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Evaluation of Selected Biominerals in Post-Menopausal Women in Nnewi, South-East Nigeria

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Abstract

Background: The post-menopausal state, as a feminine physiological aging event, may have effects on the homeostatic mechanisms of some biominerals, which can be inimical to bone health and may result to osteoporosis. There seems to be paucity of data on this important public health challenge among South-Eastern Nigeria Women. ***Objectives:*** The present study evaluated the levels of the biominerals calcium (Ca), magnesium (Mg), zinc (Zn) and selenium (Se) and their association with osteoporosis, among post-menopausal women in Nnewi, South-East, Nigeria. ***Methods:*** Thirty (30) post-menopausal women aged 49-60years, 30 peri-menopausal women aged 44-48 years, and 25 pre-menopausal subjects, aged 20-34 years, which served as controls, were recruited from patients attending the out-patient and obstetrics and gynaecology clinics of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi and apparently healthy subjects within NAUTH suburb, between June and September, 2023. Serum samples collected from the participants were analyzed for Mg, Zn, and Se, using fast-sequential atomic absorption spectrometry (AA 220/FS) while Ca, and inorganic phosphate were measured by photometric methods. ***Results:*** Results showed that serum Mg was significantly lower in post-menopausal subjects compared with their corresponding pre-menopausal counterparts (0.73+ 0.26) ($P = 0.020$). On the other hand, serum Se and Zn levels were significantly higher in post-menopausal subjects (168.70 + 58.98) and (19.66 + 5.67) compared with their corresponding control values (118.16 + 14.00 and 14.72 + 2.83) ($P = 0.000$, Cl = 95%). Additionally, a higher serum Ca level was observed in the post- menopausal subjects compared with their pre-menopausal counterparts (2.38+0.14 and (2.36+0.17). However, this difference was not significant ($P > 0.05$). ***Conclusion:*** The study opines that post-menopausal hypomagnesemia can occur and may be linked to osteoporosis. The present study also demonstrates that Zn and Se, which are metallo-enzymes of the antioxidants -superoxide dismutase and glutathione peroxidase, may be elevated in the post-menopausal state. Therefore, a routine monitoring of these important biominerals in the post-menopausal population and larger population-based prospective studies involving nutritional interventions are recommended.

Keywords: ***Post-menopausal, Biominerals, Hypomagnesemia.***

Introduction

Menopause is a feminine watershed, due to ovarian aging. It is characterized by the depletion of the oocytes in the ovaries and loss of the cyclical activity of the gonadotropins, peptides and steroids and occurs when the number of primordial follicles has fallen to a critical value [1]. This phenomenon is diagnosed retrospectively after twelve consecutive months of amenorrhea, without any other medical causes and with hormonal changes and clinical symptoms occurring over a period leading up to and immediately following it [2]. It occurs typically between the age of 40-60years [3]. The involved hormones are those of the hypothalamic-pituitary-gonadal axis and changes in these hormones cause a wide range of undesirable effects and disorders including central-nervous, metabolic, cardiovascular and musculo-skeletal, sexual and urogenital [4,5].

The post-menopausal state has musculo-skeletal effects through its impact on the homeostatic mechanisms of some biominerals such as Ca, Mg, Zn and Se. These homeostatic disturbances are majorly related, but not limited to estrogen deprivation, [4] and notable symptoms of menopause include hot flushes, night sweats, vaginal dryness, insomnia, depression and cognitive difficulties [6]. Moreover, long-term deficiencies of some biominerals are associated with certain metabolic disorders of the bone, among which osteoporosis is a major public health challenge [4,6].

Some studies on nutrition and post-menopausal osteoporosis among Nigerian women had concentrated on calcium because of its

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key role in bone health. However, with more recent studies by Adeniji et al., 2023 [7], on the nutritional status and post-menopausal complications among adult women in Ogbomoso, South-West Nigeria, it is believed that micronutrients, the biominerals inclusive, could influence the risk of osteoporosis in this population of women. Osteoporosis is a medical condition, characterized by increased bone fragility, with an increased susceptibility to fracture [8]. It is a metabolic bone disease, characterized by low bone mass and micro architectural deterioration of bone tissue leading to increased bone fragility and a consequent increase in the risk of fracture [9]. It should be noted that bone loss occurs more rapidly in women after menopause and bone mass (bone density) decreases after 35 years of age [10]. Therefore, suggesting that post-menopausal women are more prone to osteoporosis.

To avert this metabolic bone disorder and for the maintenance of a healthy bone mass in the peri and post-menopausal states, monitoring of indices like the serum levels of these biominerals should be prioritized.

Materials and Methods

Study Design: The study was undertaken in the Department of Chemical Pathology, Nnamdi Azikiwe University Teaching Hospital, Nnewi. It was a case-control study, comprised of 85 apparently healthy post-menopausal, peri-menopausal and pre-menopausal women, attending the out-patient and obstetrics and gynaecology clinics of Nnamdi Azikiwe University Teaching Hospital, Nnewi as subjects. Other apparently healthy subjects within the hospital suburb were also recruited for the study. Among them, 30 were post-menopausal women aged between 49-60years, 30 were peri-menopausal women aged between 44-48years and 25 were pre-menopausal women aged between 20-34 years, to serve as controls. All study participants were interviewed in person by the investigator using a questionnaire, which was structured to reflect the health matters that were relevant to the study objectives. Data collected with questionnaire among others included age, recall age at menarche, recall age at menopause and anthropometric indices like height and weight.

Inclusion and Exclusion Criteria: Post-menopausal women aged between 49-60years, who have experienced at least 12 consecutive months of amenorrhea, with no other medical causes, were recruited for the study. Peri-menopausal women aged between 44-48years who have started experiencing menstrual irregularities, with history of no use of hormonal contraceptives for at least 12 consecutive months were recruited for the study. Pre-menopausal women with regular cycle, non-lactating and with history of no use of hormonal contraceptives for at least 12 consecutive months were also recruited for the study. Subjects with history of chronic illnesses e.g. diabetes mellitus, tuberculosis, AIDS, liver diseases, kidney failure, cardiovascular and endocrine disorders were excluded from the study. Other subjects excluded from the study include patients on hormone replacement therapy, subjects on supplementation of the selected biominerals. 7mls of fresh fasting blood samples was aseptically collected from the ante-cubital vein of each subject and was transferred into a clean plain labeled tube. Sample was allowed to clot and then centrifuged at 6,000 rpm for 5 minutes at room temperature. The clear serum was separated and kept at -20oC, until assayed for Calcium, Phosphate, Magnesium, Zinc and Selenium.

Ethical Consideration: Ethical approval was sought and obtained from the ethics committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi. (NAUTH/CS/ 66/ VOL16/ VER.3/ 152/ 2023/031).

Estimation of Serum Calcium: Serum calcium was estimated using a colorimetric method of O-cresolphthalein complexone, without deproteinization, as described by Raysarkar and Chauhan, Barnett [11].

Estimation of Serum Inorganic Phosphate: Inorganic phosphorus was estimated using a colorimetric method of Ammonium molybolate in Sulphuric acid medium, using Ferrous Ammonium sulphate as reducing agent, as described by Tietz [12].

Estimation of Magnesium, Zinc and Selenium: The serum levels of these biominerals were determined using spectra A220/FS (fast sequential) atomic absorption spectrometer, after preparation of the samples by method of APHA, 1998.

Statistical Analysis: Data entry and analysis were carried out using the statistical package for social sciences (SPSS) version 21.0. The values were expressed as the mean + standard deviation, analysis of variance (ANOVA) was used to compare the means and proportions between the groups. Values were regarded as significant if P was less or equal to 0.05.

Results

Table I: Comparison of Age and BMI in Post- Menopausal, peri-menopausal and pre-menopausal subjects (Mean+SD)

Groups	Age (years)	BMI (kg/m^2)	N
Post-menopausal (Model A)	56.20±3.58	29.50±5.45	30
Peri-menopausal (Model B)	46.43±1.74	27.60±3.79	30
Premenopausal (Model C)	25.00±4.83	24.71±4.05	25
f-Value	549.769	7.686	
p-value	0.000	0.001	
A vs B	0.000	0.321	
A vs C	0.000	0.001	
B vs C	0.000	0.062	**N = 85**

In table I, the mean age of the post-menopausal subjects was 56.20+2.0, that of the peri-menopausal subjects was 46.43+1.74, while 25.00+4.83 was obtained for the pre- menopausal participants. Also, the mean body mass index (BMI) for the post-menopausal subjects was 29.50+5.45, that of the peri- menopausal group was 27.60 + 3.79, while that of the pre-menopausal controls was 24.71+4.05. Interestingly, a comparison of the mean age and BMI of the three groups showed a statistically significant difference (p=0.000 and 0.001) respectively.

Table II: Comparison of Serum Levels of selected Biominerals in Post-menopausal, peri-menopausal and pre-menopausal subjects (Mean+SD)

Groups	Calcium (mmol/L)	Phosphate (mmol/L)	Magnesium (mmol/L)	Selenium (ug/L)	Zinc (umol/L)	N
Postmenopausal (A)	2.38±0.14	1.24±0.29	0.73+0.26	168.70±58.98	19.66±5.67	30
Peri-menopausal (B)	2.29±0.14	1.21±0.22	0.76±0.09	126.27±30.61	14.68±2.61	30
Pre- menopausal (C)	2.36±0.17	1.30±0.28	0.86±0.09	118.16±14.00	14.72±2.83	25
f-Value	2.795	0.727	4.017	13.028	14.850	
p-value	0.067	0.487	0.020	0.000	0.000	
A vs B	0.073	1.000	1.000	0.000	0.000	
A vs C	1.000	1.000	0.022	0.000	0.000	
B vs C	0.339	0.706	0.096	1.000	1.000	

In table II, we observed that Serum Mg was significantly lower in the post-menopausal subjects, compared with their pre-menopausal counterparts (0.73 + 0.26) (P = 0.020). Conversely, the present study observed a significantly higher mean levels of serum Zinc and Selenium in the post-menopausal subjects, compared with the pre-menopausal participants (P = 0.000). On the other hand, there was no significant difference in serum phosphate and calcium levels of the post-menopausal subjects compared with their pre-menopausal counterparts (P > 0.05).

Table III: Mean recall age of attainment of menarche and menopause (for post-menopausal subjects) and mean recall age of attainment of menarche for pre-menopausal subjects.

Groups	N	Minimum	Maximum	Mean	Standard Deviation
Post menopausal: Age at menopause	30	42	58	48.93	3.84
Age at menarche	30	11	16	14.10	1.32
Pre-menopausal: Age at menarche	25	12	15	13.28	1.02

In table III, our study observed a mean recall age of attainment of menarche of 14.10 + 1.32 years for the post-menopausal subjects, which was higher than 13.28 + 1.02years, observed for the pre-menopausal subjects. The present study also obtained a mean recall age of attainment of menopause of 48.93 + 3.84 years.

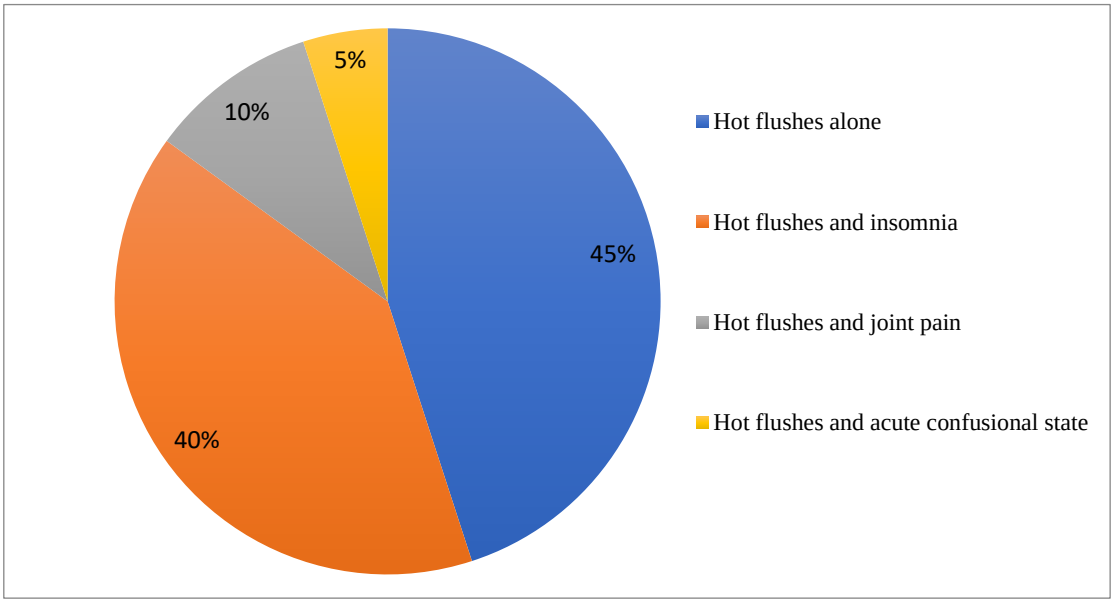

Fig. 1: Pie chart showing notable symptoms experienced by the post-menopausal subjects.

From the statistical analysis of notable symptoms of post-menopausal subjects, the present study obtained some symptoms experienced by the symptomatic post-menopausal subjects, which included hot flushes alone (45%), insomnia in addition to hot flushes (40%), joint pain in addition to hot flushes (10%), confusional state in addition to hot flushes (5%).

Discussion

Magnesium is the fourth most-abundant element in the human body and the second most abundant cation within the body cells, after potassium. This biomineral which is a co-factor to over six hundred enzymes and activator to an additional two hundred enzymes [13], influences bone quality by decreasing hydroxyapatite crystal size and involvement in other mechanisms that prevent the formation of brittle bone [14].

Hypomagnesemia is serum Mg level less than 0.75 millimoles per litre as normal plasma Mg concentrations range between 0.75-0.95 millimoles per litre [13]. Although, Mg deficiency is more common than its toxicity, both too low and too high Mg may be harmful to bone health, therefore a tight control of Mg homeostasis is very essential for the maintenance of healthy bone [15]. While Ca and vitamin D have been the master focus on nutritional prevention of osteoporosis, several additional food

constituents, including the biominerals like Mg, Cu, Zn, Se and Fe (Iron) are of great importance. Fiorentini et al., [13] earlier regretted that in spite of the fact that Mg has been recognized as an essential element, its availability is not generally determined and monitored in patients, especially post-menopausal women, therefore Mg has been referred to as the 'forgotten cation'.

Castiglioni et al., had previously maintained that Mg deficiency contributes to osteoporosis directly by acting on hydroxyapatite crystal formation and on bone cells and indirectly by impacting on the secretion and activity of parathyroid hormone and by promoting low grade inflammation [16]. A relationship exists between inflammation and bone loss, as inflammatory cytokines stimulate bone remodeling and osteopenia [16]. Again, Forentini et al., [13] agreed that serum Mg concentrations are strongly related to bone metabolism, as bone surface Mg is constantly exchanged with blood Mg, therefore consequences of hypomagnesemia are accelerated bone loss and a decline in bone formation, as Mg induces the proliferation of osteoblasts [13].

Another mechanism through which Mg plays a structural role in bone health is the complex interplay between it and Vitamin D [13]. Razzaque, had earlier stated that this important metal is required for the activity of hepatic 25-hydroxylase and renal 1-α hydroxylase, both crucial in the conversion of inactive vitamin D into its biologically active form and also facilitates the transfer of Vitamin D to target tissues [17]. Moreover, Kolanu et al., [18] has earlier reported a fall in the concentration of Mg, and hence concluded that women attaining menopause could suffer from hypomagnesemia. The present study also gives credence to the study by Odabasi et al.,[19] which recorded a slightly lower red cell Mg in osteoporotic post-menopausal women, compared with the control subjects. The present study therefore infers that the observed post-menopausal hypomagnesemia could be due reduced intake of Mg, a decreased intestinal absorption and or increased renal excretion of Mg in the post-menopausal subjects.

Comparably, this study partly agrees with Manafa et al.,[20] which observed a significant increase in selenium level among the post-menopausal subjects. Manafa et al.,[20] however, observed a significant decrease in the mean level of zinc in the post-menopausal subjects, compared with the pre-menopausal group ($P < 0.01$). However, the present study disagrees with Oloruntoba et al., [21] which observed no significant difference in the serum levels of Zinc, Iron and Selenium between the case and control groups.

Wu et al., had earlier stated that despite the reported positive effect of selenium on the bone, this bio-element is not always positive in humans, hence adverse health effects have been observed with selenium levels higher than 90-120ug/L. [22]. Blood Selenium correlates positively with 8-oxo-dG, a biomarker of oxidative stress, when plasma Selenium concentrations were greater than 110ug/L [23].

Ansar et al., previously reported that estrogen deficiency in the post-menopausal state causes the development of oxidative stress, which is the imbalance between antioxidants and free radicals, especially reactive oxygen species (ROS) and reactive nitrogen species (RNS)[24]. Antioxidant enzymes like superoxide dismutase, peroxidase, glutathione reductase are part of the intrinsic enzymatic mechanism for free radical neutralization and are located throughout the cell and act to protect the integrity of the DNA, enzymes, hormones, cell wall and membranes and other organelles from the damaging effect of ROS [25]. Interestingly, Zinc is a co-factor of the anti-oxidant, superoxide dismutase (SOD). The biomineral, selenium is also an important component of the antioxidant glutathione peroxidase (GPx). It is also note-worthy that the present study observed hypomagnesaemia, which promotes oxidative stress, partly due to inflammation and reduced anti-oxidant defences.

Moreover, several studies [23-25] agreed that oxidative stress could occur in the post-menopausal state. Therefore, the significantly higher serum levels of Zinc and Selenium, observed by the present study could be attributed to a compensatory response to oxidative stress, as the anti-oxidant enzymes, like the SOD and GPx appear to be overwhelmed, hence the increase in the levels of their co-factors Zinc and Selenium.

Surprisingly, the present study observed a higher level of serum Calcium, in the post-menopausal subjects, compared with the pre-menopausal counterparts. However, this difference was not significant ($P > 0.05$). This study disagrees with some past studies by Bhale and Ansariv [26], Patwa et al.,[27], which recorded a significantly lower calcium level in the post-menopausal subjects, compared with the pre-menopausal participants. The present study also differs from the study by Achie et al., [28] which reported a lower mean serum Calcium level in the post-menopausal subjects, compared with the pre-menopausal group. However, the difference was not significant ($P > 0.05$).

The present study therefore attributes the slightly higher mean serum Calcium level observed in the post-menopausal subjects, to a possible oxidative stress-induced bone resorption, causing the mobilization of calcium from the bone into the blood. On the other hand, the mean recall age of attainment of menarche for the pre-menopausal subjects, observed by the present study, (13.28 + 1.02 years) corroborates the study by Anikwe et al., [29] which obtained 13.0 + 1.0. Hence, the lower mean recall age at menarche observed in the pre-menopausal subjects, compared with the post-menopausal group, could be as a result of improvement in sex education and demystification of the physiological phenomenon of menarche, unlike what was obtainable several decades ago. Also the mean age of attainment of menarche for the post-menopausal subjects, obtained from the present study agrees with an earlier study by Ramraj et al., [30] which observed the value 14. 10 + 1.10 years, but differed from the pre-menopausal subjects as the value was 12.5 + 1.42years.

In the same vein, the mean recall age of attainment of menopause of 48.93 + 3.84years obtained from the present study approximately corroborates the study by Ozumba et al., [1] which reported a mean recall age of attainment of menopause of 49.4 + 3.0 years. It is also agrees with the study by Oloyode and Obajimi, [31] that obtained a mean recall age of attainment of menopause of 49.0 + 4.54 years. The present study therefore infers that South-Eastern Nigeria women attain menopause at an age comparable with that of other women from other geopolitical zones in Nigeria.

The present study observed that the commonest post-menopausal symptom experienced by the symptomatic post-menopausal subjects was hot flushes, followed by insomnia. Hot flushes is a sensation heat of unknown cause in the upper body, face and neck, lasting for about three to five minutes, accompanied by a higher heart rate and peripheral blood flow, with a rise in skin temperature [3].

Porri et al., had earlier stated that this sensation is caused by an imbalance in serotonin and norepinephrine in the brain, causing vasomotor instability, with Mg being known to be neuro-active, affecting serotonin in many body cells, including the brain and therefore opined that Mg deficiency is likely to be a reasonable causal link between vasomotor symptoms and menopause [32]. Additionally, Kolanu et al.,[18] and Fiorentini et al.,[13] previously observed that insomnia and mood swings, were the major post-menopausal symptoms and attributed them to the role of Mg in tryptophan metabolism, where it is involved in the formation of

serotonin and melatonin from the amino acid tryptophan. Serotonin acts as both a neurotransmitter and a hormone. In fact, it is one of the 'feel good' hormones and functions to improve mood. It is also a chemical precursor to melatonin, the major hormone that induces sleep. Kolanu et al.,[18] further agreed that Mg is required in the synthesis of gamma amino butyric acid (GABA), which functions, as a relaxant. It can therefore be adduced that the major post-menopausal symptoms observed by the present study - hot flushes and insomnia could be hypomagnesemia induced.

Conclusion

The current study suggests that post-menopausal women could suffer from magnesium deficiency, which could be linked to post-menopausal osteoporosis. The study also demonstrates that post-menopausal women could also have elevated Zinc and Selenium levels, a pointer to possible oxidative stress in the post-menopausal state.

Declarations

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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None

Conflict of Interest

The authors declared that there are no conflicts of interest

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Not applicable

References

[1] Ozumba, B.C., Obi, S.N., Obikili, E., Waboso, P. (2004) Age, Symptoms and perception of Menopause among Nigerian Women. Journal of Obstetrics and Gynaecology 54(6) :575-578.

[2] Coney, P. (2018) Menopause. Retrieved from emedicine.medscape.com. February, 6, 2023.

[3] Honour, J.W. (2018), Biochemistry of the Menopause. Annals of Clinical Biochemistry 55(1) :18-33.

[4] Monteleone, P., Mascagn,i G, Giannini, A., Genazzani, A.R., Simoncini, T. (2018). Symptoms of menopause, global prevalence, physiology and implications. Nature Reviews Endocrinology 14:199-215.

[5] Meenakshi, K., Komal, S., Priyanka, C., Seema, V., Pankaj, K., Tarun, S. (2020). Prevalence of menopausal symptoms and its effect on Quality of life among Rural Middle- Aged Women (40-60years) of Haryana India. International Journal of Applied and Basic Medical Research 10(3): 183-188.

[6] Santoro, N., Epperson, C.N., Mathews, S.B. (2015). Menopausal Symptoms and their management. Endocrinol. Metab Clin Journal of North America. 44(3): 497-515.

[7] Adeniji, A.O., Ogunleye, E.A., Olawuyi, Y.O. (2023). Nutritional Status and menopausal complications Among Adult women in Ogbomoso South-West, Nigeria. African Journal of Biomedical Research 26(1): 53-58.

[8] Shiel Jr, W.C. (2020). Osteoporosis, Treatment, Symptoms and Causes. Retrieved from https://www.medicinenet.com July, 21,2021.

[9] Atiase, Y. and Quarde, A. (2020). A call to action for osteoporosis research in sub-Saharan Africa. Ghana Medical Journal 54(1) :58-67.

[10] Bass, M.A., Sharma, A., Nahar, V.K., Chelf, S., Zeller, B., Pham, L., Ford, M.A. (2019). Bone Mineral Density among men and women Aged 35 to 50years. Journal of American osteopathic Association119(6):357-363.

[11] Ray Sarkar, B.C. and Chauhan, U.P.S. (1967). Analytical Biochemistry 20;155.

[12] Tietz, N. (1983). Clinical Guide to Laboratory Tests. W.B Saunders Company. Philadelphia USA: 384.

[13] Fiorentini, D., Cappadone, C., Farrugia, G., Prata, C. (2021). Magnesium: Biochemistry, Nutrition, Detection and Social impact of Diseases linked to its deficiency. Nutrients 13(4): 1136.

[14] Peters, B. and Martin L.A. (2010). Nutritional aspects of the Prevention and Treatment of Osteoporosis. de Arquivos Braisileiros Endocrinologica e Metabologia 54(2):179-185.

[15] Al-khakani, M.F., Radhi, S.W., Almohanna, A.M. (2018). Assessment of Serum Calcium, Phosphorus, Magnesium, Iron and Zinc in Osteoporotic Post-menopausal women. Iraq Medical Journal (2): 51-56.

[16] Castiglioni, S., Cazzaniga, A., Albisetti, W., Maier, J. (2013). Magnesium and osteoporosis: Current state of knowledge and future research directions. Nutrients 5(8): 3022-3033.

[17] Razzaque, M.S. (2018). Magnesium: Are we consuming enough? Nutrients. 10(12) :1863.

[18] Kolanu, B.R., Vadakedeth, S., Boddula, V., Kand,V. (2020). Evaluation of Activities of Serum Magnesium and Thyroid hormones in Pre, Peri and Post- menopausal women. Cureus.12(1):e6554.

[19] Odabasi, E., Turan, M., Aydin, A., Akay, C. (2008). Magnesium, Zinc, Copper, Manganese and Selenium levels in post-menopausal women with Osteoporosis: Can magnesium play a key role in osteoporosis? Annals of the Academy of Medicine 37(7): 564-567.

[20] Manafa, P.O., Nna, C.D., Chukwuma,G.O., Onyenekwe, C.C., Ihim, A.C., Iloghalu, E.U., Okor, L.O., Akulie, J.C. (2016). Cobalt, Copper, Selenium and Zinc levels in pre-menopausal and post-menopausal women in Nnewi, South- East Nigeria. Orient Journal of Medicine. (27): 3-4.

[21] Oloruntoba, O.F., Agbebaku, S.O., Idonije, B.O., Oluba, O.M. (2020). Comparison of serum Iron, Zinc and Selenium levels in Pre- Menopausal and Post- menopausal women in Ekpoma, Nigeria: A descriptive study. Avicenna Journal of Medical Biochemistry 8(2) 128-132.

[22] Wu, M.and Cheng, W.H. (2013). Selenium, Selenoproteins and Age-Related disorders. (Bioactive food as Dietary interventions for the Aging population). Retrieved from Researchgate.net July, 21,2021.

[23] Wu, C., Xiao, Y., Jiang, Y. (2023). Associations of blood trace elements with bone mineral density, a population - based study in US adults. Journal of orthopaedic surgery and Research 18(1):827.

[24] Ansar, S., Alhefdi, T., Aleem, A.M. (2015). Status of trace elements and anti-oxidants in pre- menopausal and post-menopausal phase of life: a comparative study. International journal of clinical and experimental medicine 8(10) 19486-19490.

[25] Kimball, J.S., Johnson, J.P., Carlson, A.D. (2021). Oxidative stress and Osteoporosis. American Journal of bone and joint surgery. 103(15):1451-1461.

[26] Bhale, D.V and Ansariv, A.H. (2014). Study of Serum Calcium levels in Post Menopausal women. International Journal of Recent Trends in Science and Technology. 9(3):332-333.

[27] Patwa, C.K., Jindani, N.J., Afroz, S. (2017) Study of Serum Calcium levels in pre-menopausal and post-menopausal women. Med. Pulse Internal Journal of Physiology 4(2):14-16.

[28] Achie, L.N., Mohammed, Y.Z., Lawal, J., Igashi, K.V., Olorun Shola (2020) Serum Calcium level of pre, peri and post-menopausal rural women of Zuturung District, Kaduna State, Nigeria. Journal African Association of Physiological Science 8(2) :133-139.

[29] Anikwe, C.C, Mamah, J.E., Okorochukwu, B.C., Nnadozie, U.U., Obarezi, C.H., Ekwedigwe, K.C. (2020). Age at menarche, menstrual characteristics and its Associated morbidities among secondary school's students in Abakaliki, South-East, Nigeria. Heliyon: Cell Press 6(5): e04018.

[30] Ramraj, B., Subramanian, V.M., Vijaya Krishnan, G. (2021). Study on age of Menarche between generations and the factors associated with it. Clinical Epidemiology and Global health 11(2):100758.

[31] Oloyode, O.A and Obajimi, G.O. (2018), Symptomatology of Menopause among Sub urban Nigerian women. Tropical Journal of Obstetrics and Gynaecology 35(1) :188-191.

[32] Porri, D., Biesalski, H., Limitone, A., Bertuzzo, L., Cena, H. (2021). Effects of Magnesium supplementation on Women's Health and well-being. NFS journal 23 (1): 30-36.