*Available online at* www.derpharmachemica.com

Scholars Research Library

**Der Pharma Chemica, 2015, 7(1):241-247**
*(http://derpharmachemica.com/archive.html)*

**ISSN 0975-413X**
**CODEN (USA): PCHHAX**

# Cyclooxygenase-2 and selenium in breast cancer

**Faten Z Mohamed[a]*, Talaat M. El-Mokadem[b], Marwa S. Sabea[a] and Ahmed H. Attia[a]**

[a]*Biochemistry Division, Chemistry Department, Faculty of Sciences, Zagazig University, Zagazig, Egypt*
[b]*Clinical Oncology & Nuclear Medicine, Faculty of Medicine, Zagazig University, Zagazig, Egypt*

---

**ABSTRACT**

*Breast cancer is a global problem that accounts for nearly a quarter of all cancers in women. Recent studies implicated expression of cyclooxygenase-2 as a marker to identify precursor cells for breast cancer. The purpose of the present work is to investigate the association of genotypic polymorphisms in cyclooxygenase-2 and selenium with breast cancer. Methods: investigation of genotypic polymorphisms of cyclooxygenase-2, measurement of selenium by electrothermal atomic absorption spectrometry (ET-AAS) and investigation of calcium and alkaline phosphatase spectrophotometry in 500 patients with breast cancer and 100 healthy controls from Zagazig University Hospital in Egypt. The results showed significant differences in distribution between the breast cancer and controls. We found 169G allele carriers had a higher risk of breast cancer and results also showed significant decrease in Se and Ca and significant increase in Alk. In conclusion: the G allele of COX-2 gene may be a good marker in breast cancer and evaluation of serum Se, Ca and AlP give a red light in early detection of breast cancer.*

**Keywords**: Breast cancer, Cylooxygenase-2, Selenium, Calcium, Alkaline phosphatase.

---

## INTRODUCTION

Breast cancer is a global problem. Worldwide, breast cancer accounts for nearly a quarter of all cancers in women [1]. In 2013, it is estimated that more than 296,000 women and 2,240 men will be diagnosed with breast cancer [2]. Recently, researchers at the National Cancer Institute (NCI) projected that the overall breast cancer incidence rate will stay the same through 2016 [3]. Most risk factors are not modifiable, including age, family history, reproductive history, obesity reduction, avoidance of use of combined estrogen and progestin menopausal hormones, reduced alcohol consumption, smoking, and increased physical activity [4]. The high prevalence of breast cancer and the limited options for treatment provide a strong rationale for identifying new molecular targets that can be nutritionally or pharmacologically modulated and offer a potential for chemoprevention. Among the regulatory molecules that have been characterized as holding great promise for breast cancer treatment is cyclooxygenase-2 (Cox-2) [5].

Cyclooxygenases also known as prostaglandin endoperoxide H synthases or prostaglandin G/H synthases, are key enzymes in mediating the conversion of free archidonic acid into prostaglandin H2. These active products are important regulators of many biologic processes such as inflammation, immune function, cell proliferation, and angiogenesis, which are all relevant to cancer development and progression [5]. There are two isoforms of cyclooxygenase (COX-1 and COX-2). COX-1 is expressed in many tissues and COX-2 is responsible for

prostaglandins produced in sites of inflammation and is induced by various carcinogens [6]. Studies of human breast tumor tissues demonstrate that upregulation of COX-2 has been detected in approximately 40% of human breast tumor tissues. Epidemiologic investigations suggest that use of nonsteroidal antiinflammatory drugs or selective COX-2 inhibitors reduces breast cancer risk [7].

Selenium (Se) is well known as an essential trace mineral and an essential cofactor for glutathione peroxidases (GPx), selenoprotein P, and thioredoxin reductase, which are involved in scavenging free radicals and maintaining the redox balance. Growing evidence indicated that Se protects mammary epithelial cells from oxidative DNA damage, inhibits the initiation phase of carcinogenesis, stimulates DNA repair, regulates apoptosis, and prevents cells from angiogenesis. Furthermore, the relationship between the Se status and breast cancer risk has been documented from clinical observations. Compared to healthy subjects, patients with cancer exhibit markedly lower plasma concentrations of Se and GPx activity. Decreased Se intake status and lower toenail Se levels were associated with breast cancer risk in previous research [8]. Epidemiologic and experimental evidences indicate that selenium, an essential trace element, can reduce the risk of a variety of cancers. Protection against certain types of cancers, is closely associated with pathways involving cyclooxygenase-2 (COX-2). We found that AMP-activated protein kinase (AMPK), which functions as a cellular energy sensor, mediates critical anticancer effects of selenium via a COX-2/prostaglandin E(2) signaling pathway [9].

Deficiency of selenium and calcium may contribute to mammary carcinogenesis due to the roles of these elements in regulating cell proliferation, differentiation and apoptosis. Additionally, selenium has immune-enhancing and antioxidant effects [10].

Alkaline phosphatase (ALP) is a serum enzyme whose total levels reflect the combined activity of several isoenzymes found in the liver, bone, kidney, and intestinal lining. The skeletal isoenzyme originates in osteoblasts that release large amounts of the enzyme when bone repair activity occurs, for example with bone metastases. In cancer patients, ALP is a sensitive indicator of mild biliary obstruction, thus being a very sensitive indicator of liver progression. In a study conducted by the International Breast Cancer Study Group (IBCSG), ALP, aspartate transaminase (AST) and c glutamyltransferase (GGT) were examined for their sensitivity in detecting breast cancer recurrence. ALP alone was abnormal in a high proportion of breast cancer patients with bone metastases and/or liver metastases, and was more effective than AST and GGT in distinguishing patients with relapse from those without [11].

The aim of the present work is to investigate the association of genotypic polymorphisms in cyclooxygenase-2 with breast cancer, measure Selenium by electrothermal atomic absorption spectrometry (ET-AAS) and investigate calcium and alkaline phosphatase spectrophotometry.

## RESULTS

### Genetic analysis

The genotype and allele distribution for the COX-2 169C/G polymorphism by study group was shown in table (1). The digested product was separated on a 3% agarose gel electrophoresis at a 120 V constant voltage for 0.5 h and then visualized under ultraviolet light. The variant C allele has a Mae II restriction site while the G allele lacks the Mae II restriction site, so the CC homozygote results in two bands (88 bp and 118 bp), the GC heterozygote produces three bands (88 bp + 118 bp + 206 bp) and the GG homozygote produces a single 206 bp band.

**Table (1): COX-2 genotypes distribution in two groups n, %**

| SNP | Breast Cancer | Control |
|---|---|---|
| **GG** | 235 ( 47 ) | 10 ( 10 ) |
| **G/C** | 75 ( 15 ) | 40 ( 40 ) |
| **CC** | 190 ( 38 ) | 50 ( 50 ) |
| **allele** | | |
| **G** | 545 ( 54.5) | 60 (30) |
| **C** | 455 (45.5) | 140 (70) |

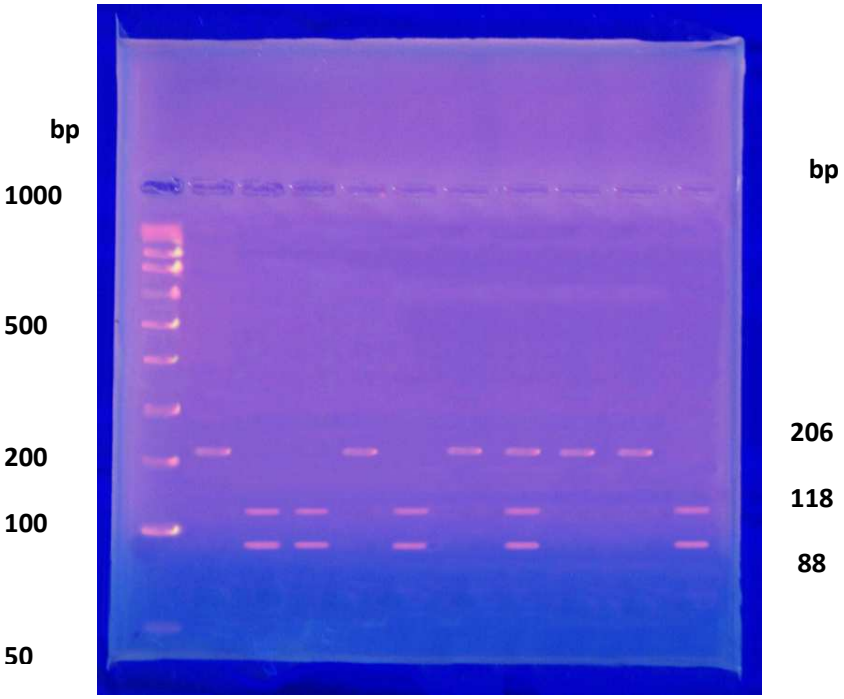
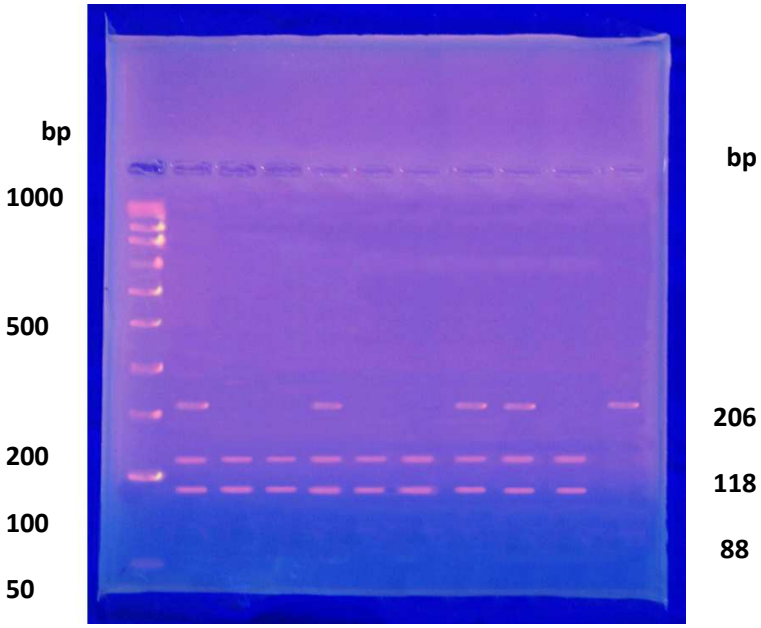



**Fig. 1): Agarose gel electrophoresis of polymerase chain reaction (PCR) products in group II (Breast cancer patients). Lan 1: 50-500 bp DNA marker; lan 2: PCR product ( 206 bp); lan 3,4,6: CC homozygote ( 88 bp + 118 bp); lan 5,7: GG homozygote ( 206 bp); lan 8: GC heterozygote ( 88 bp + 118 bp + 206 bp)**




**(Fig. 2): Agarose gel electrophoresis of polymerase chain reaction (PCR) products in group I (Control). Lan 1: 50-500 bp DNA marker; lan 2,5,8,9: GC heterozygote ( 88 bp + 118 bp + 206 bp); lan 3,4,6,7,10: CC homozygote ( 88 bp + 118 bp); lan 11: GG homozygote ( 206 bp)**

The 169 G allelic frequencies in two groups were 545 (54.5%) and 60 (30%), respectively; and 169 C allelic frequencies were 455 (45.5%) and 140 (70%), respectively (Table 1). 169G alleles carriers had a higher risk for Breast cancer and compared with control group.

**Table 2: Serum levels of Selenium, Calcium and Alk in cases and controls**

| Markers | Healthy (n=100) | BC (n=500) | *P value |
|---|---|---|---|
| **Age** | 33.95±13.2 | 52.72±10.77 | <0.0001 |
| **Alk** | 85.45±23.4 | 168.41±76.4 | <0.0001 |
| **Se** | 111.2±6.1 | 73.07±10.6 | <0.0001 |
| **Ca** | 9.23±0.423 | 8.91±0.742 | <0.0001 |

**Biochemical analysis**

As regard the obtained results of the mean value of serum Alk, a significant increase was detected in BC group (168.41±76.4) than that found in control subjects (85.45 ± 23.4), the mean value of serum Se, a significant decrease was detected in BC group (73.07±10.6) than that found in control subjects (111.2±6.1) while the mean value of serum Ca, a slightly significant decrease was detected in BC group (8.91±0.742) than that found in control subjects (9.23±0.423).

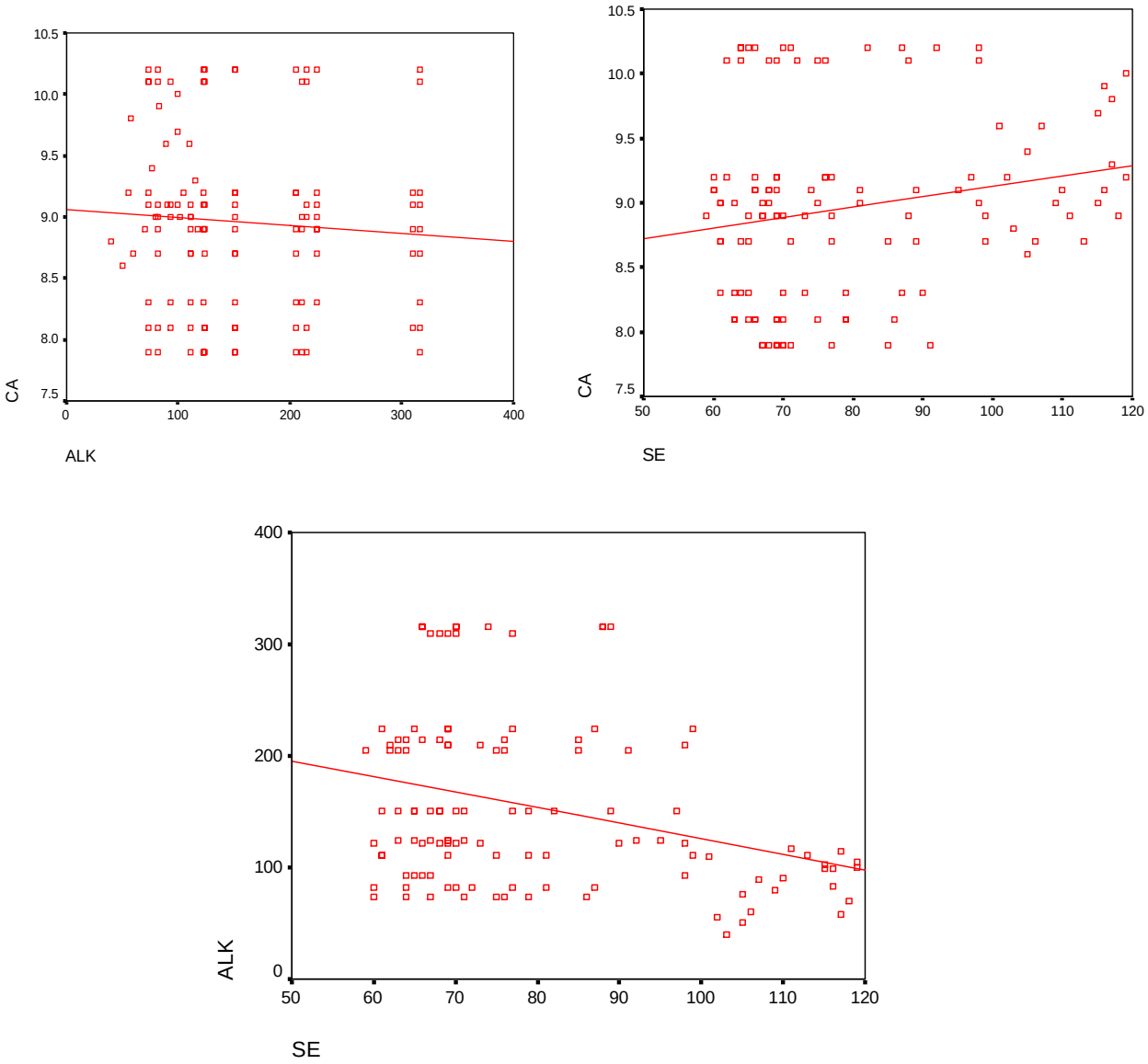



**Fig 3: Correlation between serum ALP& Se& Ca in studied individuals**

## DISCUSSION

Identification of a molecular marker is a central goal in breast cancer biology. Expression of cyclooxygenase-2 (Cox-2) as a marker to identify breast cancer. Although an increasing number of genetic markers have been used in the diagnosis of early specific nature. It has been well accepted that the Cox-2 gene is chronically over-expressed in many premalignant, malignant tissues, and metastatic cancers, including breast cancer [17]. Overexpression of cyclooxygenase 2 (COX-2) has been suggested to be associated with breast carcinogenesis [18].

SNPs are one of the most common forms of human genetic variation. SNPs in the promoter region of genes may affect either the expression or the activity of enzymes and therefore may be mechanistically associated with cancer risk [19].

By using PCR-technique, we analyzed one SNP: 169C/G. For 169C/G polymorphism, genotype frequencies in our healthy control subjects [GG: 10 (10%); GC: 40(40%); CC: 50(50%)] were similar to those reported in Fawzy: [GG, 6.6%; G/C, 36.6%; CC, 56.6%; n= 110] [20]. 169G alleles carriers had a higher risk for breast cancer when compared with control group in our study and on the contrary, C allele of COX-2, G-765C was associated with a decreased risk of breast cancer in Taiwan [18] and also C allele has a risk factor for breast cancer among Caucasian subjects[21]. This means that genetic polymorphisms may have ethnic and geographic variability.

As seen in the present work, there was significant decrease in serum Se and Ca, deficiency of selenium, and calcium may contribute to mammary carcinogenesis due to the roles of these elements in regulating cell proliferation, differentiation, and apoptosis. Additionally, selenium has immune-enhancing and antioxidant effects and this is agreement with Yan, who concluded that levels of zinc, calcium, and selenium are associated with a decrease in breast cancer risk [22] and with Vinceti et al., who found limited evidence suggesting that individuals observed to have higher selenium levels have a lower incidence of cancer. However, it is not possible to conclude from these studies that selenium was the reason for the lower cancer risk, because a high selenium level might be associated with other factors that reduce cancer risk, such as a healthier diet or lifestyle. Also, selenium comes in many different chemical forms that have different biological activity, and these studies did not identify which chemical forms were being measured [23] and with Guo, who concluded that Se plays a vital role in antioxidant enzyme GPx, which exerts cancer-preventive effects and anti-tumorgenic activity. Reduced plasma GPx activity and Se concentrations have been found in patients with metastatic cancer. Moreover, reduced GPx activity is inversely related to cancer progression and Se is thus a potential risk factor for progression of breast tumor and metastasis. Therefore, Se supplementation may be needed for the maintenance of Se homeostasis that is beneficial to patients with breast cancer. [8]. Søren Skov., who concluded that "You can say that the stimulating molecules over-activate the immune system and cause it to collapse, and we are, of course, interested in blocking this mechanism. We have now shown that certain selenium compounds, which are naturally found in, e.g., garlic and broccoli, effectively block the special immunostimulatory molecule that plays a serious role for aggressive cancers such as melanoma, prostate, breast cancer and certain types of leukaemia [24].

There was significant increase in serum alkaline phosphatase (AlP) and this is agreement with Keshaviah et al., who concluded that AlP alone was abnormal in a high proportion of breast cancer patients with bone metastases and/or liver metastases, and was more effective than AST and GGT in distinguishing patients with relapse from those without [11] and with Nathaniel et al., who reported that the skeletal invasion and destruction by tumor induced by tumor-production of various cytokines such as transforming growth factor-á (TGF-á), tumor necrosis factor.á (TNF-á), TNF-â, interleukin-1 and interleukin.2, leads to increasing bone osteolysis20 and modification of the reabsorption, excretion and resorption of calcium and phosphate ion and ALP has many isoenzymes localized in the liver, bones and in lesser amounts the intestines, placenta kidney and leucocytes. Another isoenzyme of ALP termed Regan isoenzyme has also been identified in various malignancies29 and this may be contributing to increased ALP activity seen in breast cancer patients. The increased activity of this enzyme seen in subjects of the study may also be due to osteolytic bone metastases in breast cancer leading to increased osteoclastic activity and bone resorption. Increase in serum ALP levels is however non-specific as it is also frequently associated with a variety of other diseases. Also, the elevation of ALP activity to less than three times the normal level is usually not considered significant [25].

**Subjects and methods**

The present study includes the following groups:

1-Healthy control group consisted of 100 healthy female individuals. Age ranged from 19 to 66 years.
2- 500 female patients of Breast cancer (BC) group. Age ranged from 21 to 72 years from Clinical Oncology Outpatient clinics, Faculty of Medicine, Zagazig University Hospital.

Five ml of blood sample was taken from every participant under complete aseptic condition and was divided into 2 portions; 2 ml of whole blood was collected in sterile EDTA containing tubes for DNA extraction, and the rest was left for 30–60 min for spontaneous clotting at room temperature then centrifuged at 3,000 rpm for 10 min. Serum samples were separated into another set of tubes and kept frozen at –20 C for determination of Se which measured using electrothermal atomic absorption spectrometry (ET-AAS)[12] and Ca and ALP were measured spectrophotometric [13,14].

**Genotyping analysis**
The amplification was carried out using thermal cycler PTC-100 machine (MJ Research, Inc., Watertown, Mass. USA), cycling conditions for each gene according to (Li et al., 2009) [15] as follows:

The cycling conditions were: 30 s at 94°C, 30 s at 57°C, 60s at 72°C. The 304-base pair (bp) product was amplified, and then digested with MaeII (New England Biolabs, UK) for 4h in a 37ºC water bath. The digested product was separated on a 2% agarose gel electrophoresis at a 120 V constant voltage for 0.5 h and then visualized under ultraviolet light. The variant C allele has a Mae II restriction site while the G allele lacks the Mae II restriction site, so the CC homozygote results in two bands (88 bp and 118 bp), the GC heterozygote produces three bands (88 bp + 118 bp + 206 bp) and the GG homozygote produces a single 206 bp band. More than 10% of the samples were randomly selected for repeated assays, and the results were 100% concordant. Restriction fragment length polymorphism (RFLP) analysis was confirmed by PCR-based sequencing with an Applied Biosystems 3730 DNA sequencer.

The amplified products were run in 2.3% agarose gel using the EC 360 Submarine Gel electrophoresis system (Maxicell, EC 360 M-E-C apparatus Cooperation St. Petersurg. Florida USA).

**Statistical analysis**
Data were analyzed using SPSS 19.0 for windows (SPSS Inc., Chicago, IL, USA). The tested parameters were compared using t-test student. The correlation coefficient was obtained by the parametric Pearson correlation test. P values less than 0.05 were considered to be significant. The non-parametric parameters were compared using Mann-Whitney U test. The correlation coefficient of non-parametric parameters was obtained by Spearman's correlation test [16].

## CONCLUSION

In summary, the 169C>G polymorphism of the COX-2 gene was associated with the risk of BC in Egyptian females. The current results support that COX-2 169C>G polymorphism was associated with elevated serum alkaline phosphatase (ALP) activity in females carrying GG genotype. Moreover, a decrease of selenium and calcium levels may be closely related to tumor progression and may serve as a predictor of good prognosis in patients with breast cancer.

**Acknowledgements**
We thank the patients, physicians, nurses, and data managers for their help and data management. Clinical Oncology Outpatient clinics, Faculty of Medicine, Zagazig University Hospital, Biochemistry department , Faculty of Science, Zagazig University, Egypt.

## REFERENCES

[1] Department of Defense Breast Cancer Research Program, May **2013**.
[2] American Cancer Society. *American Cancer Society*, Inc,**2013**.
[3] Anderson W, Katki H, and Rosenberg P, *J Natl Cancer Inst*, **2011**, 103(18), 1397-1402.
[4] Chlebowski RT, McTiernan A, Wactawski-Wende J, et al, *J Clin Oncol*, **2012**, 30(23), 2844-2852.
[5] Lin WL, Lee YJ, Wang SM, Huang PY and Tseng TH, *European Journal of Pharmacology*, **2012**, 680, 8-15.
[6] Donkert C, Winzer KJ and Hauptmann S, *Clinical Breast Cancer*, **2004**, 4(6), 428-433.

[7] Jing-Yi Chen1, Chien-Feng Li2, Cheng-Chin Kuo3, Kelvin K Tsai1, Ming-Feng Hou456 and Wen-Chun Hung, *Breast Cancer Research*, **2014**, 16, 410.
[8] Chih-Hung Guo, Simon Hsia and Pei-Chung Chen, *Nutrients*, **2013**, 5, 594-607.
[9] Jin-Taek Hwang, Young Min Kim, Young-Joon Surh, Haing Woon Baik, Seong-Kyu Lee, Joohun Ha,Ock Jin Park, *Cancer Res*, **2006**, 66(20), 10057-10063.
[10] Yan Cui, Stefan Vogt, Neal Olson, Andrew Glass and Thomas Rohean, *Cancer Epidemiol Biomarkers Prev*, **2007**, 16(8), 1682-1685.
[11] A. Keshaviah, S. Dellapasqua, N. Rotmensz, J. Lindtner, D. Crivellari, J. Collins, M. Colleoni, B. Thu¨ rlimann, C. Mendiola, S. Aebi, K. N. Price,, O. Pagani, E. Simoncini, M. Castiglione Gertsch, R. D. Gelber, A. S. Coates & A. Goldhirsch, *Annals of Oncology*, **2007**, 18, 701–708.
[12] A.O.A.C., Association of official Analytical chemists, official methods of Analysis. 15t. ed. Vol. 1 Arlington, Virginia. USA. 247pp, **1999**.
[13] Zak B, Epstein E, Babinski E, *Annals of Clinical and Laboratory Science*, 5, 195-212, **1975**.
[14] Tietz Textbook of Clinical Chemistry. Second Edition, Burtis-Ashwood ,**1994**.
[15] Li, F.; Ren, G.S.; Li, H.Y.; Wang, X.Y.; Chen, L. and Li, J, *Clinical Oncology*, **2009**, 21, 302-305.
[16] Levesque, R, SPSS Programming and Data Management: A Guide for SPSS and SAS Users, Fourth Edition, SPSS Inc., Chicago, IL 60606-6412, **2007**.
[17] Zhang X., Miao X., Tan W., Ning B., Liu Z., Hong Y., Song W., Guo Y., Zhang X., Shen Y., Qiang B., Kadlubar F.F., Lin D, *Gastroenterology*, **2005**, 129, 565-576.
[18] Su CH, Hsiao CL, Chang WS, Liu LC, Wang HC, Tsai CW, Li LY, Tsai CH, Bau DT, *Anticancer Res*, **2014**, 34(11), 6711-6716.
[19] Gao J., Ke Q., Ma H.X., Wang Y., Zhou Y., Hu Z.B., Zhai X.J., Wang X.C., Qing J.W., Chen W.S., Jin G.F., Liu J.Y. Tan Y.F., Wang X.R., Shen H.B, J. *Toxicol. Environ. Health A*, **2007**, 70, 908-915.
[20] Fawzy MS, Aly NM, Shalaby SM, El-Sawy WH, Abdul-Maksoud, *Z.U.M.J*, **2013**, 19(2), 180-188.
[21] Zhi-Jun Dai, Yong-Ping Shao, Xiao-Bin Ma, Dan Xu,2 Wei Tang, Hua-Feng Kang, Shuai Lin, Meng Wang, Hong-Tao Ren, and Xi-Jing Wang, *Disease Markers*, **2014**.
[22] Yan Cui, Stefan Vogt, Neal Olson, *Cancer Epidemiol Biomarkers Prev*, **2007**, 16, 1682-1685.
[23] Vinceti M, Dennert G, Crespi CM, Zwahlen M, Brinkman M, Zeegers MPA, Horneber M, D'Amico R, Del Giovane C, Selenium for preventing cancer. Cochrane Database of Systematic Reviews, **2014**, Issue 3. Art. No.: CD005195. DOI: 10.1002/14651858.CD005195.
[24] Søren Skov, Selenium compounds boost immune system to fight against cancer. Faculty of Health and Medical Sciences. University of Copenhagen. Blegdamsvej 3B, 2200 København N, **2014**.
[25] Nathaniel I. Usoro,Maxwell C. Omabbe, Chinyere A. O. Usoro & Augusta Nsonwu, *African Health Sciences*, **2010**, 10(1), 9-13.