www.jmscr.igmpublication.org **Impact Factcor-1.1147**

ISSN (e)- 2347-176x

IGM Publication **Journal Of Medical Science And Clinical Research**

An Official Publication Of IGM Publication

REDUCED MANGANESE, SELENIUM AND ZINC IN NEWLY DIAGNOSED BREAST CANCER SUBJECTS IN SOUTH WESTERN NIGERIA.

Authors

[1]Dolapo P. Oparinde, [2]Adetunji S. Oguntola, [3]Adeniran S. Atiba*, Olabamiji A Ajose,[4] Adekemi A Adeoye[5]

1 Department Of Chemical Pathology, , Ladoke Akintola University Of Technology Teaching Hospital, PMB 5000, Osogbo, Osun State, Nigeria

2Department Of Surgery, Ladoke Akintola University Of Technology Teaching Hospital, PMB 5000, Osogbo, Osun State, Nigeria

3 Department Of Chemical Pathology, Ekiti State University, Ado-Ekiti, Nigeria

4 Department of Chemical Pathology, Obafemi Awolowo University, Ile-ife, Nigeria

5 Department Of Chemical Pathology, , Ladoke Akintola University Of Technology Teaching Hospital, PMB 5000, Osogbo, Osun State, Nigeria

Email: piusdollars@yahoo.com, asoguntola@yahoo.com, atiadesam08@yahoo.com, Abiodun_ajose57@yahoo.com, Adekemi.adeoye@yahoo.com

ABSTRACT

Background*: Micronutrients, dyslipidemia and body mass index (BMI) have been established to be risk factors for breast cancer development.*

Aim*: To determine the influence of breast cancer on serum Manganese (Mn), Selenium (Se) and Zinc (Zn) level as well as its association with plasma lipid profile.*

Materials and Methods*: Fasting plasma Total-cholesterol (T-Chol), HDL-cholesterol (HDL-Chol), LDL-cholesterol (LDL-Chol), Triglyceride (Tg) and serum Mn, Se and Zn levels were determined in 50 newly diagnosed breast cancer subjects (NDBC) and 50 age matched mammography screened controls, using commercial kits from Randox laboratory and Atomic Absorption Spectrophotometric methods respectively. BMI was calculated using Weight (Kg)/Height (m^2). Data obtained were subjected to statistical analysis*

using SPSS statistical package version 17.0.

Key words: Manganese, Selenium, Zinc, Breast-cancer, Nigerians.

INTRODUCTION

There are strong indications from previous studies that the development, morbidity and mortality associated with breast cancer are also linked to the dietary and metabolic profile of breast cancer subjects[1,2]. Various risk factors such as advancing age, genetic predisposition, elevated levels of reactive oxygen species (ROS), obesity, gene mutations, has been associated with the risk of breast cancer development[3,4,5,6,7]. However, in recent time, a lot of study has gone into the nutritional aspect of breast cancer development, progression and prognosis. Two important dietary risk factors that have received wide attention are studies on disturbances of lipids and lipoprotein fractions[8,9,10,11] as well as derangements in the homeostasis of some trace elements in the etiopathogenesis of breast cancer[12,13,14]. Notable among these trace elements are derangements found in the serum levels of Zinc (Zn)[15,16], Manganese (Mn)[17,18] and Selenium (Se)[19,20]. These three important trace metals have also being associated with immunological stability and maintenance of ROS levels in the body.

Despite the fact that there are divergent reports from different parts of the world with regards to the role of micronutrients in breast cancer development and progression, there is an additional paucity of data in this regards in Nigeria. This study was therefore designed to determine the serum levels of Manganese, Selenium and Zinc in Nigerians with newly diagnosed breast cancer. In addition, this study also was to determine any association which may exist among serum levels of these micronutrients, plasma Total Cholesterol (T-Chol), Triglyceride (Tg), High Density Lipoprotein Cholesterol (HDL-Chol), Low Density Lipoprotein Cholesterol (LDL-Chol) and Body Mass Index (BMI) in Nigerian subjects with newly diagnosed breast cancer.

SUBJECTS, MATERIALS AND METHODS

This study was carried out at the surgical out-patient clinic of Ladoke Akintola University of Technology Teaching Hospital, Osogbo, Osun State, Nigeria. Informed consent was sought and obtained both from the Ethical Committee of the hospital, subjects and controls, prior to commencement/inclusion into the study. Fifty newly diagnosed breast cancer (NDBC) subjects (all females) with clinical and histological diagnosis of breast cancer were successively recruited into the study. Fifty aged matched, apparently healthy female volunteers were

recruited **from women reported back to surgical out-patient clinic after mammographic screening** and were found to be breast cancer free as controls. Individuals with chronic illness such as diabetes mellitus and hypertension were excluded from this study.

Subjects and controls were weighed, shoes off and minimal clothing, with the help of an electronic weighing scale from Adams Company U.S.A. Their heights were measured with a standard stadiometer and body mass index (BMI) calculated using; BMI = Weight (Kg)/ Height (m^2). Eight milliliters of fasting venous sample was obtained from the antecubital fossa of subjects and controls, 4mls each were dispensed into a Na- EDTA container and an acid washed plain glass tubes for lipid profile and trace metal estimation respectively. Samples in the glass tubes were allowed for 30minutes to clot and retract and then centrifuged along with those in Na-EDTA bottles at 3500rpm for five minutes. The harvested serum and plasma were stored frozen at -20^c until analysis.

Trace metals; Selenium (Se), Zinc (Zn), and Manganese (Mn) were analyzed using Flame Atomic Absorption spectrophotometric method with the aid of a Beck 200 Atomic Absorption Spectrophotometer serial number 3183048010, from Milton Roy Company, United State of America.

Plasma Total Cholesterol (T-chol), and High Density Lipoprotein cholesterol (HDL-chol) were analyzed with the aid of commercial kits from Randox Laboratory Ltd, 55 Diamond Road, Crumlin, Co. Antrim, United Kingdom. The kits were based on enzymatic endpoint methods. Plasma Triglyceride (Tg), was also estimated using commercial kits based on GPO-PAP method from Randox Laboratory. Standards and controls were supplied along with the kits and were included in each batch of analysis. Plasma Low Density Lipoprotein cholesterol (LDL-chol) were estimated using Frieldwald formular[21].

Statistical Analysis

Data obtained were subjected to statistical analysis using statistical package for social sciences (SPSS) version 17.0 setting p at ≤0.05. Significance testing was done using either Student's t-test, or Chi-square as appropriate Correlations were determined using Pearson correlation coefficient.

RESULTS

50 newly diagnosed female subjects with established breast cancer between the ages of 29-70yrs and 50 female controls whose ages ranges between 30- 68years were studied. There was no significant age difference in the two population,p>0.05.The mean BMI in breast cancer subjects were significantly higher than those in controls, 28.40±4.64kg/m2 vs 21.71±2.09kg/m2, p<0.01, see table I.

Table I: Mean ±2SD of Parameters in Breast Cancer Subjects and Controls

PARAMETER	SUBJECTS N=50 MEAN±SD	CONTROLS N=50 MEAN±SD	T-VALUE	P-VALUE
BMI(Kg/m2)	28.40±4.64	21.71±2.09	9.30	P<0.01
AGE (years)	42.64±12.94	41.26±10.37	3.57	P>0.05
Tg (mmol/L)	2.62±0.23	2.01±0.19	14.18	P<0.01
T-chol (mmol/L)	6.19±1.16	4.3±1.17	8.33	P<0.01
HDL-chol (mmol/L)	0.51±0.30	1.27±0.11	16.91	P<0.01
LDL-chol (mmol/L)	4.48±1.38	2.06±1.17	9.47	P<0.01
Mn (ug/dl)	2.18±1.10	9.38±3.02	15.83	P<0.01
Se (ug/dl)	3.76±1.96	17.24±6.35	14.34	P<0.01
Zn (ug/dl)	41.96±14.86	106.18±28.06	14.30	P<0.01

As shown in table I, the mean plasma levels of T-chol, LDL- chol, and Tg were significantly higher in breast cancer subjects when compared with the controls, 6.19±1.16 (mmol/L), 4.48±1.38 (mmol/L) and 2.62±0.03 (mmol/L) vs 4.3±1.17 (mmol/L), 2.06±1.17 (mmol/L) and 2.01±0.19 (mmol/L), p<0.01 respectively. On the contrary, plasma HDL-chol levels were significantly lower in breast cancer subjects compared with controls, 0.51±0.30 (mmol/L) vs 1.27±0.11 (mmol/L), p<0.01. The mean serum levels of Mn, Se, and Zn were significantly lower in breast cancer subjects compared with controls, 2.18±1.10 µg/dl, 3.76±1.96 µg/dl and 41.96µg/dl vs 9.38±3.02 µg/dl, 17.24±6.35 µg/dl and 106.18±28.06µg/dl, p<0.01 respectively.

Pre or postmenopausal status does not have any significant effect on any of the parameters in

breast cancer subjects and in controls as comparison of mean in each group gave p>0.05 respectively.

In table II, Pearson correlation reveals a strong inverse correlation between Mn and Tg, T-chol and LDL-Chol,r= -0.73, -0.55, -0.59,(p<0.01) respectively, however, Mn demonstrates a strong positive correlation with HDL-chol r=0.74, p<0.01. Also, there was a strong inverse relationship between serum Se and Tg, T-chol and LDL-chol r= -0.72, -0.57, -0.60, p<0.01 respectively. Selenium demonstrates a strong positive correlation with plasma HDL-chol r= 0.72, p<0.01. A similar pattern of correlation was observed between serum Zn levels and plasma Tg, T-chol and LDL-Chol r= -0.66, -0.52, -0.57 p<0.01 respectively. Serum Zn levels demonstrates a significantly strong positive correlation with plasma HDL-chol levels, r= 0.73, p<0.01. Moreover, serum Mn demonstrates a significantly strong positive correlation with zn and Se, r= 0.66 and 0.73, p<0.01. Similarly, a significantly strong positive correlation exist between serum Se and Zn, r= 0.75, p<0.01.

Also in table II, BMI in breast cancer subjects, demonstrates a strong positive correlation with Tg, T-chol and LDL-chol, r= 0.64, 0.63 and 0.67, p<0.01 respectively. BMI also demonstrates a strong negative correlation with HDL-chol, Mn, Se and Zn in breast cancer subjects, r= -0.74, -0.57, -0.54 and -0.62, p<0.01 respectively.

Age in breast cancer subjects demonstrates a fairly strong but significant positive correlations with BMI, Tg, T-chol and LDL-chol, r= 0.35, 0.38, 0.33 and 0.34, p<0.01 respectively. On the other hand, Age demonstrates a fairly strong but significant inverse correlation with HDL-chol, Mn, Se, and Zn, r= -0.36, -0.35, -0.30 and -0.21, p<0.01 respectively.

Table II: Pearson Correlation of Physical and Biochemical Parameters in Breast Cancer Subjects.

	BMI(Kg/m2	AGE (years)	Tg (mmol/L)	T-chol (mmol/L)	HDL-chol (mmol/L)	LDL-chol (mmol/L)	Mn (ug/dl)	Se (ug/dl)	Zn (ug/dl)
BMI(Kg/m2)	1	0.35*	0.64*	0.63*	-0.74*	0.67*	-0.57*	-0.54*	-0.62*
AGE (years)	0.35*	1	0.38*	0.33*	-0.36*	0.34*	-0.35*	-0.30*	-0.21*
Tg (mmol/L)	0.64*	0.38*	1	0.68*	-0.84*	0.70*	-0.73*	-0.72*	-0.66*
T-chol (mmol/L)	0.63*	0.33*	0.68*	1	-0.76*	0.99*	-0.55*	-0.57*	-0.52*
HDL-chol (mmol/L)	-0.74*	-0.36*	-0.84*	-0.76*	1	-0.83*	0.74*	0.72*	0.73*
LDL-chol (mmol/L)	0.67*	0.34*	0.70*	0.99*	-0.83*	1	-0.59*	-0.60*	-0.57*
Mn (ug/dl)	-0.57*	-0.35*	-0.73*	-0.55*	0.74*	-0.59*	1	0.73*	0.66*
Se (ug/dl)	-0.54*	-0.30*	-0.72*	-0.57*	0.72*	-0.60*	0.73*	1	0.75*
Zn (ug/dl)	-0.62*	-0.21*	-0.66*	-0.52*	0.73*	-0.57*	0.66*	0.75*	1

* Correlation is significant at the 0.01 level (2-tailed)

DISCUSSION:

Breast cancer is a major problem world-wide. Incidence of breast cancer has been on a gradual increase in both developed and developing countries. Therefore, efforts have been focused on the identification of associated risk factors on daily basis.

In this study, we aimed at identifying the relationship which may exist among some known independent risk factors for breast cancer development. The main focus is on the dietary and metabolic risk associated with breast cancer especially in the area of lipid and trace metal homeostasis.

This study has been able to establish that increased BMI and increasing age are associated factors in Nigerian women with breast cancer. This is in line with previous work from various parts of the world where obesity/increased BMI and age have been implicated as independent risk factors for the development of breast cancer[4,5,6]. Age and BMI demonstrates a strong positive correlation with the development of hyperlipidemia, while on the contrary, they demonstrate a strong negative correlation with serum levels of manganese, selenium and zinc and plasma HDL cholesterol, therefore, this study has not only confirm the association between dyslipidemia, increased BMI and breast cancer but also confirm an association between increased BMI, dyslipidemia and low levels of Mn, Se and Zn in established breast cancer. In a previous study, decreased levels of HDL-chol have been demonstrated as an independent risk factor for breast cancer development[22,23], our work is in support of this finding because of the significantly reduced level of plasma HDL-chol observed in our breast cancer subjects. Since increased BMI/ obesity are modifiable risk factors, the importance of dietary modification and exercise should be a major focus in the area of breast cancer prevention and management.

From the findings in this study, it is clear that hyperlipidemia is also an associated factor in Nigerian women with breast cancer. Apart from plasma HDL – cholesterol that was significantly reduced, the T- chol, LDL-chol and Tg were significantly elevated in our breast cancer subjects. This is in line with previous findings from different parts of the world[8,9,10,11]. With the presence of increase BMI, elevated T-chol, LDL-chol, Tg and significantly reduced HDL-chol, the development or association of breast cancer and metabolic syndrome cannot be ruled out. Metabolic syndrome is characterized by visceral obesity, glucose intolerance, hypertension and dyslipidemia (low HDL-chol and high Tg). Some studies have already implicated increase level of insulin and insulin-like growth factor I (IGF-I), found in metabolic syndrome as casually linked to breast cancer[22,23]. Therefore, emphasis should also be placed on the assessment of cardiovascular status of breast cancer subjects because increased BMI, obesity and

hyperlipidemia have all been implicated as independent risk factors for the development of coronary heart disease (CHD) and risk of developing myocardial infarction (MI).

Trace metals Manganese (Mn), Selenium and Zinc were significantly lower in breast cancer subjects compared with controls. These findings have also being documented in previous studies[15,24,25]. However, in this study, we have also found a strong inverse correlation between these trace metals and BMI and also with plasma T-chol, LDL-chol and Tg. Judging by the fact that these trace metals (Mn, Se and Zn) have powerful antioxidative and immunoprotective potentials, their low levels can further jeopardize the body's immune status and handling of free radicals which have also being implicated as an associated risk factor for breast cancer development. The presence of hyperlipidemia and low levels of antioxidant enzymes may contribute to increased membrane lipid peroxidation and free radical injury in breast cancer subjects, therefore, emphasis should also be placed on the monitoring of free radical level and periodic assessment of important metalloenzyme such as Mn, Se and Zn to determine when intervention or replacement therapy may be needed. Manganese functions mainly in the form of manganese superoxide dismutase (MnSOD), a powerful antioxidant enzyme whose polymorphism especially MnSOD Val- 9Ala and MnSOD Ala-16Val genotypes have been associated with the risk of breast cancer development in premenopausal women who had low consumption of antioxidants. Zn is an essential trace metal that is an integral to many proteins and transcription factors, that regulate key cellular functions such as the response to oxidative stress, DNA replication, DNA damage repair, cell cycle progression and apoptosis, it's deficiency have recently being shown to alter DNA damage response genes in normal human prostate epithelial cells[26]. Selenium, though a powerful antioxidant has recently being shown not to fight breast cancer via it's antioxidative mechanism but rather as a pro oxidant inducing apoptosis via a mitochondrial pathway which involves protein kinases, tumor necrosis factor, activation of caspases and reactive oxygen species. Therefore, the role of selenium in breast cancer has been proposed as a two edge sword, prevention of breast cancer on one side, and treatment of breast cancer on the other side. Studies on selenium nanoparticles are now attracting a wide attention especially in the area of cancer chemotherapy, diagnosis and prevention.

Conclusion

Strong, significant negative correlations exist between BMI and serum levels of Mn, Se and zn. Also, a strong negative correlation exist between serum levels of Mn, Se, Zn and plasma T-chol, LDL-chol and Tg. This implies that the presence of hyperlipidemia and or increased BMI is a strong pointer to Mn, Se and Zinc deficiencies, especially in breast cancer patients. HDL cholesterol

demonstrates a strong positive correlation with serum levels of Mn, Se and Zn, therefore, decreased levels of plasma HDL cholesterol alone may also be a strong pointer to Mn, Se and Zinc deficiency in breast cancer. Breast cancer subjects with increased BMI and or hyperlipidemia should be specially considered for trace element and antioxidant replacement, dietary modification and exercise.

Acknowledgement: We sincerely thank the staff and management of Surgical Outpatient Clinic, LAUTECH Teaching Hospital, Osogbo, Osun State, Nigeria for their support during this study.

REFERENCES

1. Ong KR, Sims AH, Harvie M, Chapman M, Dunn WB, et al.: Biomarkers of dietary energy restriction in women at risk of breast cancer. *Cancer Prev Res* **2,** 720-31, 2009.
2. Lopez-Saez JB, Martinez-Rubio JA, Alvarez MM, Carrera CG, Dominquez Villar M, et al.: Metabolic profile of breast cancer in a population of women in southern Spain. *Open Clin Cancer J* **2,** 1-6, 2008.
3. Pan SY, Dewar R, Dryer D, Kreiger N, Kliewer E, et al.: Antioxidants and breast cancer risk-a population-based case-control study in Canada. *BMC Cancer* **11,** 372 doi 10.1186/1471-2407-11-372, 2011.
4. Morimoto LM, White E, Chen Z, Chiebowski RT, Hays J, et al.: Obesity, body size, and risk of postmenopausal breast cancer: the Women's Health Initiative (United States). *Cancer Causes Contrl* **13,** 741-51, 2002.
5. Kaaks R, Van Noord PA, Den Tonkelaar I, Peeters PH, Riboli E, et al.: Breast-cancer incidence in relation to Height, weight and body-fat distribution in the "DOM" cohort. *Int J Cancer* **76,** 645-51, 1998.
6. Carmichael AR: Obesity and prognosis of breast cancer. *Obes Rev* **7,** 333-40, 2006.
7. Harvie M, Hooper L and Howell AH: Central obesity and breast cancer risk: a systemic review. *Obes Rev* **4,** 157-73, 2003.
8. Shah FD, Shukla SN, Shah PM, Patel HR and Patel PS: Significance of alterations in plasma lipid profile levels in breast cancer. *Integr Cancer Ther* **7,** 33-41, 2008.
9. Chang SJ, Hou MF, Tsai SM, Wu SH, Hou LA, et al.: The association between lipid profile and breast cancer among Taiwanese women. *Clin Chem Lab Med* **45,** 1219-23, 2007.
10. Aliev DA, Azizov VA, Sadygova TA, Zeinalov RS and Musaev IN: lipid metabolism disorders at the breast cancer

patients receiving hormonotherapy. *Geogian Med News* **168,** 44-7, 2009.

11. Owiredu WK, Donkor S, Addai BW and Amidu N: Serum lipid profile of breast cancer patients. *Pak J Biol Sci* **12,** 332-8, 2009.

12. Kotsopoulos J, Suklennicki G, Muszynska M, Gackowski D, Kaklewski K, et al.: Plasma micronutrients, trace elements, and breast cancer in BRCA 1 mutation carriers: and exploratory study. *Cancer Causes Control* **23,** 1065-74, 2012.

13. Silva MP, Soave DF, Ribbeiro-Silva A and Poletti ME: Trace elements as tumor biomarkers and prognostic factors in breast cancer: a study through energy dispersive x-ray fluorescence. *BMC Res Notes* **5**, 194 doi:10.1186/1756-0500-5-194, 2012.

14. Panjehpour M, Taher M and Bayesteh M: The growth inhibitory effects of cadmium and copper on the MDA-MB468 human breast cancer cells. *J Res Med Sci* **15,** 279-286, 2010.

15. Ajayi GO: Copper and Zinc concentrations in Nigerian women with breast cancer. *Eur J Gynaecol Oncol* **32**, 307-8, 2011.

16. Bobrowska-Korczak B, Skranjnowska D and Tokarz A: The effect of dietary zinc – and polyphenols intake on DMBA –induced mammary tumorigenesis in rats. *J Biomed Sci* **19,** 43. doi:10.1186/1423-0127-19-43, 2012.

17. Ennen M, Minig V, Grandemange S, Touche N, Merlin JL, et al.: Regulation of the high basal expression of the manganese superoxide dismutase gene in aggressive breast cancer cells. *Free Radic Biol Med* **50**, 1771-9, 2011.

18. Tsai SM, Hou MF, Wu SH, Hu BW, Yang SF, et al.: Expression of manganese superoxide dismutase in patients with breast cancer. *Kaohsiung J Med Sci* **27,** 167-72, 2011.

19. Yazdi MH, Mahdavi M, Varastehmoradi B, Faramarzi MA and Shahverdi AR: The Immunostimulatory Effect of Biogenic Selenium Nanoparticles on the 4T1 Breast Cancer Model: an in Vivo Study. *Biol Trace Elem Res* **149**, 22-28, 2012.

20. Luo H, Wang F, Bai Y, Chen T and Zheng W: Selenium nanoparticles inhibit the growth of HeLa and MDA-MB-231 cells through induction of S phase arrest. *Colloids Surf B Biointerface* **94**, 304-8, 2012.

21. Frieldwald WT, Levi RI and Friedericksn DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative

ultracentrifuge. *Clin Chem* **18**, 499-502, 1972.

22. Furberg AS, Jasienska G, Bjurstam N, Torjesen PA, Emaus A, et al.: Metabolic and Hormonal Profiles: HDL Cholesterol as a Plausible Biomarker of Breast Cancer Risk. The Norwegian EBBA Study. *Cancer Epid Biom Prev* **14**, 33-40, 2005.

23. Furberg AS, Veierod MB, Wilsgaard T, Bernstein L and Thune I: Serum High-Density Lipoprotein Cholesterol, Metabolic Profile, and Breast Cancer Risk. *Jour National Cancer Instit* **96**, 1152-60, 2004.

24. Rejali L, Jaafar MH and Ismail NH: Serum selenium level and other risk factors for breast cancer among patients in a Malaysian hospital. *Environ Health Prev Med* **12**, 105-10, 2007.

25. Sharma K, Mittal DK, Kesarwani RC, Kamboj VP and Chowdhery: Diagnostic and prognostic significance of serum and tissue trace elements in breast malignancy. *Indian J Med Sci* **48,** 227-32, 1994.

26. Yan M, Song Y, Wong CP. Wong, Hardin k, and Ho E: Zinc Deficiency Alters DNA Damage Response Genes in Normal Human Prostate Epithelial Cells. *The J Nutr* **138,** 667-73, 2008.