ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF NURSING AND MIDWIFERY

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Prevalence of Vitamin D Deficiency and Associated Risk Factors Among Patients with Nonspecific Musculoskeletal Pain Symptoms Attending Tikur Anbessa Specialized Hospital, Addis Ababa , Ethiopia

BY: Amanuel Dubale

Advisors: 1. Mistire Wolde   (MSc, PhD)

2. Tatek G/Egzeabher (MSC)

3. Seid Ali (MD Neurologist)

A thesis submitted to the Department of Medical Laboratory Sciences, School of Nursing and Midwifery, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Clinical Chemistry track)

January, 2019

Addis Ababa, Ethiopia

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Amanuel Dubale, entitled: ***"Prevalence of Vitamin D deficiency and associated factors among patients with nonspecific musculoskeletal pain symptoms attending Tikur Anbesa Specialized Hospital in Addis Ababa; Ethiopia"*** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Clinical chemistry) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

External Examiner_____ Signature _____Date _____

Internal Examiner_____ Signature _____ Date _____

Advisor_____Signature _____ Date _____

Advisor_____Signature _____Date _____

Advisor_____Signature _____Date _____

_____

Chairman of the Department or Graduate Program Coordinator

Acknowledgment

Table of Contents

IV

List of tables

List of figures

Abbreviations and acronyms

1, 25 (OH) $_2$ D: 1, 25-dihydroxy vitamin D

25OHD: 25-hydroxy vitamin D

ALP: Alkaline phosphatase

BMI: Body man index

EPHI: Ethiopian public health institute

FGF-23: fibroblast growth factor 23

IGF1: insulin like growth factor 1

IGFBP3: insulin like growth factor binding protein 3

NCX1: sodium calcium ion exchanger 1

NPT2b: sodium phosphate transporter 2b

PMCA1b: plasma membrane calcium ATPase 1b

PTH: parathyroid hormone

RANK: receptor associated nuclear factor κβ

RANKL: receptor associated nuclear factor κβ ligand

SOCC: source operated channel

SOP: standard operational procedure

TASH: Tikure Anbesa Specialized Hospital

TRPV6: transient receptor protein cat ion channel subfamily V member 6

UV: ultraviolet

VDBP: vitamin D binding protein

VDCC: voltage dependent calcium channel

VDR: vitamin D receptor

VDRE: vitamin D response elements

WC: waist circumference

Abstract

Background: Vitamin D deficiency causes rickets in children, and osteomalacia in children and adults. In 2016 musculoskeletal conditions are second cause of disability worldwide. Vitamin D deficiency and insufficiency can be both corrected and prevented safely through supplementation therapy. Therefore assessing prevalence of vitamin D deficiency and associated risk factors among patients with nonspecific musculoskeletal pain symptoms is crucial in Ethiopian context.

Objective: To determine the prevalence of vitamin D deficiency and its associated risk factors in patients with nonspecific musculoskeletal pain symptoms in Tikure Anbessa Specialized Hospital (TASH), Addis Ababa ; Ethiopia.

Methods: A cross-sectional study was conducted among patients with nonspecific musculoskeletal pain symptoms from March to May 2018 at TASH. Demographic data were collected using a structured questionnaire. Clinical data were obtained from medical records. Anthropometric indices were measured. Alkaline phosphatase (ALP) was determined in TASH laboratory with Mindray-200E. Vitamin D was determined with Mini Vidas hormone analyzer in Ras Desta Memorial hospital laboratory. Calcium (Ca) and Phosphorous (P) were analyzed in EPHI laboratory with Cobas 6000 c501 module. The data were entered into SPSS version 20 for analysis.

Results: Among the total 75 study participants with nonspecific musculoskeletal pain symptoms, 66 (88%) had vitamin D deficiency and 9 (12%) had vitamin D insufficient. Age, body mass index (BMI), waist circumference (WC) and occupation were associated with vitamin D deficiency. However, ALP, Ca, and P levels were not significantly correlated with vitamin D level. BMI were identified as independent risk factors for vitamin D deficiency.

Conclusion: The prevalence of vitamin D deficiency was high among patients with nonspecific musculoskeletal pain symptoms in this tertiary care. Obesity was independent risk factors for vitamin D deficiency.

Keywords: Nonspecific musculoskeletal pain symptoms, vitamin D deficiency, prevalence, risk factors

1. Introduction

1.1. Background

Vitamin D (D_2 and D_3) is cholesterol resembling molecule that physiologically regulates Calcium, Phosphorous, and bone metabolism. Vitamin D is gained from exposure of skin to sunlight, diet, and dietary supplements(1). Few foods naturally contain or are fortified with vitamin D meanwhile the main source is exposure to sun(2).

The ultraviolet (UVB) spectrum of sunlight (wave length 290-315 nm) radiates on human skin and facilitates the conversion of 7-dehydrocholesterol to pre-vitamin D, which undergo thermal isomerization to vitamin D in skin(3, 4). Vitamin D from dietary sources is incorporated into chylomicrons and transported by the lymphatic system into the venous circulation. With vitamin D made in the skin dietary vitamin D can be stored in and released from fat cells. Once vitamin D is ejected out of the plasma membrane in to the extracellular space, it bounds to the vitamin D binding protein (VDBP)(5). In the liver, vitamin D is converted by 25-hydroxalase (CYP2R1) to 25-hydroxyvitamin D (25OHD(4). 25OHD is the major circulating form of vitamin D that is used to measure vitamin D status(6). This immediate precursor metabolite is hydroxylated to the active form of vitamin D [1, 25- dihydroxyvitamin D (1, 25(OH)$_2$D)] by the mitochondrial 25OHD 1α-hydroxaylase (CYP27B1) enzyme in the kidney(4).

25OHD 1α-hydroxaylase is also expressed outside kidney in different tissues and enables the production of 1, 25 (OH)$_2$D. This active form of vitamin D is locally active and exerts auto- or paracrine effect(7).

The renal synthesis of 1, 25(OH)$_2$D is regulated by several factors including serum phosphorus, calcium, fibroblast growth factor 23 (FGF-23), parathyroid hormone (PTH) and vitamin D itself(2). 1,25(OH)$_2$D induces its own destruction by rapidly inducing the 25-hydroxyvitamin D-24-hydroxylase (CYP24A1), which leads to the multistep catabolism of both 25(OH)D and 1,25(OH)$_2$D into biologically inactive, water-soluble metabolite(6).

Vitamin D exerts its genomic effect on target tissues that express VDR and non-genomic effects on tissues that express membrane bound vitamin D receptors. The target tissues include the classical tissues like intestine, bone, kidneys and Parathyroid gland and other non-classical tissues through the body that express its receptor including muscle and neurons (7).

Vitamin D prevents hypocalcaemia by increasing intestinal and renal calcium absorption and regulates the PTH levels. The role of vitamin D in metabolism of calcium and phosphorous leads to an adequate calcium-phosphorus product ($Ca2+ \times HPO42-$) resulting in an effective bone mineralization hence keep bone integrity and structure(8). In muscles 1, 25(OH)$_2$ D regulate gene transcription and rapid non-genomic pathways that affect the muscle cell regeneration and calcium handling(9).

Although there is no consensus on optimal levels of 25-hydroxyvitamin D as measured in serum, vitamin D deficiency is defined by most experts as a 25-OH D level of less than 20 ng /ml (50 nmol/L) and vitamin D insufficiency as 25-OHD of 20-29 ng/ml(50-75 nmol/L)(4). Symptoms of vitamin D deficiency can be nonspecific and include fatigue, altered mood and depression, insomnia, non-radicular back pain, arthralgia, proximal muscle weakness, headache, and hair loss(10).

Nonspecific musculoskeletal pain refers a pain for which no specific causes such as trauma, infection, tumor, and inflammatory disorders could be identified. The symptoms includes low back pain, knee pain and diffuse pain(11).

Vitamin D deficiency can lead to musculoskeletal pain due to bone demineralization and muscle weakness associated with hypocalcaemia. Direct effect of 1, 25(OH)$_2$D on muscle cells, neurons, neurotrophins, prostaglandin and nitric oxide is also another mechanism(8).

Different factors are associated with vitamin D deficiency which includes factors that decrease coetaneous synthesis of the vitamin, dietary intake and factors that decrease absorption of the vitamin(1).

1.2. Statement of the problem

The prevalence of Vitamin D deficiency in world general population is estimated to be between 25% and 50%(1). In developing countries, the prevalence of Vitamin D deficiency ranges between 30–90%. Despite ample sunshine throughout the year, one-third to one half of individuals living in Sub-Saharan Africa and the Middle East have serum 25-hydroxyvitamin D levels <25 nmol/l(12).

Even though Ethiopia is located to equator, Vitamin D insufficiency was reported to be 84.2% among women in southern Ethiopia(13). This result shows living near the Equator does not guarantee adequate vitamin D status.

Vitamin D deficiency causes rickets in children and osteomalacia in children and adults(2). The prevalence of rickets is high in many parts of Asia, Africa, and the Middle East and resurgence of rickets has been recorded even among the developed countries like Northern European countries. The global prevalence of osteomalacia in adults is assumed in regions where rickets is prevalent in children especially among pregnant women and the elderly(14).

A meta-analysis of observational study published in 2015 by Ming-Yen Hsiao and colleagues summarized that there was significantly higher risk of vitamin D deficiency in patients with chronic widespread pain than the control group(15). Another systematic review and meta-analysis of 29 studies published in the year 2017 by Joshua Zadro and colleagues summarized that there is strong association between vitamin D deficiency and low back pain(16).

In 2016 musculoskeletal disorders are second cause of disability worldwide with back pain as most frequent condition. The disability due to musculoskeletal condition is increased by 19% from 2006 to 2016 and is expected to rise in sedentary and aging people(17). One form of musculoskeletal disorder, low back pain's prevalence among Africans is rising and is of concern(18).

Musculoskeletal conditions produce severe long-term pain. It is well known that prolonged untreated or under-treated chronic pain can have significant negative physical impacts like long term activity limit, sleep disturbance and physical fatigue, psychological impacts like pain related anxiety and fear, and social effects like reduced time in leisure activities with family and the loved ones(19).

Vitamin D status has become an essential aspect of primary care. Vitamin D deficiency and insufficiency can be both corrected and prevented safely through supplementation(20). An endocrine society recommended screening for vitamin D deficiency in individuals at risk for deficiency and recommended dietary and supplement intakes of vitamin D for patients at risk for vitamin D

deficiency(21). In resource limited countries like Ethiopia it is difficult to screen for vitamin D deficiency for every individuals but screening for at list patients with symptoms of vitamin deficiency can identify patients with nonspecific musculoskeletal pain symptoms who can benefit from vitamin D supplementation.

Vitamin D deficiency and its associated risk factors among patients with nonspecific musculoskeletal pain symptoms are largely under studied in Africa despite the continent having a high burden of vitamin D deficiency (18) and Ethiopia is no exception. Therefore assessing prevalence of vitamin D deficiency and associated risk factors in nonspecific musculoskeletal pain symptoms is crucial in Ethiopian context.

1.3.   Significance of the study

Prevalence of Vitamin D deficiency and its associated risk factor among patients with non-nonspecific musculoskeletal pain symptoms is under studied and to our knowledge there is no study done so far in Ethiopia. The data generated from this research showed the prevalence of vitamin D deficiency and its associated risk factors in patients with nonspecific musculoskeletal pain symptoms in TASH. Therefore, determining prevalence and risk factors of Vitamin D deficiency will alert clinicians and health policy makers to give attention to this problem and initiate the need for further research, appropriate intervention and its inclusion as part of routine diagnosis in patients with nonspecific musculoskeletal pain symptoms.

Vitamin D is measured rarely in Ethiopia in spite of suggestion by Endocrine society guideline to screen for vitamin D deficiency for individuals at risk of deficiency. Measuring vitamin D among patients with nonspecific musculoskeletal pain symptoms is important to identify patients who can benefit from vitamin D supplementation therapy.

Furthermore, the finding of this study will also provide background data for future research among this population with large sample size.

2. Literature review

2.1. Vitamin D and the Regulation of Calcium, Phosphorus, Bone and muscle function

Vitamin D increases the efficiency of intestinal calcium absorption from 10-15% to 30-40% and absorption of phosphorus from 60% to 80%(4).

In intestine calcium is absorbed through paracellular and transcellular transport pathway. In paracellular transport pathway Calcium enters enterocytes by passive diffusion while in the transcellular transport pathway calcium enters by active transport mediated by transporter proteins that are expressed due to stimulation by $1,25(OH)_2D(22)$. The binding of $1,25(OH)_2 D$ to VDR modulates the transcription of genes encoding transporter proteins such as transient receptor protein cat ion channel subfamily V member 6 (TRPV6), calbindin, plasma membrane calcium ATPase 1b (PMCA1b) and sodium calcium ion exchanger 1 (NCX1)(23).

TRPV6 on the luminal surface of enterocytes facilitates the entry of calcium into the enterocytes and calbindin transports calcium from the luminal surface to the basal surface while PMCA1b and NCX1 extrudes the calcium from the basal surface to the extracellular circulation [Fig 1a](3).

Even though intestinal phosphate absorption is not tightly regulated as serum calcium, in severe hypophosphatemia, phosphorus is absorbed through active transport, which is facilitated by $1, 25(OH)_2 D$. A large fraction of dietary phosphate intake is considered to be transported by a passive parcellular pathway. The transcellular pathway consists of sodium phosphate transporter 2b (NPT2b) [Fig 1b]. $1, 25(OH)_2D$ increases the expression of NPT2b transporter at the intestinal brush border membrane(22).

Vitamin D in kidney enhances the re-absorption of calcium by initiating expression of TRPV5 and calbindin transporter proteins(23). By negative feedback mechanism, vitamin D also regulates its production by inhibiting renal expression of 1α-hydroxylase(6).

PTH is one of the hormones involved in calcium homeostasis by inducing bone resorption and renal expression of 1α-hydroxylase. Vitamin D suppresses the proliferation of parathyroid gland cells and secretion of PTH by up regulating calcium receptors (CaR) on parathyroid gland cells. Calcium binds with CaR and suppresses the release of PTH(2).

Vitamin D maintains bone health by involving both in bone formation and resorption processes. In bone formation process vitamin D provides Ca and P to bone mineralization from intestine and kidney(24). The suppression of PTH by vitamin D, favors the bone formation process(3). Direct involvement of vitamin D in gene expression of bone forming proteins such as calcium binding

proteins and transcription factors for osteoblast differentiation is another mechanism of vitamin D involvement in bone formation(25). ALP is one of the bone differentiation transcription factors whose expression is stimulated by1, 25(OH)$_2$D (26) Though, measurement of increased ALP expression is taken as a reliable indication of the osteoblastic function the mechanism of this elevation in ALP expression is considered less clear. ALP was postulated to increase the local concentration of inorganic phosphate(27).

b)
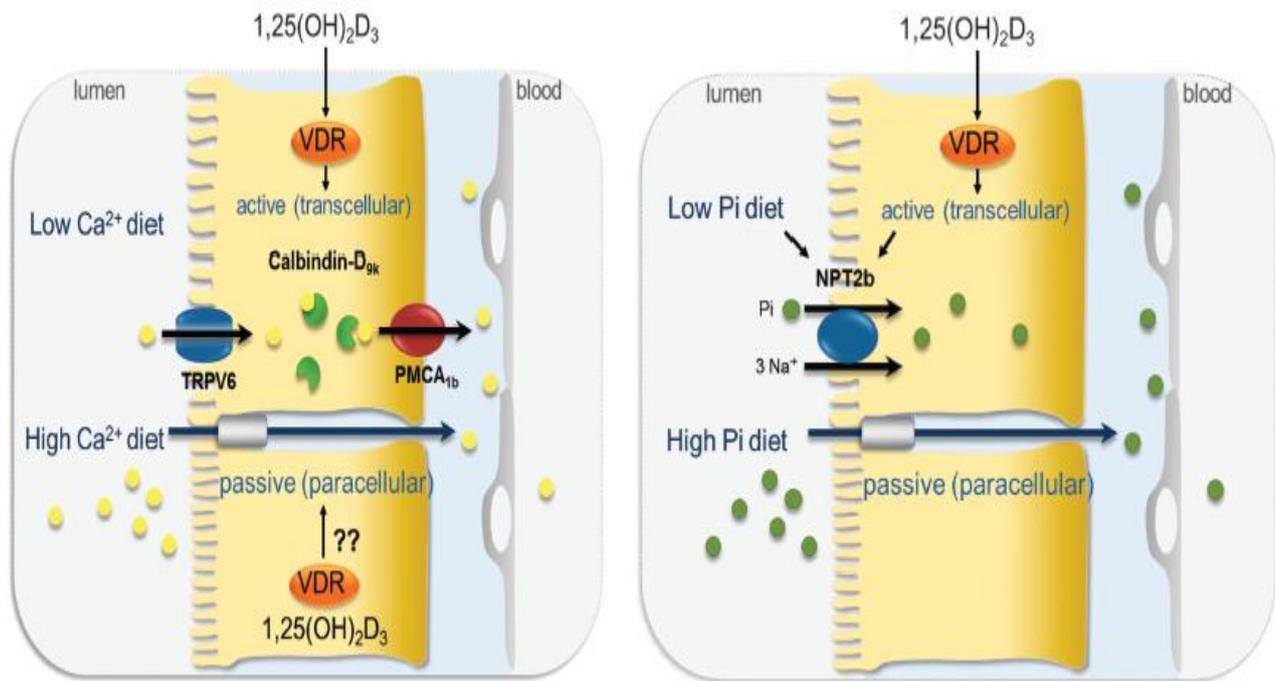
Figure 1: The role of vitamin D on intestinal absorption of a) calcium and b) phosphate (adapted from Christakos S, et al 2014)

To maintain serum calcium homeostasis vitamin D is also involves in mobilization of Ca and P from bone by stimulating osteoclastogenesis. Vitamin D up regulates expression of receptor associated nuclear κβ transcription factor ligand (RANKL) in osteoblast progenitors and osteoblasts(3). By cell to cell contact between RANKL expressing osteoblasts and receptor associated nuclear κβ transcription factor (RANK) expressing osteoclast progenitors, mature osteoclasts are differentiated. The mature osteoclasts dissolve the bone collagen matrix and release Ca and P to blood circulation. Vitamin D also down regulates osteoprotegerin which is the hormone that antagonizes RANKL(25).

Muscle cells are one of the cells known to express VDR(9). The binding of 1, 25(OH)$_2$D to the VDR results in enhanced transcription of a range of proteins(26), including proteins involved in calcium metabolism, insulin like growth factor 1(IGF 1) and insulin like growth factor binding protein 3 (IGFBP 3) . IGF 1 induces proliferation, differentiation and hypertrophy of skeletal muscle and is a

key component in muscle regeneration. IGFBP 3 binds IGF-1 in the serum. The formation of IGF-1–IGFBP-3 complex may block the binding of IGF-1 to its receptors, thereby mitigating its effect on DNA synthesis, growth and glucose regulation, but also prevent its rapid clearance(7).

Calcium is necessary for normal muscle contraction and relaxation. Vitamin D enhances muscle cell intracellular calcium homeostasis by indirect serum calcium homeostasis and direct non genomic calcium influx. The non-genomic calcium influx to muscle cell cytosol relies on vitamin D dependent activation of store operated calcium channel (SOCC) and voltage dependent calcium channel (VDCC) component of signal transduction. The SOCC component of signal transduction results in instol phosphate 3 (IP3) –dependent calcium release from sarcoplasmic reticulum while VDCC results in phosphokinase A (PKA) dependent extracellular calcium influx(28).

2.2. Association of vitamin D deficiency with nonspecific musculoskeletal pain symptoms

Vitamin D deficiency results in abnormalities in calcium, phosphorus, and bone metabolism. Specifically, vitamin D deficiency causes a decrease in the efficiency of intestinal absorption of dietary calcium and phosphorus, resulting in an increase in PTH levels. PTH maintains serum calcium in the normal range at the expense of mobilizing calcium from the skeleton and increasing phosphorus wasting in the kidneys(19).

Vitamin D deficiency in children causes growth retardation and rickets. In adults, vitamin D deficiency causes a skeletal mineralization defect. The bone tissue with mineralization defect provides little structural support for the periosteal covering. As a result osteopenia, osteoporosis, and increase in the risk of fracture and osteomalacia; often complain of isolated or global bone discomfort along with aches and pains in their joints and muscles(23).

Deficiency of 1,25 $(OH)_2D$ that maintains calcium homeostasis, modulates neuronal excitability, up regulates the synthesis of neurotrophins, influences prostaglandin action by inhibiting COX-2 expression and stimulates 15-prostaglandin dehydrogenase (15-PGDH) expression, and inhibiting synthesis of nitric oxide synthentase is another mechanism of nonspecific musculoskeletal pain symptoms(8).

A meta-analysis of observational study published in 2015 by Ming-Yen Hsiao and colleagues which included twelve studies comprising 1,854 patients with chronic widespread pain summarized that there was significantly higher risk of vitamin D deficiency than the control group(15). Another systematic review and meta-analysis of 29 studies published in the year 2017 by Joshua Zadro and colleagues summarized that there is strong association between vitamin D deficiency and low back pain(16).

A 2010 descriptive, analytical, cross-sectional study in Sao Paulo by Santos F C and colleagues found a high prevalence of vitamin D deficiency and insufficiency of 49 and 38%, respectively; however such levels were not significantly correlated to chronic pain(29).

Paul S. McCabe et al. recruited 3369 men aged 40–79 between 2003-2005 from eight European centers for a longitudinal study of male ageing and after adjustment for age and centers, physical performance and number of co morbidities, compared to those in upper quintile of 25-(OH) D (≥36.3 ng/mL), those in the lowest quintile (<15.6 ng/mL) were more likely to develop chronic wide spread pain(30).

Demiryurek BE and Gundogdu AA. in their case control study included 36 patients with mild carpel tunnel syndrome and 40 without carpel tunnel syndrome in 2016 found significantly lower vitamin D levels in the mild carpel tunnel syndrome patients than those electro-physiologically normal subjects(31).

Sarah E. Tague et al conducted experimental study on rats. The rats were offered vitamin D-deficient diets for 2– 4 weeks and the rats showed mechanical deep muscle hypersensitivity but not coetaneous hypersensitivity. This result indicated that vitamin D deficiency can lead to selective alterations in target innervations, resulting in presumptive nociceptor hyper-innervations of skeletal muscle, which in turn is likely to contribute to muscular hypersensitivity and pain(32).

Noha T. Abokrysha studied 30 women diagnosed with fibromyalgia in Saudi Arabia in 2011. The result showed mean vitamin D level of 4.76 +/- 1.46 ng/mL and significant negative correlation between vitamin D level and widespread pain index(33). Another hospital based case control study conducted in 2010 in Saudi Arabia by Ahmed et al showed that a mean serum ALP, PTH and serum 25 (OH) vitamin D levels were significantly difference in patients than controls(34).

A prospective, observational, triple-arm case-control study which included a total of 250 chronic low back pain, 177 sub-acute low back pain cases, and 248 controls conducted in south India by Ajay Panwar et al from November 2016 to January 2017 showed no significant difference in the prevalence of vitamin D deficiency among chronic low back pain, sub-acute low back pain, and controls which was 53.6, 50.8, and 51.6% respectively, in the three arms. However, the categorical analysis revealed that chronic low back pain and sub-acute low back pain cases had a significantly higher prevalence of worse categories of vitamin D deficiency(35).

A study done in south Australia by Jill Benson and colleagues in 2005 on eight cases with muscle pain and eight age and sex matched controls showed that all the eight patients with muscle pain had lower vitamin D levels than those without muscle pain(36).

2.3. Prevalence of vitamin D deficiency among patients with nonspecific musculoskeletal pain symptoms

A retrospective study done by Turner et al involved 267 of 313 patients admitted to the Mayo Comprehensive Pain Rehabilitation Center in USA for whom Vitamin D levels were available, revealed the prevalence of vitamin D deficiency as 26%(37). Another cross sectional study in USA by Gregory A. et al reported prevalence of vitamin D as 93 % among patients with persistent, nonspecific musculoskeletal pain(38).

Gorter EA et al conducted a cross sectional study in Netherland from 2012 to 2013 which showed out of 208 men and 319 females with fracture, 166 patients (31 %) had insufficient levels (50–75 nmol/l) and 210 patients (40 %) had a vitamin D deficiency (calcidiol <50 nmol/L), of whom 58 patients (11 % of the total group) had a severe vitamin D deficiency(39). A cross sectional study on 174 Switzerland chronic patients by Von Känel P et al reported 92% of the participants had vitamin D level below 30ng/ml from which 71% were vitamin D deficient and 21% vitamin insufficient(40).

Of 218 premenopausal women with common musculoskeletal pain and unknown etiology studied by Halim Yilmaz and colleagues in Turkey, 79.8% were detected with vitamin D deficiency(41). Another cross-sectional study by Muharrem Çidem and colleagues involved 8457 patients with widespread musculoskeletal pain showed the prevalence of vitamin D deficiency of 73.9%(42). A retrospective study on 145 Turkey chronic low back pain by Yalkın Çalık and Ümit Aygün reported 22.8% prevalence of vitamin deficiency and 22.8% insufficiency(43).

Among sixty Saudi males with musculoskeletal pain complains studied by Hala Lotfy Fayed and Amel Hamdy Saleh, fifty-four (90%) had vitamin D inadequacy; 42 (70%) deficiency and 12 (20%) had insufficiency and 6 (10%) had adequate 25(OH)D(44). Another similar study by Sadat-Ali M and colleagues on 187 Saudi females with nonspecific musculoskeletal pain showed 27.27% vitamin D insufficiency and 49.19% deficiency(45).

A descriptive analytic study performed on Sixty five adult patients with musculoskeletal pain symptoms in Qazvin city, Iran by Mahnaz Abbasi and his colleagues showed vitamin D deficiency of 95.4 %(46). Another cross sectional study by Alireza Jalili and colleagues in Iran on 438 patients nonspecific musculoskeletal pain showed 86% of the patients had vitamin D less than 30ng/ml and deficiency of 47%(11). A cross sectional study performed on 355 Iraq patients with chronic patients nonspecific musculoskeletal pain reported vitamin D prevalence of 85.35%(47).

Among forty female patients with fibromyalgia included in the study by Shaheen Ayuob Bhatty et al. in Karachi, Pakistan thirty two (80%) patients had Vitamin D deficiency, with mean levels of 15.855± 4.918 ng/ml and 8(20%) had Vitamin D insufficiency, with mean levels of 23.64 ± 2.39 ng/ml(48). Another study conducted by Sobia Qamar and colleagues in Karachi, Pakistan from October 2008 to September 2009 included hundred children aged 5-12 years, presenting with limb pains and fulfilling the diagnostic criteria of growing pains showed the prevalence of vitamin D deficiency of 72% and insufficiency of 22%(49). A case control study which involved 60 cases with persistent non-specific musculoskeletal pains and 60 controls reported 86.6% prevalence of vitamin D deficiency among the cases(50).

A cross sectional by Afsar SS. and collegues in Pakistan on 500 patients with low backache showed 88.4% had vitamin D level below 30ng/ml and deficiency of 63%(51). Another cross sectional study in Pakistan by Humaira Achakzai and colleagues which involved 400 participants, reported 80% prevalence of vitamin D deficiency(52).

A study by Babita Ghai et al. in India was included 328 patients in an open label, single arm clinical trial aimed to assess the effectiveness of vitamin D supplementation in patients with chronic low back pain and determined prevalence of vitamin D as 86%(53).

Another cross sectional study was conducted among 281 adult patients above the age 20 years with non-specific complaints of general body pain/back pain/tiredness/weakness on working and walking with no other symptoms and no relief of the pain symptoms with routine treatments with analgesics and rest and physiotherapy by A Manoharan and colleagues in India. The result showed that out of 281 patients tested, 201 (71.5%) of the study population had below normal vitamin D of them 40.2% had deficiency, 31.3% had insufficiency and 28.5 had sufficient vitamin D level(24).

A cross sectional study conducted on North Korean refuges in South Korea reported all 386 participants had vitamin D level below normal (30ng/ml) of which 87% had vitamin D deficiency(54).

2.4. Risk factors associated with vitamin D deficiency

Factors associate with vitamin D deficiency includes, deprivation of sunlight, decline in coetaneous synthesis of vitamin D due to aging, and decrease in renal hydroxylation, melanin pigmentation, the solar zenith angle which depends on latitude, season, time of the day, low intake of foods fortified with vitamin D, obesity, smoking, pollution, exercise, use of drugs (e.g. anti-epileptic drugs and anti-tuberculous drugs) or diseases that accelerate vitamin D metabolism (e.g. reticulo-endothelial

malignancies, sarcoidosis, tuberculosis), renal insufficiency, mal-absorption syndromes, and chronic liver disease(1).

A review by A. Mithal et al summarized as older age; female sex; darker skin pigmentation; factors that determine sunlight exposure, such as clothing and cultural practices; dietary habits; and national policies of vitamin D fortification are risk factors for vitamin D deficiency(55).

BMI is associated with low vitamin D status. A cross-sectional study on US 18-year-old children by Christy B. Turer and colleagues showed Vitamin D deficiency is highly prevalent in overweight and obese children and severely obese (56). In another study in Singapore by Xinyan B and collegues, BMI was significantly differ between those who were vitamin D deficient and those who were without Vitamin D deficiency (P=0.047) (57). Another study by Merlo C. and colleagues showed negative correlation between vitamin D and BMI (r= -0.156, 95%CI= -0.202 to -0.108)(58). On other side Keyeong Kim et al found Underweight participants (body mass index (BMI) <18 kg/m2) had significantly lower 25(OH) D levels than individuals with normal BMI(P=0.04) (54).

In a survey conducted by the National Center for Health Statistics in US population, Vitamin D deficiency was significantly more common in not college educated(p<0.0001) and obese (p<0.0001)(59). In another study by Catherine M. Gordon et al in USA on 307 healthy adolescents serum 25OHD levels were inversely correlated and body mass index (r=−0.29) (60).

A hospital based case-control conducted in Saudi Arabia from September 2010 to June 2011 by Ahmed M. S. Hegazy et al showed mean serum ALP and serum 25 (OH) vitamin D levels were significantly difference in patients with low back pain than controls without low back pain (p<0.001)(34).

In Qatar healthy school children studied by Abdulbari Bener and colleagues there was a significant difference between groups with deficient vitamin D and those with sufficient vitamin D in terms of physical activity, exposure to sunlight and duration of time spent outside under the sun(P<0.05). The mean values of vitamin D serum concentration, calcium, alkaline phosphates, and phosphorus were very low in vitamin D-deficient children(61). On another hand a study by Sima Hashemipour et al in Tehran showed Vitamin D serum levels had no significant statistical relation with the duration of exposure to sunlight, kind of clothing and BMI(62).

A cross sectional study conducted by Silva Hovsepian and colleagues in Isfahan city, Iran showed Vitamin D deficiency was more prevalent among women (p=0.001) and younger age-group

(p=0.001)(63). Another study by McCarty D. E and colleagues found age as independent risk factor for vitamin D deficiency(64).

Sobia Qamar and colleagues conducted a study in Karachi, Pakistan which showed that hypocalcaemia was found to be more common in patients with vitamin D deficiency but this was not found to be statistically significant (p=0.863) and ALP was elevated in 38 patients (38%)(49).

A study by Kapil U et al in India also showed significantly high vitamin D deficiency in females(p<0.001)(65). On the other hand a study done in India by Babita Ghai and colleagues found that men were significantly more prone to have deficiency as compared to women(OR = 1.78 (1.10 – 2.88), P = 0.02)(53).

Tae-Hwan Kim et al identified a significantly higher prevalence of vitamin D deficiency in medical co- morbidity, and lower score for sunlight exposure(p<0.005)(66).

Fadheela T Al-Mahroos and colleagues conducted study in Bahrain which showed significant association of high income (P-value 0.020), smoking (P-value 0.021), lack of sun exposure (P-value 0.001) and high body mass index (P-value 0.022) with vitamin D deficiency(67).

A retrospective study in Turkey by Atalay G S and colleagues showed that there was significant mean vitamin D difference between females and males(p=0.004). However, the study reported no significant association between serum vitamin D level and Ca, P and ALP(68). Another study in Denmark by Brot C. et al also reported no relation between serum vitamin D metabolites, Ca and P (69).

A study in India by Lodh M and colleagues showed positive correlation between Vitamin D and Ca, P and ALP (70). Another cross sectional study in Qatar by Bener A and colleagues also reported significantly low mean vitamin D, Ca, P and ALP in vitamin D deficient group (61)

2.5. Conceptual framework

Socio-demographic risk factors	Anthropometric indices	Vitamin D deficiency associated factors
• Age • Sex • Education • Occupation • Income • Household size	• Waist circumference • BMI	• Pregnancy • Number of pregnancy • Lactation • Smoking habit • Alcohol consumption, • Exposure to sun light • Use of umbrella

Vitamin D (25-OH) level

Medical history

- Duration of compliant
- Physical activity
- Co- morbidity (mal-absorption, renal diseases and liver diseases)
- Drugs

✓ Nonspecific musculoskeletal pain symptoms
✓ Non musculoskeletal diseases
  • Respiratory infections
  • Autoimmune diseases
  • Diabetes mellitus
  • Cardiovascular diseases
  • Cancer

Figure 2: Conceptual framework

3. Objectives of the study

3.1. General objective

To determine the prevalence of vitamin D deficiency and its associated risk factors in patients with nonspecific musculoskeletal pain symptoms in TASH in Addis Ababa, Ethiopia from March to May 2018.

3.2. Specific objectives

- To determine the prevalence of vitamin D deficiency among patients with nonspecific musculoskeletal pain symptoms

- To compare the level of biochemical parameters among patients with nonspecific musculoskeletal symptoms

- To determine potential risks factors associated to vitamin D deficiency among patients with nonspecific musculoskeletal symptoms

4. Material and methods

4.1. Study design and period

A Cross sectional study design was conducted from March to May 2018.

4.2. Study area

This study was conducted in Tikur Anbessa Specialized Hospital. TASH is located in Addis Ababa. Addis Ababa is the capital and largest city of Ethiopia. TASH is referral and teaching hospital that serves approximately 370,000- 400,000 patients a year. The hospital has 800 beds, with 169 specialists, 65 non-teaching doctors. The hospital provides service with different clinics including neurology and rheumatology clinic(71).

4.3. Population

4.3.1. Source population

All patients who were visited TASH neurology and rheumatology clinic

4.3.2.Study population

All patients who were visited TASH neurology and rheumatology clinic for nonspecific musculoskeletal pain symptoms during the study period and met the inclusion criteria.

4.4. Exclusion and inclusion criteria

4.4.1. Inclusion criteria

- Patients who were with nonspecific musculoskeletal pain symptoms in TASH neurology
- Patients who were rheumatology clinic and capable of independent communication to give informed consent.

4.4.2. Exclusion criteria

- Patients with age of less than 18 years old

- Patients on vitamin D supplementation therapy

- Patients on calcium supplementation therapy

- Patients on multivitamin therapy

- Patients with known history of chronic diseases like liver failure and/or kidney failure

- Patients with rheumatologic diseases, trauma and anatomic skeletal malformations

4.5. Study variables

4.5.1. Dependent variables

Vitamin D deficiency

4.5.2. Independent variables

- Socio-demographic factors; age, sex, education, occupation, household size and income level

- Vitamin D deficiency associated factors; smoking habit, alcohol consumption, exposure to sun, dietary intake of fish and/egg, use of sunscreen and menopause state.

- Anthropometric indices; waist circumference and body mass index (WC, BMI)

- Medical history; duration of compliant, physical activity and co- morbidity

4.6. Measurement and data collection procedure

4.6.1. Sample size determination

The actual sample size for the study was determined using the formula for single population proportion by assuming 5% marginal error (d), 95% confidence interval (α=0.05). The value of p is 95.4% from previous study conducted in Iranian patients with musculoskeletal pain symptoms(46). Based on the above information the total initial sample size is calculated by using the following formula.

$$Ni = \frac{[(Z\ \alpha/2)^2\ pq]}{d^2}$$

Where; Ni= required initial sample size

Zα/2 = critical value for normal distribution at 95% confidence interval which equals to 1.96 (Z value at alpha=0.05).

p = the prevalence of vitamin D insufficiency in Iranian nonspecific musculoskeletal symptom patients= 95.4%

q = Proportion of Iranian nonspecific musculoskeletal symptom patients without deficiency of vitamin D= 0.46%

d = marginal error =0.05

Ni = (1.96)2 (0 .46 x 0.954) / [(0.05)2] =68

Taking 10% non-response rate, a total sample size is calculated to be 75.

4.6.2. Sampling method

Consecutive convenient sampling method was used to select 75 study participants.

4.6.3. Demographic and clinical data collection procedure

The demographic characteristics and associated risk factors data such as; age, gender, smoking habit, alcohol consumption, exposure to sun, education, occupation, level of income, household size, dietary intake of fish and/egg, were collected using a structured questionnaire by trained data collectors (**Refer Annex II**).

Duration of compliant, physical activity, and other co- morbidity were collected from the medical records by using checklist forms (**Refer Annex III section A**).

Anthropometric characteristics of study participants: height and weight were measured. BMI was calculated as weight (kg) divided by height squared (m2). WC was measured at a point midway between the inferior border of the costal margin and the iliac crest in mid auxiliary line to the nearest 0.5 cm. Collection of these medical records and clinical measurements were done by professional nurse to minimize bias. BMI of less than 18.5kg/m2 was considered underweight, BMI between 25 and 29.9 kg /m2 was considered overweight, whereas an adult who has a BMI of 30 kg /m2 or higher was considered obese. Central obesity was assessed by WC and subjects with WC ≥94 cm was regarded as obese, according to the international guideline(72).

The questioner was translated into Amharic to make it easily to understand.

Vitamin D deficiency was defined as a 25-hydroxyvitamin D level of less than 20 ng per milliliter and vitamin D insufficiency as 25(OH) D of 20-29 ng/ml(4).

4.6.4. Blood sample collection, processing and laboratory analysis

Whole blood sample was collected from study participants using serum separator tube. After collection of 4 ml of blood sample, the sample was allowed to clot for 30 minute and serum was separated by centrifugation at 1500 rpm for 10 minutes. Then serum samples were stored in nunc tubes for biochemical test and immunoassay at -20 ^{0}C until analysis (**Refer Annex IV section A**). The activity of alkaline phosphatase was measured at TASH laboratory. Then the stored sample was transported to

Ras Desta Memorial Hospital Laboratory for analysis of Vitamin D and to Ethiopian public Health Institute clinical chemistry referral laboratory for analysis of calcium and phosphate.

Vitamin D (25- OH) was analyzed with mini Vidas hormone analyzer, by combined competitive enzyme immunoassay method with final fluorescent detection. To measure Vitamin D the solid phase receptacle serves as solid phase as well as pipetting device. The sample was mixed with pretreatment reagent to separate vitamin D from its binding protein. The pretreated sample is then collected and transferred to anti-vitamin D-ALP conjugate coated well. Then vitamin D in the sample was allowed to compete with the vitamin D in the solid phase for anti-vitamin D-ALP conjugate. During the detection the conjugate enzyme catalyzes the substrate in to fluorescent product which was measured at 450 nm. The intensity of the fluorescence is inversely proportional to the concentration of vitamin D in the sample. (**Refer Annex IV-section B**).

Alkaline phosphetase (ALP) was analyzed in TASH clinical chemistry laboratory with mindery by spectrophotometeric method in which ρ-Nitrophenyl phosphate is hydrolyzed to p-nitrophenol and inorganic phosphate. The rate at which the ρ-Nitrophenyl phosphate is hydrolyzed measured at 405nm, was directly proportional to the alkaline phosphatase activity. (**Refer Annex IV-section C**)

Calcium (Ca) and Phosphate (P) were analyzed in EPHI national clinical chemistry laboratory with Cobas 6000 c501 module. Photometeric method was used to analyze the analytes in the serum samples. Calcium ion reacts with 5-nitro-5-methyl-1, 2-bis (o-aminophenoxy) ethane-N, N, N, N-teteraacetic acid under alkaline condition to form a complex and this complex reacts with ethylendiamineteteraacetic acid. Change in absorbance is measured photometerically at 340 nm (**Refer Annex IV section D**). P reacts with ammonium molybdate complex in the presence of sulfuric acid and the amount of phosphomolybdate formed was measured photometrically at 340 nm which was directly proportional to the P in sample (**Refer Annex IV section E**)

4.7. Data quality assurance

The questioner was translated into Amharic to make it easily to understand when administered to study participants being assisted by data collectors. The data collectors were professional nurses who work in neurology clinic. The data collection instruments were pre-tested for coherence and data collectors were trained and supervised by principal investigator during the data collection period. The data collected by questionnaire were checked for completeness.

Pre-analytical: Blood sample quality was ensured during collection and processing by following standard operating procedures. Samples were stored in appropriate refrigerator temperature at -20ºC until analysis in Ras Desta Memorial Hospital and EPHI laboratories.

An ice box was used for transportation of the sample to Ras Desta Memorial Hospital and EPHI

Analytical: the automated clinical chemistry analyzers (mini Vidas hormone analyzer, mindery and cobas) were calibrated and their performances were checked by running quality controls.

Post analytical: results were printed out after checking appropriateness of all the test results and the data were carefully entered into Microsoft Excel worksheet and saved for statistical analysis. The leftover samples were discarded according to the protocol of waste disposal of the respective laboratories.

4.8. Data processing, presentation and analysis

Data which were obtained from medical records, using questioners and findings from laboratory analysis were checked for completeness and consistency, coded and entered into Microsoft Excel and were exported to SPSS for analysis.

Before data analysis was carried out, all continuous variables were assessed whether they are normality distributed or not visually using histogram. Data were also explored and tested by Kolmogorov-Smirnov (K-S) and Shapiro-Wilk test for normality. If $p \geq 0.05$, the data were considered as normally distributed.

Descriptive statistics were used to summarize Qualitative variables and were reported with number and percentages. Graphs and tables were used to present results. All parametric values were expressed as the mean ± SD whereas for non-parametric values median were reported with interquartile range. Fisher's exact (Ψ) test was also used to compare qualitative variables. Pearson's and Spearman's correlation for parametric and nonparametric data were used to establish correlations respectively. Independent sample t-test was used to compare parametric data. Multiple logistic regressions were used to identify independent risk factors for vitamin D deficiency. P value < 0.05 was considered statistically significant.

4.9. Ethical considerations

Ethical clearance was obtained from the Departmental Research and Ethics Review Committee of the Department of Medical Laboratory Sciences, School of Nursing and Midwifery of Addis Ababa University. This ethical clearance and support letter was submitted to the Department of Neurology of

Addis Ababa University College of health sciences; the department was further evaluated the proposal of this research and allowed the start of the research by a letter which was written to TASH neurology and rheumatology clinic.

Written consent was obtained from each study subjects before collection of blood samples and other relevant clinical information. Study participants were recruited only based on their consent and the inclusion and exclusion criteria apart from any form of discrimination. Security and confidentiality of detail clinical and laboratory finding data was kept carefully by using code for identification.

The participants were ensured to be free from any coercion, under inducement, influence or intimidation. The only potential risk was a little pain due to vein puncture and consumption of time of participant during the data collection.

The participants had the right to withdraw from the study whenever they feel inconveniences.

The results of participants with vitamin D deficiency were linked to the attending physician for appropriate treatment and management.

The leftover sample of the participants were decontaminated and disposed according to waste disposal protocol of respective laboratories immediately after investigation of the research parameters.

4.10. Result dissemination

The result of this study will be presented for thesis defense, conferences, stakeholders, and publication on peer reviewed journals.

4.11. Operational Definitions

Nonspecific Musculoskeletal pain symptoms: nonspecific pain for which no specific causes such as trauma, infection, tumor, and inflammatory disorders could be identified.

Central obesity: subjects with WC ≥94 cm

Obese: who has a BMI of 30 kg/m2 or higher.

Overweight: BMI between 25 and 29.9 kg/m2

Underweight: BMI of less than 18.5kg/m2

Vitamin D deficiency: a 25-hydroxyvitamin D level of less than 20 ng per milliliter (50 nmol per liter)

Vitamin D insufficiency: a 25(OH) D level of 20-29 ng/ml

Vitamin D sufficiency: a 25(OH) D level of >30 ng/ml

5. Results of the study

5.1. Socio-demographic and anthropometric characteristics of study participants

The minimum and the maximum age of study participants were 18 and 80 year respectively. The mean age was 48.89 years (**Table 1**).

Table 1: Socio-demographic and anthropometric characteristics of patients with nonspecific musculoskeletal pain symptoms at neurology clinic of TASH from March to May 2018 (n=75)

No.	Variables	Categories	Frequency	Percent
1.	**Age (Years)**	18-35	13	17.3
		36-55	39	52
		≥55	23	30.7
2.	**Sex**	Male	24	32
		Female	51	68.0
3.	**Menopause stage**	No	20	26.7
		Yes	31	41.3
4.	**Level of Education**	Illiterate	10	13.3
		primary school	28	37.3
		Secondary school	18	24.0
		College/University	19	25.3
5.	**Occupation**	Government employee	22	29.3
		Private business	17	22.7
		Unemployed	6	8.0
		Housewife	19	25.3
		Farmer	2	2.7
		Retired	9	12
6.	**Monthly income(ETB)**	<2100	50	66.7
		≥2100	25	33.3
7.	**Household size**	≤5	48	64.0
		>5	27	36.0
8.	**BMI (Kg/m2)**	<18.5	35	46.7
		25-29	21	28.9
		>29	14	18.7
9.	**WC**	<94	48	64
		≥94	27	36

BMI: body mass index, WC: waist circumference

5.2. Life style and clinical characteristics of study participants

Of 75 study participants 54(72%) had sun exposure of less than or equal to 2.4 hours per day. The majority of study participants72 (96%) didn't use sun screen. Of the participants 62(92%) were never smoked and 25(33.3%) were drinking alcohol currently. However, 46(61.3%) were reported never drunk. Majority of the study participants 50(66.7%) had less than three years of duration of pain complaint (**Table 2**).

Table 2: Life style and clinical characteristics of patients with nonspecific musculoskeletal pain symptoms at neurology clinic of TASH from March to May 2018. (n=75)

No.	Variables	Categories	Frequency	Percent
1.	**Time of sun exposure(hours)**	≤2.4	54	72
		>2.4	21	28
2.	**Use of Sun screen**	Yes	3	4
		No	72	96
3.	**Fish intake per month**	Never	53	70.7
		<1	2	22.7
		1	4	5.3
		>1	1	1.3
4.	**Egg intake per week**	Never	31	41.3
		<1	23	30.7
		1	12	16
		>1	9	12
5.	**Habit of cigarette smoking**	Current	1	1.3
		Past	5	6.7
		Never	69	92
6.	**Habit of alcohol consumption**	Current	25	33.3
		Past	4	5.3
		Never	46	61.3
7.	**Duration of the pain**	<3	50	66.7
		3-6	19	25.3
		>6	6	8
8.	**Other co-morbidity**	No	42	56
		HIV AIDS	4	5.3
		Hypertension	15	20
		Diabetes Mellitus	10	13.3
		Others[*]	4	5.3

*breast cancer, coronary artery disease and asthma

5.3. Biochemical characteristics of study participants

The mean calcium and phosphorous was 2.08±.021 and 1.13±0.192 respectively whereas the mean Vitamin D was 15.051±4.67. The median ALP activity was 204 with inter quartile range of 161-288. (**Table 3).**

Table 3: Biochemical characteristics of patients with nonspecific musculoskeletal pain symptoms at neurology clinic of TASH from March to May, 2018 (n=75)

No.	Variables	Categories	Frequency	Percent	Mean ±SD	Range	Median (IQR)
1.	**ALP (IU/L)**	<23	1	1.3		1142	204(161-288)
		23-129	12	16			
		>129	62	82			
2.	**Ca (mmol/l)**	<2.15	2	2.7	2.08±.021		
		2.15-2.5	73	97.3			
3.	**P (mmol/l)**	<0.81	34	45.3	1.13±0.19		
		0.81-1.45	41	54.7			
4.	**Vitamin D (ng/ml)**	<20	66	88	15.05±4.67		
		20-29	9	12			

ALP: alkaline phosphatase, Ca: calcium, P: phosphorous, IQR: inter quartile range

5.4.   Prevalence of vitamin D deficiency and insufficiency among study participants

Vitamin D Deficiency is defined as a 25-OH D level of less than 20 ng /ml and vitamin D insufficiency as 25-OHD of 20-29 ng/ml. Among the study participants with nonspecific musculoskeletal pain symptoms 66(88%) [Male 19(25.33%) and female 47(62.67%)] were with Vitamin D deficiency and 9(12%) [Male 5(6.67%) and female 4(5.33%)] were vitamin D insufficient.

5.5.   Comparison of mean of biochemical parameters between vitamin D deficient and vitamin D insufficient groups of study participants

There was no statically significant difference between the vitamin D deficient and sufficient groups in their ALP, Ca and P levels (p>0.05) (**Table 4**).

Table 4: Comparison of biochemical parameters of study participants between vitamin D deficient and vitamin D insufficient groups among patients with nonspecific musculoskeletal pain symptoms at neurology clinic of TASH from March to May, 2018 (n=75)

	Parametric values	Vitamin deficient group(N=66)	Vitamin insufficient group(N=9)	P value
		Mean ± SD	Mean ± SD	
1.	P (mmol/l)	1.14 ± 0.194	1.10 ± 0.19	0.591
2.	Ca	2.09 ± 0. 21	2.0544 ± 0.17	0.67
	Non Parametric values	**Median**	**Median**	**P value**
3.	ALP	206.50	204.0000	0.546

P: phosphorus, Ca: calcium and ALP: alkaline phosphatase

5.6. Correlation and multiple logistic regression analysis result of independent variables with vitamin D deficiency

The bivariate correlation analysis showed Age (r= -0.251, p=0.030), BMI (r= -0.388, P=0.001) and WC (r =-0.35; *p*=0.002) were inversely correlated with Vitamin D level (**figure.5).**

A.
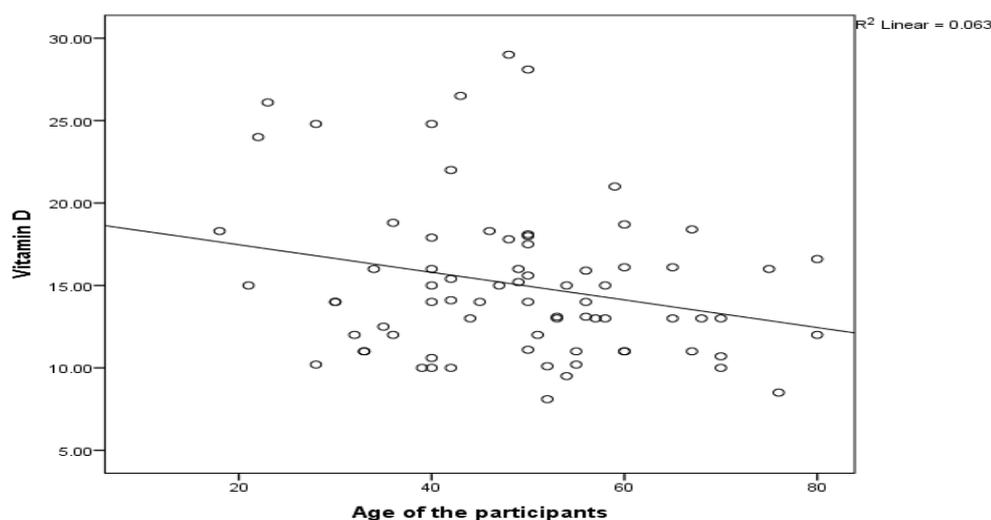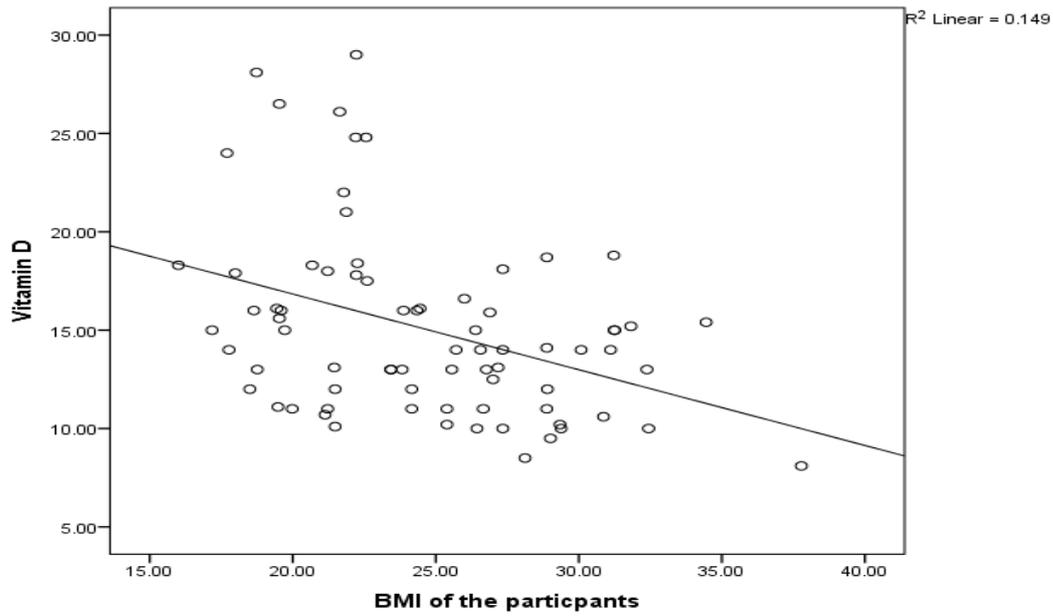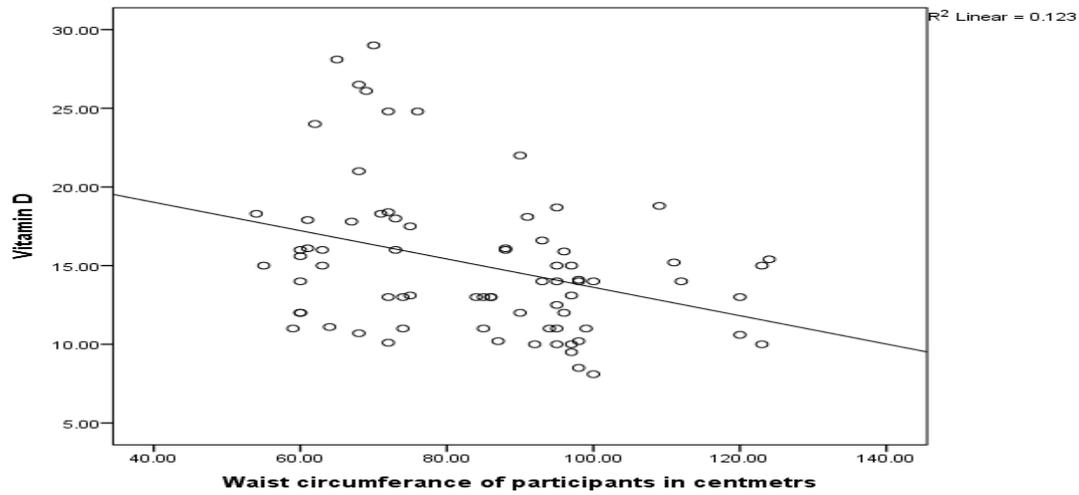
Figure 3: Scatter Plots that show relationship between vitamin D level and Age (A), BMI (B) and WC (C)

Simple logistic regression result showed only age (COR=1.063, p=0.037), occupation (COR=5.455, P=0.046), BMI (COR=1.302, p=0.001) and WC (COR=1.059, p=0.002) were significantly associated with vitamin D deficiency (**Table 5**).

Table 5: Binary logistic regression of demographic and anthropometric variables with vitamin D deficiency among patients with nonspecific musculoskeletal pain symptoms at neurology clinic of TASH from March to May, 2018 (n=75)

No.	Variables		Vitamin D deficient (N=66)	Vitamin D insufficient (N=9)	COR	95% CI	P value
1.	Age(years)		66	9	1.063	1.00-1.13	0.037[*]
2.	Gender	Male	19	5	Ref.		
		Female	47	4	3.092	0.75-2.78	0.119
3.	Menopause stage	Yes	29	2	1.611	0.21-2.45	0.648
		No	18	2	Ref.		
4.	Level of education	Illiterate	9	1	2.4	0.23-4.96	0.464
		primary school	26	2	3.45	0.57-1.23	0.179
		Secondary school	16	2	2.133	0.34-13.4	0.419
		College/University	15	4	Ref.		
5.	Occupation	Farmer	2	0	-	-	
		Government employee	20	2	5.455	0.94-1.75	0.059
		Private business	11	6	Ref.		
		Unemployed	6	0	-	-	
		Housewife	18	1	9.818	1.04-2.78	0.046[*]
		Retired	9	0	-	-	
6.	Monthly income(ETB)		66	9	1	0.23-4.38	0.684
7.	Household size		66	9	1.143	0.262-1.621	0.859
8.	BMI (Kg/m2)		66	9	1.302	1.05-1.62	0.001[*]
9.	WC		66	9	1.059	1.005-1.15	0.002[*]

*statistically significant difference, COR: crud odds ratio, ETB: Ethiopian birr, BMI: body mass index, WC: waist circumference

Table 6: Binary logistic regression analyses result of life style and clinical data with vitamin D deficiency among patients with nonspecific musculoskeletal pain symptoms at neurology clinic of TASH from March to May, 2018 (n=75)

No.	Variables Categories		Vitamin D deficient (N=66)	Vitamin D insufficient (N=9)	COR	95% CI	P value
1.	Time of sun exposure(hours)		66	6	0.802	0.62-1.04	0.095
2.	Use of sun screen	Yes	3	0	Ref.		
		No	63	9	0.00	0.00	0.999
3.	Fish intake/month	Never	45	8	Ref.		
		<1	16	1	2.84	0.33-24.56	0.342
		1	4	0	-	-	
		>1	1	0	-	-	
4.	Egg intake/week	Never	29	2	Ref.		
		<1	20	3	0.46	0.07-3.00	0.417
		1	9	3	0.21	0.03-1.44	0.111
		>1	8	1	0.55	0.04-6.89	0.644
5.	Cigarette smoking habit	Yes	6	0	-	-	
		No	60	9	Ref.		
6.	Alcohol consumption habit	Yes	23	3	1.3	0.29-5.67	0.727
		No	40	6	Ref.		
7.	Duration of the pain		66	9	0.982	0.87-1.109	0.772
8.	Other co-morbidity	No	34	8	Ref.		
		HIV AIDS	4	0	-	-	
		Hypertension	14	1	3.294	0.38-28.85	0.282
		DM	10	0	-	-	
		Others	4	0	-	-	

COR: crud odds ratio, HIV AIDS: human immune virus acquired immunodeficiency syndrome, DM: diabetes mellitus

Table 7: Binary logistic regression analyses result of biochemical parameters with vitamin D deficiency among patients with nonspecific musculoskeletal pain symptoms at neurology clinic of TASH from March to May, 2018 (n=75)

No.	Variables Categories	Vitamin D deficient (N=66)	Vitamin D insufficient (N=9)	COR	95% CI	P value
1.	ALP	66	9	1.003	0.99-1.01	0.32
2.	Ca	66	9	1.517	0.06-97.21	0.62
3.	P	66	9	2.879	0.03-246.2	0.641

COR: crude odds ratio, ALP: alkaline phosphatase, Ca: calcium, P: phosphorous

Finally, multivariate model with forward Wald elimination showed only BMI (AOR=1.302 p=0.018) was remained as independent risk factor of vitamin D deficiency. One kg/m^2 increase in BMI increase odds of developing vitamin D deficiency by 1.302 times. (**Table 8**)

Table 8: Multiple logistic regression analyses result of predictor of vitamin D deficiency among patients with nonspecific musculoskeletal pain symptoms at neurology clinic of TASH from March to May, 2018

No.	Variables		Vitamin D deficient (N=66)	Vitamin D insufficient (N=9)	AOR	95% CI	P value
10.	Age(years)		66	9	1.063	1.00-1.13	0.107
11.	Gender	Male	19	5	Ref.		
		Female	47	4	3.092	0.75-2.78	0.139
12.	Level of education	Illiterate	9	1	2.4	0.23-4.96	0.564
		primary school	26	2	3.45	0.57-1.23	0.189
		Secondary school	16	2	2.133	0.34-13.4	0.489
		College/University	15	4	Ref.		
13.	Occupation	Farmer	2	0	-	-	
		Government employee	20	2	5.455	0.94-1.75	0.129
		Private business	11	6	Ref.		
		Unemployed	6	0	-	-	
		Housewife	18	1	9.818	1.04-2.78	0.086
		Retired	9	0	-	-	
14.	BMI (Kg/m2)		66	9	1.302	1.05-1.62	0.018[*]
15.	WC		66	9	1.059	1.00-1.15	0.062

[*] Statistically significant association, AOR: adjusted odds ratio, BMI: body mass index, WC: waist circumference, CI: confidence interval

6. Discussion

We assessed Vitamin D deficiency and its associated risk factors among 75 Ethiopian participants with nonspecific musculoskeletal pain symptoms attending treatment follow up at TASH. It was the first study performed in this population at this tertiary setting aiming to determine prevalence of Vitamin D deficiency and its associated risk factors and compared differences in biochemical parameters in Vitamin D deficient and Vitamin D insufficient groups of this population.

Prevalence of Vitamin D deficiency

In this study all the participants had vitamin D level below normal (<30 ng/ml). Among these participants with nonspecific musculoskeletal pain symptoms 66(88%) [Male 19(25.33%) and Female 47(62.67%)] were with Vitamin D deficiency and 9(12%) [Male 5(6.67%) and female 4(5.33%)] were vitamin D insufficient. Dark skin might be the reason for the relatively high vitamin D inadequacy in this study. Melanin is known to block UVB radiation(73). Other possible reason might be due to eating diet poor in vitamin D among Ethiopians(13).

A similar cross sectional study conducted in USA on patients with persistent nonspecific musculoskeletal pain symptoms with age ranging from 10 to 60 years reported consistent prevalence (93%) to our study(38). Another study with smaller sample size of 62 in Iran on adult patients with nonspecific musculoskeletal pain symptoms reported comparable prevalence of 95.4 % (46). A case control study done in Pakistan on 60 cases with nonspecific musculoskeletal pain symptoms and 60 controls showed consistent prevalence of vitamin D among cases (86.6%) (50).

A cross sectional studies done in Pakistan(48, 49) with small sample sizes of 40 and 100 reported slightly lower prevalence of 80%, and 72% respectively compared to our study. The possible explanation may be the study subjects' age difference between study participants. Our study participants mean age was 48.9 while the former's study mean age was 37.65+/-11.5 and the latter's study participants were children from 10 to 12 years old. Cutaneous vitamin D synthesis declines with age(55). Another studies done in Saudi 60 patients with chronic and diffuse nonspecific musculoskeletal pain symptoms also identified slightly lower (70%) prevalence of vitamin D deficiency and 20% vitamin D insufficiency(44). A similar study in Switzerland 174 patients with chronic pain reported slightly lower (71%) prevalence of vitamin D deficiency and 21% vitamin D insufficiency(40). Unlike to our study a cross sectional study done with small sample sizes in Turkey on 145 chronic low back pain patients identified lower (22.8%) prevalence of vitamin D deficiency, 42.8% vitamin D insufficiency(43). Another study in Singapore on 114 participants showed 42% prevalence of vitamin D deficiency(57). This may be due to difference in life style, dietary habits and availability of vitamin D supplementation therapies in European countries.

A cross sectional studies conducted on large scale sample sizes as a study in Iraq reported prevalence of vitamin D deficiency 85.35% in a sample of 355 patients with chronic nonspecific musculoskeletal pain symptoms(47). Another study on 386 North Korean refuges in South Korea found prevalence of vitamin D deficiency as 87%(54). A controlled study in India reported 86% prevalence of vitamin D deficiency among 328 chronic low back pain patients(53). These findings are consistent with our study's result.

Another cross sectional studies conducted on large scale sample sizes as in studies done in Turkey(42) and Pakistan(52) reported slightly lower prevalence of vitamin D deficiency as 71.7 % and 80% respectively. This could be possibly explained by the difference in mean age between our study participants and in these studies.

However, a studies done in USA(37), India(24), Iran(11), and Saudi(45) on larger sample size found lower prevalence of 26%, 40.2%, 47% and 49.19% respectively. This may be due to difference in life style, skin color, and living condition.

Association of vitamin D level with independent variables: We observed that gender, menopause stage, level of education, use of sun screen, monthly fish intake, weekly egg intake, history of cigarette smoking, duration of cigarette smoking, number of cigarette, alcohol consumption, household size, income level, duration of pain compliant, duration of sun exposure, other co-morbidity, Ca, P and ALP were not significantly associated with vitamin D level.

In contrast to our study, a study in Bahrain(67) showed that there was association of vitamin D deficiency with high income level (P-value 0.020) and habit of smoking (P-value 0.021).

In line to our study, a study conducted in Turkey(41) sowed no association of vitamin D deficiency with duration of pain compliant, level of income, level of education and duration of sun exposure. Similarly a study in Bahrain(62) showed no association of vitamin D deficiency with duration of sun exposure. In contrast to this a study in USA(59) showed vitamin D deficiency was common among those who were not collage educated (P <0.05). Another contrasting studies in Qatar(61), Bahrain(67), USA(37), India(53) and Korea(66) showed association of vitamin D deficiency with duration of sun exposure.

In our study age, BMI, WC and occupation were associated with vitamin D deficiency. In line to our study, a studies conducted in Turkey(42), India(24), Qatar(61), Iran(11, 62), Iraq(47) and USA(64) showed association of vitamin D deficiency with age. In contrast to these studies, studies in Saudi Arabia(33) and Turkey(41, 68) showed no association of vitamin D deficiency with age. It is well established that cutaneous vitamin D synthesis declines with age(55)

In line with our study, studies in South Korea(54), Singapore(57), USA(37, 56, 59, 60, 64), Bahrain(67) and Qatar(61) showed association between BMI and vitamin D deficiency. In contrast to this finding, a study in Teheran(62), Turkey(41) and Switzerland(58) showed no association of vitamin D deficiency with BMI.

In this study housewives were more vitamin D deficient compared to those who were private company owners and employers. This might to be due to limited outdoor activities by housewives compared to private company owners and employers. Sedentary way of life limits the exposure of skin to UVB radiation and hence related to vitamin D deficiency(1). In line to our study a study done in Iraq(47) showed association of vitamin D deficiency with occupation. In contrast to this a study in Turkey(41) showed no association of vitamin D deficiency and occupation.

Comparison of biochemical parameters between vitamin D deficient and Vitamin D insufficient groups: In our study, P, Ca and ALP were not significantly differs between vitamin D deficient and vitamin D insufficient group.

In line to our study a study in Turkey(41) showed no difference in levels of Ca, P and ALP between those with vitamin D level of <20ng/ml and those with vitamin D level of >20ng/ml. Another study in Denmark(69) and Turkey(68) also showed no relationship between levels of Ca and P ALP and vitamin D metabolites. Similarly, a study conducted in Pakistan(49) reported no significant difference in P and Ca levels but with regard to ALP showed significantly higher activity in vitamin D deficient group.

In contrast to our study, a studies in Qatar(61) showed that level of Ca, ALP and P were low in vitamin D deficient children. Similarly a study in India(70) reported level of Ca, ALP, P and vitamin D were positively correlated.

Risk factors of Vitamin D deficiency: According to result of multiple logistic regression analysis of the association between independent variables and vitamin D deficiency, BMI was remained as independent risk factor of vitamin D deficiency. The relationship of obesity and vitamin D deficiency may be due to increased volume of distribution to fat of lipid soluble vitamin D as it leaves the general circulation after being synthesized in the skin or obtained through the diet. Moreover the vitamin also retains in those fat stores(74). Studies conducted in USA(59, 64), Singapore(57) and Bahrain (67) reported BMI as independent risk factor of vitamin D deficiency.

7. Strength and limitations of the study

7.1. Strength of the study

- To our knowledge this study is new among our study participants with nonspecific musculoskeletal pain symptoms.
- Moreover, Ca and P levels were determined in accredited referral clinical chemistry of Ethiopian Public Health Institute.

7.2. Limitations of the study

- Convenient sampling technique was used to select the study participants
- This study was conducted on small sample size in a tertiary referral neurology clinic where most of the patients had many complications and numerous co morbidities. Because of this fact, results of this study cannot therefore be generalized to the majority of patients with nonspecific musculoskeletal pain symptoms who follow up at primary health care.
- Some of the information like the use of sun screen, fish intake, egg intake, hours of sun exposure, habits of smoking and alcohol consumption were subjectively obtained from patients.
- Total ALP in serum was analyzed without identifying bones specific ALP.
- Moreover, our inability to measure PTH which is a better bone mineral density indicator with ALP, Ca and P due to unavailability of these assays and cost.

Conclusions

In conclusion, this cross sectional study indicated the Prevalence of vitamin D deficiency as 88% and vitamin D insufficiency as 12%.

Age, BMI, WC and occupation are associated with vitamin D deficiency. This study demonstrated that obesity is independent risk factor for vitamin D deficiency.

Recommendations

Based on our findings, we would like to put the following recommendation:

1. Patients with nonspecific musculoskeletal pain symptoms need to have evaluation of Vitamin D levels in order to have a better picture about their disease status.

2. Further study should be conducted to:

   - Establish the local reference range of vitamin D level for Ethiopians, and then the prevalence of vitamin D deficiency and associated risk factors among patients with nonspecific musculoskeletal pain symptoms should be studied based on the established reference range.

   - Determine the association of vitamin D with biochemical marker by testing the participants serum for PTH and bone specific ALP

   - Determine the effect of vitamin D supplementation therapy on pain symptoms among patients with patients with nonspecific musculoskeletal pain symptoms.

References

1. Yilmaz R, Ozkayit S. Vitamin-D-deficiency and chronic widspread pain. EMJ Rheumatol. 2017;4(1):104-11.

2. Henry HL. Regulation of vitamin D metabolism. Best practice & research Clinical endocrinology & metabolism 2011;25(4):531-41.

3. Kochupillai N. The physiology of vitamin D : current concepts The Indian journal of medical research. 2008;127(3):256-62.

4. Holick M. Vitamin D deficiency. The New England journal of medicine. 2007;357(3):266-81.

5. Holick M. Cancer, sunlight and vitamin D. Journal of clinical & translational endocrinology. 2014;1(4):179-86.

6. Wacker M, Holick M. Vitamin D - effects on skeletal and extraskeletal health and the need for supplementation. Nutrients. 2013;5(1):111-48.

7. Bikle D. Vitamin D metabolism, mechanism of action, and clinical applications. Chemistry & biology. 2014;21(3):319-29.

8. Shipton EA, Shipton EE. Vitamin D and pain: vitamin D and its role in the aetiology and maintenance of chronic pain states and associated comorbidities. Pain Research and Treatment. 2015;2015.

9. Capiati D, Benassati S, Boland R. 1,25(OH)2-vitamin D3 induces translocation of the vitamin D receptor (VDR) to the plasma membrane in skeletal muscle cells. J Cell Biochem. 2002;86(1):128-35.

10. Khan QJ FC. How I Treat Vitamin D Deficiency. Journal of oncology practice. 2010; 6(2).

11. Jalili A, Bahrabadi M, Zare S. Prevalence of Vitamin D Deficiency in Nonspecific Musculoskeletal Pain. Shafa Orthopedic Journal. 2015;2(4).

12. Arabi A, El Rassi R, Fuleihan GE-H. Hypovitaminosis D in developing countries—prevalence, risk factors and outcomes. Nature Reviews Endocrinology. 2010;6(10):550.

13. Gebreegziabher T, Stoecker BJ. Vitamin D insufficiency in a sunshine-sufficient area: southern Ethiopia. Food and nutrition bulletin. 2013;34(4):429-33.

14. Prentice A. Vitamin D deficiency: a global perspective. Nutrition reviews. 2008;66(suppl_2):S153-S64.

15. Chang K. Is serum hypovitaminosis D associated with chronic widespread pain including fibromyalgia? A meta-analysis of observational studies. Pain physicia. 2015;18:E877-87.

16. Zadro J, Shirley D, Ferreira M, Carvalho-Silva AP, Lamb SE, Cooper C, et al. Mapping the association between Vitamin D and low back pain: A systematic review and meta-analysis of observational studies. Pain physician. 2017;20(7).

17. Vos T, Allen C, Arora M, Barber RM, Bhutta ZA, Brown A, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. The Lancet. 2016;388(10053):1545-602.

18. Louw QA, Morris LD, Grimmer-Somers K. The prevalence of low back pain in Africa: a systematic review. BMC Musculoskeletal disorders. 2007;8(1):105.

19. Tüzün EH. Quality of life in chronic musculoskeletal pain. Best Practice & Research Clinical Rheumatology. 2007;21(3):567-79.

20. Stechschulte SA, Kirsner RS, Federman DG. Vitamin D: bone and beyond, rationale and recommendations for supplementation. The American journal of medicine. 2009;122(9):793-802.

21. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. The Journal of Clinical Endocrinology & Metabolism. 2011;96(7):1911-30.

22. Christakos S, Lieben L, Masuyama R, Carmeliet G. Vitamin D endocrine system and the intestine. BoneKEy reports. 2014;3(496).

23. Hwang I, Jung E-M, Yang H, Choi K-C, Jeung E-B. Tissue-specific expression of the calcium transporter genes TRPV5, TRPV6, NCX1, and PMCA1b in the duodenum, kidney and heart of Equus caballus. Journal of Veterinary Medical Science. 2011;73(11):1437-44.

24. Manoharan A, Selvaraj P, Vasanthamani P. Vitamin D level among patients with non specific musculoskeletal pain attending tertiary care hospital in Tamil Nadu. International Journal of Orthopaedics. 2016;2(4):94-6.

25. Khosla S. Minireview: The opg/rankl/rank system. Endocrinology. 2001;142(12):5050-5.

26. Haussler MR, Jurutka PW, Mizwicki M, Norman AW. Vitamin D receptor (VDR)-mediated actions of 1α, 25 (OH) 2vitamin D3: genomic and non-genomic mechanisms. Best practice & research Clinical endocrinology & metabolism. 2011;25(4):543-59.

27. Maehata Y, Takamizawa S, Ozawa S, Kato Y, Sato S, Kubota E, et al. Both direct and collagen-mediated signals are required for active vitamin D3-elicited differentiation of human osteoblastic cells: roles of osterix, an osteoblast-related transcription factor. Matrix biology. 2006;25(1):47-58.

28. Girgis CM, Clifton-Bligh RJ, Hamrick MW, Holick MF, Gunton JE. The roles of vitamin D in skeletal muscle: form, function, and metabolism. Endocrine reviews. 2012;34(1):33-83.

29. Santos FC, Moraes NSd, Pastore A, Cendoroglo MS. Chronic pain in long-lived elderly: prevalence, characteristics, measurements and correlation with serum vitamin D level. Revista Dor. 2015;16(3):171-5.

30. McCabe PS, Pye SR, Mc Beth J, Lee DM, Tajar A, Bartfai G, et al. Low vitamin D and the risk of developing chronic widespread pain: results from the European male ageing study. BMC musculoskeletal disorders. 2016;17(1):32.

31. Demiryurek B, Gundogdu A. The effect of vitamin D levels on pain in carpal tunnel syndrome. Orthopaedics & Traumatology: Surgery & Research. 2017;103(6):919-22.

32. Tague SE, Clarke GL, Winter MK, McCarson KE, Wright DE, Smith PG. Vitamin D deficiency promotes skeletal muscle hypersensitivity and sensory hyperinnervation. Journal of Neuroscience. 2011;31(39):13728-38.

33. Abokrysha NT. Vitamin D deficiency in women with fibromyalgia in Saudi Arabia. Pain Medicine. 2012;13(3):452-8.

34. Hegazy A, Salama B, Elgaml A, Alzyat AR. Association of low back pain with vitamin D deficiency and other common risk factors: A hospital based case–control study. European Journal of Preventive Medicine. 2015;3(1):1-5.

35. Panwar A, Valupadas C, Veeramalla M, Vishwas HN. Prevalence of vitamin D deficiency in chronic and subacute low back pain patients in India: a triple-arm controlled study. Clinical rheumatology. 2017:1-8.

36. Benson J, Wilson A, Stocks N, Moulding N. Muscle pain as an indicator of vitamin D deficiency in an urban Australian Aboriginal population. Medical journal of Australia. 2006;185(2):76.

37. Turner MK, Hooten WM, Schmidt JE, Kerkvliet JL, Townsend CO, Bruce BK. Prevalence and clinical correlates of vitamin D inadequacy among patients with chronic pain. Pain Medicine. 2008;9(8):979-84.

38. Plotnikoff GA, Quigley JM, editors. Prevalence of severe hypovitaminosis D in patients with persistent, nonspecific musculoskeletal pain. Mayo clinic proceedings; 2003:78:1463-1470

39. Gorter E, Krijnen P, Schipper I. Vitamin D deficiency in adult fracture patients: prevalence and risk factors. European Journal of Trauma and Emergency Surgery. 2016;42(3):369-78.

40. von Känel R, Müller-Hartmannsgruber V, Kokinogenis G, Egloff N. Vitamin D and central hypersensitivity in patients with chronic pain. Pain medicine. 2014;15(9):1609-18.

41. Yilmaz H, Bodur S, Karaca G. The Association between Vitamin D Level and Chronic Pain and Depression in Premenopausal Women. Turkish Journal of Physical Medicine & Rehabilitation/Turkiye Fiziksel Tip ve Rehabilitasyon Dergisi. 2014;60(2).

42. Çidem M, Kara S, Sarı H, Özkaya M, Karacan İ. Prevalence and risk factors of vitamin D deficiency in patients with widespread musculoskeletal pain. Journal of Clinical and Experimental Investigations. 2013;4(4):488-91.

43. Çalık Y, Aygün Ü. Evaluation of vitamin D levels in patients with chronic low back-leg pain. Acta orthopaedica et traumatologica turcica. 2017;51(3):243-7.

44. Fayed HL, Saleh AH. Frequency of vitamin D inadequacy among Saudi males visiting a Rheumatology Outpatient Clinic of a tertiary hospital in Al-Qassim region: Effect of vitamin D supplementation. The Egyptian Rheumatologist. 2017;39(4):249-54.

45. Sadat-Ali M, Al Essa ON, Alani FM, Al Omar HK, Ebrahim WY. Correlation of symptoms to serum vitamin D levels? Clinical nutrition ESPEN. 24 (2018) 31-34

46. Abbasi M, Hashemipour S, Hajmanuchehri F, Kazemifar AM. Is vitamin D deficiency associated with non specific musculoskeletal pain? Global journal of health science. 2013;5(1):107.

47. Anoar KA, Amin BAM. Hypo Vitaminosis D in Chronic Muscular Skeletal Pain. Int J Curr Microbiol App Sci. 2015;4(9):696-703.

48. Bhatty SA, Shaikh NA, Irfan M, Kashif SM, Vaswani AS, Sumbhai A, et al. Vitamin D deficiency in fibromyalgia. JPMA-Journal of the Pakistan Medical Association. 2010;60(11):949.

49. Qamar S, Akbani S, Shamim S, Khan G. Vitamin D levels in children with growing pains. J Coll Physicians Surg Pak. 2011;21(5):284-7.

50. Alam HMA, Zaidi SBH, Kamran M, Khan DA, Rehman SU, Hussain K. Association of" Hypovitaminosis D" With Persistent Non-Specific Musculoskeletal Pains. Pakistan Armed Forces Medical Journal. 2017(3):386.

51. Afsar SS, Idrees M, Gulzar M. Levels of vitamin D in patients with low backache. Gomal J Med Sci 2014; 12:151-5.

52. Achakzai H, Shah H, Zahid SB, Zuhaid M. Hypovitaminosis-D: Frequency and association of clinical disease with biochemical levels in adult patients of RMI Medical OPD. Pakistan journal of medical sciences. 2016;32(2):394.

53. Babita Ghai M, Dipika Bansal M, Raju Kanukula M. High prevalence of hypovitaminosis D in Indian chronic low back patients. Pain Physician. 2015;18:E853-E62.

54. Kim KJ, Kim YJ, Kim SH, An JH, Yoo HJ, Kim HY, et al. Vitamin D status and associated metabolic risk factors among North Korean refugees in South Korea: a cross-sectional study. BMJ open. 2015;5(11):e009140.

55. Mithal A, Wahl DA, Bonjour J-P, Burckhardt P, Dawson-Hughes B, Eisman JA, et al. Global vitamin D status and determinants of hypovitaminosis D. Osteoporosis international. 2009;20(11):1807-20.

56. Turer CB, Lin H, Flores G. Prevalence of vitamin D deficiency among overweight and obese US children. Pediatrics. 2013;131(1):e152-e61.

57. Bi X, Tey SL, Leong C, Quek R, Henry CJ. Prevalence of vitamin D deficiency in Singapore: its implications to cardiovascular risk factors. PloS one. 2016;11(1):e0147616.

58. Merlo C, Trummler M, Essig S, Zeller A. Vitamin D deficiency in unselected patients from swiss primary care: a cross-sectional study in two seasons. PloS one. 2015;10(9):e0138613.

59. Forrest KY, Stuhldreher WL. Prevalence and correlates of vitamin D deficiency in US adults. Nutrition research. 2011;31(1):48-54.

60. Gordon CM, DePeter KC, Feldman HA, Grace E, Emans SJ. Prevalence of vitamin D deficiency among healthy adolescents. Archives of pediatrics & adolescent medicine. 2004;158(6):531-7.

61. Bener A, Al-Ali M, Hoffmann GF. Vitamin D deficiency in healthy children in a sunny country: associated factors. International journal of food sciences and nutrition. 2009;60(sup5):60-70.

62. Hashemipour S, Larijani B, Adibi H, Javadi E, Sedaghat M, Pajouhi M, et al. Vitamin D deficiency and causative factors in the population of Tehran. BMC Public health. 2004;4(1):38.

63. Hovsepian S, Amini M, Aminorroaya A, Amini P, Iraj B. Prevalence of vitamin D deficiency among adult population of Isfahan City, Iran. Journal of health, population, and nutrition. 2011;29(2):149.

64. McCarty DE, Reddy A, Keigley Q, Kim PY, Cohen S, Marino AA. Nonspecific pain is a marker for hypovitaminosis D in patients undergoing evaluation for sleep disorders: a pilot study. Nature and science of sleep. 2013;5:37.

65. Kapil U, Pandey RM, Goswami R, Sharma B, Sharma N, Ramakrishnan L, et al. Prevalence of Vitamin D deficiency and associated risk factors among children residing at high altitude in Shimla district, Himachal Pradesh, India. Indian journal of endocrinology and metabolism. 2017;21(1):178.

66. Jin-Oh Park M, Hak-Sun Kim M, Seok Woo Kim M, Seong-Hwan Moon M. Prevalence of vitamin D deficiency in patients with lumbar spinal stenosis and its relationship with pain. Pain Physician. 2013;16:165-76.

67. Al-Mahroos FT, Al-Sahlawi HS, Al-Amer EA, Radhi HT, Khalaf S. Prevalence and Risk Factors of Vitamin D Deficiency among Men. Bahrain Medical Bulletin. 2013;158(749):1-4.

68. Atalay SG, Atalay R, Alkan BM, Fidan F, Bozkurt S, Aksekili H, et al. Vitamin D Deficiency in Adults with Musculoskeletal Pain. Turk Osteoporoz Dergisi. 2015;21(3).

69. Brot C, Jørgensen N, Madsen O, Jensen L, Sørensen O. Relationships between bone mineral density, serum vitamin D metabolites and calcium: phosphorus intake in healthy perimenopausal women. Journal of internal medicine. 1999;245(5):509-16.

70. Lodh M, Goswami B, Mahajan RD, Sen D, Jajodia N, Roy A. Assessment of vitamin D status in patients of chronic low back pain of unknown etiology. Indian Journal of Clinical Biochemistry. 2015;30(2):174-9.

71. Derebew M, Bekele A. providing sustainable medical education andcare in african countries. [updated march 01 2007;cited 2018 December 20]. Available from: https://www.ameca.org.uk/directory/listing/black-lion-specialized-hospital-ethiopia

72. Alberti, George K, Mayer M, Zimmet, Paul, Shaw, et al. Metabolic syndrome—a new world-wide definition. A consensus statement from the international diabetes federation. Diabetic medicine. 2006;23(5):469-80.

73. Holick MF, Chen TC, Lu Z, Sauter E. Vitamin D and Skin Physiology: A D-Lightful Story. J Bone Miner Res 2007;22(S2):V28–V33. .

74. Adams JS, Hewison M. Update in Vitamin D. J Clin Endocrinol Metab 2010;95: 471–8.

Annexes

Annex I: Information sheet
A. **English version of participant information sheet and informed consent**.

Addis Ababa University College of Health Sciences Department of Medical Laboratory Sciences

Participant information sheet

You are invited to participate in a study to be conducted by MSc student Amanuel Dubale at Addis Ababa University, College of Health Sciences and Department of Medical Laboratory Science. Please read the following statements and ask any unclear points before you agree to participate.

Introduction: The topic of this study is "Prevalence of Vitamin D deficiency and associated risk factors in Patients with nonspecific musculoskeletal pain symptoms attending Tikure Anbesa Specialized Hospital". Participation in this study is exclusively voluntarily. If you are not interested to participate, there will be no consequences.

What is expected from me as participant of the study?

As a participant of this study, you will be asked to give 5ml of blood sample, which will be used to determine serum Vitamin D, PTH, Calcium, ALP and P. You will also let us measure your waist circumference, weight and height. You are also expected to respond to questions included in the questioner and interviews which will be used as relevant data for this particular research.

Potential benefits to participant and/ or to the society

1. You will get laboratory test result of total Vitamin D, PTH and Ca, P and ALP which are costly for free.

2. The study will determine prevalence of Vitamin D deficiency and knowing prevalence is important to the clinician to look for vitamin D deficiency in patients with nonspecific musculoskeletal symptoms and carryout appropriate management. In case you will be vitamin D deficient the result will be reported to your attending clinician for appropriate management.

3. It will also identify major risk factors for vitamin D deficiency which correlate to nonspecific musculoskeletal symptoms to facilitate prevention, early diagnosis, and early treatment as some of them are modifiable.

42

4. Additionally the study will recommend inclusion of these analytes (Vitamin D, PTH, Ca, P and ALP) in a routine test for patients with nonspecific musculoskeletal symptoms presenting symptoms of vitamin D deficiency. Hence, you are directly or indirectly benefiting yourself, other patients and the society at large.

Compensation for participation: You will not receive any payment for your participation in this research study.

Confidentiality: On the request paper your name or your identities will not be mentioned.

Samples and information given by the participants will serve only for this research not for any other purpose.

Person to contact: Please direct any questions you may encounter during this study to the principal investigator.

Amanuel Dubale: Email: zamuyeeadk@gmail.com or mobile +251916702777

Department of Medical Laboratory Science research ethics office +251 11 275 5170

Consent form

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction.

I voluntarily consent that I would participate in this study to collect my blood and be a participant in this study and understand that I have the right to withdraw from the study at any time.

 Print name of participant, date and signature or thumb impression of participant

_____   ____ /____ /____ (dd/mm/yy) _____

If illiterate;

Print name of independent literate witness, date and signature of witness (if possible, this person should be selected by the participant and should have no connection to the research team)

_____   ____/____/_____ (dd/mm/yy) _____

Phone number_____

Print name of researcher, date and signature of researcher

_____   _____/____/_____ (dd/mm/yy) _____

B. የተሳታፊዎች መረጃ መሰጨ እና የስምምነት መጠየቂያ ቅጽ

በአዲስ አበባ ዩኒቨርሲቲ፤ የጤና ሳይንስ ኮሌጅ የህኪምና ላቦራቶሪ ትምህርት ክፍል በሁለተኛ ዲግሪ ተማሪ አማኑኤል ዱባሌ የሚካሄደውን የመመረቂ ጥናት ላይ እንድሳተፉ ተጋብዘዋል። እባክዎ በዚህ ጥናት ላይ ከመሳተፍዎ በፊት ከዚህ ቀጥሎ የሚገኘውን ጽሁፍ በጥሞና ያንብቡና ይመልሱ፤ ግልጽ ያልሆነ ነገር ካጋጠሞት ይጠይቁ።

መግቢያ

የጥናቱ ርዕስ " በጥቁር አንበሳ ሰፔሻላይዝድ ሆስፒታል ካሉ የአጥንት ና ጡንቻ ህመምተኞች ውስጥ የቫታሚን ዲ ማነስ እና ተያያዥ አስጊ ሁኔታዎችን ይገመግማል"። እርስዎ በዚህ ጥናት ላይ የሚኖርት ተሳትፎ ሙሉ በሙሉ በበጎ ፈቃዳነትዎ ላይ የተመሰረተ ነው። በዚህ ጥናት ውስጥ ላለመሳተፍ ከወሰኑ በዚህ የህኪምና ቦታ ውስጥ የሚስጥዎት አገልግሎት አይቀረጥም። በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጽሁፍ ወይም በጣት አሻራ ፈራማዎትን ማስቀመጥ ይጠበቦታል።

የጥናቱ ተሳታፊ በመሆን ምን ይጠበቅብኛል?

በዚህ ጥናት ውስጥ ለመሳተፍ ፍቃደኛ ከሆኑ 5 ሚሊ (አንድ የሻይ ማንኪያ የሚሆን) የደም ናሙና የሰጣሉ። ይህም የደም ናሙና በደምዎ ውስጥ የሚገኘውን የቫይታሚን ዲ ፣የካልሲየሚ ና የፎስፈረስ መጠን እንዲሁም የአልካላይን ፎሰፈታስ እንዛይም እንቅስቃሴ በላቦራቶሪ ለመመርመር ይረዳል። የወገብዎ ስፋት፣ ቁመትዎ እና ክብደትዎን እንዲለካ ይጠየቃሉ። ለጥናቱ ምርጃ የሚሰጡ በመጠየቁ ውስጥየተካተቱ ጥቄዎች ተከክለኛውን መረጃ ይሰጣሉ።

በዚህ ጥናት ውስጥ መሳተፍ የሚያስገኝቸው ጥቅሞች

1. የላቦራቶሪ ምርመራ የቫይታምን ዲ፣ የካልሰየም እና የፎስፈረስ መጠን እንዲሁም የአልካላይን ፎሰፈታስ እንዛይም እንቅስቃሴ ጥናቱ ስያልቅ በነጻ ያገኛሉ።

2. የቫይታሚን ዲ ማነስ ጥናቱ ስለሚጠቀም ሀኪሞች ቸግሩን ትኩረት ሰጥተው እንድያዩ ና የተከታታይ ህኪምና አካል አድርገው መፍተሄ እንድፈልጉላት መረጃ ይሰጣል።

3. ከዚህ የቫይታሚን ዲ ማነስ ጋር ተያያዝነት ያላቸው አስጊ ሁነታዎችን ለይቶ ሳይባባሱ ለመከላከል እና ለማከም ይረዳል።

4. ጥናቱ የቫታምን ዲ ማነስ ላለባቸው የአጥነት እና የጡነቻ ህመምተኞች በህኪምና ክትትላቸው ወቀት የቫይታምን ዲ፣ካልሰየምና የፎስፈረስ መጠን እንዲሁም የአላካላይን ፎስፈተስ እንዛም እንቅስቃሴ እንድላካቸው ለሆስፒታሉ ይጠቁማል። ስለዚህ በጥናቱ በመሳተፍዎ በቀጥታም ሆነ በተዘዋዋሪ መንገድ ለራሶ፣ ለሌሎች ሁሙማን ብሎም ለማህበረሰቡ ይጠቅማሉ ማለት ነው።

በዚህ ጥናት ላይ በመሳተፍዎ የሚከፍሉትም ሆነ ሚከፈሉት ክፍያ የለም።

የተሳታፊዎችን ምስጢር ስለመጠበቅ፤ በመጠየቂያው ወረቀት ላይ የተሳታፊዎች ስም ወይም ማንንነት አይገለጽም ። በተሳታፊዎች የሚሰጥ ናሙና ለዚህ ጥናት ጥቅም ብቻ የሚያገለግል ይሆናል።

ጥያቄ ካሎት ፤ ይህ ጥናት በተመለከተ ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ ችግሮች ወይም ጥያቄ በሚከተለው አድራሻ ይጠቁሙ።

ስም ፥ አማኑኤል ዱባለ

ሞባይል: +251- 916702777

ኢ.-ሜይል:zamuyeeadk@gmail.com

በአድስ አበባ ዩኒቨርሲቲ፤ የጤና ሳይንስ ኮሌጅ የህኪምና ላቦራቶሪ ት/ክፍል የጥናት ስነ ምግባር ክፍል ፤ ስ.ቁ. +251 11 275 5170

የስምምነት መጠየቂያ ቅጽ

ኮድ. ቁ._____

ከላይ የተሰጠውን መረጃ አንብቢያለሁ ወይም ተነቦልኛል። ጥያቄን እንድጠይቅ እድል ተሰቶኝ ጠይቄ አርኪ መልስ ተመልሶልኛል።

ጥናቱ ላይ ተሳትፌ የደም ናሙና እና አስፈላጊውን መረጃ ለመስጠት በፍቃዴ እየተስማማሁ በማንኛውም ሰአት ከጥናቱ ለመውጣት መብት እንዳለኝ አረጋግጣለሁ።

የተሳታፌው ስም _____

ቀን____ /____ /___

ፊርማ ወይም የጣት አሻራ _____

ያልተማሩ እንደሆን

የተማረ ሰው የአይን ምስክር _____

ቀን____ /____ /___

ፊርማ _____

ሰ.ቁ _____

የአጥኝው ስም _____

ፊርማ_____

ሰ.ቁ_____

Annex II Data collection questionnaire

A. English version

ADDIS ABABAUNIVERSITY COLLEGE OF HEALTH SCIENCE SCHOOL OF ALLIED HEALTH SCIENCE DEPARTEMENT OF MEDICAL LABORATORY SCIENCE

Instruction

I. Please write your hospital Card number here _____

II. Please try to answer all

Instruction: Please write your choice in the space provided in response column and write shortly for items that requires you to give short answer

Part IA: Socio-demographic characteristics

1. Age (years) _____

2. Sex: A. Male   B. Female

3. If female

   Menopause: A. Yes   B. No

4. Level of education? A. Informal/illiterate   B. Primary

   C. Secondary   D. College/university

5. Occupation? A. Farmer   B. Government employee   C. Private Sector employee

   D. Self-employment   E. Unemployed

6. Level of income per month_____

7. Household size:_____

Part IIa: Questions for patients to assess risk factors for vitamin D deficiency

1. How much time you are exposing to sun light (in hour) per day? _____

2. Do you use sun screen?   A. Yes   B. No

3. How many times you eat fish per month? _____

4. How many times you eat fish per week? _____

5. Have you ever smoked cigarettes? A. Yes   B. No   C. Stopped

6. If yes, for how long? _____years

7. If yes, how much _____/day (quantify)

8. Do you take alcohol?   A. Yes   B. No   C. Stopped

B: የአማርኛ ቃለ መጠየቅ

ኮድ _____

<u>እባክዎ አጭር መልስ ለሚፈልጉ ጥያቄዎች መልስዎትን ይጻፉ ። ምርጫ ላላቸው ደግሞ የመረጡትን መልስ ያክብቡ።</u>

Part Ib: <u>የተሳታፊዎች አጠቃላይ መረጃ</u>

1. ዕድሜዎት ስንት ነው?_____

2. ጾታ   ሀ. ወንድ   ለ. ሴት

3. ሴት ከሆኑ

                እርግዝት ደረሰዋል?   ሀ. አዎ   ለ. አይደለም

4. የትምህርት ደረጃ?   ሀ. ያልተማሪ/ች   ለ. አንደኛ ደረጃ   ሐ.ሁለተኛ ደረጃ   መ. ኮልጅ/ዩኒቨርሲቲ

5. የስራ ሁኔታ? ሀ. ገበሬ   ለ. የመንግስት ሰራተኛ   ሐ.የግል ድርጅት   መ. የቤት እመቤት ሠ. ስራ አጥ

6. የወር ገቢ_____

7. የቤተሰብ ቁጥር_____

Part IIb: <u>ለሻይታሚን ዲ ማነስ አስጊ ሁኔታዎችን ለመለየት የሚረዱ ጥቄዎች</u>

1. በቀን ምን ያክል ሰአት ጸሀይ ያገኛሉ? _____

2. ጸሀይ ለመከላከል የሚቄባ ቅባት ይጠቀማሉ? ሀ. አዎ   ለ.አይደለም

3. በወር ስንተ ዓሳ ይመገባሉ? _____

4. በሳምንት ስንተ እንቁላል ይመገባሉ? _____

5. ስካራ አጭሰው ያውቃሉ? ሀ. አዎ   ለ. አይደለም   ሐ.አቁሜያለሁ

6. ካጨሱስ ለምን ያህል ጊዜ(በአመታት)? _____

7. ካጨሱስ በቀን ምን ያህል ሲካራ ያጨሳሉ? _____

8. አልኮል ይጠጣሉ? ሀ. አዎ   ለ. አይደለም   ሐ.አቁሜያለሁ

Annex III: Data collection checklists

    A. Checklist to gather medical history and current medication from medical records

No.	Parameter	Record	Remark
1.	Duration of compliant in months		
3.	Other co-morbidity	Diabetes mellitus_____ Autoimmune diseases(specify)_____ Other(specify)_____	

    B. Checklist to record anthropometric data

No	Variables	Value	Remark
1.	Body weight		
2.	Height		
3.	BMI		
4	Waist circumference		

    C. Checklist to record laboratory findings

No	Laboratory parameters	Value	Remark
1.	Vitamin D(OH-25)		
3.	Ca		
4.	P		
5.	ALP		

Annex V. Standard operating procedures (SOP)
A. Sample collection

Take blood samples from the anti-cubital vein of the arm by using syringes after proper antisepsis with alcohol and sterile cotton swabs. Then transfer the blood from each participant to serum separator tube and allowed to stand for 30 minutes. Separate serum by centrifugation at 1500 rpm. Follow safety precaution.

Procedure for serum separation

1. Drawn 5 ml whole blood into serum separator tube containing no anticoagulant.

2. Kept in upright position at room temperature for 30-45 min to allow clotting.

3. Centrifuged for 5 min at manufacturer's recommended speed.

4. Carefully aspirate the serum at room temperature and pool into a centrifuge tube, taking care not to disturb the cell layer or transfer any cells. Use clean pipette for each tube.

5. Inspected serum for turbidity. Centrifuge turbid samples and aspirate again to remove remaining insoluble matter.

6. Separate serum sample

7. Aliquot into nunc tubes and stored at –20 °C. Label nunc tubes with patient identification number.

B. SOP for Vitamin D determination

Clinical relevance

This assay is intended for the quantitative determination of total vitamin D (25-OH) in human serum and plasma, as an aid in the assessment of vitamin D sufficiency in patients with bone disorders, in children with rickets, in elderly with risk of falling, diabetes, cancer, cardiovascular disease, and autoimmune diseases.

Test principle and procedure of vitamin D determination

Test principle: the assay principle combine an enzyme immunoassay competition method with a final fluorescent detection (ELFA). The solid phase receptacles (SPR) serve as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready to use and pre dispensed in the sealed reagent strips. All the assay steps are performed automatically by the instrument. The reaction

medium is cycled in and out of the SPR several times. The sample is mixed with pretreatment reagent to separate vitamin D from its binding protein. The pretreated sample is then collected and transported into the well that contains an alkaline phosphatase (ALP) labeled anti vitamin D antibody conjugates. The vitamin D antigen in the sample and the vitamin D antigen coated in the interior of the SPR compete for binding site on the anti-vitamin D antibody-ALP conjugate.

During final detection step, the substrate (4-Methyl-umberferyll phosphate) is cycled in and out of the SPR. The conjugate enzyme catalysis the hydrolysis of this substrate in to a fluorescent product, 4-Methyl-umberferone, the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is inversely proportional to the concentration of vitamin D antigen present in the sample. At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in the memory, and then print out.

Sample: human serum or lithium heparin plasma

Sample preparation: after thawing frozen sample were homogenized by vortex mixer before testing. Samples with fibrin clot or erythrocyte stroma were centrifuged before testing.

Procedure

1. Remove one VITD strip and one VITD SPR for each sample, control and calibrator to be tested.

2. The test is identified by the VITD code on the instrument. The calibrator must be identified by S1 and tested in duplicate and the control by C1.

3. Mix the calibrator, control and samples using vortex type mixer for serum or plasma separated from pellet.

4. Add 100 ul of calibrator, control and sample in to the VTID reagent strip.

5. Before pipetting make sure that the sample, control and calibrator are free of bubbles.

6. Insert the VITD SPR and VITD strips in to the instrument. Check to make sure the color abeles with the assay code on the SPRs and the reagent strips match.

7. Initiate the assay, the assay steps are performed automatically by the instrument.

8. Reclose the vials and return them to 2-8^{0}c after pipetting.

9. The assay will be finished within approximately 40 minutes. After the assay is completed remove the SPRs and strips from the instrument and dispose of in to appropriate recipient.

10. Results are determined via an instrument-specific calibration curve which is generated by 2-point calibration and a calibration master curve provided via the reagent barcode.

Limitation of the assay: interference may be encountered with certain sera containing antibodies directed against reagent components.

Range of expected values: vitamin D deficient (<20ng/ml), vitamin D insufficient (20-29ng/ml), vitamin D sufficient (30-100ng/ml) and vitamin D potential toxicity (>100ng/ml).

C. SOP for ALP determination

Clinical relevance: Serum alkaline phosphatase estimations are of interest in the diagnosis of two groups of conditions; hepatobiliary disease and bone disease associated with increased osteoblastic activity.

Test principle and procedure of ALP determination

Test principle:

$$P\text{-}NPP + H_2O \xrightarrow{\text{ALP}} \rho\text{-Nitro phenol} + H_3PO_4$$

P-Nitro phenyl phosphate is hydrolyzed to ρ-nitro phenol and inorganic phosphate. The rate at which the p-NPP is hydrolyzed, measured at 405 nm, is directly proportional to the alkaline phosphatase activity.

Specimen: Serum samples should be stored at 2-8°C and run within two days.

Reference range:

Adults

Males 40-129 IU/L   Adults 35-123 IU/L

Limitations:

1. This methodology measures total alkaline phosphatase irrespective of tissue or organ of origin. Further tests may be necessary to assist in differential diagnosis.
2. Samples with values exceeding 1000 IU/L should be diluted with an equal volume of saline and re-assayed multiplying the results by two.

D. SOP for Ca determination

Clinical relevance: Serum calcium levels and hence the body content are controlled by parathyroid hormone (PTH), calcitonin, and vitamin D. An imbalance in any of these modulators leads to alterations of the body and serum calcium levels. Increases in serum PTH or vitamin D are usually associated with hyperkalemia. Increased serum calcium levels may also be observed in multiple myeloma and other neoplastic diseases. Hypocalcaemia may be observed in hyperparathyroidism, nephrosis, and pancreatitis.

Test principle and procedure of Ca determination

Calcium ions react with 5-nitro-5'-methyl-BAPTA (NM-BAPTA) under alkaline conditions to form a complex. This complex reacts in the second step with EDTA. The change in absorbance is directly proportional to the calcium concentration and is measured photo metrically at 340 nm.

Specimen: Serum or heparinized plasma

Reference range:

Serum/plasma: 2.15-2.5 nmol/l

E. **SOP for P determination**

Clinical relevance: Phosphorus occurs in blood in the form of inorganic phosphate and in organically bound phosphoric acid. The small amount of extracellular organic phosphorus is found almost exclusively in the form of phospholipids. The ratio of phosphate to calcium in the blood is approximately 6:10. An increase in the level of phosphorus causes a decrease in the calcium level. The mechanism is influenced by interactions between parathyroid hormone and vitamin D. Hyperparathyroidism, vitamin D intoxication and renal failure with decreased glomerular phosphate filtration give rise to hyperphosphatemia. Hypophosphatemia occurs in rickets, hyperparathyroidism and Fanconi's syndrome.

Test principle and procedure of P determination

Test principle: Molybdate UV.

Procedure of P determination

1. Inorganic phosphate reacts with ammonium phosphomolybdate complex having the formula $(NH_4)_3[PO_4 (MoO_3)_{12}]$ with ammonium molybdate in the presence of sulfuric acid.

2. The concentration of phosphomolybdate formed is directly proportional to the inorganic phosphate concentration and is measured photometrically.

Reference range: 0.81-1.45nmol/l

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Amanuel Dubale (B.Sc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: Mistre wolde (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Tatek Gebreegzeabeher (MSc, PhD candidate)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.