ORIGINAL ARTICLE

Cobalt, copper, selenium and zinc levels in pre-menopausal and post-menopausal women in Nnewi, South-East Nigeria

Patrick O **MANAFA**[1]
Chinedu D **NNA**[1]
George O **CHUKWUMA**[1]
Charles C O**NYENEKWE**[1]
Augustine C **IHIM**[1]
Emmanuel U **ILOGHALU**[1]
Livinus O **OKOR**[2]
Justina C **AKULUE**[1]

[1]*Dept of Med Lab Sciences, Faculty of Health Sciences and Technology, Nnamdi Azikiwe University Nnewi Campus Anambra State, NIGERIA*
[2]*Dept of Obstetrics and Gynaecology, Nnamdi Azikiwe University Teaching Hospital Nnewi, Anambra State NIGERIA*

Author for Correspondence
Dr George O **CHUKWUMA**
Dept of Med Lab Sciences Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus, Anambra State NIGERIA

Email: georgechuma@yahoo.com
Phone: +234 -803-410-1608

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ABSTRACT

Background: The effect of oestrogen deficiency after menopause on the trace elements may give rise to some metabolic disturbances leading to osteoporosis, cardiovascular disease, arterial hypertension, thyroid disorders and many other pathological conditions.
Objective: This study was meant to unravel the possible effects of menopause on some select trace elements.
Methodology: A total of 100 subjects were selected for this study which comprised 50 pre-menopausal (control) and 50 post-menopausal (test). The subjects were divided into different age categories and duration. Determination of the concentrations of trace elements was performed using the FS-240 atomic absorption spectrophotometry method.
Results: There was a significant decrease in the mean concentrations of zinc and cobalt in the post-menopausal subjects (test), compared with the pre-menopausal group *($p<0.01$)*. However, a significant increase was observed in the mean levels of selenium in the post-menopausal group (test), compared with that of the pre-menopausal individuals *($p<0.01$)*, while there was no significant difference in the mean concentration of copper in the post-menopausal subjects, compared with the pre-menopausal group *($p=0.428$)*.
Conclusion: Oestrogen deficiency after menopause may be the cause of some fluctuations in the serum concentrations of trace elements. This work has established that the serum concentrations of zinc and cobalt are diminished with a concomitant increase in selenium in post-menopausal women. The precise impact of this deficiency and possible influence of oestrogen therapy on trace element status in post-menopausal women need further larger-population studies.

Keywords: *Oestrogen deficiency, serum concentrations, trace elements, women*

INTRODUCTION

Menopause literally means the cessation of the monthly cycles. Menopause originates from the Greek word *pausis* (cessation) and the root men- (month).[1] Menopause is an event that usually occurs in women in mid-

life, during their late 40s or early 50s, and it signals the end of the fertile phase of a woman's life.[1]

Menopause is more accurately defined as the permanent cessation of the primary functions of the ovaries, ripening and release of ova and release of hormones that cause both the creation of the uterine lining, and the subsequent shedding of the uterine lining called menses or period.[2] The decrease in the levels of the sex steroid hormones during menopause in women causes a number of disturbances in the metabolism of different organs. In this period of life, the risk of osteoporosis, cardiovascular diseases, arterial hypertension, impairment of glucose metabolism, and degenerative cognition diseases rises.

The impact of oestrogen deficiency after menopause on the trace elements has not yet been widely studied but, the expected menopause related alterations in the trace mineral status may have an impact on the above pathologies.[3]

Trace elements are chemical elements present only in minute amounts in the living organism, usually in milligrammes or microgrammes per kilogramme of body weight. Trace elements include zinc, copper, selenium, manganese, chromium, cobalt, iron etc. Some trace elements like lead, cadmium, arsenic, aluminum, and nickel are toxic, hence monitoring of dosage is required.[4] The risk of nutritional disturbances, particularly as regards trace elements and vitamin micronutrient deficiencies is high during menopause.

Magnesium [Mg] enhances bone turnover through the stimulation of the osteoclastic function and its deficiency may play a role in post-menopausal osteoporosis. It acts as a surrogate for calcium in the transport and mineralization process, and the deficiency may lead to disturbances in the cardiac rhythm, necrotic changes, atheromatous plaques, a high value of total cholesterol and a low value of high density lipoprotein cholesterol.[5,6]

Zinc regulates the secretion of calcitonin from the thyroid gland and it has an influence on the bone turnover. Copper [Cu] induces a low bone turnover by suppression of the osteoblastic and osteoclastic functions.[5] A deficiency of Cu, as well as its abundance may increase the cholesterol content of blood serum. In Cu deficiency, formation of the crosslinks of the elastin of blood vessels is disturbed.[6]

This research is, therefore, tailored towards discovering the possible effects of the post-menopausal state on some trace elements levels in women in Nnewi since some of these trace elements are cofactors that assist in biochemical reactions.

METHODOLOGY

Study Design

This research was carried out in Nnewi. A total of 100 subjects were selected for this study which comprised 50 pre-menopausal (control) and 50 post-menopausal (test) according to different age categories and duration. About 5ml of blood sample was collected by venipuncture from each of the subjects. The samples were allowed to clot and separation performed by centrifugation at 3,000 revolutions per minute for 5minutes. The trace elements were analysed using Varian atomic absorption spectrophotometer (Model FS 240) as described by.[7]

Inclusion Criteria

1. Postmenopausal women
2. Age bracket of 45 to 85 years
3. Not on hormone replacement therapy
4. Disease asymptomatic individuals

Exclusion Criteria

1. Women who are still menstruating
2. Those on hormone replacement therapy
3. Chronically ill and symptomatic individuals

Calculation of Sample Size

Sample size was calculated using the minimum sample size for simple proportion

with 5% margin of error and 95% level of confidence as shown below.

$N=Z^2PQ/D^2$
Where Z= Standard normal deviation at 1.96(which corresponds to 95% confidence interval).
P= prevalence Q=1-P
D=Degree of accuracy/ precision expected = 0.05%
Substituting for the above formulae
N= 50

Principle

The principle of atomic absorption spectrophotometry is based on the atomization of aspirated sample in the flame when the AAS light beam was directed through the flame into a monochromator and onto the detector that measures the amount of light absorbed by the atomized element in the flame. Since metals have their own characteristic absorption wavelength, a source lamp composed of that element was used, making the method relatively free from spectral or radiation interferences. The amount of energy of the characteristic wavelength absorbed in the flame was proportional to the concentration of the element in the sample.

Statistical Analysis

Data were subjected to statistical analysis using SPSS version 20. The students' T-test and the Analysis of Variance (ANOVA).Values were considered significant if $p<0.05$.

Ethical approval was obtained from the Ethical Committee of Faculty of Health Sciences and Technology, Nnewi Campus, Nnewi. Informed consent was obtained from each participant prior to sample collection".

RESULTS

Results from this work has shown a significant increase in the mean concentration of selenium in the post-menopausal group compared with that of the pre-menopausal subjects (*p=0.00), see Table 1*. However, no significant difference was noticed in the mean concentration of copper in the postmenopausal subjects compared with the control. (*p= 0.0428*), *see Table 2*.

Table 1. Comparison of the mean concentrations of selenium in postmenopausal (test) and premenopausal (control) groups

	Mean	Std. Deviation	p-value
Postmenopausal (test) (selenium)	67.204	21.520	0.01
Premenopausal (Control)	41.000	27.642	

Table 2. Comparison of the mean concentrations of copper in postmenopausal (test) and premenopausal (control) subjects

	Mean	Std. Deviation	p-value
Postmenopausal (test) Copper	18.814	9.141	0.428
Premenopausal (Control)	27.671	4.418	

Table 3. Comparison of the mean concentrations of cobalt in postmenopausal (test) and premenopausal (control) individuals

	Mean	Std. Deviation	p-value
Postmenopausal (test) Cobalt	0.079	0.125	0.01
Premenopausal (Control)	0.249	0.206	

Table 4. Comparison of mean concentrations of zinc in post-menopausal (test) and pre-menopausal (control) groups

TEST	Mean	Std. Deviation	p-value
Postmenopausal (test) Zinc	57.203	24.669	0.01
Premenopausal (Control)	76.045	27.281	

In addition, a significant decrease was observed when the concentrations of zinc and cobalt in the postmenopausal group were compared with that of the control *(p = 0.00)*, *see Tables 3 and 4.*

DISCUSSION

Our work has shown a significant increase in the mean concentration of selenium in the postmenopausal group compared with that of the pre-menopausal subjects *(p=0.00)*. Furthermore, a significant decrease was observed when the concentrations of zinc and cobalt in the post-menopausal group were compared with that of the control *(p=0.00)*. However, no significant difference was noticed in the mean concentration of copper in the postmenopausal subjects compared with the control *(p=0.0428)*.

Bednarek-Tupikowska, *et al*, stated that serum selenium concentration showed only a slight tendency to be higher in post-menopausal healthy women than in controls.[8] Bednarek-Tupikowska, *et al*, observed that hormone therapy (HT) did not significantly alter selenium levels, it increased slightly in whole blood, whereas it rather decreased in plasma after both types of therapy.[9] Ha and Smith found in their investigation a positive relationship between plasma oestrogen and selenium; a similar relationship was found by Smith, *et al*, in a cross-sectional study of American daughters, mothers, and grandmothers.[10,11]

Previous work by Steidl and Ditmar showed that serum zinc levels in pre-menopausal subjects were significantly higher than the post-menopausal group.[12] They found that serum zinc levels were lower among post-menopausal subjects than in controls. Reginster, *et al*, reported that there is no significant difference in post-menopausal women with osteoporosis in terms of copper and zinc levels in plasma as compared to the non-osteoporotic controls.[13]

It has been known that zinc and copper are essential co-factors for enzymes involved in synthesis of various bone matrix constituents, and play a particularly important role in the regulation of bone deposition and resorption. However, there are still some unanswered questions, particularly regarding mineral status in the elderly and in those with osteoporosis.[14]

Jonathan and Wright, stated that a significant decrease existed in the mean concentration of cobalt in menopausal subjects (test) when compared with that of the pre-menopausal group (control group).[15] They also stated that a small percentage of menopausal women who start on bioidentical hormone therapy experience little or no relief of their menopausal symptoms despite using higher than average doses of estrogen and they also stated that a common mistake made by practitioners is to continually increase their estrogen dose above physiological doses in an attempt to overcome their symptoms which can be potentially dangerous. After doing some library research, in the late 1990s they found that 300-600micrograms of cobalt chloride almost always corrects this situation, although it happened very gradually over 3 to 6months.

According to one group of researchers, cobalt reduces the total number of oestrogen-metabolizing enzymes, called cytochromes, so that less oestrogen is excreted and thus more is retained in the body where it can function. With more oestrogen retained by the body, symptoms of low oestrogen decreased and ultimately disappeared.[16] Other hormones such as testosterone and cortisol may also be hyper excreted and treated the same way with cobalt chloride.

Although the results vary somewhat between different studies the average dietary daily cobalt intake (without supplementation) is around 160–580mcg. Bednarek-Tupikowska, *et al*, found that the whole-blood and serum copper concentrations were slightly, but not statistically significantly, lower than in the controls.[8] Meram, *et al*, showed that the administration of oestrogens and/or oestroprogestins during hormonal

replacement therapy caused a tendency to increase copper concentration to the level of the controls.[17]

In a study by Bureau and colleagues, hormone therapy in post-menopausal women caused a significant increase in serum copper level, while it remained unmodified by treatment in erythrocytes.[18] The recent study of a Portuguese population reported that hormonal replacement therapy does not significantly affect serum copper concentration.[19] Other studies show that the administration of sex hormones in oral contraceptives is connected with an increase in serum copper concentration.[20,21] Similarly, it has been shown in Spanish and Polish populations that serum copper level increases progressively during pregnancy, when estrogen levels are higher than in non-pregnant women.[22]

It has also been experimentally shown that estrogen deficiency leads to a decrease in copper content in rat teeth and mandible, and giving 17 beta-estradiol, positively influences the content of mineral components in these tissues.[23] The impact on copper concentration has been ascribed to an oestrogen-induced release of ceruloplasmin in the liver.[20] An adequate copper status plays a crucial role in osteoporosis.[23] Copper concentration decreases with age in women.[21]

CONCLUSION

Post-menopausal women may be exposed to a greater risk of serum biochemical changes than the premenopausal individuals. We have observed that serum concentrations of zinc and cobalt are diminished with a concomitant increase in the levels of selenium in menopausal women. We have also shown that the concentrations of these trace elements correlated positively with the ages of the subjects and the duration of menopause.

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