs at high amounts dramatically lower osteoblast numbers, bone formation rate, and osteocyte activity and numbers. GCs raise the expression of RANK-ligand and lower the expression of osteoprotegerin in osteoblastic and stromal cells. In effect, a prolonged lifespan of osteoclasts is noted (contrasting with the decrease in the lifespan of osteoblasts). Earlier, much concern had been placed on the impacts of GCs on the metabolism of calcium, because of reduction in gastrointestinal uptake of calcium and induction of renal calcium loss (6).

There is up to a six-fold rise in the possibility of developing fractures due to osteoporosis in individuals on long term steroids (7). Corticosteroids usage, an anti-inflammatory drug that decreases the output of (IL-1 and IL-6), led to osteoporosis in patients suffering from rheumatoid arthritis (RA) (8).

1.2.1.3 Alcohol

Alcohol has also indicated direct toxic impacts on the osteoblasts in in-vitro studies (9). A meta-analysis and a systematic review demonstrated that multiple risk factors were related to low bone density-associated fractures in adulthood. Statistically significant relations for less BMI, excessive consumption of alcohol (described either as everyday consumption or above 10 servings every week), and increasing age were identified (9).

Excessive consumption of alcohol affects bone structure mainly through two postulated mechanisms. It decreases the body's activated Vitamin D levels thereby reducing the absorption of calcium from the diet, increasing bone resorption to restore normal calcium homeostasis. Secondly, it reduces the production of parathyroid hormone which is centrally involved in calcium regulation.

1.2.1.4 Smoking

Studies show that women who smoke have a greater risk for hip fracture unlike those who do not smoke, the risk rises together with cigarette uptake, while smoking men have a higher loss of bone at the trochanter (10).

Recent evidence shows that an imbalance in bone turnover is caused by tobacco smoking, resulting in reduced bone mass and creating bone vulnerability to fracture and osteoporosis.

Tobacco smoke indirectly affects bone mass via bodyweight alteration, adrenal hormones, sex hormones, parathyroid hormone-vitamin D axis, and raised oxidative stress tissues that are bony. More so, it directly affects bone angiogenesis and osteogenesis.

A RANKL-RANK-OPG route is a key regulatory route for the metabolism of bone and its significance is found in its interplay with the majority of pathophysiologic processes through which bone mass is affected by smoking. Both first-hand and second-hand smoke immensely affects mass of bone; ceasing to smoke seems to reverse the impact of smoking and develop the health of bones. New research approaches on markers of bone turnover may improve scientific know-how on the processes through which smoking affects the mass of the bone (11). Risk level reduces on quitting to smoke, but not declined significantly till 10 years later on from ceasing cigarette smoking (12).

1.2.1.5 Physical activity

Generally balanced physical activity in childhood and adolescence has been shown to improve bone health through mechanical stimulation which enables skeletal tissue development. Regular physical activity also increases muscle mass, reduces blood cholesterol and triglyceride levels, decreases fatigue, improves cardiorespiratory function, and increases bone mineral density (8).

Persons who ought to be considered having most at risk of osteoporosis are those with an adolescent lifestyle that is sedentary. Adults currently having a sedentary lifestyle are at a higher risk too (8). As of Canadian Multi-Centre Osteoporosis Study (CaMos), a retrospective analysis of data on a sum of 1169 female participants of 75years of age and above gave details based on the levels of their everyday activity, plus the quantity of time used every week doing exercise in differing intensity levels. To establish the impact of the growing extent of this frequent physical activity on BMD, Multiple and linear regression analyses were applied.

The findings indicated a step raise in the quantity of exercise done every day lead to positively impacting on BMD in Ward's triangle, the hip, trochanter with the femoral neck ($B = 0.006$ to 0.008, $p < 0.05$). Likely confounding elements like usage of anti-resorptive therapy, age plus BMI were added on the investigation and implored that one's age negatively impacted on bone

density while BMI affected positively. Anti-resorptive therapy gave a protective impact from bone density loss (13).

1.2.1.6 Diet

Greater emphasis has been placed on the significance of Vitamin D and calcium. However, there is increasing evidence for the effect of other nutrients (sodium, vitamin K, vitamin C, magnesium, potassium, manganese, zinc, phosphorus, copper, and others) health of bone. More so, studies are more on foods and food groups, indicating beneficial effects from vegetables, fruits, and whole grains with dairy products too (14).

In general, the "Prudent/Healthy" dietary pattern was influenced by high intakes of fruits, whole grains, legumes, vegetables, nuts, low-fat milk, low-fat dairy products, fish and reduced intakes of sugars, soft drinks, refined grains or cereals, processed meat and red meat (14).

Table 1. Risk factors for osteoporosis (in absence of a history of fracture)

Strongest Risk Factors	**Others**
Female sex	Smoking
Family history of osteoporosis	Caucasian origin
Age > 60 years	Early menopause
	Low BMI
	Long term (≥3 months) use of corticosteroid
	Sedentary lifestyle

1.2.1.7 Vitamin D Levels

The major impact of vitamin D 1, 25(OH) 2D is promoting absorption of calcium from the bowel. Effects by vitamin D deficiency are bone loss and secondary hyperparathyroidism, leading to fractures and osteoporosis, mineralization defects, which in the long term may lead to osteomalacia and weakness of muscles, causing falls plus fractures (1).

The status of Vitamin D determines the general mineralization of bone, fracture occurrence, and rate of bone turnover. Epidemiological studies have shown relationships between lesser BMD with vitamin D deficiency. There is a higher fracture incidence and higher bone turnover vitamin D deplete state cases. Studies on supplementation of Vitamin D that improved the

status of vitamin D have shown a BMD increase, a fracture incidence decrease, and a bone turnover decrease (1).

1.2.2 Non-modifiable Risk Factors

1.2.2.1 History of a previous fracture

Statistically significant risk factors were: an individual's history of a previous fracture or a fall that occurred within one-year passing and any history of parental bone crack (15). Women and Men of 65 years or above having a vertebral fracture do possess a five-year risk for hip or femur crack of 13.3% with 6.7% respectively (7).

1.2.2.2 Age

The study was carried out in the Korea National Health and Nutrition Examination Survey (KNHANES) from 2008 to 2010, where BMD on a lumbar spine plus the femoral neck was determined using dual-energy X-ray absorptiometry. Osteoporosis and Osteopenia diagnosed as per the WHO T-score basis. Bone Marrow Density records of 17,208 people (male, 7,837; female, 9,368) were analyzed and the results were women's osteoporosis normal occurrence was greater than of men (7.8% in males versus 37.0% in females) and it did increase with age.

The age group that had maximum BMD varied across the genders. For men in 20s possessed the greatest figure in overall sites of the skeleton. Moreover, in women, the highest BMD in the femoral neck, lumbar spine, and total hip was seen in their 20s, 30s, and 40s, respectively. Osteoporosis's starting age varied between genders. Osteoporosis for women in the femoral neck started when 55 years and in men when 60 years (16). As BMD decreases, the risk for osteoporosis adds up with age. A notable rise has been demonstrated in its occurrence for a given time decade-wise after the age of 60 (16,17).

1.2.3.3 Sex

Women, having smaller bones resulting in lower total bone mass are at higher risk for osteoporosis. Besides, following menopause, women are prone to losing bone much quickly but typically live longer. Osteoporosis still is a significant problem among men but less common. The rate of losing bone in men is lower compared to women. From Framingham's Osteoporosis Study, annualized bone loss in women as a percentage (rang, 3.4-4.8%) was higher compared to the loss in men (range, 0.2-3.6%) in all sites (18,13).

1.2.2.3 Ethnicity

African women are known to possess a greater BMD compared to white women in entire ages because of a slower loss rate and a higher peak bone mass. White women do have 2.5-times increased risk for acquiring osteoporosis (18,13).

1.2.2.4 Reproductive Factors

A far on in time menopause is related to increased BMD. Evidence has consistently shown that low BMD and early menopause relates. (6). Consequently, women who ought to be regarded at a greater risk for osteoporosis compared to others at the same age are those with early menopause (8).

There lacks constant evidence to show that the number of preceding miscarriages, tubal ligation parity, or breastfeeding influence BMD (19,20). Estrogen replacement therapy's current usage is connected with an increased BMD (17). Individuals on estrogen therapy at the moment to be considered to be at a lower risk than those at a similar age.

There were 201 postmenopausal Malaysian women aged 45–71years as participants of this study. Some lifestyle, reproductive and socio-demographic factors were written down. Quantitative ultra-sonography measured Calcaneal bone mineral density. Connections of bone mineral density with reproductive factors were assessed by multiple regression analysis and Pearson's correlation test. Results: One's Age during menopause is not significantly related to BMD.

Years following menopause, menarche, number of years gravid, and total periods of lactation were found to be inversely related to it. On reproductive contributors, just a relationship across the period of lactation with BMD remained important following adjustment for BMI, age, calcium intake plus activity. Conclusion: In the Exception of a prolonged period of lactation, the results indicated that other reproductive contributors were found not to be significantly connected to bone BMD among women after menopause (7).

1.2.2.5 Family History of Osteoporosis

Lesser BMD presents in men and women with a family history on osteoporosis (explained as a history of brittle bones, or osteoporosis, or less trauma fracture following the age of 50 years as reported by an offspring). A person's BMD reduces with an increase in the number of family members having osteoporosis. General family history can best sensitively predict the risk of

osteoporosis than only paternal or maternal history. Occurrence in time for present history on sisters is the same as that reported on mothers (16,(5).

Exploration on the impact of osteoporosis parental history on BMD did indicate an important association across just paternal (and not maternal) side history with lumbar spine BMD in the two sexes and an important association across maternal (unlike paternal) side history with hip BMD just among men (18).

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Introduction

Bone Marrow Density levels can be determined through several methods, the one with superior quality being the DXA. QUS is gaining popularity since its introduction in 1984 as a good epidemiological and screening tool in resource-poor settings. QUS is a mobile, easy to perform, relatively inexpensive, and radiation-free technique that can show fractures to the exact extent as DXA (5,10,3).

2.2 Assessment of Bone Mineral Density

Table 2. World Health Definitions of Bone Marrow Density Levels

WHO Definitions as Per Bone Density Levels	
Normal	-1.0 or above
Osteopenia	The bone density between 1 and 2.5 SD below the young adult mean (−1 to −2.5 SD)
Osteoporosis	Bone density is 2.5 SD or more below the young adult mean (−2.5 SD or lower).
Acute (established) osteoporosis	Bone density more than 2.5 SD below the young adult mean, and there have been one or more osteoporotic fractures.

It is possible to measure BMD by several methods. This includes Quantitative ultrasound, Dual-energy X-ray absorptiometry, Quantitative computed tomography plus others (single photon absorptiometry, digital X-ray radio-grammetry as well as dual photon absorptiometry) (19).

2.2.1 Dual X-Ray Absorptiometry

DXA formerly referred to as DEXA scan is the benchmark method for measuring BMD. A DXA scanner delivers two X-ray beams, one high-energy, and one low-energy. Quantity of X-rays that goes throughout a bone (dependent on bone thickness) is measured for either beam. The two beams difference helps establish the density of the bone and is presented as a ratio of bone matter to the scanned area (40). It emits low radiation levels coupled with high precision and is non-invasive. Dual photon-absorptiometry (DPA) uses a radioactive substance to measure bone density. BMD can be assessed at the spine or shoulder. DPA still uses very low radiation doses but has a longer testing time than the DXA (20).

Mineral matter of the bone is measured or areal mineral density of the bone, that is, the quantity of mineral of the bone divided by the scanned bone area. Dual x-ray absorptiometry (DXA) mechanisms are now approved to do this measurement both at the two sites of the skeleton subject to osteoporotic fracture in particular, like the proximal femur and lumbar spine plus at outer skeletal part like the forearm. Hip with/or spine should be mainly looked at in diagnosis for osteoporosis. BMD reports a variance of greater than two-thirds of strength of the bone as established in vitro on far away pieces of the skeleton, like the vertebral column. An inverse relation exists between osteoporotic fracture occurrence and DXA-produced BMD figures (19).

Dual X-ray Absorptiometry (DXA) gives details on the status of mineralization but does not touch on the quality of bone. The WHO recommends the use of DXA method in determining BMD levels which have guided classifying the levels into clinically relevant outcomes following the value of standard deviations (SDs) under an average BMD to a (25–35 years) young, healthy, sex- with ethnicity-matched population reference (T-score). It practically explains osteoporosis as BMD which drops 2.5 SDs under the average for healthy same-sex young adults —also termed T-score of –2.5. Females after menopause falling at a further end of the young usual range (a T-score ≤1.0) explained as possessing little bone density likewise, and also more probable of getting osteoporosis(1,(21). Additional techniques used in determining bone mineral density are quantitative ultrasound, quantitative computer tomography, digital radio-grammatory as well as dual photon absorptiometry.

Using the DXA, BMD levels, the WHO developed guidelines for classifying levels into clinically relevant outcomes depending on the value for SDs under an average BMD of a young and healthy (25–35 years old), sex- with ethnicity-matched population reference (T-score). This classification was initially used on post-menopausal women but has now been generalized to other adult populations. Osteoporosis is described from the T-score below or equivalent to −2.5 in the spinal or hip while osteopenia is that of between −1 to −2.49 and the normal one is that of greater than -1 (5,12,3).

Radio-grammetry software can provide a BMD calculation with an error of less than 1% from digitized plain forearm and hand radiographs. Peripheral DXA (pDXA) gadgets have been developed to offer simpler and cheaper alternatives to DXA instruments that scan the central skeleton. DXA-calculated sites are a range of regions within the calcaneus or forearm (19).

2.2.2 Vitamin D Serum Levels

The usual 25(OH) D figures are still poorly-illustrated and there is no existing agreement regarding the least serum amount of 25-hydroxyvitamin D needed to assure excellent health. According to an article from Orthopedic Surgery Department, School of Medicine, University General Hospital of Alexandroupolis, Democritus Thrace University, Dragana, 68100 Alexandroupolis, Greece Usual serum 25(OH)D figures had been described as >20 ng/mL. According to Fitzpatrick deficiency was explained as when serum figures are <20 ng/mL while acute deficiency when <21–29 ng/mL. Figures of Serum of 25(OH) D >200 ng/mL are regarded as harmful (22).

From Kagotho E's study, the levels of Vitamin D classified as sufficient (>30 ng/ml), while (<20 ng/ml) as deficient while (21-29 ng/ml) as insufficient. Reference ranges for PTH were 15-65 pg/ml, total calcium 2.1–2.66 mmol/l, and inorganic phosphate 0.84–1.45 mmol/l (23).

The studies utilized varying cut-offs to describe deficiency of vitamin D making it difficult for directly comparing its prevalence. Most of them failed to relate levels of Vitamin D with surrogate markers of physiological insufficiency like phosphate, PTH, and calcium, and sample sizes were insufficient (23).

Other elements affecting skin yield of vitamin D3 are the melanin content of the skin, time of day, age, cloud cover, air pollution, and clothing extent that covered the body (23). An Increase in age decreases the quantity of 7-dehydrocholesterol in the skin resulting in a lower output of vitamin D3 (35). Time of day, Latitude, and season influence the quantity of UVB solar photons accessing the earth thereby impacting on skin's vitamin D3 output (23).

From Fitzpatrick, advocates the usage of serum circulating 25 (OH) D levels measured from a dependable trial, to assess the status of vitamin D on victims liable to deficiency of vitamin D. Vitamin D deficiency description is a 25(OH) D under 20 ng/mL (50 nmol/liter) while vitamin D insufficiency as a 25(OH) D of 21–29 ng/mL (52.5–72.5 nmol/liter) (22).

2.2.2.1 Vitamin D Sources and Metabolism

By Alshahrani, Vitamin D can be found as either cholecalciferol (vitamin D3) or as ergocalciferol (vitamin D2). Ergocalciferol, obtained in plants is changed to 25-

hydroxyvitamin D2 (25(OH) D2) by the liver, then to 1, 25-dihydroxyvitamin D2 (1, 25(OH) 2 D2) by the kidney (24).

Cholecalciferol alike, derived from animals, is changed to 25(OH) D3 thereafter to 1, 25(OH) 2 D3. Cholecalciferol is plentiful in a couple of food sources (e.g., fish liver). It is often used either with calcium or alone, as a dietary supplement, (24). Ultraviolet B (UV-B) radiation (290–315 nm) changes 7-dehydrocholesterol in extending far down surfaces of epidermal to provitamin cholecalciferol (24).

Following Holick, Vitamin D(D represents D2,D3 or both) in diet and the skin is broken down to 25-hydroxyvitamin D in the liver, whose study was on vitamin D for health; 25-hydroxyvitamin D is broken down in the kidneys by the 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1) enzyme to 1,25-dihydroxyvitamin D its active form. 1-4, Renal output of 1,25-dihydroxyvitamin D is closely controlled by hormone levels of plasma parathyroid, phosphorus, and levels of serum calcium (31).

Fibroblast growth contributor 23, derived from bone, makes a co-transporter of sodium–phosphate to be embodied by the small intestine and kidney cells and restrains synthesis of 1,25-dihydroxyvitamin D too (Fig. 1) (2,3,6). Besides, it brings about the expression of 25-hydroxyvitamin D-24-hydroxylase (CYP24) enzyme, which breaks down 25-hydroxyvitamin D with 1,25-dihydroxyvita-min D into water-soluble, biologically innate calcitroic acid (25).

2.2.2.2 Vitamin D and Intestinal Absorption of Calcium

Metabolite 1, 25(OH) 2D of active vitamin D does the following; draws open the calcium channels in the bowel, raises levels of the making of calcium-binding protein in cells of the intestine, and by so doing raises the uptake of phosphate with calcium from the bowel. This process creates Optimal circumstances for mineralization of bone (26).

2.2.2.3 Serum 25(OH) D and Serum Parathyroid Hormone Bone turnover and Bone Marrow Demineralization

Mineralization is a passive process in itself the moment sufficient Vitamin D and calcium are present. In the event of deficiency of vitamin D, the concentration of 1, 25(OH) 2D might go down and lesser calcium could be present for mineralization of the bone. The level of parathyroid hormone (PTH) will go up, raising levels of hydroxylation of 25(OH) D to 1,

25(OH) 2D in the kidney. A rise in serum PTH raises the level of bone turnover, resulting in loss of bone. Serum 1, 25(OH) 2D in the usual reference range plus uptake of calcium is put back, instead of raised resorption of bone in the new steady state.

Loss of bone is raised in the event of extended vitamin D deficiency and by so doing can result in osteoporosis (Fig. 1). High bone turnover consists of many osteoid specialized cells (yet to be bone mineralized) since much refurbishing takes place on the bone surface compared to in usual situations. More so, the bone that has undergone mineralization has got a smaller amount of mineral, since the average osteons' age is not so great and the mineral is collected for a total of 2 years on the make-up of osteon (26).

In the event of an acute deficiency of vitamin D that has existed for a long time, the capacity of the osteoid specialized cells dropped by 5%, thereby bringing about osteomalacia. Overt osteomalacia was not detected in a chain of about 119 bone biopsies on hip fractured patients, but in 20% of those patients, a great bone remodeling was noted. In this chain of patients, an estimated 80% of them possessed serum 25(OH) D under 25 nmol/l. In 0–37% in 19 chains of patients who had fractured hip, hypersteroidosis was noted although with very varying basis, like osteoid capacity, osteoid surface, osteoid thickness, and osteoid lamellae number. Osteomalacia ranged from 0 to 12% when the thickness of the osteoid was applied as a key basis (26).

According to Ravn, high turnover is relatively evident in elderly persons, mainly in hip fractured patients; surprisingly, biopsies on patients having acute vitamin D deficiency and hip fracture don't often manifest the vitamin D deficiency signs. Secondary hyperparathyroidism is mostly due to loss of bone in patients having vitamin D deficiency and is irreversible for most cases. Vitamin D deficiency is connected to added serum PTH, but between serum 25(OH)D and serum PTH, the correlation coefficient doesn't surpass 0.35, showing an average relationship (26).

In the aged population, other significant elements of serum PTH are immobility and renal function. As creatinine clearance falls under 60 ml/min, Serum PTH rises and serum PTH is restrained by immobility. Serum PTH may also be restrained by a huge intake of calcium. Relations into serum PTH serum, serum 25(OH) D and BMD are very evident in vitamin D

insufficiency patients and Vitamin D deficiency patients, yet not noted a replete state of vitamin D. Following these outcomes threshold levels of serum 25(OH) D might be accepted (26).

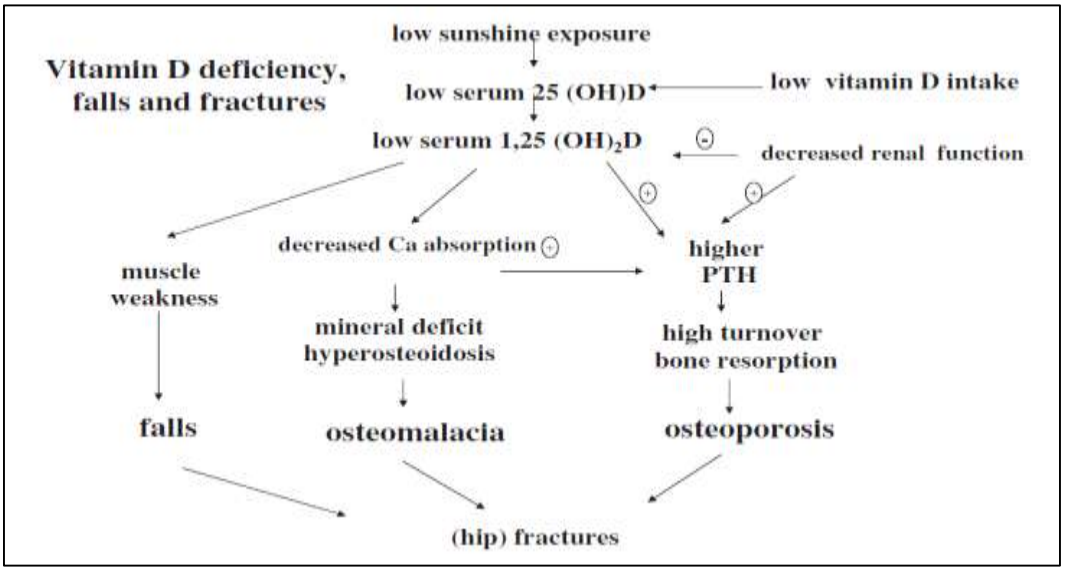

Figure 1. The Pathophysiologic routes from vitamin D deficiency to osteoporosis, osteomalacia, falls and fractures, adapted from Consensus Development Conference. Diagnosis, prophylaxis, and treatment of osteoporosis Am. J Med. 1993 (1)**.**

2.2.3 Quantitative Computerized Tomography (QCT)

This is used on appendicular and axial skeleton. Informs on tridimensional volumetric density and differentiates across the cancellous and cortical bone envelopes. Besides, it evaluates shape and architecture. To calibrate the density measurements a simultaneously scanned bone phantom is used. Cancellous bone reacts quickly to most disease healing interventions hence for monitoring treatment at the vertebral body levels, this technique could be of theoretical interest. A lesser reproducibility at the minimum for the basic assessment of radiation exposure, axial skeleton, or the instruments' cost, constitute real disfavor (19).

2.2.4 Quantitative Ultrasound (QUS)

The ultrasound is a sound wave type and has a frequency surpassing the usual auditory range for human beings (>20 kHz). A frequency applied in QUS normally falls at 200 kHz to 1.5 MHz. Waves in form of sound give rise to distinctive piezoelectric probes as released and moved horizontally or longitudinally throughout the bone in the study. Usually on QUS gadget, there exist two probes: the receiver and the emission probes. A bone segment in the study to be kept across the probes then ultrasound waves released out of emission probes will be sensed by the receiver probe along the bone (27).

There exist two QUS kinds according to an axis on which the waves of the ultrasound are used to move along in the bone. The horizontal transmission does use probes that ascertain sound speed on a bone's cortical covering at a predetermined distance. The bone segments ascertained that way include the tibia and radius. Longitudinal transmission most often is applied to measure the calcaneus segment of the bone (6). Calcaneal QUS occurs as an only approved QUS measurement and it determines the health status of the bone. This is because a bigger number of research has been conducted on it than on other segments of the bone, according to the International Society of Clinical Densitometry (ISCD) (28).

Investigations from the Laboratory have indicated that QUS measurements of excised bone samples are characterized by structural bone characteristics material properties. More so, ultrasound velocity has been applied widely to distinguish the elastic features of the cortical with trabecular bones. Taken together, these findings show that unlike BMD, QUS could assess the quality of bone, especially microarchitecture, and thereby be applied for determining predisposition to a fracture (21). The objective of many studies has been to assess the use of a heel QUS in osteoporosis which was done in elderly osteoporotic women; others have mainly preferred populations consisting of poor bone quality (10,11).

Quantitative ultrasound (QUS) technique of measurement had been introduced for the analysis of the status of the bone in osteoporosis from an evaluation of ultrasound attenuation and velocity. The calcaneus, due to its large capacity of trabecular bone and ready accessibility, the phalanges can be selected for the transmission measurements. Physical measurements are a measure of the attenuation of ultrasounds via the bone (Broadband Ultrasonic Attenuation, BUA, expressed in decibels per megahertz) and Speed of Sound (SOS). Both BUA with SOS is lesser in victims having osteoporosis (19).

The calcaneus too comprises 95% of trabecular bone with 2 sideway surfaces, which makes it easier for the ultrasound waves to travel along it (5,6). Thus, the study will stress on the calcaneal QUS technique of measurement. QUS includes placing ultrasound transducers on each bone side of interest: one functions as a wave transmitter while another as the receiver. The following gadgets evaluate three major frameworks:

- Broadband ultrasound attenuation (BUA)
-The velocity of sound or Speed of sound (SOS)
- Quantitative ultrasound stiffness index

BUA measures the dependence frequency of little reduction in amplitude of ultrasound indication that happens by the time energy is being moved from a wave, first and foremost through absorption plus scattering in both soft and bone specialized cells (27). Velocity of sound does ascertain the length traveled by the ultrasound signal for every unit time (29). Quantitative ultrasound stiffness and index are compound frameworks obtained out of BUA plus SOS or sound velocity (5,35). Ultrasound parameters are commonly lesser in osteoporotic/osteopenic bone as compare to in a bone that is healthy (29).

Both DXA and QCT involve the utilization of specialized equipment, generate ionizing radiation, are expensive, and require relative expertise. Quantitative calcaneal ultrasonography offers several benefits. It is cheaper and more portable than DEXA, there is no exposure to ionizing radiation and is as effective as DEXA at predicting femoral neck, hip, and spine osteoporotic fractures (4,5,6). With the advancement in technology, the quantitative ultrasound (QUS) is popularly becoming a proper tool for determining BMD profiles in poor resource settings (5,7,8).

2.2.5 Utility and Comparability of Quantitative Ultra-Sonography to Other Diagnostic Tools for Bone Marrow Density Assessment

Most studies have shown that the prediction of fracture through QUS is equivalent to and at times better compared to DXA. Quantitative ultrasound technology turns out as the best tool in screening for osteoporosis. It gives more details on BMD besides bone microarchitectures. From several studies, it can also estimate fractures on the two genders. In countries that are still developing and whose accessibility to DXA is poor, QUS can be an appropriate tool for testing the absence or presence of osteoporosis early enough (27).

In 2006, a meta-analysis of 25 studies concluded that QUS is non- inferior to DXA using the current WHO-recommended cut-offs (3). Quantitative ultrasound accomplished similarly to BMD ascertained at the spine and femur by DXA on assessing glucocorticoid-induced osteoporosis (36,37). Unlike DXA, other studies have indicated that QUS may have the ability to evaluate bone quality additional to BMD (30). Other studies have noted that QUS appears to have the ability to differentiate between normal and osteoporotic patients to some extent independent of BMD in several cases (31).

Other advantages of QUS over the other methods are that it is relatively cheaper, radiation-free, easier to use, and more transportable (28). Both prospective and cross-sectional studies have illustrated that QUS can differentiate normal from osteoporotic subjects closely as efficiently as traditional bone measuring approaches. These advantages, together with clinical results presenting good diagnostic sensitivity for fracture distinction, have promoted increased utilization in clinical settings.

2.3 Statement of the Problem

Africa being a diverse mainland bestrides the equator has got the Southern with Northern and mild areas. The majority of African nations enjoy sunshine throughout the year from their closeness to equator (6). There is little Data on levels of vitamin D among Africans who are healthy with a deficiency in vitamin D fluctuating from 5 to 91% and average levels of vitamin D fluctuating from 4.4–46.1 ng/ml (1).

Kenya's present population is about over 40 million: 9% (3.5 million) of these are 50 years of age or above while 5% (1.9 million) are 70 years or above. By 2050, it is approximated that 17% (14 million) of the total number of people to be 50 in years or above then 10 % (7.8 million) to be 70 in years or above, whilst the overall population shall rise to about 80 million. The osteoporosis prevalence will go up due to this rising aging population (1).

A study done in a hospital on osteoporosis in Kenya by Odawa indicated that the osteoporosis prevalence in a black population of females (50 years and above) is at 24.5% currently. In Kenya, Osteoporosis has not been considered a health concern. Clinical guidelines for treatment and prevention of osteoporosis are lacking. In men and women, it has been associated with the main risk factors connected to the demineralization of bone or underlying conditions (1).

Available screening methods to determine reduced BMD include; calcaneal QUS, DEXA, and clinical risk evaluation tools. For screening epidemiological reasons, QUS issues an effective tool for comparing BMD between varying groups and recognizing elements related to differences in bone density mainly in areas where DEXA is unavailable (3,10).

Currently, there are no studies carried out in East and Central Africa to establish the BMD prevalence in low levels of vitamin D patients using quantitative calcaneal ultrasound despite sub-Saharan Africa's poor social-economic status and a heavy burden of osteoporosis. This may be due to the low index of suspicion among clinicians, limited availability, and the prohibitive cost of DEXA for assessing bone mineral density (1).

2.4 Justification

Worldwide, Osteoporosis turns out as the commonest metabolic disease for the bone and it can lead to destructive psycho-social, economic and physical implications. Affected persons undergo pain, diminished quality of life, and disability. It is frequently undertreated and overlooked. Many times it is clinically silent before manifesting as a fracture.

In Kenya, Osteoporosis has not been regarded as a health concern. Official disease guidelines and government public programs for awareness about diagnosis, management, and prevention of osteoporosis, fragility, and fractures are lacking. Additionally, there is low awareness about the disease amongst health care professionals since it is not included in the medical school curriculum and also a majority of practitioners, (except for well-trained orthopedists), are prepared and trained poorly to diagnose and treat osteoporosis.

The Kenyan based Osteoporosis Prevention and Age Concern (OPAC) is at the moment mobilizing and organizing the aged population and raising their views on health in addition to organizing World events like Osteoporosis Day here in Kenya. The OPAC, Kenya, also organizes programs on lifestyle prevention of osteoporosis. There are inadequate tools to ascertain bone density both in Kenya and also in the East African Countries.

Following the latest developments in densitometry technology that has laid down alternative methods of determination of bone mineral density; quantitative ultrasound happens to be the most used widely, providing a cheap, efficient, and low-risk alternative for estimating the prevalence of osteoporosis in Kenya. Finally, there is a paucity of data on studies looking at bone mineral density in the adult population within Eastern Africa and the African continent at large.

2.5 Research Question

Is there a correlation between low Bone Mineral Density and low vitamin D levels in serum in adult patients aged 50 years and above attending the orthopedic clinic and wards in Kenyatta National Hospital?

2.6 Null Hypothesis

Null Hypothesis 1 (H_{01}): There is no correlation between Bone Mineral Density and vitamin D levels in adult patients aged 50 years and above in Kenyatta National and Referral Hospital.

Null Hypothesis 2 (H_{02}): The correlations of serum vitamin D levels with Bone Mineral Density in adult patients aged 50 years and above seen at the orthopedic clinic is equal to the correlations of serum vitamin D levels with Bone Mineral Density in adult patients aged 50 years and above at the wards in Kenyatta National and Referral Hospital.

2.7 Study Objectives

2.7.1 Broad Objective

To determine the correlation between Bone Mineral Density and vitamin D levels in adult patients aged 50 years and above in Kenyatta National and Referral Hospital.

2.7.2 Specific objectives

1. To determine Bone Mineral Density in adult patients 50 years and above managed at KNH.
2. To determine the levels of vitamin D in adult patients 50 years and above at KNH.
3. To compare the Bone Mineral Density and vitamin D levels among adult patients 50 years and above at Kenyatta National Hospital.

CHAPTER THREE

3. METHODOLOGY

3.1 Study Design

The study was a cross-sectional observational study design in the order establishing the correlation between bone mineral density and vitamin D levels among patients aged 50 years and above admitted in the orthopedic wards and attending the Orthopedic Clinic at the Kenyatta National Hospital. The research design enabled the comparison of a novel diagnostic approach for bone marrow demineralization with the existing "hallmark" diagnostic approach, in this case, bone mineral density, in a cross-section of both healthy and diseased respondents to determine potential usefulness. The same patient was used for comparison while assessing bone mineralization density and vitamin D levels, hence controlling for any inter participant variabilities.

3.2 Study Setting

This study was carried out in KNH orthopedic clinics plus wards. KNH is a tertiary teaching and referral hospital located at the upper hill area 5 km from CBD with an orthopedic bed capacity of 250. It is the largest public referral hospital in east and central Africa region. KNH orthopedic clinics are run from Mondays to Fridays with an average of 350 patients per week with the majority being adults aged above 30 years. The orthopedic clinics are usually run by the consultants and registrars in orthopedic surgery under guidance from the consultant orthopedic surgeons both from the University of Nairobi and KNH.

3.3 Study Population

All patients aged 50 years and above seen at KNH orthopedic clinics and admitted in orthopedic wards fulfilled the inclusion criteria.

3.4 Inclusion Criteria

1. Patients admitted to the orthopedic wards and seen at the orthopedic clinics at Kenyatta National Hospital who are aged 50 years and above.

3.5 Exclusion Criteria

1. Those who had single/double lower limb amputees

2. Those with bilateral calcaneal/foot wounds
3. The presence of persistent medical illnesses and organ dysfunction or being under medications which can affect bone mass or change the vitamin D level.
4. Pregnant, lactating, and postpartum females, within one year of delivery.
5. Patients who were unwilling to give consent.

3.6 Sample Size Determination

The sample size was determined by use of the Fishers formula:

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

Where,

n = Desired sample size

Z = value from standard normal distribution corresponding to the desired confidence level (Z=1.96 for 95% CI)

P = expected true proportion (patients with osteoporosis following ultrasound estimation of BMD. In a study conducted by Abdullah et al, where 9.1 had osteoporosis diagnosed using BMD assessment (40).

d = desired precision (0.05)

$$n_0 = \frac{1.96^2 x\ 0.09(1-0.09)}{0.05^2} = 126$$

A sample size of 126 patients was recruited for this study.

3.7 Sampling Procedure

Consecutive sampling was used to identify all the eligible participants until the sample size was arrived at. It was a non-probability sampling technique that seeks to include all accessible subjects as part of the sample.

3.8 Ethical Considerations

The KNH Ethics, Review, and Standards Committee (KNH ERC) was requested for approval for conducting the study. The necessary approval was received, the study recruited patients who met the eligibility criteria and were given written informed consent. The Department of Orthopedic Surgery, UON, and KNH Ethics and Review Committee was sought for ethical approval. In beginning the analysis, copies of this protocol, the informed consent form, and

any subsequent changes to either document were sent for written approval to the above-mentioned authorities.

The research used guidelines from WHO for the study involving human participants throughout the process. Participants received a good explanation concerning the core reason for carrying out this study to be in a good position of obtaining written informed consent before participant enrolment. They were also clarified that there was no payment and therefore it was understandable if they decline the invitation and this would affect their treatment in any form. Participants were free to decline to answer these questions if they felt them to be intrusive. Strict confidentiality was observed throughout the study by the participating investigators, research assistants, and study institutions. Participants were given study identification numbers and no personal identifiers were used. The study subjects could leave the research exercise at their own free will and that did not jeopardize their treatment.

3.9 Recruitment Strategy

Upon receiving ethical clearance and the KNH research committee to conduct the study, the clinicians attached to the department of surgery and clinics were sensitized about the study. This entailed a clear understanding of the study procedures with minimal interference with the patient flow. Records for the patients attending the clinic or admitted in the orthopedic wards and who fit within the eligibility criteria were identified and labeled using a white sticker on the morning of the clinic attendance or admission.

Patients who qualified to be enrolled were identified once they arrived at the clinic or wards and the procedure for the study explained before administration of the written consent for signing. All potential study participants were escorted to a private room within the clinic or ward. From here, the written consent, both in English and Swahili, was administered by either the PI or RA. Those who decline to further participate in the study were excluded from the study.

3.10 Study Flow

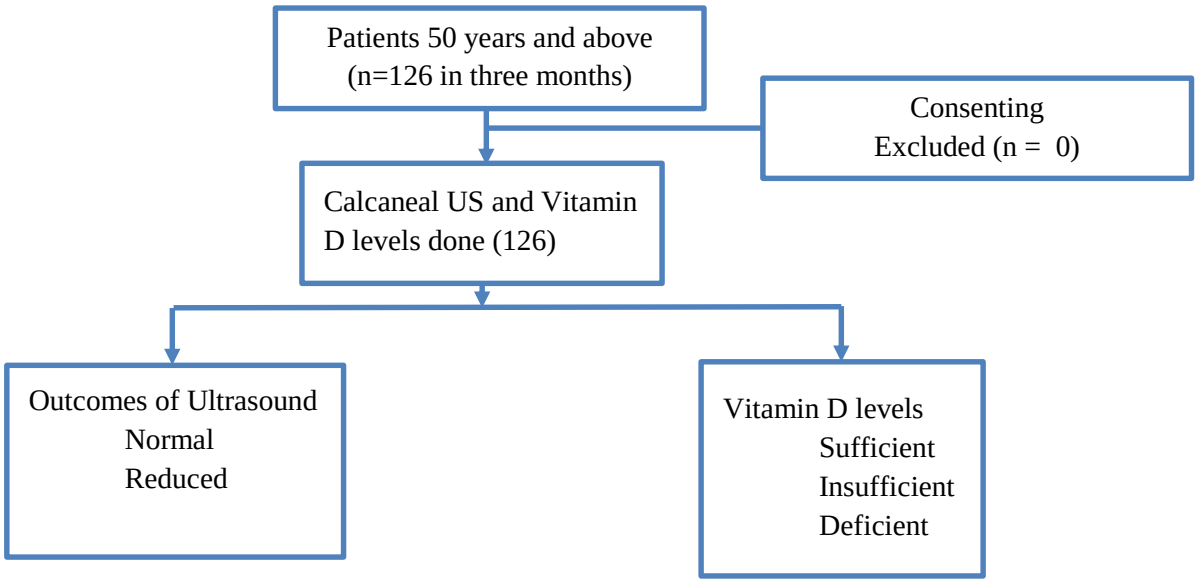

Figure 2. Study Flow Diagram

Following participant recruitment, data was collected from enrolled patients using a questionnaire administered by either the principal investigator or the research assistants, who were two (a clinical officer and a nurse) with training in research methodologies and data collection procedures. Names were not recorded instead a study number was assigned.

3.12 Study Procedures

3.12.1 Quantitative Ultrasonography

Quantitative Ultra-Sound of bone mineral density was assessed by the use of a Samsung Mysono U-6 Model ultrasound machine by a qualified sonographer. The participants were asked to remove their shoes and stand on one foot on the ultrasound machine. Two measurements were conducted on both feet for all study participants. BMD levels were expressed as normal or reduced depending on the level of shadowing of bone.

3.12.2 Assessment of Vitamin D3 levels

All participants had their blood drawn by the PI or RA on the day when they consented to participate in the study after administration of the study questionnaire. Five (5) ml of venous blood from the cubital vein of the non - dominant hand was drawn in the morning for serum

25OHD from each of the study participants using a 23-gauge needle. The samples were taken to the laboratory using a red top blood collection bottle within one hour and results collected within one day. 2 ml were required for the analysis, the excess blood sample was stored in the freezer for at least one month. The standard KNH biochemistry laboratory request form was filled and the sample and request form taken to KNH for processing.

Assessment of vitamin D3 levels was done from the KNH biochemistry laboratory. Vitamin D was measured using a Cobas E 6000 machine using reagents from Roche. The samples were put in a sample cup labeled with the patients' details and centrifuged for 3 minutes then analyzed with the reagents already on board. The cut-off points were as follows Vitamin D sufficient > 30ng/ml (70nmol/litre), deficient <20ng/ml(50nmol/litre), insufficient 21-29ng/ml(50-70nmol/litre). Excess blood was discarded using KNH laboratory infection control practices after one month.

3.12.3 Quality Assurance Procedures

The questionnaires were pre-tested and analyzed before a final draft was administered to the study participants. The research assistants were trained on appropriate interview techniques and filling the questionnaires. The recording of clinical findings was entered after thorough scrutiny. Unique identifiers were assigned to all the study participants. Study samples were taken under aseptic conditions by the two trained research assistants who were either a qualified clinical officer or nurse. Analysis of the blood samples was undertaken within the KNH biochemistry laboratory. The labeling and type of specimen bottle were confirmed after which samples were separated and quality control was done on the machine before analyzing the samples. External quality control is done monthly by submitting the results to RIQAS (Randox International Quality Assessment Scheme) online.

3.13 Data Variables

3.13.1 Dependent Variable

Reduced bone mineral density was categorized into either low, normal, or high according to the shadowing and trabecular pattern of the calcaneus bone. Serum levels of vitamin D were also categorized as low if less <20ng/ml.

3.13.2 Independent Variables

A face-to-face patient interview collected data documenting risk factors for low bone mineral density. These included the previous fracture of the bone, alcohol consumption, use of oral corticosteroids, smoking status, use of oral contraception, and physical activity. Assessment of the Physical activity was done by a short frequency questionnaire.

3.14 Data Management and Analysis

Data collection was done by the principal investigator, two trained research assistants (clinical officer and nurse), and a sonographer. Before data collection, the questionnaire was pre-tested among 10 patients randomly selected at the KNH surgical department. Data collection was done over a period of two months. The collected data was entered into a password-protected Microsoft Excel database managed by the statistician. Once data entry was complete, entries in the database were compared to the hard copies to ensure accurateness and the detected inconsistencies corrected before data analysis. The collected data was imported into R software version 4.0.2 for data management and analysis.

Descriptive data were presented as proportions and means with their standard deviations (SD) appropriately. Fisher's exact Test or Chi-square Test were used in comparison of categorical variables (age categories and bone fracture history by BMD status) while Mann-Whitney test or Kruskal-Wallis Anova were used in comparing serum vitamin D levels (continuous variables) in the age and bone fracture categories appropriately. Point biserial correlation coefficient test was used to assess the bivariate correlation between vitamin D and BMD. Stepwise linear and stepwise logistic regression were used to adjust for covariates in the relationship/correlation between vitamin D and BMD.

3.15 Dissemination of Results

Study findings were compiled and availed to:

1. Department of orthopedic surgery-UoN.
2. The University of Nairobi, Faculty of medicine, college of health sciences.
3. Board of postgraduate studies UON.
4. Kenyatta National Hospital.
5. University of Nairobi library.
6. The findings will be sent for publishing in peer-reviewed scientific journals.

3.16 Study Delimitations

Correlation of the diagnosis with history, imaging, and serum levels of vitamin D. Using the same patient to assess Bone Mineral Density and Vitamin D levels will provide a certain way of comparing the two values without the need for matching of the participants. Since ultrasonography is a large user-dependent assessment and could, therefore, introduce measurement bias, this was overcome by utilizing only one sonographer experienced in the assessment of BMD using ultrasonography.

CHAPTER FOUR

4. RESULTS

4.1 Characteristics of the study population

The mean age of the patients was 61.31 with a standard deviation (SD) of 8.18. Most of the patients were male (64.3%) and 81.7% were married. Six in ten (63.7%) had a history of having sustained a bone fracture. Of the 126 patients, 82.5% reported having had no vigorous activity in the last 7 days and 25.4% reported that they had moderate physical activity in the past 7 days, given this, the percentage of the study participants who were inpatients was 48.4% and 51.6% were from the orthopaedic clinics. Most of the patients reported that they were self-employed (47.6%). In terms of known risk factors for reduced BMD, the use of oral corticosteroids continuously for a period equal to or exceeding three months was reported by 29.4% and this related well with high number of the patients who reported history of having sustained a bone fracture. Additionally, patients who reported that they used to smoke were 36.5%. Details of the study participants are described in Table 1 below.

Table 3. Patients' characteristics, Kenyatta National and Referral Hospital (n= 126)

Total No. of Participants = 126			
Characteristics		**Frequency/ Mean ± SD[1]**	**Percentage**
Age (years), Mean ± SD		61.31± 8.18	–
Gender			
	Male	81	64.3%
	Female	45	35.7%
Marital status			
	Married	103	81.7%
	Single	3	2.4%
	Divorced	11	8.7%
	Widowed	9	7.1%
Residence			
	Urban	68	54.0%
	Rural	58	46.0%
Highest level of education			
	None	5	4.0%
	Primary	65	51.6%
	Secondary	41	32.5%
	Tertiary	15	11.9%
Occupation			
	Unemployed	47	37.3%
	Self-employed	60	47.6%

[1] SD – Standard Deviation

Table 3. Patients' characteristics, Kenyatta National and Referral Hospital (n= 126)

Total No. of Participants = 126				
Characteristics			**Frequency/ Mean ± SD[1]**	**Percentage**
	Civil servant		15	11.9%
	Retired		4	3.2%
Osteoporosis risk factors				
	Used oral corticosteroids continuously for a period equal to or exceeding three months			
		Yes	37	29.4%
		No	89	70.6%
	History of smoking cigarettes			
		Currently smoke	1	0.8%
		Used to smoke	46	36.5%
		Never smoked	79	62.7%
	Frequency of taking an alcoholic drink			
		Never	51	40.5%
		Monthly or less	14	11.1%
		Weekly	17	13.5%
		Daily	6	4.8%
		Stopped	38	30.2%
	Use of oral contraception			
		Yes	22	17.5%
		No	104	82.5%
	History of sustaining a bone fracture (n=124)			
		Yes	79	63.7%
		No	45	36.3%
Physical activity				
	Vigorous activity in the last 7 days			
		Yes	22	17.5%
		No	104	82.5%
	Moderate physical activity in the last 7 days			
		Yes	32	25.4%
		No	94	74.6%
	Walked for at least 10 minutes in the last 7 days			
		Yes	48	38.1%
		No	78	61.9%
	Time spent sitting[2] on a weekday in the last 7 days (hours/day), Mean ± SD		7.35± 6.52	–

[2] Was determined only 44 patients. The rest were mostly inpatients or those who provided time as "daily", "at night" or "on Sundays" and therefore excluded from analysis.

4.2 Bone Mineral Density in adult patients 50 years and above managed at KNH.

The prevalence of reduced bone mineral density (BMD) was 87.3% while normal was 12.7%. Figure 3 below shows BMD stratified by age. In the age category of 50–59 years, 84.7% had reduced BMD. Equally, all the patients aged 70 – 79 years had reduced BMD. The proportions were statistically significantly different across the age groups (*p*-value = 0.029).

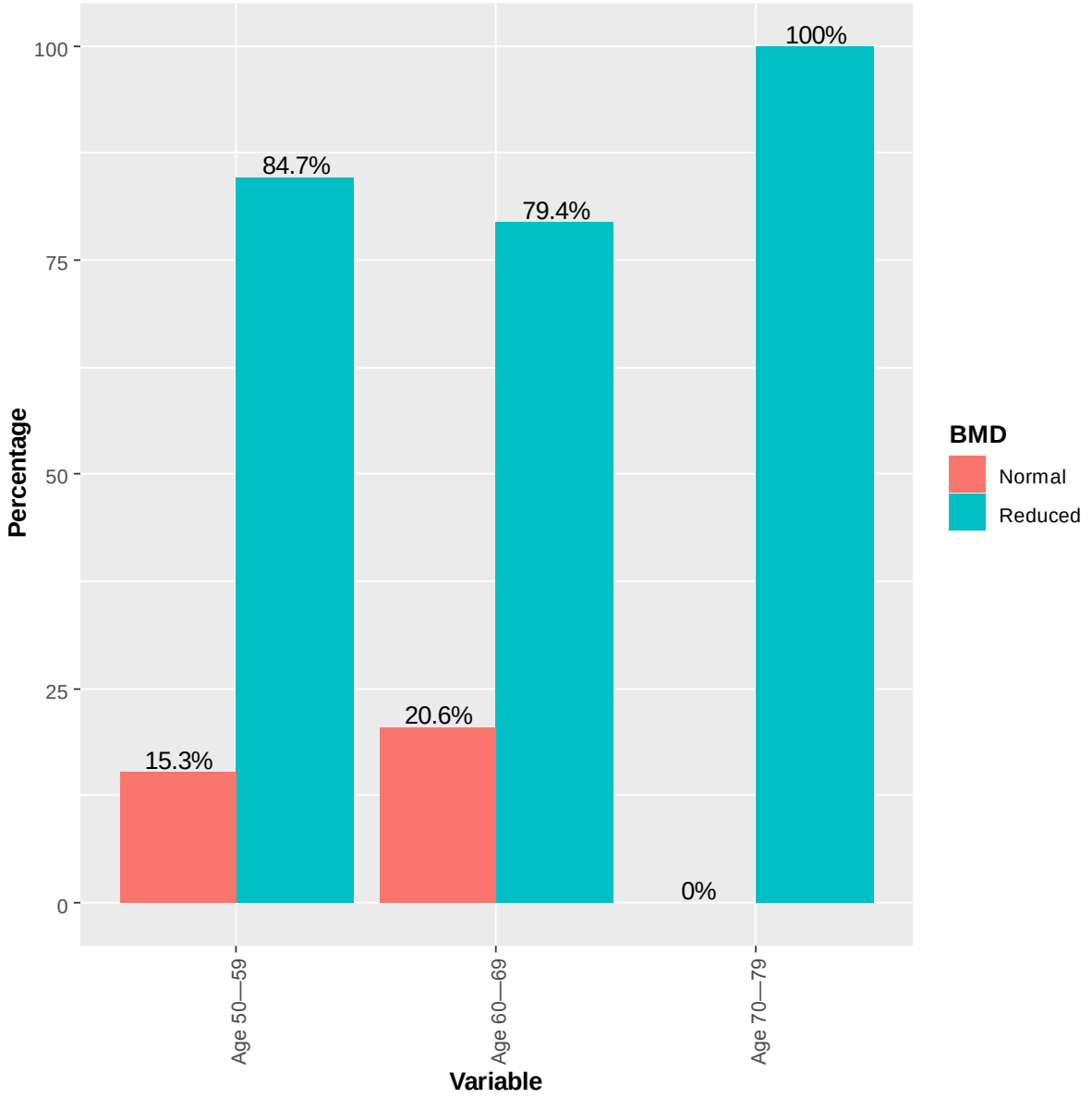

Figure 3. Prevalence of bone mineral density in adult patients 50 years and above according to age categories

Figure 4 below shows BMD stratified by having sustained a bone fracture. Among those who had a history of sustaining a bone fracture, 91.1% had reduced BMD measured by calcaneal ultrasound, however, there was no statistically significant difference in proportions between

those who reported a history of bone fracture and those with no bone fracture *p*-value = 0.161).

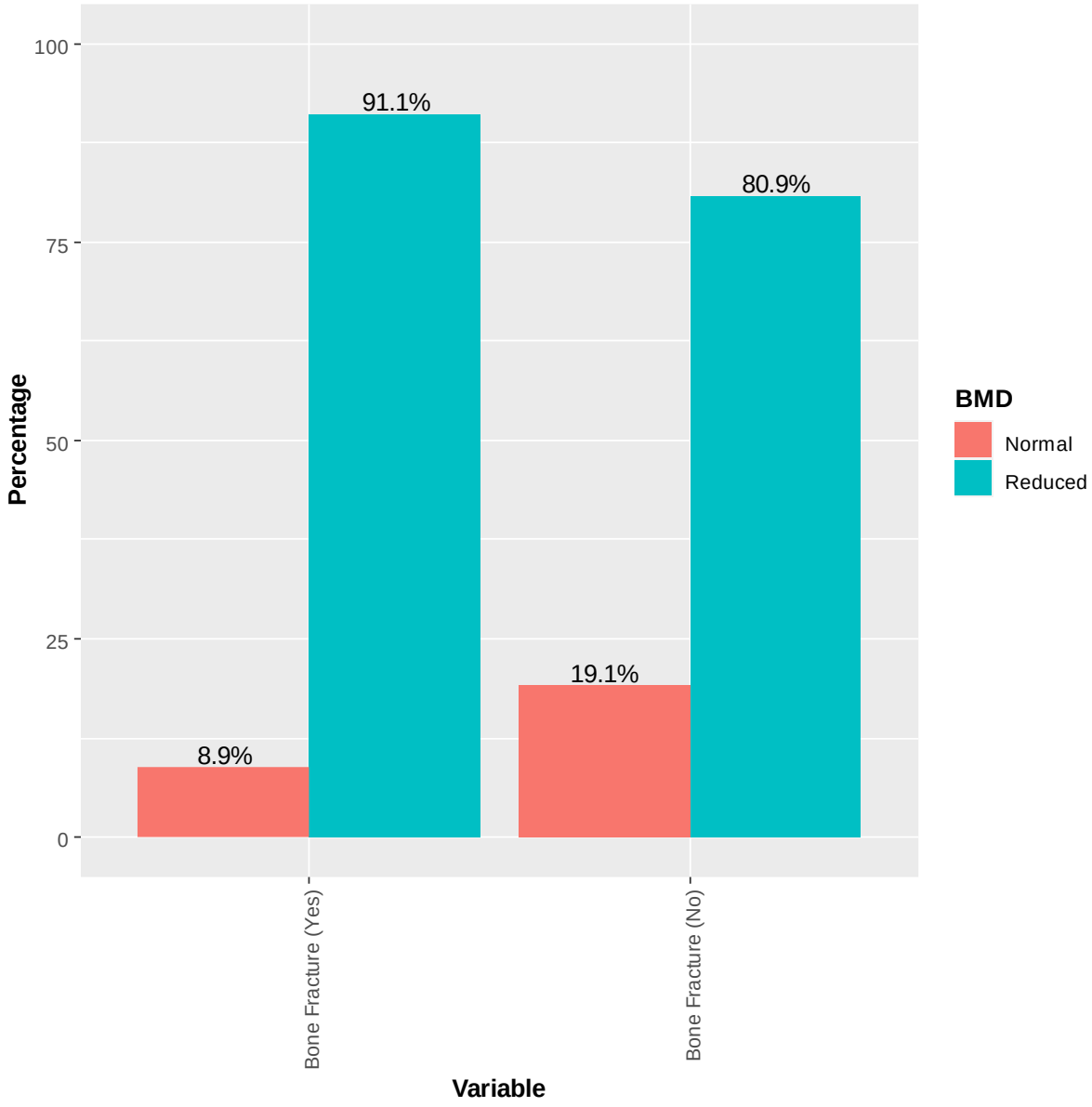

Figure 4. Prevalence of bone mineral density in adult patients 50 years and above according to history of sustaining a bone fracture

4.3 The levels of vitamin D in adult patients 50 years and above at KNH.

When the serum vitamin D levels were classified in the 126 adult patients studied, the prevalence according to these categories were found to be 10.3%, 31.7% and 57.9% for the deficient (<20 ng/mL), insufficient (20–29 ng/mL) and normal (≥ 30 ng/mL) levels respectively (Figure 5).

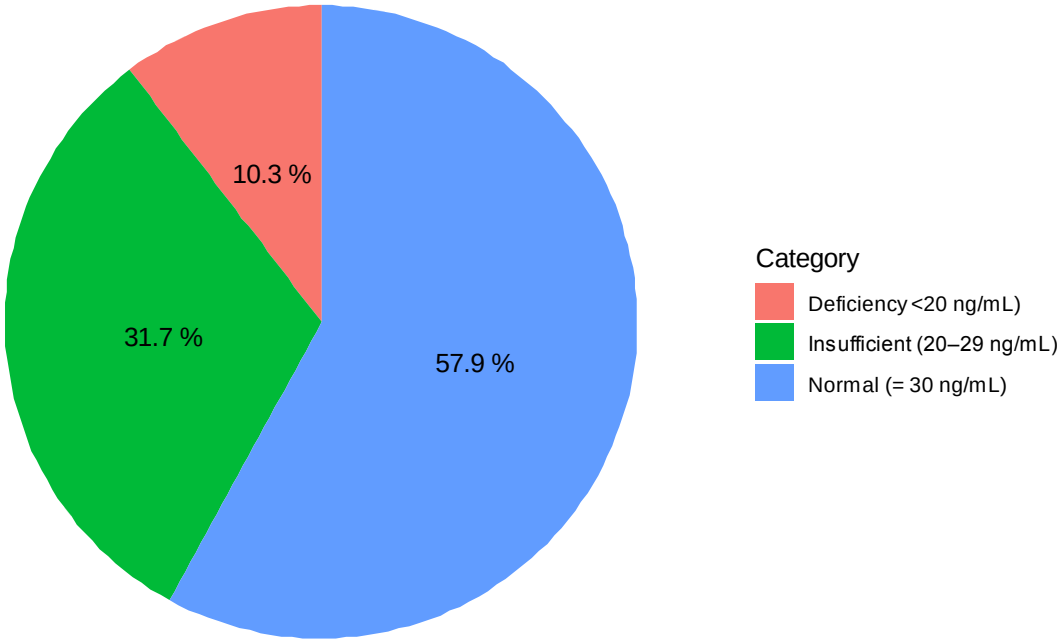

Figure 5. Pie chart of prevalence serum vitamin D

The mean values of the studied serum vitamin D are described in Table 4 below. The overall mean of serum vitamin D was 34.53 (SD, 12.02) ng/mL. There was a statistically significant difference in the mean serum vitamin D by age categories (*p*-value <0.001) implying that each age category had different mean values for serum vitamin D. The mean values increased with advancing age. Table 4 also indicates the mean serum vitamin D by age category and

categorized as deficient, insufficient, and normal levels. Patients in the age group 60 – 69 had the highest mean levels (48.86± 13.36) among those considered having normal serum levels.

Table 4. The mean and standard deviation of serum vitamin D in patients 50 years and above by age

Variable	Age category	Mean ± SD levels of serum vitamin D				
		Overall	*p* -value[3]	Deficient (<20 ng/mL)	Insufficient (20–29 ng/mL)	Normal (≥ 30 ng/mL)
Age (years)	50 to 59	32.46 ± 8.28	<0.001	16.14 ± 0.00	25.83 ± 2.03	39.38 ± 4.06
	60 to 69	32.47 ±16.49		17.82 ± 1.70	24.16 ± 1.89	48.86± 13.36
	70 to 79	40.36 ±10.58		–	25.22 ± 1.20	43.06 ± 9.08

Table 5 also shows the mean serum vitamin D categorized by bone fracture status and classified as deficient, insufficient, and normal levels. Those with reporting bone fracture history had a higher mean (36.48 ± 11.99) than those without bone fracture (*p*-value=0.012) as seen in Table 5 below.

Table 5. The mean and standard deviation of serum vitamin D in patients 50 years and above by bone fracture

Variable	Age category	Mean ± SD levels of serum vitamin D				
		Overall	*p*-value[3]	Deficient (<20 ng/mL)	Insufficient (20–29 ng/mL)	Normal (≥ 30 ng/mL)
Bone fracture	Yes	36.48 ±11.99	0.012	17.85 ± 2.27	24.95± 2.19	43.28± 8.45
	No	31.23 ±11.62		17.08 ± 0.88	25.77 ± 1.73	40.95± 10.47

4.4 Comparison of the Bone Mineral Density and vitamin D levels

4.4.1 Correlation between vitamin D and Bone Mineral Density

The patient's data shows that vitamin D correlated with reduced BMD positively. That is, there was a statistically significant but weakly positive correlation of 0.351 (Table 6).

Table 6. Correlation between vitamin D and reduced BMD

Variable	Bone Mineral Density	P-value
Serum vitamin D	0.351[4]	<0.001*[5]

[3] Mann-Whitney test or Kruskal-Wallis Anova comparing serum vitamin D levels in the age categories
[4] Point biserial correlation coefficient
[5] *Correlation is significant at the 0.05 level (2-tailed).

Table 7 below also displays the mean serum vitamin D levels of 25 (OH)D (ng/mL) and biochemical categorization by BMD. The overall mean levels of 25(OH)D (ng/mL) between the BMD groups were found statistically significantly different (*p*-value <0.001) and this might imply that high-dose vitamin D might reduce BMD (32) since the majority were inpatients.

Table 7. Vitamin D level (ng/mL) for each BMD category

Variable[6]	**Bone mineral density categorization**		***p*-value**
	Reduced	**Normal**	
25(OH)D (ng/mL)	36.09± 12.01	23.84 ± 3.98	<0.001[7]
Deficiency (<20 ng/mL)	9 (69.2%)	4 (30.8%)	<0.001[8]
Insufficient (20–29 ng/mL)	28 (70.0%)	12 (30.0%)	
Normal (≥ 30 ng/mL)	73 (100.0%)	0 (0.0%)	

4.4.2 Correlation between serum vitamin D and Age

Figure 6 below is a correlation plot between serum vitamin D levels of 25 (OH)D (ng/mL) and age (years). The data shows a statistically significant positive correlation between the two parameters (R=0.21, *p*-value = 0.018) implying the serum vitamin D increased as the age advances for the study participants.

[6] Data expressed as mean ± SD.
[7] Mann-Whitney test *p*-value
[8] Fisher's Exact Test *p*-value

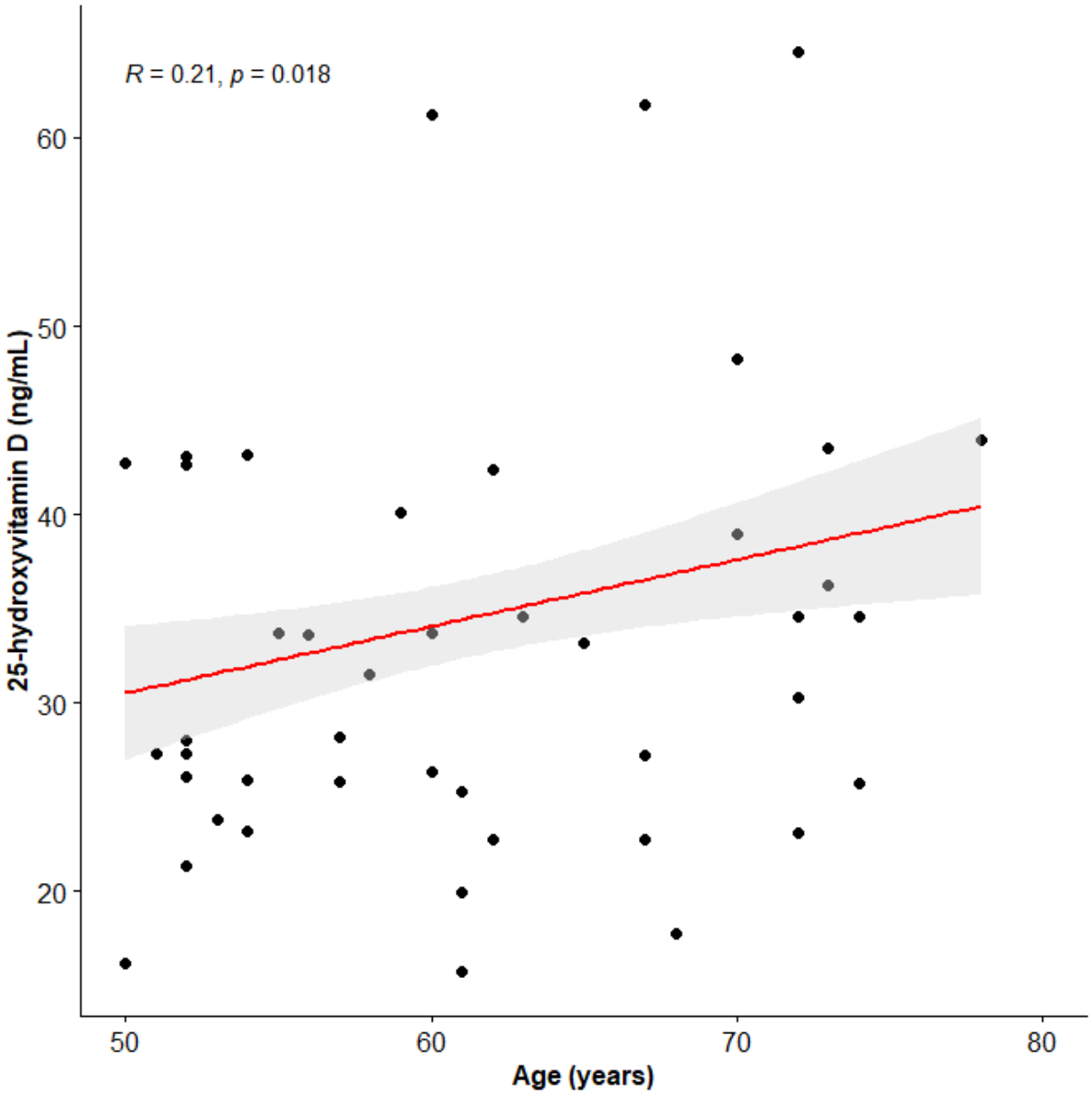

Figure 6. Correlation between serum vitamin D levels of 25 (OH)D (ng/mL) and age

4.4.3 Correlation between 25-hydroxyvitamin D and age according to bone mineral density classification

A significant correlation between serum vitamin D and age was found in both categories of BMD. In the reduced BMD category, a positive correlation of 0.24 (p-value = 0.011) while in the normal group, a significant negative correlation of -0.85 was found (p-value < 0.001). The result is displayed in Figure 7 below.

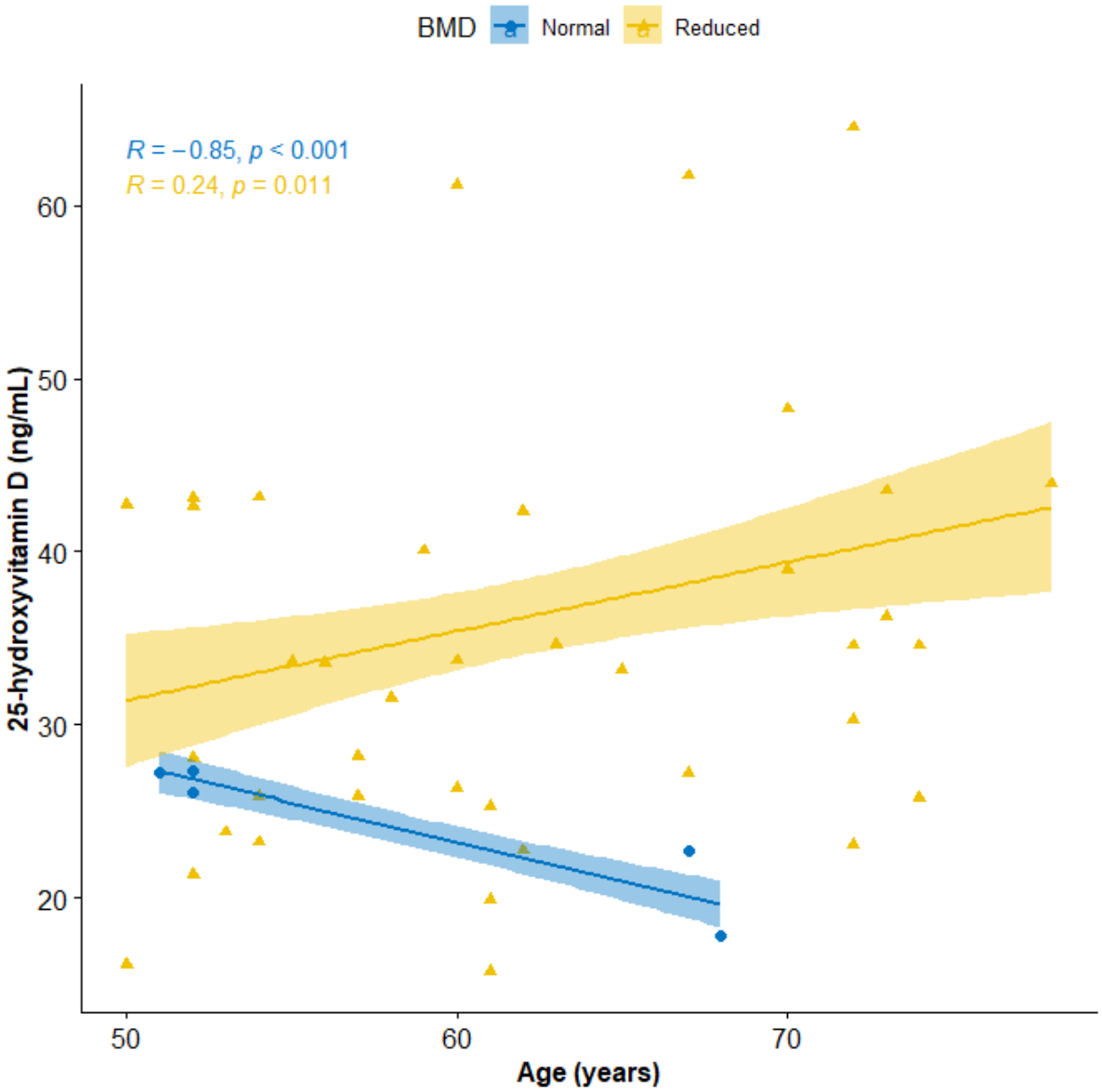

Figure 7. Correlation between 25-hydroxyvitamin D and age according to bone mineral density classification

4.4.4 Analyses for testing difference in correlation between vitamin D and bone mineral density in the orthopedic clinic (outpatients) and inpatients (wards)

Analyses find that the correlation between serum vitamin D and reduced BMD in inpatients (rho = 0.204, *p*-value = 0.1149) was not statistically significant while that in outpatients (rho = 0.419, p-value = 0.0005) was statistically significant. This result suggests that serum vitamin D was positively correlated with reduced BMD in outpatients.

This study also hypothesized that there is a difference in correlation of vitamin D levels and reduced BMD assessed using qualitative ultrasonography in adult patients aged 50 years and above seen at the orthopedic clinic and wards in KNH. A comparison test was done on the correlation coefficients of the clinic (outpatients) and ward (inpatients). The result of a comparison of the independent groups revealed that the two correlations coefficients (rho=0.204) and (rho = 0.419) was not statistically significant (*p*-value = 0.189), and therefore, the study failed to reject the null hypothesis. This implied that there was no statistical evidence that serum vitamin D correlated differently in orthopedic outpatients and inpatients with reduced BMD. Given this, at the conventionally significant level of 5%, or 0.05, it was concluded that there was no statistically significant evidence to reject the null hypothesis (H_0) of no difference. Hence the null hypothesis of no difference is not disproved.

4.4.5 Multiple regression analysis of the serum Vitamin D levels in the prediction of bone mineral density adjusted for patient factors

Linear and logistic regression models were fitted to evaluate whether any of the serum Vitamin D levels predict Bone Mineral Density and to adjust for the patient factors.

4.4.5.1 Logistic regression models evaluating whether any of the serum Vitamin D levels predict Bone Mineral Density

Unadjusted and adjusted logistic regression models were fitted to evaluate whether serum vitamin D predicts reduced BMD and the results are summarized in Table 8 below. A unit increase in Vitamin D (D 25-Hydroxy) level was associated with a 16% increase in odds of reduced BMD [crude odds ratio (cOR): 1.16, 95% confidence interval (CI): 1.1–1.22; *p*-value <0.001]. When adjusted for other age, residence, bone fracture history and physical activity (walking in the last seven days), a unit increase in serum vitamin D was associated with 1.16 times of reduced BMD compared to normal BMD as measured by calcaneal ultrasound (adjusted OR: 1.16, 95% CI: 1.08 – 1.25; *p*-value <0.001).

Table 8. Crude (unadjusted) and adjusted odds ratios of predictors reduced bone mineral density

Characteristic	**Reduced BMD n[9] (%)**	**Crude Odds Ratio (95% CI[10])**	***p*-value**	**Adjusted Odds[11] Ratio (95% CI)**	***p*-value**
Age		1.05 (0.98, 1.13)	0.175	1.06 (0.96, 1.16)	0.260
Vitamin D (D 25-Hydroxy)		1.16 (1.1, 1.22)	< 0.001***	1.16 (1.08, 1.25)	< 0.001***
Residence					
Rural	55 (50.0)	1.00		1.00	
Urban	55 (50.0)	0.23 (0.06, 0.85)	0.028*	0.26 (0.05, 1.53)	0.095
Bone fracture					
No	38 (34.5)	1.00		1.00	
Yes	72 (65.5)	2.44 (0.91, 6.52)	0.100	1.48 (0.39, 5.57)	0.545
Walked for at least 10 minutes in the last 7 days					
No	64 (58.2)	1.00		1.00	
Yes	46 (41.8)	5.03 (1.1, 22.99)	0.038*	3.96 (0.57, 27.36)	0.115

4.4.5.2 Linear regression models evaluating the prediction of serum Vitamin D levels with bone mineral density and adjusting for patient factors

Table 9 below details the linear regression analyses of serum vitamin D and reduced BMD controlling for other variables. For each year increase in age, the serum vitamin D was increased by 0.31 (95% CI: 0.03–0.58, *p*-value=0.03). Most of the study participants were inpatients. Participants with reduced BMD had 7.53 (95% CI: 1.29–13.76; *p*-value =0.018) higher serum vitamin D levels compared to those with normal. While patients reporting living in urban areas had serum vitamin D levels reduced by -6.48 (95% CI: -11.61 – -1.36; *p*-value = 0.014) compared to those in rural areas. Patients who reported a history of a bone fracture had increased serum vitamin D levels (5.87, 95% CI: 1.62 – 10.13; *p*-value = 0.007) compared to those without a history of bone fracture. Walking in for at least 10 minutes was correlated with lower levels of serum vitamin D (-7.66, 95% CI: -12.52 – -2.79; p-value = 0.002) than in those who didn't. The study participants were inpatients, and so with a

[9] n = total number of patients who experience reduced bone mineral density measured by calcaneal quantitative ultrasound.
[10] CI = Confidence Interval and *p*-values are from Logistic regression model; significant codes: * $p < 0.05$; ** $p < 0.01$; *** $p < .001$; Variables excluded during backward stepwise regression: education, marital status, occupation, use of oral corticosteroids, smoking history, alcohol use, use of oral contraceptives, vigorous and moderate activity.
[11] *p*-value = 0.25 was used as a threshold for a covariate to both enter and exit the final model.

prevalence of 87.3%, it is likely that the outcomes of serum vitamin D and BMD presented in this study have some virtual certainties, that is, it was like almost all patients had loss of BMD as measured by calcaneal ultrasound.

Table 9. Correlates of serum vitamin D with reduced bone mineral density (N = 126), displayed as a multivariate-adjusted mean difference

Characteristic	**Mean[12] level**	**Mean change (95% CI[13])**	***p*-value**	**Adjusted Mean change[14] (95% CI)**	***p*-value**
Age, per year increase	–	0.35 (0.10, 0.61)	0.007	0.31 (0.03, 0.58)	0.030
Marital status					
Divorced	42.13 ± 4.63	1.00		1.00	
Married	34.65 ±12.46	-7.49 (-14.7, -0.25)	0.346	-1.61 (-10.6, 7.35)	0.722
Single	15.78 ± 0.00	-26.4 (-41.2, -11.5)	0.001	-34.4 (-47.6, -21.2)	< 0.001
Widowed	30.17 ± 4.18	-12.0 (-22.2, -1.72)	0.022	-4.24 (-16.45, 7.97)	0.493
Education					
None	33.69 ± 0.00	1.00		1.00	
Primary	30.93± 8.98	-2.76 (-13.3, 7.83)	0.607	-8.82 (-23.14, 5.51)	0.225
Secondary	38.85± 13.20	5.16 (-5.65, 16.0)	0.346	-3.43 (-19.59, 12.73)	0.674
Tertiary	38.59± 17.06	4.90 (-6.90, 16.7)	0.412	11.29 (-5.61, 28.18)	0.188
Occupation					
Civil servant	29.06 ± 9.82	1.00		1.00	
Retired	34.70 ± 1.77	5.64 (-7.55, 18.82)	0.399	-3.53 (-15.9, 8.87)	0.574
Self-employed	37.12 ±14.57	8.05 (1.29, 14.82)	0.020	3.60 (-5.67, 12.87)	0.443
Unemployed	32.96 ± 8.35	3.90 (-3.05, 10.85)	0.269	0.86 (-7.82, 9.53)	0.845
Residence					
Rural	35.04± 10.87	1.00		1.00	
Urban	34.09± 12.98	-0.95 (-5.22, 3.31)	0.659	-6.48 (-11.61, -1.36)	0.014
Smoking history					
Current smoker	27.21	1.00		1.00	
Never smoker	32.02± 9.40	4.81 (-18.32, 27.93)	0.681	24.00 (5.11, 42.87)	0.013
Used to smoke	39.01 ±14.67		0.317	28.61 (9.61, 47.61)	0.004
Bone mineral density					
Normal	23.84 ± 3.98	1.00		1.00	
Reduced	36.09 ±12.01	12.25 (6.24, 18.26)	<0.001	7.53 (1.29, 13.76)	0.018
Bone fracture					
No	31.26 ±11.62	1.00		1.00	
Yes	36.48 ±11.90	5.22 (0.92, 9.52)	0.018	5.87 (1.62, 10.13)	0.007

[12] Mean serum vitamin D

[13] CI = Confidence Interval and *p*-values are from Linear regression model; significant codes: * $p < 0.05$; ** $p < 0.01$; *** $p < .001$; Variables excluded during backward stepwise regression: use of oral corticosteroids, alcohol use, use of oral contraceptives, vigorous and moderate activity.

[14] *p*-value = 0.05 was used as a threshold for a covariate to both enter and exit the final model.

Table 9. Correlates of serum vitamin D with reduced bone mineral density (N = 126), displayed as a multivariate-adjusted mean difference

Characteristic	**Mean[12] level**	**Mean change (95% CI[13])**	***p*-value**	**Adjusted Mean change[14] (95% CI)**	***p*-value**
Walked ≥10 minutes in the last 7 days					
No	34.77± 12.54	1.00		1.00	
Yes	34.13± 11.23	-0.64 (-5.02, 3.74)	0.773	-7.66 (-12.52, -2.79)	0.002

CHAPTER FIVE

5. DISCUSSION AND CONCLUSION

5.1 Discussion

The biological process of aging has been studied and found to be complex and guided by category of molecules as well as pathways that influence the general decline of physiological functioning resulting in an increased risk of age-linked illnesses (33). The study aimed to determine the correlation between bone mineral density and vitamin D levels in adult patients aged 50 years and above in Kenyatta National and Referral Hospital.

Calcaneal quantitative ultrasound was used to evaluate BMD in these patients. Comparable studies using a similar machine have been conducted elsewhere (34–36) to determine the prevalence of BMD. The analyses from this current study showed that a higher proportion of the patients had reduced BMD (87.3%), and among postmenopausal women, the prevalence was 100%. In Kenya, Sitati et al. (37) reported a proportion of 26.4% among postmenopausal women in a household survey – unlike this current study – in which they associated osteoporosis with increasing age.

The study participant's profile corroborates this high prevalence of reduced BMD (87.3%) given that a high proportion (63.7%) reported a history of sustaining a bone fracture. Besides, nine in ten (91.1%) patients with a history of sustaining a bone fracture had reduced BMD measured by calcaneal ultrasound. The sociodemographic profile results on bone fracture is quite consistent with most studies that report a reduced BMD rate of 70 –89% after fragility fracture (38) and incident hip fracture (39). Besides, other previous studies have even reported reduced BMD ranges of 69 –100% across some studies (40,41).

Moreover, in terms of known risk factors for reduced BMD, 29.4% reported that they used oral corticosteroids continuously for a period equal to or exceeding three months, and this can be triangulated with the high proportions of the patients who reported history of having sustained a bone fracture as well as those who reported that they used to smoke (36.5%) to have a comprehensive understanding of high proportions of reduced BMD. Systemic corticosteroids have been reported previously to induce osteoporosis and also increase the risk of bone fractures in adults (42). The high proportions of reduced BMD may also be discussed in terms of lack of vigorous activity in the last 7 days (82.5%) with about a quarter of the study population (25.4%) reporting they engaged in moderate physical activity in the past 7 days.

Additionally, about half of the study participants were inpatients (48.4%) and the other half (51.6%) came from orthopaedic clinics, this suggests that most participants were susceptible to BMD or bone disorders hence the high percentage of reduced BMD.

This study reports a weak positive correlation between vitamin D and reduced BMD. Looking at the high prevalence estimate of reduced BMD, it is very easy to establish that the outcomes of serum vitamin D and reduced BMD have some sort of virtual certainty – it suggests that all the patients were at risk of BMD loss/reduction hence the high percentage of 87.7%. Other studies have found an inverse correlation between serum vitamin D and BMD (43,44). The overall mean levels of 25(OH)D (ng/mL) between the classified as deficient, insufficient and normal were statistically significantly different in the BMD reduced versus normal groups implying the patients were dependent on support or living in the institutional care of KNH. It would be proper to suggest that some patients were under vitamin D supplementation/diet – however, this data was not collected since it was beyond the scope of the study and could have possibly had confounding effect (32), and is something that can be considered in future studies.

The role of vitamin D 25 (OH)D in aging is well acknowledged. The 25 (OH)D plays an indispensable role in mineralization and formation of bone, its deficiency leads to osteoporosis in the aged population (33). This study found an increasing prevalence of serum vitamin D from deficient (<20 ng/mL), insufficient (20–29 ng/mL), and to normal (≥ 30 ng/mL) levels. This means that a higher proportion of patients had a normal 25 (OH)D. And has already been described above, this high proportion can only be explained by the care and management the patients are get at KNH since a high proportion of them had reduced BMD. However, the relationship between vitamin D and BMD including osteoporosis are controversial with evolving evidence (45).

This study also found a significant bivariate correlation between serum vitamin D levels and age (years), overall, and a positive and negative correlation in BMD categories (reduced and normal). Previous studies have also presented a similar positive correlation between reduced BMD and serum vitamin D as this current study (46–50). However, a comparative analysis indicated that two correlation coefficients of serum vitamin D and reduced BMD between independent groups of patients from the orthopedic clinics and orthopedic wards was not different, implying a lack of statistical evidence that serum vitamin D correlated differently in between these two patient groups.

After controlling for other factors in a multivariable logistic regression model, this study found that Vitamin D (D 25-Hydroxy) level was associated with increased odds of reduced BMD, age was associated with reduced BMD most likely due to the exclusion criteria of only those 50 years and above.

Although reduced BMD was proportionately the same (50% each) among patients who reported living either in urban areas and rural areas, multivariable linear regression model revealed that patients who lived in urban areas had lower serum vitamin D levels compared to those who reported living in rural areas. However, patients who reported a history of a bone fracture had increased serum vitamin D levels in the adjusted linear regression model.

Generally, reduced BMD was linked to higher serum vitamin D levels compared to those with normal, and this can only be explained in terms of high-dose vitamin D that might have been given, during orthopaedic care, to the patients to correct vitamin D level since a half of the patients were inpatients – and even outpatients might be eligible for such kind of management. In fact, Burt et al. (32) found no benefit of high-dose vitamin D supplementation for bone health/BMD and recommended further studies be done to ascertain if it is harmful. Given the aforesaid, this study attributes the high levels of serum vitamin D to the treatment of the patients; this is particularly the case in the institutionalized population such as the one presented in this study. So in this current study, the results may suggest that patients who had vitamin D supplements had higher levels of vitamin.

5.2 Conclusion

The conclusions to this study are presented with reference to the broad and specific objectives. Guided by the broad objective, there was a positive weak bivariate correlation between serum vitamin D with reduced BMD, and in a multivariate analysis, serum vitamin D was strongly associated with reduced BMD. Therefore, vitamin D can be used in predicting reduced BMD among patients 50 years and above at KNH. In terms of specific objective one, the prevalence of reduced BMD was high (above 80%) in patients 50 years and above in KNH set up.

Regarding specific objective two, the prevalence of serum vitamin D was high and was deficient in only 10.3% of patients, insufficient in 31.7% and normal in 57.9% of the patients due to institutional care at KNH. For specific objective three, there was significant positive bivariate correlation between the serum vitamin D levels and age, in which case a positive

correlation of serum vitamin D was found in patients with normal BMD group and a negative correlation in patients with reduced BMD group.

When adjusted for other factors, a unit increase in serum vitamin D was associated with 16% increase in odds of reduced BMD compared to normal BMD as measured by calcaneal ultrasound – this implies that serum vitamin D can be used to predict BMD. However, age was not associated with reduced BMD but with increased serum vitamin in the adjusted model. Patients who reported a history of a bone fracture had increased serum vitamin D levels compared to those without a history of bone fracture.

Finally, there was no evidence that the correlation between vitamin D and reduced BMD were different in patients at the orthopedic clinics and those at the orthopedic wards, given this, the null hypothesis of no difference in correlation of vitamin D levels and BMD assessed using qualitative ultrasonography in adult patients aged 50 years and above seen at the orthopedic clinic and wards in KNH was not disproved.

5.3 Recommendations

Since there was a high proportion of patients with reduced BMD, a more thorough understanding of physiological mechanisms that could have led to high proportions of patients with reduced BMD but higher levels of serum vitamin D is needed. Given that the study participants were patients under KNH institutional care, this study recommends that orthopaedic physicians might need to consider that higher dose of vitamin D supplementation might be correlated with substantial BMD reduction (loss) with no further improvement, especially in patients with risk factors such as systemic corticosteroids use, lack of physical activity, among others. The percentage of those having physical activity or weight-bearing exercises like walking was very low, so this study recommends exercise-based interventions such as progressive resistance exercises to manage the reduced BMD.

From the prevalence estimates, the orthopaedic physicians assessing patients with bone fractures may also expect that almost nine in ten patients presenting at the orthopedic clinic or wards above the age of 50 years will have reduced BMD as measured by the calcaneal quantitative ultrasound. Because of this, they may need to assess the risks of osteoporosis in these patients and follow justified strategies to manage the BMD. Given the profile of study participants, patients and the general population also need to be made aware of the risks of reduced BMD and loss of quality of life.

This study highlights present evidence about the importance vitamin D have in diseases associated with aging and calls for well-designed prospective clinical studies with a sufficient sample size are needed to assess the correlation between BMD and serum Vitamin D level. Further prospective clinical research with a gold standard such as DEXA may be needed to reveal why even with high levels of vitamin D, patients still have reduced BMD.

5.4 Limitations to the study

The results from this study should be interpreted with respect to the limitations. The results should be interpreted with caution since a correlation does not imply causation. Several variables such as BMI were not collected that could be potential confounders of vitamin D and BMD. Absence of BMI data might have affected the relationship between vitamin D and BMD when adjusting/controlling for covariates in a multivariable analysis hence results should be interpreted bearing this in mind.

Additionally, vitamin is affected by the length of stay in a hospital and dependence on institutional care (Calcium/vitamin D supplementation) which were all not factored in the multivariable analysis since it was beyond the scope of this study hence the absence of all these variables might have had an influence of the results presented in this study and bring out a significant effect when there is none and vice versa. Data on comorbid conditions such as diabetes were not collected to control for the relationship between serum vitamin D and BMD.

Calcaneal quantitative ultrasound was used for BMD assessment while dual-energy X-ray absorptiometry (DEXA) is the gold standard. The dichotomy of calcaneal quantitative ultrasound results of “reduced” versus “normal” BMD results might have influenced results since the analyses were between a continuous variable (serum vitamin D) and a dichotomous variable (BMD). Additionally, ultrasonography is a large user-dependent assessment and could, therefore, introduce measurement bias.

Recall bias from the patients might have had an impact, this is because some participants might have given wrong information, for instance, past medical history. In addition, being a hospital-based study, there could be selection bias of the participants, who by having come to the hospital for treatment are likely to be on follow up for chronic conditions that may further influence bone mineral density and vitamin D levels, the results could therefore not be generalizable.

5.5 Strength of the study

The study population was a homogenous patient group and this was a key strength of this study. Moreover, the use of patients from clinics and wards provided sufficient power to identify the correlation of vitamin D with reduced BMD between the clinic and the ward groups. Consequently, while the study didn’t find a difference in correlation coefficients between the patients in the clinics/outpatients and the inpatients' groups, the computed sample size gave an acceptable quantity of type II error.

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ANNEXES

Annex 1: Data Collection Sheet

Correlation between Vitamin D Level and Bone Mineral Density in Kenyan Adults aged 50 Years and above at Kenyatta National Teaching and Referral Hospital

Socio-Demographic characteristics of the respondent:

Patient Identification number……..

Age (Years)..... (To the nearest year)

Marital status

1. ▢ Married
2. ▢ Single
3. ▢ Divorced
4. ▢ Widowed

Residence

1. ▢ Rural
2. ▢ Urban

Highest level of education

1. ▢ None
2. ▢ Primary
3. ▢ Secondary
4. ▢ Tertiary

Occupation

1. ▢ Unemployed
2. ▢ Student
3. ▢ Self-employed
4. ▢ Civil servant
5. ▢ Other (Specify)…….

Osteoporosis risk factors

2) Have you used oral corticosteroids continuously for a period equal to or exceeding three months?

1) ▢ Yes

2) ▢ No

3) Have you ever smoked cigarettes?

1) ▢ currently smoke

2) ▫ Used to smoke. ……. (Pack years)

3) ▫ never smoked

4) How often do you take an alcoholic drink?

1. ▫ Never
2. ▫ Monthly or less
3. ▫ Weekly
4. ▫ Daily

5) Have you used or are you currently using oral contraception?

1. ▫ Yes
2. ▫ No

6) Have you ever sustained a bone fracture?

1. ▫ Yes
2. ▫ No

Physical activity

We are interested in finding out about the kinds of physical activities you do as part of your everyday life. The questions are about the time you spent being physically active in the last seven (7) days. They include questions about activities you do at work, as part of your house work, to get from place to place, and in your spare time as exercise or sport.

In answering the following questions;

- Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal.
- Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat/ slightly harder than normal.
- Minimal/no activities refer to activities that fall below moderate physical activity

7) During the last seven days, have you had any vigorous physical activity like, heavy lifting, digging, aerobics, or fast cycling? (Activities for at least 10 minutes)

1. ▫ Yes
2. ▫ No

If yes, how many days per week? ……..

If yes, how many hours cumulatively per day? ……

8) During the last seven days, have you had any moderate physical activity like carrying light loads, or riding a bike at a regular pace (activities for at least 10 minutes)

1. ▫ Yes

2. ▫ No

If yes, how many days per week? ……..

If yes, how many hours cumulatively per day? ……

9) During the last seven days, have you walked for at least 10 minutes?

1. ▫ Yes

2. ▫ No

If yes, how many days per week? ……..

If yes, how many hours cumulatively per day? ……

10) In the last seven days, approximately how much time did you spend sitting on a weekday?

……hours/day

11) Serum vitamin D level ……

12) Bone mineral density status (Congenicity of bone) …….

13) Weight in kilograms……..

14) Square of height in meters……..

Kiambatanisho nambari 1
Uwiano wa kiwango cha vitamini D na wiani wa madini katika mifupa kwa watu wazima wenye asili ya Kenya wenye umri wa miaka 50 na zaidi katika hospitali ya taifa Kenyatta.

Taarifa za kijamii na demografia za mhojiwa
Namba ya utambulisho ya mgonjwa…….
Umri (kwa miaka).....
Hali ya ndoa

1. Nimeoa/kuolewa
2. Sijaoa/kuolewa
3. Mtalaka
4. Mjane

Makazi

1. Mjini
2. Kijijini

Kiwango cha elimu (cha juu)

1. Sijasoma
2. Elimu ya msingi
3. Elimu ya sekondari
4. Elimu ya juu

Kazi

1. Sina ajira/sijaajiriwa
2. Mwanafunzi
3. Nimejiajiri
4. Mtumishi wa umma
5. Nyingineo(eleza hapa).

Sababu hatarishi za ugonjwa wa mifupa
2. Umewahi kutumia dawa za jamii ya ‘steroid’ kwa kipindi cha au kuzidi miezi mitatu

1. Ndio.
2. Hapana

3. Umewahi kuvuta sigara

1. Ndio na bado ninavuta
2. Nilivuta ila nimeacha(Kiasi.....’pack years’)
3. Sijawahi kuvuta

4. Je matumizi yako ya pombe yakoje?

1. sijawahi kutumia
2. Kwa mwezi mara moja
3. Kwa wiki mara moja
4. Kila siku

5. Je umewahi kutumia dawa(tembe) za uzazi wa mpango/kupanga uzazi

1. Ndio
2. Hapana

6. Je umewahi kupiga mvunjiko wa mfupa

1. Ndio

2. Hapana

3.Shughuli za mwili

Tuna nia ya kutambua aina ya shughuli za mwili unazifanya katika siku yako ya kawaida. Maswali haya yatalenga katika kujua muda unaotumia katika shughuli hizi kwa kipindi cha siku saba. Yatauliza kuhusu shughuli za mwili unazofanya ukiwa kazini, ukiwa nyumbani, unapotoka sehemu moja kwenda nyingine na pia katika muda wako wa ziada kama katika mazoezi na michezo.
Katika kujibu maswali haya

- Shughuli za kiwango cha juu yamaanisha zile shughuli zinazohitaji nguvu nyingi na zinazokufanya kuhema kwa nguvu kuliko kawaida
- Shughuli za kati yamaanisha zike shughuli zinazohitaji nguvu za wastani/kiwango cha kati na hukusababisha kuhema ila kwa wastani na sio kwa nguvu
- Shughuli za kiwango cha chini/kutoshughulika itamaanisha shughuli zisizotumia nguvu ama hutumia nguvu chini ya wasatani/kiwango kati

7. Kwa siku saba zilizopita umewahi kuwa na shughuli za mwili za kiwango cha juu mfano kubeba vitu vizito, kulima, mazoezi viungo au kuendesha baisikeli kwa kasi (kwa muda usiopungua dakika 10)
1. Ndio
2. Hapana
    a. Kama ndio siku ngapi kwa wiki
    b. Kama ndio masaa mangapi kwa siku

8. Katika siku saba zilizopita umekuwa na shughuli gani za kiwango cha kati kama kubeba vitu vyepesi au kuendesha baiskeli kwa mwendo wa kawaia (kwammuda usiopungua dakika kumi)
1. Ndio
2. Hapana
    a. Kama ndio siku ngapi kwa wiki
    b. Kama ndio masaa mangapi kwa siku

9. Katika siku saba zilizopita umetembea kwa muda usiopungua dakika kumi?
1. Ndio
2. Hapana
    a. Kama ndio siku ngapi kwa wiki
    b. Kama ndio masaa mangapi kwa siku

10. Katika siku saba zilizopita unakadiria kutumia muda gani kuketi katika siku za juma Masaa...../siku

11. Kiwango cha vitamini……..

12. Wiani wa madini katika mifupa.....

13. Uzito wa kilo…….

14. Urefu kwa mita za mraba…….

Annex 2: English Version of the Consent Form

CONSENT FORM

DEPARTMENT OF ORTHOPEDIC SURGERY,

FACULTY OF MEDICINE,

SCHOOL OF HEALTH SCIENCES,

UNIVERSITY OF NAIROBI.

Study Title: Correlation Between Vitamin D Level and Bone Mineral Density In Kenyan Adults Aged 50 Years and above At Kenyatta National Teaching And Referral Hospital

This is a form of agreement for enrollment to a study titled **"Correlation between Vitamin D Level and Bone Mineral Density in the Kenyan Adults Aged 50 years and above at a Tertiary Referral Hospital in Kenya"**

Broad Objective

To determine the correlation between Bone Mineral Density and vitamin D levels in adult patients aged 50 years and above in Kenyatta National and Referral Hospital.

Benefits

The study will provide health care workers and policy makers with knowledge on Correlation between Vitamin D Level and Bone Mineral Density, which is a measure of how strong your bones are. Those patients who, after assessment, will be found to have low bone mineral density will be recommended to have vitamin D supplements.

Risks

There are no immediate or later risk of suffering any complication while taking part in this study.

Voluntarism

This consent is not sort under any coercion of any kind and you reserve the right to withdraw from the study at any particular point.

Research procedure

This will involve measurement of vitamin D level in serum and bone mineral density using calcaneal ultrasound (this is a non-painful procedure where a machine is placed at the heel of your foot and a picture of the bone taken for assessment.

Confidentiality

Your information will be handled with utmost confidence. All details will be stored under lock and key only accessible to the principle investigator and research assistants.

Right of Withdrawal

Kindly note that you have the right to withdraw from the study at whatever point; feel free to seek any clarifications from the principal investigator and make any inquiries from the KNH-UoN ERC at any time.

Researcher's information

Principal investigator: Dr. David Masenge, phone 0723738118, mail- davidmasenge33@gmail.com

KNH-UON ethics and research committee

Tel: (254-020) 2726300 Ext 44355, mail- uonknh_erc@uonbi.ac.ke, web- www.erc.uonbi.ac.ke

Consent by the patient/ next of kin for participation in the study

I ……do hereby give consent of data collection regarding my illness for purposes of research.

I understand that the information given is primarily for research purposes and I do not expect any material or financial gain from it.

I have been duly informed that utmost confidentiality will be maintained and the information I will give will not be used against me or prejudice my treatment.

SIGNATURE…….. DATE……..

WITNESS

NAME…….

SIGNATURE…… DATE…….……

Annex 3: Swahili Version of the Consent Form

Kibali Fomu

Idara ya Upasuaji, Kitivo Cha Tiba, Shule Ya Sayansi Ya Afya, Chuo Kikuu Cha Nairobi.

Hii ni aina ya mkataba kwa ajili ya uandikishaji kwa utafiti wenye kichwa "uwiano kati ya Vitamin D ngazi na mfupa madini wiani katika watu wazima wa Kenya zaidi ya miaka 50 katika hospitali ya rufaa ya juu nchini Kenya.

MALENGO

kuamua na kulinganisha tofauti katika maambukizi ya mfupa madini wiani (BMD) abnormalities kutumia mzunguko ultrasound na Serum ya vitamini D ngazi katika wagonjwa watu wazima kuonekana katika kliniki Orthopedic na wadi katika hospitali ya Taifa ya Kenyatta.

FAIDA YA UTAFITI

Itatoa wafanyakazi wa huduma za afya na watunga sera na maarifa juu ya uwiano kati ya vitamini D ngazi na mfupa madini wiani. Wale wagonjwa na kuhusishwa na mfupa wa chini wiani madini itakuwa ilipendekeza kuwa na vitamini D virutubisho.

MADHARA

Hakuna madhara ya haraka na ya baadaye itakupata ukishiriki kwa utafiti.

UHURU WA KUSHIRIKI

Hautashurudishwa na mtu yeyote kutoa idhini ya utafiti na una haki ya kujiondoa wakati wowote.

USIRI

Maelezo yako itawekwa kwa usiri mkubwa. Itahifadhiwa chini ya ulinzi tafiti.

TAARIFA JUU YA WATAFITI

Mtafiti mkuu: Dr David Masenge 0723738118, barua pepe- davidmasenge33@gmail.com

KNH-UON MAADILI NA UTAFITI **WA KAMATI**

Simu: (254-020) 2726300 Ext 44355, barua pepe-uonknh_erc@uonbi.ac.ke, mtandao-www.erc.uonbi.ac.ke.

IDHINI KUTOKA KWA ANAYESHIRIKI KWA UTAFITI AMA JAMAA YAKE

Mimi…….nimekubali kupeana ruhusa ili utafiti ufanyiwe dhidi yangu.

Sitarajii manufaa yeyote ya kifedha kutokana na utafiti huu.

Nimeelezwa kwa kina yakwamba utafiti unaofanywa hautatumika kunikandamiza au kuhujumu matibabu yangu.

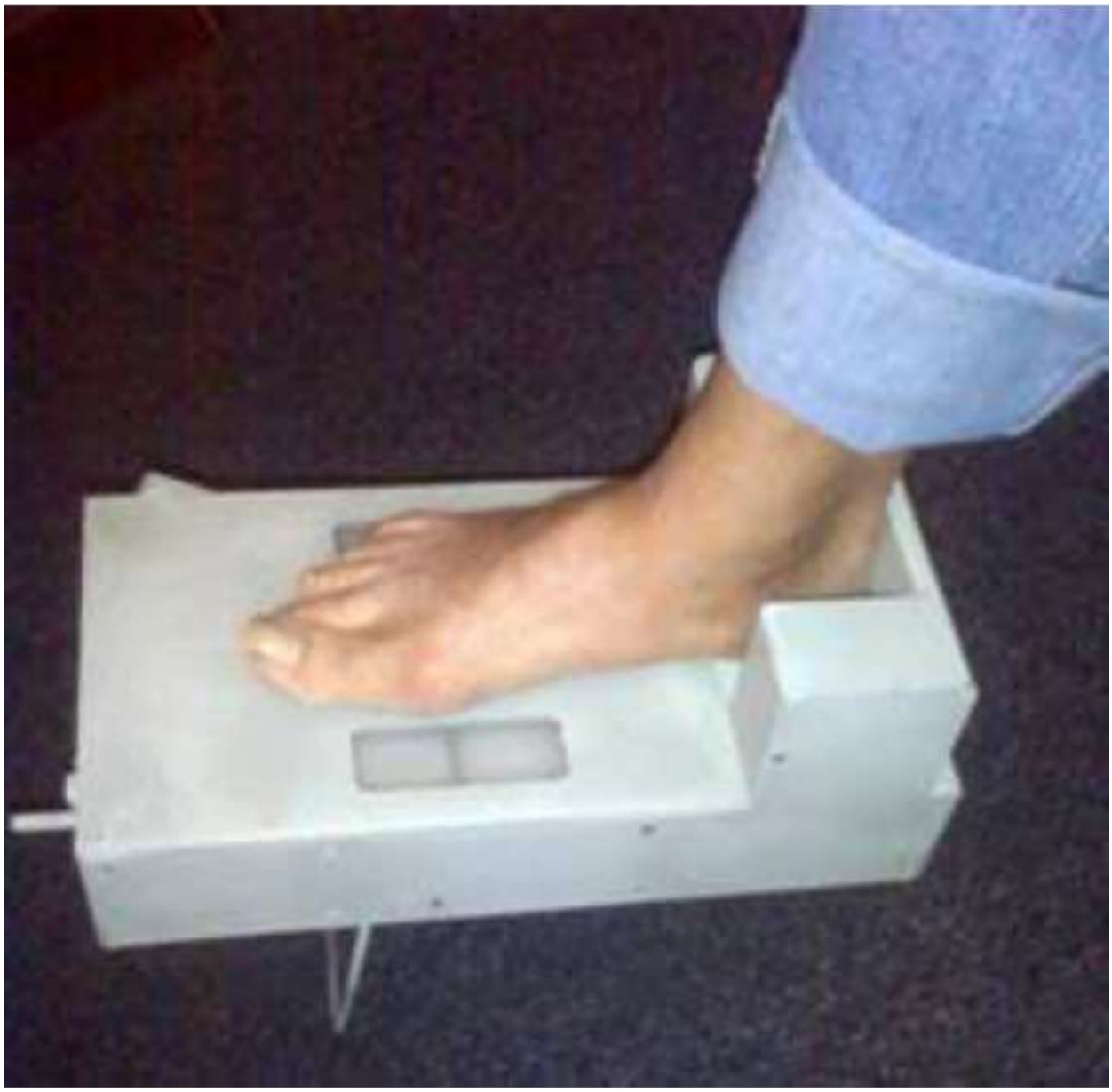
SAHIHI…….. TAREHE……..

MDHIBITISHI

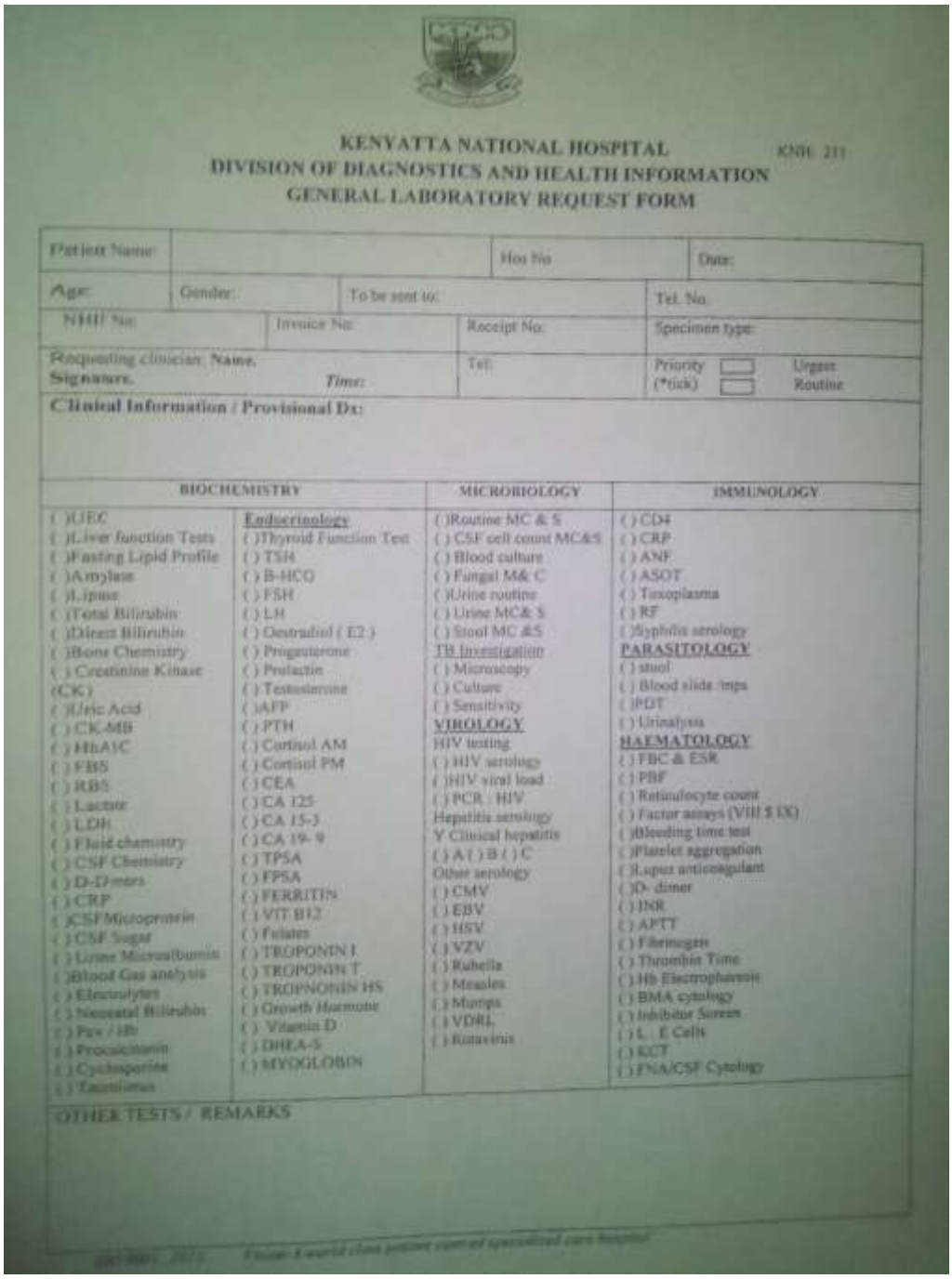
JINA…….……..

SAHIHI…….. TAREHE……

Annex 4: Calcaneal Quantitative Ultra Sound Picture



Annex 5: Sample Laboratory Request Form



KENYATTA NATIONAL HOSPITAL KNH: 211
DIVISION OF DIAGNOSTICS AND HEALTH INFORMATION
GENERAL LABORATORY REQUEST FORM

Patient Name:		Hosi No	Date:
Age:	Gender:	To be sent to:	Tel. No.
NHIF No:	Invoice No:	Receipt No:	Specimen type:
Requesting clinician: Name. Signature. Time:		Tel:	Priority (*tick) Urgent Routine

Clinical Information / Provisional Dx:

BIOCHEMISTRY		MICROBIOLOGY	IMMUNOLOGY
( )U/E/C	Endocrinology	( )Routine MC & S	( ) CD4
( )Liver function Tests	( )Thyroid Function Test	( ) CSF cell count MC&S	( ) CRP
( )Fasting Lipid Profile	( ) TSH	( ) Blood culture	( ) ANF
( )Amylase	( ) B-HCG	( ) Fungal M& C	( ) ASOT
( )Lipase	( ) FSH	( )Urine routine	( ) Toxoplasma
( )Total Bilirubin	( ) LH	( ) Urine MC& S	( ) RF
( )Direct Bilirubin	( ) Oestradiol (E2)	( ) Stool MC &S	( )Syphilis serology
( )Bone Chemistry	( ) Progesterone	TB Investigation	PARASITOLOGY
( ) Creatinine Kinase (CK)	( ) Prolactin	( ) Microscopy	( ) stool
	( ) Testosterone	( ) Culture	( ) Blood slide /mps
( )Uric Acid	( )AFP	( ) Sensitivity	( )PDT
( ) CK-MB	( ) PTH	VIROLOGY	( ) Urinalysis
( ) HbA1C	( ) Cortisol AM	HIV testing	HAEMATOLOGY
( ) FBS	( ) Cortisol PM	( ) HIV serology	( ) FBC & ESR
( ) RBS	( ) CEA	( )HIV viral load	( ) PBF
( ) Lactate	( ) CA 125	( ) PCR : HIV	( ) Reticulocyte count
( ) LDH	( ) CA 15-3	Hepatitis serology	( ) Factor assays (VIII $ IX)
( ) Fluid chemistry	( ) CA 19- 9	Y Clinical hepatitis	( )Bleeding time test
( ) CSF Chemistry	( ) TPSA	( ) A ( ) B ( ) C	( )Platelet aggregation
( ) D-Dimers	( ) FPSA	Other serology	( )Lupus anticoagulant
( ) CRP	( ) FERRITIN	( ) CMV	( )D- dimer
( )CSF Microprotein	( ) VIT B12	( ) EBV	( ) INR
( ) CSF Sugar	( ) Folates	( ) HSV	( ) APTT
( ) Urine Microalbumin	( ) TROPONIN I	( ) VZV	( ) Fibrinogen
( )Blood Gas analysis	( ) TROPONIN T	( ) Rubella	( ) Thrombin Time
( ) Electrolytes	( ) TROPNONIN HS	( ) Measles	( ) Hb Electrophoresis
( ) Neonatal Bilirubin	( ) Growth Hormone	( ) Mumps	( ) BMA cytology
( ) Pro / Hb	( ) Vitamin D	( ) VDRL	( ) Inhibitor Screen
( ) Procalcitonin	( ) DHEA-S	( ) Rotavirus	( ) L. E Cells
( ) Cyclosporine	( ) MYOGLOBIN		( ) KCT
( ) Tacrolimus			( ) FNA/CSF Cytology

OTHER TESTS / REMARKS

Annex 6: Study Timelines

Activity	2020								
	Jan	Feb	Mar	Apr	May	Jun	Jun	Jul	Aug
Proposal Development									
Proposal Presentation									
Ethics Committee Review									
Data Collection									
Data Analysis									
Results Presentation									
Publication									

Annex 7: Budget

Item	Quantity	Unit Cost	Total
KNH/ERC fees	1	@2500	2500
Printing	1page printing 1page photo photocopying	@ksh 10x33pages @ksh 5x33	500
Statistician	1	@30,000	30,000
Stationaries	Rims		3,000
Research assistant	2	@2x10,000	20,000
Printing thesis	10	@500x7	3,500
Miscellaneous	Binding, anti-plagiarism check, flash,		20,000
Calcaneal ultrasound	126 patients	@500 x 81	63,000
Vitamin D kit	1	@50,000	50,000
Vitamin D claset	1	@25,000	25,000
Vitamin D control (variable)	1	@20,000	20,000
Total			**237,500**