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Evaluation of micronutrients and vitamins in patients diagnosed with osteoarthritis

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ABSTRACT

Objective: Osteoarthritis (OA) is a degenerative joint disease, a leading cause of pain and disability worldwide. The hallmark of OA is pathological changes of the joint structure, such as cartilage erosion and synovial inflammation. The study aimed to evaluate the micronutrients and vitamins in patients diagnosed with osteoarthritis (OA) in Edo State, Nigeria.

Material and Methods: A total of 300 patients comprising one hundred and fifty OA subjects and 150 non-osteoarthritis subjects were recruited for this study. The levels of micronutrients (Ca, Cu, Zn, Se) were analyzed using AAS, while Vitamin D and K were measured using HPLC and spectrophotometer, respectively.

Results: The levels of copper, zinc, calcium, and vitamin K were significantly lower ($p<0.05$) except vitamin D ($p>0,05$) among osteoarthritis than non-osteoarthritis subjects. The selenium level was markedly higher in osteoarthritis than non- osteoarthritis subjects ($p<0.05$). The levels of trace elements were positively correlated with vitamin D, selenium($r= 0.23$, $p<0.05$), calcium($r= 0.35$, $p<0.05$), copper($r= 0.09$, $p>0.05$). Blood levels of vitamin D, K, and zinc were negatively correlated with age. Vitamin D($r= -0.01$, $p>0.05$), vitamin K($r = -0.02$, $p>0.05$) and zinc($r = - 0.01$, $p>0.05$).

Conclusion: Exposure to free radicals may be a predisposing factor to impaired synthesis of antioxidants that might be involved in the mechanical induction of osteoarthritis. Therefore, it is believed that strict metabolic control delays the development of late complications in osteoarthritis (OA). Therefore, adequate supplementation of trace elements and vitamins (D, K) in diet should be encouraged to lower the risk associated with osteoarthritis.

Keywords: Osteoarthritis, trace elements, antioxidant, disability

INTRODUCTION

Osteoarthritis (OA) is a type of joint disease that results from the breakdown of joint cartilage and underlying bone (1). The most common symptoms are joint pains and stiffness (2). Initially, symptoms may occur only following exercise, but over time may become constant (2). Other symptoms may include joint swelling, decreased range of motion, weakness, or numbness of the arms and legs (2). The total economic burden for arthritis is estimated to be 1%–2.5% of the gross national product in Western countries. Osteoarthritis is a leading cause of disability, affecting 60 - 70% of people aged $\geqslant$ 60 years. Multiple etymologies are suspected of contributing to the formation of OA, including defective articular cartilage structure, biosynthesis, joint trauma, joint instability, inflammatory conditions, congenital and developmental abnormalities (2).

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It is sometimes called a degenerative joint disease or degenerative arthritis; osteoarthritis is the most common chronic condition of the joints. The prevalence of osteoarthritis in Nigeria is estimated to be 0.4% of the population among adults aged 65years across Africa (3). OA is found as the most prevalent arthritis in urban settings; this was found to be 55.1%, and in rural settings, all were found in South Africa ranged from 29.5%, 29.7%, up to 82.7% among adults aged 65years (4). Other urban Hospital-based studies reporting OA of the knee are Burkina Faso, with a prevalence of 0.5% among adults. Tunisia reported a prevalence of 4.7% of knee osteoarthritis among elderly subjects and 9.9% prevalence with the musculoskeletal condition in Cameroon (5). Approximately 27 million Americans are affected by osteoarthritis. It is the most common form of arthritis, affecting about 237 million (3.3% of the population) (6). About 10% of males and 18% of females are affected (7). It is the cause of about 2% of years lived with disability. In Australia, about 1.9 million people are affected, and in the United States, 30 to 53 million people are affected (8). It becomes more common in both sexes as people become older (2). OA can affect any joint, but it occurs most often in knees, hips, lower back and neck, small joints of the fingers, and the bases of the thumb and big toe. The pain is naturally made worse by prolonged activity and relieved by rest. Stiffness is most common in the morning and typically lasts less than thirty minutes after beginning daily activities but may return after periods of inactivity. Osteoarthritis can cause a crackling noise (called "crepitus") when the affected joint moves, especially the shoulder and knee joints. A person may also complain of joint locking and joint instability. As osteoarthritis progresses, movement patterns (such as gait) are typically affected (9). Osteoarthritis is the most common cause of joint effusion of the knee (10). In smaller joints, such as the fingers, hard bony enlargements, called Heberden's nodes (on the distal interphalangeal joints) or Bouchard's nodes (on the proximal interphalangeal joints), may form, and though they are not necessarily painful, they do limit the movement of the fingers significantly. Osteoarthritis of the toes may be a factor causing the formation of bunions (10), rendering them red or swollen. In normal joints, a firm, rubbery material called cartilage covers the end of each bone. Cartilage provides a smooth, gliding surface for joint motion and cushion between the bones. The most commonly involved joints are those near the fingers' ends, at the base of the thumb, neck, lower back, knee, and hips (2). The risk factor is more significant in overweight, having one leg of a different length, and having jobs that result in high levels of joint stress (2, 7, 11). Osteoarthritis is believed to be caused by mechanical stress on the joint and low-grade inflammatory processes (12). It develops as cartilage is lost and the underlying bone becomes affected (2), as pain may make it difficult to exercise, muscle loss may occur (13, 7). Diagnosis is typically based on signs and symptoms, with medical imaging and other tests occasionally used to support or rule out other problems (2). In contrast to rheumatoid arthritis, which is primarily an inflammatory condition, in osteoarthritis, the joints do not become hot or red (2).

Damage from mechanical stress with insufficient self-repair by joints is the primary cause of osteoarthritis (14). Sources of this stress may include misalignments of bones caused by congenital or pathogenic causes; mechanical injury; excess body weight; loss of strength in the muscles supporting a joint; and impairment of peripheral nerves, leading to sudden or uncoordinated movements (14). However, exercise, including running in the absence of injury, has not been found to increase the risk of knee osteoarthritis (15).

Several studies have shown a greater prevalence of the disease among siblings and mainly identical twins, indicating a genetic basis (16). Although a single factor is not generally sufficient to cause the disease, about half of the variations in susceptibility have been assigned to genetic factors (17).

The development of osteoarthritis is correlated with a history of previous joint injury and obesity, especially concerning knees (18). Since the correlation with obesity has been observed not only for knees but also for non-weight bearing joints and the loss of body fat is more closely related to symptom relief than the loss of body weight, it has been suggested that there may be a metabolic link to body fat as opposed to just mechanical loading (19). Changes in sex hormone levels may play a role in the development of osteoarthritis as it is more prevalent among postmenopausal women than among men of the same age (20,21). A mice study found natural female hormones to be protective while injections of the male hormone dihydrotestosterone reduced protection (22).

Increased risk of developing knee and hip osteoarthritis was found among those who work with manual handling (e.g., lifting), have physically demanding work, walk at work, and have climbing tasks at work (e.g., climb stairs or ladders) (11). In particular, with hip osteoarthritis, an increased risk of development over time was found among those who work in the bent or twisted positions (11). For knee osteoarthritis, in particular, the increased risk was found among those who work in a kneeling or squatting position, experience heavy lifting combined with a kneeling or squatting posture, and work standing up (11). Women and men have similar occupational risks for the development of osteoarthritis (11).

Osteoarthritis is a degenerative joint disease which may cause gross cartilage loss and morphological damage to other joint tissues. Basal biochemical changes occur in the earliest stages of osteoarthritis progression. The water content of healthy cartilage is finely balanced by compressive force driving water out and hydrostatic and osmotic pressure drawing water in (23). Collagen fibers exert compressive strength, whereas the Gibbs–Donnan effect and cartilage proteoglycans create osmotic pressure, which tends to draw water in (23). However, during the onset of osteoarthritis, the collagen matrix becomes more disorganized, and there is a decrease in proteoglycan content within cartilage. The breakdown of collagen fibers results in a net increase in water content (24). This increase occurs because while there is an overall loss of proteoglycans (and thus a decreased osmotic pull) (25, 26), it is outweighed by a loss of collagen (23 25). Without the protective effects of the proteoglycans, the collagen fibers of the cartilage can become susceptible to degradation and thus exacerbate the degeneration. Inflammation of the synovium (joint cavity lining) and the surrounding joint capsule can also occur, though often mild (compared to the synovial inflammation in rheumatoid arthritis).

Other structures within the joint can also be affected (27). The ligaments within the joint become thickened and fibrotic, and

the menisci can become damaged and wear away (28). Menisci can be completely absent when a person undergoes a joint replacement. New bone outgrowths, called "spurs" or osteophytes, can form on the margins of the joints, possibly in an attempt to improve the unity of the articular cartilage surfaces in the absence of the menisci. The subchondral bone volume increases and becomes less mineralized (hypomineralization) (29). These changes can cause problems functioning and subchondral bone lesions (30).

Globally, as of 2010, approximately 250 million people had osteoarthritis of the knee (3.6% of the population) (31). Hip osteoarthritis affects about 0.85% of the population (31). As of 2004, osteoarthritis globally causes moderate to severe disability in 43.4 million people (32). Together, knee and hip osteoarthritis had a ranking for disability globally of 11th among 291 disease conditions assessed (31).

As of 2012, osteoarthritis affected 52.5 million people in the United States, approximately 50% of whom were 65 years or older (8). The rate of osteoarthritis in the United States is forecast to be 78 million (26%) adults by 2040 (8).

There are ongoing efforts to determine if agents modify outcomes in osteoarthritis. Sprifermin is one candidate drug. There is also tentative evidence that strontium ranelate may decrease degeneration in osteoarthritis and improve outcomes (33).

As well as attempting to find disease-modifying agents for osteoarthritis, there is emerging evidence that a system-based approach is necessary to find the causes of osteoarthritis (34). Changes may occur before the clinical disease is evident due to abnormalities in biomechanics, biology, or structure of joints that predispose them to develop clinical disease. Thus, research focuses on defining these early pre-osteoarthritis changes using biological, mechanical, and imaging markers of osteoarthritis risk, emphasizing multi-disciplinary approaches and looking into personalized interventions that can reverse osteoarthritis risk in healthy joints before the disease becomes evident.

Guidelines outlining requirements for inclusion of soluble biomarkers in osteoarthritis clinical trials were published in 2015 (35), there are no validated biomarkers for osteoarthritis. One problem with using a specific type II collagen biomarker from the breakdown of articular cartilage is that the amount of cartilage is reduced (worn away) over time with the progression of the disease.

As a result, a patient can eventually have very advanced osteoarthritis with none of this biomarker detectable in their urine. Another problem with a systemic biomarker is that a patient can have osteoarthritis in multiple joints at different stages of disease simultaneously, so the biomarker source cannot be determined. Some other collagen breakdown products in the synovial fluid correlated with each other after acute injuries (a known cause of secondary osteoarthritis) but did not correlate with the severity of the injury (36).

Osteoarthritis occurs in people of all ages; osteoarthritis is most common in people older than 60 years of age. Common risk factors include increasing age, obesity, previous joint injury, overuse of the joint, weak thigh muscles, and genes. Metal concentrations in bones reflect long-term exposure, yet no evidence would determine whether the mobilization of bone stores could occur so quickly that it may result in poisoning. The characteristics and long recovery time of bone tissue may reflect a chronic level of exposure and serve as a basis for an indirect assessment of environmental exposure. Among the elements necessary for life, zinc (Zn) and Copper (Cu) concentrations are often determined in highly mineralized tissues. It was found that zinc accelerates the bone formation and is essential for the correct ossification and mineralization of the skeleton, especially the femoral epiphysis. Zinc and copper are involved in the formation and metabolism of bone tissue. Naturally occurring minerals such as Calcium (Ca), Copper (Cu), selenium (Se), and zinc (Zn) have shown anti-inflammatory effects in both animal and human studies.

Animal model of OA, a deficiency of dietary Mg was established to accelerate cartilage damage (37). Copper is an essential cofactor in enzymes such as superoxide dismutase (SOD) that also needs Zn and Mn as cofactors. Many studies revealed a role for oxidative stress in the pathogenesis of OA, whereby ROS generation and impaired antioxidant status of the joint might degrade cartilage joint remodelling (38).

Selenium is also an essential cofactor for glutathione peroxidase, which may have a role in reducing the incidence of osteoarthritic lesions (39). It is unknown whether trace element status leads to disease or whether diseases are set in due to the deficiency of trace elements. However, it is generally believed that a strict metabolic control delays the development of late complications OA.

Many trace elements (micronutrients) have been recognized to play an essential role in the pathogenesis and progression of many diseases, including osteoarthritis. However, prevalence data on arthritis in Africa is very scarce despite the overwhelming report on the rising prevalence of the musculoskeletal disease. More studies are needed to address this disease's prevalence and true burden in Africa. Hence, investigating changes in the metabolism of these elements was the major reason for this research.

Recently, there has been increased interest in the incidence of osteoarthritis in elderly subjects resulting in progressive degenerative changes in the cartilage and articular tissues. Multiple etiologies are suspected of contributing to the formation of OA, including defective articular cartilage structure and biosynthesis, joint trauma, joint instability, congenital and developmental abnormalities, and inflammatory conditions. Measuring the levels of Ca, Cu, Zn, Se, and some essential vitamins necessary for Oxidative damage is essential in understanding cell dysfunction and degradation caused by oxygen free radicals in the pathobiology of degenerative joint disease.

MATERIAL and METHODS

This is a cross-sectional study of diagnosed osteoarthritis patients attending the orthopedic clinic in Central Hospital, Benin – City, Edo State, Nigeria. The study was carried out in Benin - city, an urban area, the capital of Edo state, with a population of 1147188 according to the 2006 Nigeria census. It is located at latitude 6.340 N and longitude 5.600E with 87.88m.

The Hospital serves an estimated population of 450,000 and serves as a reference center for orthopedic and treatment and

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management of patients with disabilities. Participants are educated, aged between 51-90 years, dark complexion, normal, overweight, obese, married, and unmarried.

The sample size was determined according to the method of (40). The prevalence of knee osteoarthritis in Nigeria is 8.9% (41)

A random sampling method was used for the collection of three hundred samples. Blood samples comprised one hundred and fifty (150) osteoarthritis patients and one hundred and fifty (150) blood samples of non-osteoarthritis patients. The samples were collected from the blood bank of the screened patient following all legal and professional ethical documentation.

Blood samples were collected into 5ml capacity plain plastic bottles with the help of health personnel in the Hospital. All patients included were aged 51 and above with knee injury, Hip OA, non-smoker, and non-alcoholic patients. All patients excluded had Inflammatory arthritis, uncontrolled D.M., hypertension, chronic kidney disease, and uncorrected Hypo/hyperthyroidism.

Ethical clearance was obtained from Edo State Ministry of Health, Benin –City. Also, written informed consent was sort from the participants and gave assurance that the health history of the patients obtained will not be linked with the true identity of the patient when recording the outcome of my findings. The whole blood sample collected into anticoagulant and plain bottles were centrifuged using a refrigerated centrifuge at a speed of 10000 rpm for 15mins. The distinct layer was obtained, i.e., Plasma and Serum, where the plasma was kept at low temperature for vitamin D analysis. The blood serum intended for analysis was stored under a low temperature of about -20 ^{0}C.

Measurement of trace elements, vitamin k and vitamin d

The trace elements content of the digested samples (Zinc, Selenium, Copper, and Calcium) were assayed using atomic absorption spectroscopy (Buck Scientific Model VGP-210, Germany) at the University of Benin, Benin - City, Edo State, Nigeria. Their concentrations were obtained in duplicate from the absorbance read.

Vitamin K and Vitamin D were analyzed using Spectrophotometer and HPLC (C18), respectively.

Statistical analysis: All data obtained were analyzed using SPSS Version 23.0. The comparison between the osteoarthritis and non- osteoarthritis subjects was performed using the student's unpaired t-test, correlation, and chi-square. The statistical significance was beset at p<0.05.

RESULTS

The socio-demographic variables of the three hundred (300) subjects in the study revealed that 19.3% were between the ages of 51-60years, 57.3% were between 61-70years, 7.3% were between 71-80years and 16.0% were between 81-90 years. Of the total subjects, 39.6% were males, and 60.3% were females. In addition, 52.6% were singles, and 47.3% were married (Table1). Of the total subjects, 33.3% had a primary level of education, 50.0% had a secondary level of education, and 16.6% had a tertiary level of education. In addition, 1.0% were underweight (BMI of 16.5 kg/m2), 66.6% were normal (BMI of 23.6kg/m2), 30.0% were overweight (BMI of 28.6 kg/m2 and 2.3% were obese (BMI of 33.5 kg/m2). Of the total subjects, 20.0% were Bini, 13.3% Ibos and 66.6% Yoruba (Table 2)

Table 3 shows the blood calcium levels, trace elements, vitamin K, and D of osteoarthritis patients and the control group. It was observed that the level calcium, trace elements (Copper, zinc, and selenium), vitamin K were statistically significant (p<0.05) in osteoarthritic patients when compared with the control group.

Table 4 shows a post–hoc (Bonferroni) multiple comparisons of calcium and copper. It was observed that calcium levels in female osteoarthritic subjects were statistically significantly higher than in female control (p<0.05), while copper was non-significant across the group.

Table 5 shows a post –hoc (Bonferroni) multiple comparisons of zinc and selenium. It was observed that the level of selenium in male osteoarthritic subjects was statistically significantly higher than the female and male control group (p<0.05), while zinc was higher in the female control group than male subjects (p<0.05).

Table 6 shows a post –hoc (Bonferroni) multiple comparisons of vitamin D and K. It was observed that levels of vitamin D in female osteoarthritic subjects were statistically significantly higher than male osteoarthritic subjects (p<0.05) while vitamin K was non-significant across the group.

Table 7 shows correlation of vitamin D, K, age, calcium and trace elements. It was observed that there was positive significant relationship between vitamin D, calcium(r =0.35,p<0.05), selenium(r=0.22, p<0.05), vitamin K (r=0.48, p<0.05) except zinc (r=-0.19, p>0.05) which was negatively correlated. In addition, age was negatively correlated with vitamin D (r= -0.01, p>0.05), vitamin K(r= -0.02, p>0.05), zinc(r= -0.01, p>0.05) except calcium(r= 0.04, p>0.05) that was non- significant positively correlated.

Table 1: Distribution of demographic factors of osteoarthritis

Demographic factors	Total (n=300)	Osteoarthritis (n=150)	Control (n=150)	X^2	*p-value*
Age (years)					
51-60	58(19.3%)	30(20%)	28(18.6%)		
61-70	172(57.3%)	84(56%)	88(58.6%)		
71-80	22(7.3%)	8(5.3%)	14(9.3%)	176.48	<0.05
81-90	48(16%)	28(18.6%)	20(13.3%)		
Sex					
Male	119(39.6%)	59(39.3%)	60(40.0%)		
Female	181(60.3%)	91(60.6%)	90(60.0%)	12.81	<0.05
Marital status					
Single	158(52.6%)	50(33.3%)	108(72%)		
Married	142(47.3%)	100(66.3%)	42(28%)	2.18	P=0.140

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Table 2: Distribution of demographic factors of osteoarthritis

Demographic factors	Total (n=300)	Osteoarthritis (n=150)	Control N=150	X^2	p-value
Educational status					
Primary	100(33.3%)	80(53.3%)	20(13.3%)		
Secondary	150(50.0%)	60(40.0%)	90(60.0%)	62.07	<0.05
Tertiary	50(16.6%)	10(6.6%)	40(26.6%)		
Body mass index (kg/m^2)					
Underweight	3(1.0%)	3(2.0%)	0(0.0%)		
Normal	200(66.6%)	100(66.6%)	100(66.6%)		
Overweight	90(30.0%)	45(30.0%)	45(30.0%)	45.53	<0.05
Obese	7(2.3%)	2(1.33%)	5(3.3%)		
Ethnicity					
Bini	60(20.0%)	30(20.0%)	30(20.0%)		
Igbo	40(13.3%)	10(6.6%)	30(20.0%)		
Yoruba	200(66.6%)	110(73.3%)	90(60.0%)	275.78	<0.05

Values in parenthesis are percentages.

Table 3: Level of calcium, trace elements, vitamin d and k among osteoarthritis and non osteoarthritis patients

Variable	Osteoarthritis (n=150)	Control (n= 150)	p-value
Calcium (mmol/L)	1.56 ± 0.01	1.96 ± 0.02	**<0.05****
Copper (mmol/L)	8.68 ± 0.30	9.84 ± 0.35	**<0.05****
Zinc (µm/L)	12.71 ± 0.15	13.61 ± 0.19	**<0.05****
Selenium (ng/mL)	141.13 ± 4.39	106.07± 3.01	**<0.05****
Vitamin K (mm/L)	1.59 ± 0.02	1.67 ±0.02	**<0.05****
Vitamin D (ng/L)	50.59 ± 1.28	51.75 ± 1.31	**>0.05***

Table 4: Multiple comparison of calcium and copper among sex

Bonferroni

Dependent Variable	(I) SEX	(J) SEX	Mean Difference (I-J)	Std. Error	P-value.
CALCIUM(mmol/l)	male subjects	female subject	-.06	.04	1.000
		male control	.06	.05	1.000
		female control	.08	.04	.452
	female subject	male subjects	.06	.04	1.000
		male control	.12	.04	.052
		female control	.14*	.04	.004
	male control	male subjects	-.06	.05	1.000
		female subject	-.12	.04	.052
		female control	.02	.04	1.000
	female control	male subjects	-.08	.04	.452
		female subject	-.14*	.04	.004
		male control	-.02	.04	1.000
COPPER(umol/l)	male subjects	female subject	.70	.68	1.000
		male control	-.83	.75	1.000
		female control	-.65	.68	1.000
	female subject	male subjects	-.70	.68	1.000
		male control	-1.54	.67	.145
		female control	-1.35	.61	.156
	male control	male subjects	.83	.75	1.000
		female subject	1.54	.68	.145
		female control	.18	.67	1.000
	female control	male subjects	.65	.68	1.000
		female subject	1.35	.61	.156
		male control	-.18	.68	1.000

*The mean difference is significant at the 0.05 level.

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Table 5: Multiple comparison of Zinc and Selenium among sex

Bonferroni

Dependent Variable	(I) SEX	(J) SEX	Mean Difference (I-J)	Std. Error	P-value
ZINC (umol/l)	male subjects	female subject	-.24	.36	1.000
		male control	-1.01	.39	.066
		female control	-1.08*	.36	.019
	female subject	male subjects	.24	.36	1.000
		male control	-.77	.35	.193
		female control	-.84	.32	.057
	male control	male subjects	1.01	.39	.066
		female subject	.77	.36	.193
		female control	-.07	.36	1.000
	female control	male subjects	1.08*	.36	.019
		female subject	.84	.32	.057
		male control	.07	.36	1.000
SELENIUM (ng/ml)	male subjects	female subject	3.99	7.74	1.000
		male control	36.06*	8.49	.000
		female control	38.45*	7.76	.000
	female subject	male subjects	-3.99	7.74	**1.000**
		male control	32.06*	7.70	.000
		female control	34.45*	6.88	.000
	male control	male subjects	-36.06*	8.49	.000
		female subject	-32.06*	7.70	.000
		female control	2.38	7.71	1.000
	female control	male subjects	-38.44*	7.6	.000
		female subject	-34.45*	6.88	.000
		male control	-2.39	7.71	1.000

*. The mean difference is significant at the 0.05 level.

Table 6: Multiple comparison of vitamin D and K among sex

Dependent Variable	(I) SEX	(J) SEX	Mean Difference (I-J)	Std. Error	P-value
VITAMIN K(mmol/l)	male subjects	female subject	-.02	.048	1.000
		male control	-.08	.052	.828
		female control	-.09	.05	.356
	female subject	male subjects	.02	.05	1.000
		male control	-.06	.05	1.000
		female control	-.07	.04	.498
	male control	male subjects	.08	.05	.828
		female subject	.06	.04	1.000
		female control	-.01	.05	1.000
	female control	male subjects	.09	.05	.356
		female subject	.07	.04	.498
		male control	.01	.05	1.000
VITAMIN D(ng/L)	male subjects	female subject	-9.78*	2.61	.001
		male control	-5.03	2.85	.474
		female control	-5.12	2.61	.306
	female subject	male subjects	9.78*	2.61	.001
		male control	4.73	2.59	.412
		female control	4.66	2.32	.271
	male control	male subjects	5.04	2.89	.474
		female subject	-4.74	2.59	.412
		female control	-.08	2.59	1.000
	female control	male subjects	5.12	2.61	.306
		female subject	-4.66	2.31	.271
		male control	.08	2.59	1.000

Table 7: Correlation of age with trace elements and vitamin D and K

PARAMETERS	R- VALUE	P-VALUE
AGE/VITAMIN D	-0.01	P>0.05
AGE/ VITAMIN K	-0.02	p>0.05
AGE/ZINC	-0.01	p>0.05
AGE/SELENIUM	0.04	P>0.05
AGE/COPPER	0.02	p>0.05
AGE/CALCIUM	0.04	p>0.05
VITAMIN D/ ZINC	-0.19	P<0.05

p<0.05(significant) , p>0.05(non-significant)

DISCUSSION

Osteoarthritis is a chronic progressive degenerative disorder of synovial joints affecting the articular cartilage and the underlying subchondral bone (42). The cause of OA is multifactorial, including genetic, endocrine, function and exercise, and nutritional consideration (43, 44). Bone formation and metabolism are also modulated by trace elements (zinc, copper, and selenium), vitamin D, and K, in addition, calcium and phosphorus. Trace elements are essential for bone growth and development because the components interact with the bone matrix and affect bone metabolism (45). These minerals are also implicated in the pathology, diagnosis, and treatment of osteo-disorder like OA (46). To further clarify the relationship between trace elements and effect on bone matrix density in OA, we measured serum levels of zinc, copper, selenium, vitamin D, and K in OA and analyzed their correlation with bone matrix density. Few studies have concentrated their efforts on the functional status and periodic measurement of some trace elements that help in the lubrication of vital joints ensuring holistic care and maintenance of the elderly subjects. This research was aimed at establishing the effect of antioxidant and vitamin (D and K) on bone mineralization by evaluating the level of calcium, trace elements (Copper, selenium, and zinc), and vitamin (D and K) in osteoarthritic subjects. A total of three hundred (300) subjects were recruited in this study; one hundred fifty (150) diagnosed osteoarthritic subjects and (150) healthy non-osteoarthritic subjects within the age of 51-90 years in Edo state.

In this study, it was observed that there was a significant difference across the age ($p<0.05$), which suggest that osteoarthritis is independent of age, but as age increase, there is a tendency of bone demineralization resulting in osteoarthritis. It was observed that 60.6% of females had osteoarthritis, which agrees with previous work carried out by (47), which stated that a higher prevalence of physical disability for the basal activity of daily living was more common in women than men.

In this study, it was observed that the levels of calcium, copper, zinc, vitamins K and D were low in osteoarthritis compared with the control group($p<0.05$) except vitamin D, which was not statistically significant ($P>0.05$). It was also observed that selenium level was higher in OA than in the control group ($p<0.05$). The significantly lower calcium, copper, zinc, vitamin K, and D levels could be attributed to endothelial injury, resulting from oxidative damage of the amino acid needed for tissue lubrication, repair, cell signal, and growth (48). Biochemically, reduced serum levels of trace elements might expose the individual to damages mediated through oxidative stress.

Oxidative stress and the cellular antioxidant defence system are regulated in a coordinated fashion during inflammation. It is known that reactive oxygen species (ROS) such as hydrogen peroxide and superoxides are released and scavenged during wound healing. Glutathione is another prominent player in the cellular antioxidant function (48). Increasing glutathione suppresses Hepatic stellate cells (HSCs) growth and activation (48). It also stimulates TGF-β and suppresses glutamate-cysteine ligase (GCL), the rate-limiting enzyme in glutathione biosynthesis (48).

In this study, it was observed that the level of copper and zinc was lower ($p<0.05$). Zinc is an activator of numerous metal enzymes that can stimulate bone metabolic enzymes such as alkaline phosphatases, collagenase, and sulforicolyases. Zinc also influence 1,25 –OH vitamin D3 and calcitonin concentration (49,50). It can stimulate gene expression of transcription factors such as runt-related transcription factor 2, which is related to differentiation forming osteoblastic cells; zinc can inhibit osteoclastic bone resorption by inhibiting osteoclastic-like cells formation from bone marrow cells by stimulating apoptotic cells death of mature osteoclasts (51). Bone growth retardation is common in various conditions associated with dietary zinc deficiency, suggesting that zinc compound may be a novel supplement factor in preventing and treating osteo-disorder like OA (51). The low level of zinc found in this study could be attributed to the role of oxidative stress in the pathogenesis of OA due to ROS generation and impaired antioxidant status of the joint resulting in the degradation of cartilage joint remodelling (38). Arikan et al. (52) found that zinc is positively correlated with the bone matrix density of the lumbar vertebrates, but different was found in this study ($r= -0.19$, $p<0.05$), which indicate dietary zinc deficiency and malabsorption was sufficient in OA. Roughly 2 - 4grams of zinc (53) are distributed throughout the human body, mainly the brain, muscles, bones, kidney, and liver, with the highest concentrations in the prostate and parts of the eye (54). Zinc homeostasis also plays a critical role in the functional regulation of the central nervous system (55,56). Dysregulation of zinc homeostasis in the central nervous system results in excessive synaptic zinc concentrations and is believed to induce neurotoxicity resulting in mitochondrial oxidative stress (e.g., by disrupting certain enzymes involved in the electron transport chain, including complex I, complex III, and α-ketoglutarate dehydrogenase), the dysregulation of calcium homeostasis, glutamatergic neuronal excitotoxicity, and interference with intraneuronal signal transduction (57,55).

Zinc deficiency is usually due to reduced or insufficient dietary intake. Still, it can be associated with malabsorption, acrodermatitis enteropathica, chronic liver disease, chronic renal disease, sickle cell disease, diabetes, malignancy, and other chronic illnesses such as osteoarthritis, especially in the elderly (58). In this study, the copper level was low in OA compared with the control group($p<0.05$), which could be attributed to oxidative stress from reactive oxygen species(ROS); but contrast in other studies that found increased in blood serum Cu concentration, was even considered to be a marker of clinical activity of this disease (59)

Copper plays an important role in metabolism in the nervous system, hematogenesis, skeleton construction, connective tissue, and cross-linkage of elastin and collagen protein; thus, copper is implicated in bone development and repair (60). Rodriguez et al. (61) conclude that copper stimulates MSC differentiation preferentially toward the osteogenic lineage. Copper deficiency may influence the synthesis and the stability of bone collagen and may induce skeleton development disorder resulting in osteo- disorder like OA copper supplement may be a potential strategy to treat and prevent involutional osteo- disorder (62).

In this study, the level of selenium was higher in OA patients than in control ($p<0.05$) which could be attributed to the mild severity and duration of the disease whose mechanism is to alleviate the pain generated due to reactive oxygen species (ROS) from oxidative stress, but the contrast in other studies which state that decreased levels of selenium and the activity of selenium-dependent enzymes have also been studied in other diseases, including epilepsy, which showed a strong correlation between their reduction and severity of the disease (63). Selenium is also an essential cofactor for glutathione peroxidase, which may reduce the incidence of osteoarthritic lesions (39); in addition to a strong association of disease duration and severity with serum concentrations of Se. However, selenium is an essential constituent of the glutathione peroxidase enzyme. Its deficiency results in a marked decline in glutathione peroxidase activity of many tissues, leading to increased oxidative stress. In humans, decreased serum Se levels are unlikely to happen, but maybe the etiological factor of some severe disorders such as Keshan disease (endemic cardiomyopathy) and Kashin-Beck disease (endemic osteoarthritis).

In this study, the level of vitamin K was lower in OA compared with the control group ($p<0.05$), which reflects the severity of the disease and oxidative damage (51). Increased supplement of vitamin K will prevent the progression of OA and lower the risk associated with OA. Vitamin K is a cofactor of gamma-glutamyl carboxylase, which plays an important role in the activation of gamma- glutamate (gla) containing protein that negatively regulates calcification. This vitamin deficiency status might be associated with OA, in which cartilage calcification plays a role in the pathogenesis of the disease. Much evidence had agreed that a sufficient level of vitamin K is associated with a lower risk of OA and pathological joint features. Despite a plausible biological rationale and a positive observational study, many findings do not support a significant effect of vitamin K supplementation on osteoarthritis for all persons. Of note, despite previous studies demonstrating an association between poor vitamin K status and bone health (64), the parent trial did not demonstrate a significant effect of vitamin K supplementation on bone mineral density in the hip or spine (65).

In this study, the level of vitamin D was not significant in OA compared with the control group ($p>0.05$); the reason for this was unclear, which will be elucidated in further study. Vitamin D has a significant role in calcium homeostasis and metabolism. Its discovery was due to an effort to find the dietary substance lacking in children with rickets (the childhood form of osteomalacia) (66). However, vitamin D deficiency has become a global problem in the elderly and remains common in children and adults (67,68). Low blood calcifediol (25-hydroxy-vitamin D) can result from avoiding the sun (69). Deficiency results in impaired bone mineralization and bone damage, leads to bone-softening diseases (70), including rickets, osteomalacia, and osteoarthritis. Vitamin D supplementation in the general population is inconsistent (71,72). The effect of vitamin D supplementation on mortality is not clear, with one meta-analysis finding a small decrease in mortality in elderly people (73) and another concluding no clear justification exists for recommending supplementation for preventing many diseases, and that further research of similar design is not needed in these areas (74).

For older people with osteoporosis, taking vitamin D with calcium may help prevent hip fractures, but it also slightly increases the risk of stomach and kidney problems (75). Supplementation with higher doses of vitamin D in those older persons than 65 years may decrease fracture risk (76). The effect may be smaller for people living independently than in institutions (77). It helps in regulating calcium in the human body (78). The active vitamin D metabolite calcitriol mediates its biological effects by binding to the vitamin D receptor (VDR), principally located in the nuclei of target cells. The binding of calcitriol to the VDR allows the VDR to act as a transcription factor that modulates the gene expression of transport proteins (such as TRPV6 and calbindin), which are involved in calcium absorption in the intestine (79). The vitamin D receptor belongs to the nuclear receptor superfamily of steroid/thyroid hormone receptors, and VDRs are expressed by cells in most organs, including the brain, heart, skin, gonads, prostate, and breast.

VDR activation in the intestine, bone, kidney and parathyroid gland cells leads to the maintenance of calcium and phosphorus levels in the blood (with the assistance of parathyroid hormone and calcitonin) and to the maintenance of bone content (80). One of the most important roles of vitamin D is to maintain skeletal calcium balance by promoting calcium absorption in the intestines, promoting bone resorption by increasing osteoclast number, maintaining calcium and phosphate levels for bone formation, and allowing proper functioning of parathyroid hormone to maintain serum calcium levels. Vitamin D deficiency can result in lower bone mineral density and an increased risk of reduced bone density (osteoporosis) or bone fracture because a lack of vitamin D alters mineral metabolism in the body.

The VDR may be involved in cell proliferation and differentiation. Vitamin D also affects the immune system, and VDRs are expressed in several white blood cells, including monocytes and activated T and B cells (81). Apart from VDR activation, various alternative mechanisms of

action are under study, such as inhibition of signal transduction by hedgehog, a hormone involved in morphogenesis (82).

In this study, it was observed that age was negatively correlated with vitamin D, K, and zinc($p>0.05$) which could be attributed to a decrease in trace elements with increasing age, which is in agreement with the previous study (83) which state that decreases trace elements(Copper, zinc), vitamin K and bone matrix density decrease with age therefore, we believe enriching the diet with trace elements should be considered in the elderly population. Although, Oxidative damage to essential cell components caused by oxygen free radicals results in the pathobiology of degenerative joint disease (84). It is generally believed that a strict metabolic control delays late complications in OA.

CONCLUSION

Osteoarthritis is a degenerative joint disease with an age-associated increase in incidence and prevalence. The hallmark of OA is the pathological changes of the joint structure, such as cartilage erosion and synovial fluid inflammation. Thus far, no efficient treatment can alter the progression of OA except the non-pharmacological prevention through lifestyle and nutrition. It is good to know that some trace elements like zinc, selenium, copper, and calcium are conducive to the prevention and treatment of OA. To some extent, the status of trace elements depends on the external environment (nutrition) and internal factors (individual absorption and metabolism of trace elements, genetic tendency, age, and gender). However, knowledge in this area is much needed to elucidate the effect of trace elements on OA. The protective effect has been found. However, theoretical and practical issues on supplementation in the prevention and treatment of OA are still unknown. Despite the efficacy of trace elements, the impact factor is still limited due to the obtainment of these elements in combination. Further studies are needed to confirm the dosage, concentration, and interaction of individual trace elements on OA, thereby preventing and treating OA. This study shows that OA subjects have significantly lower levels of copper, calcium, zinc, vitamin D, vitamin K, and elevated selenium levels than control subjects. The low level of trace elements in OA resulted from oxidative stress generated from reactive oxygen species (ROS) and an increase in age. This result showed that OA subjects were prone to oxidant stress, which led to altered antioxidant levels.

Exposure to free radicals may predispose to impaired synthesis of antioxidants. Therefore, it is believed that strict metabolic control delays the development of late complications in osteoarthritis (OA). Therefore, adequate supplementation of trace elements and vitamins (D, K) in diet should be encouraged to lower the risk associated with osteoarthritis.

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