Trace Element Analysis of Cancerous and Non-cancerous Breast Tissues of African Women in Southwest Nigeria Using Particle-Induced X-ray Emission Technique

David O Olaiya[1], Olusegun I Alatise[2], Oyebamiji O Oketayo[3], Olawale E Abiye[4], Eusebius I Obianjunwa[4] and Fatai A Balogun[4]

[1]Department of Physics and Engineering Physics, Obafemi Awolowo University, Ile Ife, Nigeria. [2]General Surgery Department, Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile Ife, Nigeria. [3]Department of Physics, Federal University Oye-Ekiti, Oye-Ekiti, Nigeria. [4]Center for Energy Research and Development, Obafemi Awolowo University, Ile Ife, Nigeria.

ABSTRACT: In this study, we applied particle-induced X-ray emission (PIXE) spectroscopy to investigate the levels of trace elements in breast tissues and whole blood (cancerous and non-cancerous) of selected African women in Ile-Ife, Southwest Nigeria. Freeze-dried and homogenized specimens obtained through mastectomy from clinically diagnosed patients were made into 11-mm-diameter pellets. The pellets were irradiated with 2.5 MeV proton beam energy from a 1.7 MV 5SDH Tandem accelerator. The PIXE analytical system was calibrated with certified reference matrices of Bovine Liver and Animal Blood: NIST 1577a and IAEA-A-13, respectively. A total of 23 elements: Na, K, Ca, Cl, S, Al, P, Si, Zn, Pb, Br, Rb, Zr, Se, Sr, Mn, V, Ti, Cu, Fe, Ni, Cr, and Mg were detected. The results indicated that the levels were within 0.9-5288 and 0.6-2320 ppm in breast tissues and 0.3-17228 and 2.0-2475 ppm in the whole blood of cancerous and non-cancerous subjects, respectively. At the .05 level of significance, significant differences exist between these levels in the cancerous and non-cancerous breast tissues ($t = 0.008$) as well as the whole blood ($t = 0.041$). The results gave the baseline concentration of the observed trace elements in the normal and malignant subjects and indicated PIXE as a powerful tool for such investigation.

KEYWORDS: trace elements, cancerous, non-cancerous, breast tissues, blood, PIXE

Introduction

The human populace in sub-Saharan Africa is increasingly threatened by overwhelming reports of cancer incidences. In particular, recent results from population-based cancer registries have shown that cancer incidence in Nigeria is quite alarming.[1,2] More disturbing is the fact that over 60% of all incidences were found among women with breast cancer accounting for the highest figure.[1–3] In 2012, for instance, approximately 14 million new cancer cases were reported globally and up to 8.2 million deaths recorded from cancer in the same year, with 57% of the cases and 65% of the deaths occurring in less developed countries,[4] 6% of the world total of cancer cases and 7.2% of the deaths occurring in Africa which may increase by at least 70% in 2030.[5] In Nigeria, some 100 000 new cases of cancer occur every year, with high case fatality ratio.[6] With approximately 20% of the population of Africa and slightly more than half the population of West Africa, Nigeria contributed 15% to the estimated 681 000 new cases of cancer that occurred in Africa in 2008.[7] World Health Organization estimates that the incidence of cancer in Nigerian men and women by 2020 will be 90.7/100 000 and 100.9/100 000 and the deaths rates will be 72.7/100 000 and 76.0/100 000, respectively.[8]

Despite its significance to public health, studies investigating chemical signatures of cancerous tissues are rare in Nigeria and perhaps in West Africa.[1,9] The reasons are directly linked to lack of research facilities required for such studies, inadequate and commensurate skilled manpower, and ultimately lack of research funding. Currently, Nigeria has no national policy or a comprehensive document on cancer control, neither is there any organized national programme for cancer prevention.

As a positive contribution towards the pressing need to improve the quality of services for cancer diagnosis and control in Nigeria, this study aimed at applying an advanced and highly sophisticated facility (a Tandem 1.7 MV 5SDH accelerator) hosted at the Center for Energy Research and Development (CERD), Obafemi Awolowo University to investigate the chemical compositions of cancerous and non-cancerous breast tissues as well as the whole blood of selected African women in the region. The objective is to obtain tendencies, in terms of concentration levels, for trace elements present in breast tissues as possible biomarkers for cancer occurrence and signals for early prevention therapy.

Table 1. Reference values of calibrating standards and experimental results (ppm).

ELEMENTS	NA	MG	P	S	K	CA	MN	FE	CU
NIST 1577a (Bovine Liver)	2400	600	11 100	7800	9960	120	8.5	194	158
Experimental values obtained	2420	602	11 300	7801	9950	120	8.6	194	124
System efficiency (%)	100.8	100.3	101.8	100.0	100.0	100.0	100.0	101.2	78.5

ELEMENTS	NA	MG	P	S	K	CA	FE	ZN
IAEA-A-13 (Animal Blood)	12 600	99	940	6500	2500	286	2400	13
Experimental values obtained	12 610	104	940	6480	2506	286	2402	13
System efficiency (%)	100.1	105.1	100	99.7	100.2	100	100.1	100

Materials and Methods

Sample collection and preparation

In total, 10 specimens, each of paired cancerous and non-cancerous breast tissues, and 10 whole blood samples were obtained from mastectomy operations on patients with confirmed breast cancer diagnosis. Non-cancerous tissues were taken from regions of the breast defined by the safety margin from the same patients.[10] The paired-sample methodology adopted in this study was to avoid demographic uncertainty which may occur due to differences in age, diet, sexual orientation, hormonal status, medication, genetics, and other environmental factors. Deionized and double-distilled water was used to remove blood stains from the breast tissue specimen and then freeze-dried at –54°C for 32 hours. To minimize crystal formation and morphological damage, cryo-sectioned samples were quickly frozen in liquid nitrogen (–176°C) for 72 hours. Final freeze drying was carried out with a *Christ BETA1-8 LD plus* machine at 6.1 mbar for 72 hours. The specimens were then converted to a homogeneous fine powder by pulverizing in an agate mortar and subsequently pelletized into 11-mm-diameter samples required as targets in the particle-induced X-ray emission (PIXE) analytical setup.

PIXE analysis

A 1.7 MV Pelletron 5SDH tandem accelerator hosted at the CERD, Obafemi Awolowo University, Ile-Ife, Nigeria was used for PIXE analysis of the blood and breast tissues. The accelerator end station (the section where the sample was irradiated) was equipped with a multipurpose end station consisting of an aluminium chamber of about 150 cm diameter and 180 cm height. The chamber was maintained under vacuum for Rutherford backscattering (RBS), proton-induced gamma ray emission (PIGE), and PIXE analysis. For the PIXE technique employed in this study, a proton beam of energy 2.5 MeV extracted from a radiofrequency charge-exchanged ion source was focused on the samples through a 4-mm collimator and irradiation was maintained at a beam current not more than 6 nA for an average of 20 minutes. Characteristic X-ray signals emitted from the bombarded samples were collected on a highly sensitive Canberra Si-Li detector (Model ESLX 30-150, beryllium thickness of 25 μm), inclined at an angle of 135° from the incident beam. The detector has an active area of 12.5 mm^2 and a resolution of 145 eV FWHM (full width at half maximum) at 5.9 keV. Detailed configuration parameters of the CERD accelerator have been reported elsewhere.[11,12] The PIXE spectrum processed by a Canberra Genie 2000 (3.1) multi-channel analyser was analysed using GUPIXWIN software. GUPIXWIN provided a non-linear least-squares fitting of the spectrum, together with subsequent conversion of the fitted X-ray peak intensities into elemental concentrations utilizing the fundamental parameter method for quantitative analysis. Sample matrix was taken into account in the GUPIXWIN analysis based on the iterative matrix element solution.

System calibration/quality assurance

Two certified and appropriate reference standards: NIST 1577a (Bovine Liver) and IAEA-A-13 (Animal Blood) irradiated under the same experimental condition with the specimens and analysed with consistent fundamental parameters were used to calibrate the PIXE system for quality assurance. The efficiency of the system to replicate reference values for 10 elements (Na, Mg, P, S, K, Ca, Mn, Fe, Cu, and Zn) in the 2 standards was considered.

Results and Discussion

Analytical results obtained for the 2 reference materials used for the PIXE system are presented in Table 1, while Table 2 depicts trace element levels in breast tissues and whole blood (ppm). From Table 1, it is clear that the experimental analyses of the bovine liver and animal blood standards adequately reproduced their certified reference values ($r = 0.999$) within and acceptable system efficiency ranging from 78.5% (Cu) to 105.1% (Mg). Figure 1 also shows the levels of trace element distributions in cancerous and normal tissues of clinically diagnosed patients with breast cancer.

The levels of the observed trace elements were in the range of 0.9-5288 and 0.6-2320 ppm in breast tissues and 0.3-17 228 and 2.0-2475 ppm in the whole blood of cancerous and non-cancerous subjects, respectively. Generally, high positive

Table 2. Trace element levels in breast tissues and whole blood (ppm).

	BREAST TISSUES		WHOLE BLOOD	
	MALIGNANT	NORMAL	CANCER PATIENTS	NON-CANCER PATIENTS
Na	5288 ± 277	2320 ± 215	12 123 ± 499	1787 ± 28
Mg	428 ± 78	134 ± 68	72 ± 20	36 ± 7
Al	564 ± 41	442 ± 30	275 ± 8	a
Si	97 ± 25	111 ± 19	30 ± 5	a
P	1577 ± 20	242 ± 14	1761 ± 44	451 ± 7
S	2312 ± 21	804 ± 14	6993 ± 43	1221 ± 7
Cl	4140 ± 18	2226 ± 13	17 228 ± 52	2475 ± 9
K	2863 ± 11	493 ± 6	7633 ± 26	1182 ± 6
Ca	509 ± 7	348 ± 4	365 ± 20	91 ± 4
Cr	0.9 ± 0.4	4 ± 1		
Mn	5 ± 4	3 ± 1	a	2 ± 1
Fe	182 ± 3	25 ± 1	2128 ± 18	394 ± 8
Ni	a		1.89 ± 1.75	13.00 ± 5.09
Cu	3 ± 1	2 ± 1		
Zn	30 ± 6	14 ± 4	59 ± 16	31 ± 9
Se	5 ± 3	4 ± 2	19 ± 7	3 ± 2
Br	17 ± 10	26 ± 4	63 ± 30	4 ± 3
Rb	21 ± 9	3 ± 1	0.3 ± 0.1	4 ± 4
Sr	3 ± 1	0.6 ± 0.4		
Zr	10 ± 5	4 ± 1		
Pb	17 ± 7	4 ± 2		

PIXE, particle-induced X-ray emission.
[a]Values are below the PIXE system detection limits.

correlations were obtained between the levels (mean values) of these trace elements in breast tissues of cancerous and non-cancerous patients ($r = 0.938$) as well as in the whole blood ($r = 0.995$). The elements: Na, Mg, Al, Si, P, S, Cl, K, Zn, Br, Si, Ca, Rb, and Zr were elevated in the malignant breast tissues of the cancer patients compared to the normal tissues obtained at a safety margin from the same patients as shown in Figure 1. However, at the .05 level of significance, significant differences exist between the levels of Br, Mn, Se Pb, and Zn in the cancerous and non-cancerous breast tissues ($t = 0.02$-0.04).

The elevated level of electrolytes Na, Cl, K, and Ca reported by Stephen[10] as shown in Figure 3 are also in good agreement with our study. In addition, the disparity in the levels of Na detected in this study compared with the result obtained by

Stephen[10] could be an indication of variation in dietary habit, location, or other factors. Also, the difference in concentrations of Sr and As in the subjects' malignant breast tissues compared with the 2 studies could be due to the same varying factors. However, both levels were higher than those in the normal tissues in the studies. Zirconium (Zr) detected in this study has not been reported in the literature regarding its contribution to cancer but could find its way into the tissues as a result of its use as jewelleries.

Figure 2 shows the PIXE spectrum of the blood, while Figure 3 depicts the trace element distribution in the blood of cancer and non-cancer patients.

The levels of electrolytes Ca, Cl, K, and Na were higher in the blood as obtained in the malignant breast of patients with
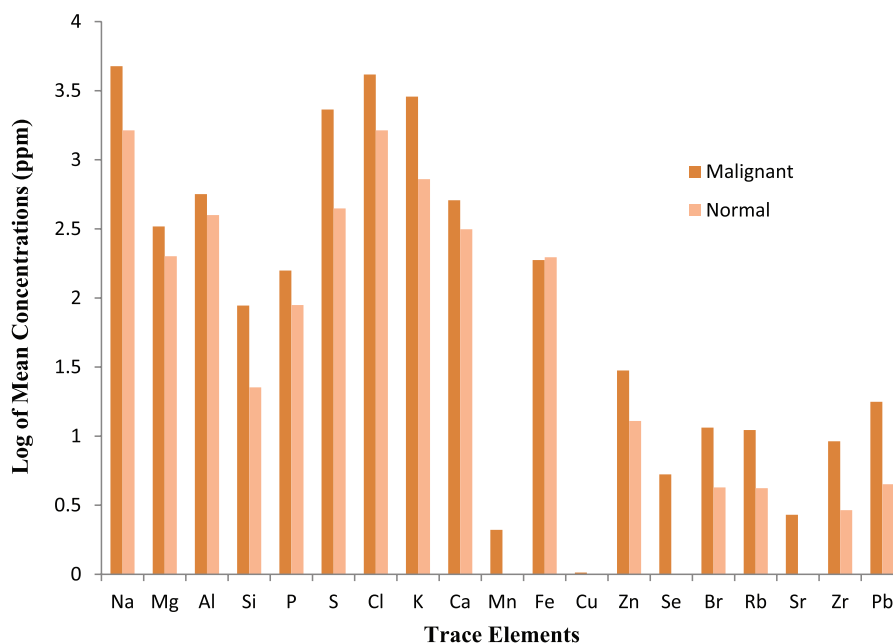
Figure 1. Trace element distributions in cancerous and normal tissues of clinically diagnosed patients with breast cancer.
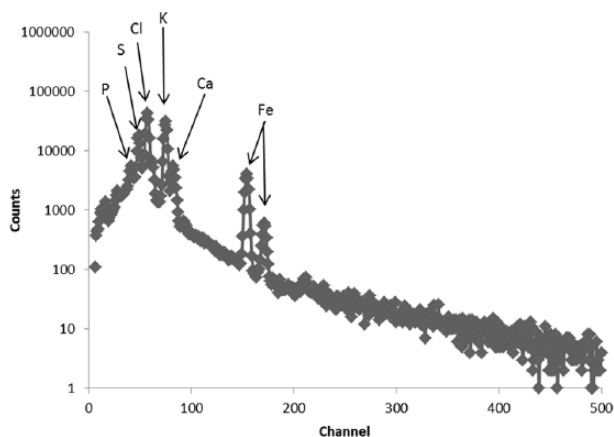

Figure 2. PIXE spectrum of blood.
PIXE, particle-induced X-ray emission.

cancer (Figure 3). Perhaps, this phenomenon could be used as an indicator for cancer. Virtually, all the elements found to be significantly elevated in the cancerous breast tissues were equally elevated in the blood of the subjects. The levels of Nickel were below the detection limit in the breast tissues. Also, no significant difference exits in the levels of this element in the blood of cancerous and non-cancerous patients ($t = 0.42$, at the .05 level of significance).

From Table 1, chromium was detected in a small amount and there was no appreciable difference between its levels in malignant and normal tissues. Cr toxicity is commonly associated with exposure to hexavalent Cr compounds rather than to the low toxic trivalent Cr compounds.[13] Cr(VI) has a characteristic of ease of absorption by the body cells and, once in the cell, it is reduced to the trivalent state which produces genotoxic effects.

The availability or deficiency of some of these trace elements goes a long way in the formation or inhibiting cancer.

Mo, Mn, and Zn have been found to prevent the formation of experimentally induced cancer, whereas the deficiency of Mo and Zn has been cited as a possible factor in the causation of oesophageal cancer.[14,15]

According to the study conducted by Alatise and Schrauzer,[16] there could also exist an association of Se with its antagonistic elements. In their study, correlation calculations revealed that elements like Cr, Pb, and other oxidants interact with Se, thereby suppressing the antioxidant effects of Se. In metal-exposed subjects, a state of latent Se deficiency may exist resulting in depressed immune functions and increased cancer susceptibility. Evidence has also been shown that Pb and other metals interact with iodine, another vitally important essential trace element that is believed to protect against breast cancer development.[16] These observed correlations suggest that the levels of Se and other anticancer elements significantly influence the concentration of the carcinogenic or toxic elements in the affected part or the whole body.

The elevated level of Al found in this study agreed with Christopher Exley who also confirmed the presence of Al in breast tissue and its possible regional distribution within the breast.[17] A higher content of Al in the outer breast tissues might be due to its proximity to the underarm where the highest density of application of antiperspirant could be assumed. There is evidence that skin is permeable to Al, even when applied as an antiperspirant.[18]

Phosphorus (P) was elevated in the malignant tissues relative to normal. The same trend was observed in the blood of cancer patients. High levels of phosphorus can affect body's ability to effectively use other minerals such as iron, calcium, magnesium, and zinc. It can combine with calcium resulting in mineral deposits in the muscles. It is rare to have too much phosphorus in the blood. Typically, only people with kidney
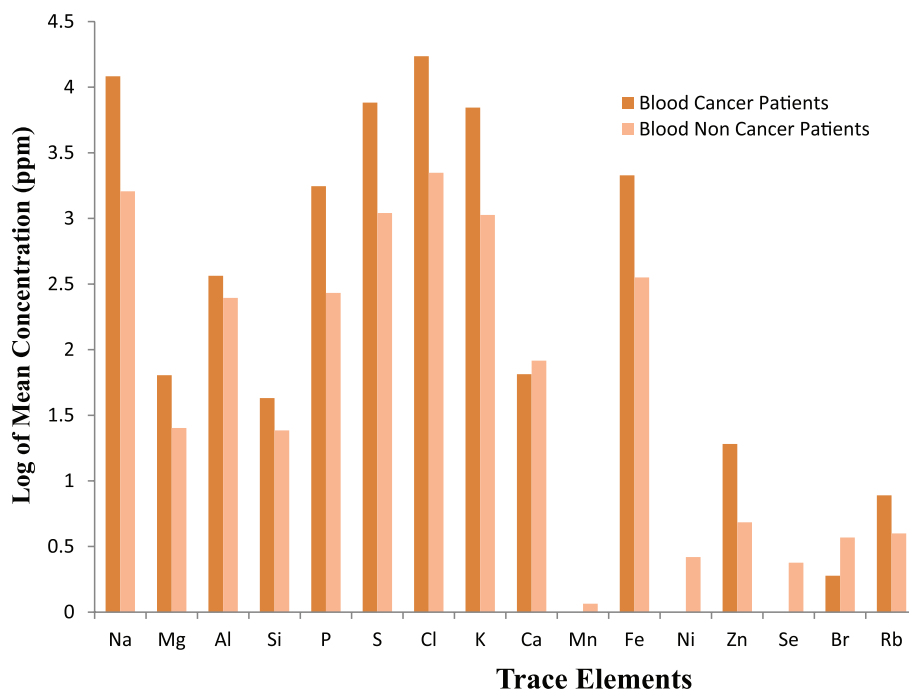
Figure 3. Trace element distribution in the blood of cancerous and non-cancerous patients.

problems or those who have problems regulating their calcium level develop this problem. A similar result was obtained by Sivakumar and Mohankumar in patients with breast cancer which was attributed to the elevation of cellular activity in malignant tissue and active enzymatic systems.[19,20]

Conclusions

The study was undertaken to determine the variation in trace elemental concentrations between normal and malignant human breast tissue specimens and to understand the effects of altered homeostasis of these elements in the cause of breast cancer. Using PIXE, the observed trace elements were found within the range of 0.9-5288 and 0.6-2320 ppm in breast tissues and 0.3-17228 and 2.0-2475 ppm in the whole blood of cancerous and non-cancerous subjects, respectively. Trace element concentrations in cancerous whole blood and malignant breast tissues were significantly or relatively higher (in most cases) than the normal ($t = 0.008$-0.041). The results established the baseline levels and indicated that PIXE is a powerful technique to investigate and evaluate trace element concentration in human blood and breast tissues, despite its advantages (being a multi-elemental technique with high levels of precision and accuracy).

Cancer disturbs the physiological functions of biological cells which are manifested in the variation of trace element concentrations in malignant and normal tissues obtained from the subjects. The difference in the concentration of some of these elements in malignant tissues in comparison with normal tissue samples indicated the possibility of using these elements as indicators or biomarkers. The elements that can be used for differentiation could be Se, Zn, and Cr which were significantly elevated in malignant tissues. Moreover, the low or high levels of some other trace elements in the observed malignant or non-malignant tissues could also be used to determine an earlier indicator of cancer and carcinogenic role, ie, the formation of free radicals or other reactive oxygen species that adversely affect DNA, thereby causing cancer-related diseases. Although some trace elements have controversial results, there is a need for more correlation investigations regarding their association with one another. For result reliability and correct assessment of the role of trace elements in initiation, promotion, progression, or inhibition of cancer in various organs, there is a need for acquisition of more data from different regions using differentials like age, gender, dietary habit, and lifestyle.

REFERENCES

1. Jedy-Agba E, Curado MP, Ogunbiyi O, et al. Cancer incidence in Nigeria: a report from population-based cancer registries. *Cancer Epidemiol*. 2012;36:e271–e278.
2. Ekanem IA, Parkin DM. Five year cancer incidence in Calabar, Nigeria (2009–2013). *Cancer Epidemiol*. 2016;42:167–172.

3. Salako O, Robert AA, Okunade KS, et al. Utilization of cancer information system for breast cancer control in Lagos, Nigeria. *Pan Afr Med J*. 2016;24:323.

4. Ferlay J, Soerjomataram I, Ervik M, et al. *GLOBOCAN (20120 v1.0). Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 11*. Lyon, France: International Agency for Research on Cancer. http://globocan.iarc.fr; https://www.123rf.com/stock-photo/nigeria_map.html. Accessed April 22, 2017.

5. Parkin DM, Bray F, Ferlay J, Jemal A. Cancer in Africa 2012. *Cancer Epidemiol Biomarkers Prev*. 2014;23:953–966.

6. Ferlay J, Shin H-R, Bray F, et al. Estimates of worldwide burden of cancer in 2008:GLOBOCAN 2008. *Int J Cancer*. 2010;127:2893–291.

7. Sylla BS, Wild CP. A million Africans a year dying from cancer by 2030: what can cancer research and control offer to the continent? *Int J Cancer*. 2012;130:245–250.

8. World Health Organization. *The Global Burden of Disease: 2004 Update*. Geneva: WHO; 2008.

9. Curado MP, Edwards B, Storm H, et al. Cancer incidence in five continents, vol IX. IARC scientific publication no. 160. http://www.iarc.fr/en/publications/pdfs-online/epi/sp160/CI5vol9-A.pdf. Published 2008. Accessed March 18, 2017.

10. Mulware SJ. Comparative trace elemental analysis in cancerous and noncancerous human tissues using PIXE. *J Biophys*. 2013;2013:192026.

11. Olise FS, Owoade OK, Olaniyi HB. An optimization of PIXE procedure for high-Z species in a lower Z matrix. *Appl Radiat Isot*. 2010;68:1030–1034.

12. Ezeh GC, Obiajunwa EI. Multi-elemental analysis of colonial and post-colonial Nigerian coins by Particle induced X-ray emission (PIXE) spectrometry. *J Fundam Appl Sci*. 2012;9:499–508.

13. Raju GJN, Sarita P, Kumar MR. Trace elemental correlation study in malignant and normal breast tissue by PIXE technique. *Nucl Instrum Meth B*. 2006;247:361–367.

14. Eric R, Braverman BA, Pfeifter CC. Essential trace elements and cancer. *Orthomol Psychiatry*. 1982;2:28–41.

15. Taccioli C, Chen H, Jiang Y, et al. Dietary zinc deficiency fuels esophageal cancer development by inducing a distinct inflammatory signature. *Oncogene*. 2012;31:4550–4558.

16. Alatise OI, Schrauzer GN. Lead exposure: a contributing cause of the current breast cancer epidemic in Nigerian women. *Biol Trace Elem Res*. 2010;136:127–139.

17. Exley C, Charles LM, Barr L, et al. Aluminium in human breast tissue. *J Inorg Biochem*. 2007;101:1344–1346.

18. Guillard BMH, Reiss D, Gombert J. Physiologie et pathologie du zinc. *Pathol Biol (Biol)*. 2004;28:469–478.

19. Ng KH, Bradley DA, Looi LM. Elevated trace element concentrations in malignant breast tissues. *Br J Radiol*. 1997;70:375–382.

20. Sivakumar S and Mohankumar N. Mineral Status of female breast cancer patients in Tami Nadu. *Int J Res Pharm Sci*. 2012;3(4):618–621.