

# Selenium: As A Diagnostic Marker For Malignant Neoplasm

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**Abstract:** The deficiency of selenium has been correlated with the accelerated development of prostate cancer in transgenic mice whereas supplementary selenium has been found to reduce progression of lung cancer in human. This study aims at exploring any association or otherwise of the blood selenium level in cancer patient with the cancer occurrence and the investigation of the selenium as a biomarker for malignancy. The 368 samples were collected including healthy and cancer patients. Results shows that the concentration levels of selenium in the blood of 16 to 70 years males and females both combined together weighted averages for control group are  $0.121 \pm 0.03 \mu\text{g per ml}$  ( $n= 170$ ). Besides for lung and breast cancer patients fall in the range of  $0.057 \pm 0.017 \mu\text{g per ml}$  ( $n= 119$ ) and  $0.056 \pm 0.018 \mu\text{g per ml}$  ( $n= 198$ ) respectively. In cancer patients the selenium levels were significantly lower than in control with a  $P > 0.05$  and critical values are  $> 0.009$  and  $> 0.01$  for lung and breast cancer patients respectively. Studies also revealed that there is a sharp decline in the blood selenium level of the cancer patients irrespective of the gender, age, and type of cancer as compared to controls.

**Index Terms:** Selenium, human blood, malignant neoplasm, trace elements, cancer, Thioredoxins (Trx), selenoproteins, p53, chemopreventive.

## 1. INTRODUCTION

Selenium is vital for mammals and plays a critical role of fundamental importance to human health. It is present in body as an essential trace element and is involved in several metabolic pathways such as thyroid hormone metabolism, antioxidant protection system, immune system, cardiovascular diseases, inflammation and cancer related diseases. A number of epidemiological studies have been performed to determine serum selenium levels in cancer patients in different parts of the world. Selenium is incorporated in proteins through two vicinal cysteines to form a separate class of proteins called selenoproteins e.g. Thioredoxins Trx. Thioredoxin reductase TrxR, and glutathione peroxidase Gpx4. These proteins provide protection to the cell against reactive oxygen species (ROS) produced endogenously by xenobiotics, macrophages and neutrophils etc, and act as a chemopreventive reagent against cancerous transformation of the cell. Polymorphonuclear leukocytes and macrophages secrete ROS including hydrogen peroxide which is a major source of inflammation to the adjacent cells and cause lesions in DNA. Blood cells contain Gpx4 which regulate the redox state of the cell proteins and indicate the presence of an effective antioxidant system which maintains redox balance in the cells.

The method employed here in this study does not have limitation for the determination of selenium in the whole blood or serum. The aim of this study was to explore that the blood selenium level in cancer patient has an association with the cancer occurrence and if so, can selenium be used as a biomarker for malignancy. Further more determination of selenium directly in the whole blood requires fewer steps which make it less prone to errors and at the same time more efficient for the routine tests. In addition smaller quantity of blood is required for such analysis. One of the trace elements selenium is considered to be an essential trace element with both oxidant and antioxidant properties [1, 2]. The diagnosis of cancer at early stage plays a pivotal role and becomes the key factor in devising a strategy for the treatment of the patients. Selenium is ubiquitously present in every cell, incorporated in proteins through cysteine /s to form selenoproteins or selenocysteine proteins and the physiological effects of selenium mediated through these selenoproteins[3]. A group of 25 selenoproteins or selenocysteines including Trx, TrxR and Gpx4 have been characterized for their sequences and higher level structures which have been crystallized. The deficiency of selenium affects the cells ability to synthesize selenoproteins, Trx act as a proton donor to peroxidases and ribonucleotide reductases with reversible oxidation of two cysteines to form a disulfide bridge, the active site with two vicinal cysteine residues (cys-gly-pro-cys) is conserved. Supplemental selenium in the form of high selenium containing yeasts has been reported [4] to reduce lung cancer risk in humans. Interestingly, selenoproteins play a protective role against oxidative stress, inflammation and apoptosis and also take part in DNA repair, DNA demethylation by inhibiting cytosine-DNA-methyltransferase, in regulation of transcription factors, growth and fertility [3-6]. However, what triggers this role of selenoproteins from antiapoptosis to proapoptosis in cancer cells is a subject of great interest. The selenoprotein deficient mice have been shown to exhibit accelerated development of lesions associated with prostate cancer progression, suggesting that selenium prevents by modulating the level of these selenoproteins [5]. Significantly lower selenium levels have been detected [7, 8] in serum of the cancer patients as compared to the serum of healthy individuals.

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## 2. EXPERIMENTAL DESIGN OF THE STUDY

This is a short term randomized controlled study comprising determination of selenium concentration in the blood of control ( healthy), lungs and breast cancer patients, started on March 20, 1993 and continued until November 25th, 2000, the day on which last sample was drawn for analysis. Altogether 368 healthy and cancer patients donated their blood. For this study, 5 ml each of 170 controls, lung cancer 119 and breast cancer 79 cases were taken. The samples were divided in to two groups on the basis of gender and into three groups on the basis of age 16- 30 years, 31- 50 years, and 51- 70 years. In cancer patients the samples were further divided into two groups depending upon the type of cancer; lungs or breast cancer. For statistical analysis the student "t" test, ANOVA and extended tukey test were used where variables fulfilled parametric conditions and Kruskal Wallis test was used where these were non parametric.

## 3. SELENIUM METABOLISM

Inorganic selenium, sodium selenite  $\text{Na}_2\text{SeO}_3$ , is found in six stable isotopes having atomic weights ranging from 74 to 82. The amount of selenium absorbed through gastrointestinal tract, the main site of absorption, depends upon the chemical form and the amount ingested. When selenium is absorbed it is first carried by plasma proteins. After wards selenium is released and bound by other selenium proteins namely alpha- 2 and beta-1 globins. When selenite form of selenium is used for ingestion, it is rapidly taken up by the cells (50-70%) and delivered to tissues, bones, hair and red blood cells. It has been reported that 60% of selenium in plasma is incorporated to selenoprotein P which contains 10 selenium atoms per molecule of selenoprotein [9-11]. In a recent report it has been suggested that selenium is stored preferentially in prostate as compared to seminal vesicle [12].

## 4. ELIGIBILITY FOR BLOOD DONOR

Healthy blood donors were nonsmokers, non-addict, not suffering from any other illness. Therefore they were not on medications, between the age of 16-70 years and they did not have any family history of cancer incidences in two generations. The cancer patients were diagnosed positively for lung and breast cancer therefore, they were taking medicines on regular basis. Blood donors were informed accordingly about the purpose of the study and they all signed up a "consent form" prepared in view of the rules and regulations delineated by the Ministry of Health Islamabad, Pakistan. In different geographical regions of the world there are different rates of occurrences of cancer, which may be attributed to the genetic factors, environmental pollutions, living conditions and presence of essential nutrients in the food. For this study, we selected three cities of Pakistan; Rawalpindi, Islamabad and Lahore, nevertheless, subjects of the neighboring regions were included for the collection of blood samples. These regions are known for agriculture produce for the whole country therefore analysis of blood selenium level of the people living in these regions in principle could represent the intake of essential minerals, vitamins and trace elements of the people residing in far remote areas of the country. The intake of selenium depends upon the concentration of selenium in agriculture produce of that region, therefore

regional variation in the intake of selenium by the inhabitants of that area may be expected. A concomitant decline in Se status based on analysis of blood and serum has been reported, for UK population and may be at risk from an increased prevalence of certain health disorders [13-16]. Significantly low level of selenium was detected among the British wheat grain as compared to the US and Canadian wheat grain. It is a result of differences in the underlying geology and consequent higher Se concentrations in the North American soils [17].

## 5. MATERIAL AND METHODS

The blood samples of the healthy and cancerous patients were collected from a large group of individuals and these were analyzed for the determination of selenium concentration by using atomic absorption spectrophotometer model 932AB of GBC Scientific Equipment pty led, Australia coupled with hydride generator. The absorption was recorded at 196 nm with spectral bandwidth of 1.0 nm. The method described in [18, 19] was used to determine selenium concentration in the whole blood and modifications were made accordingly. The 5ml blood was drawn by using a plastic needle from the antecubital, vein of the left arm after overnight fasting of the subjects under the supervision of paramedic staff. The blood samples were stored in Venoject in the presence of anticoagulant. Approximately 0.5 ml blood was taken in 50 ml flask fitted with more than 30cm long air condenser and 3.0 ml triple distilled nitric acid of analar grade was added to the sample and then this mixture was heated for 30 min at 80°C. The mixture was cooled, and 1.5 ml triple distilled concentrated perchloric acid of analar grade was added and was heating at 250°C till white fumes evolved. This mixture was cooled and transferred to 10ml measuring flask. Through out deionized water was used in the sample preparations. Same procedure was adopted for the digestion of standard reference material; A- 2- 1974, NBS bovine liver SRM- 1577 and orchard leaves SRM-1571.

## 6. RESULTS AND DISCUSSION

Standards selenium samples A- 2- 1974, NBS bovine liver SRM- 1577 and orchard leaves SRM-1571 were subjected to analysis for the determination of selenium concentration and to test the precision and sensitivity levels of the procedure. The selenium concentrations determined for these standard samples were  $0.58 \pm 0.071 \mu\text{g}$  per ml for A- 2- 1974,  $1.13 \pm 0.11 \mu\text{g}$  per ml for SRM-1577 and  $0.081 \pm 0.009 \mu\text{g}$  per ml for SRM-1571 which was not significantly different P, 0.05, from expected values  $0.59 \pm 0.09 \mu\text{g}$  per ml,  $1.1 \pm 0.1 \mu\text{g}$  per ml and  $0.08 \pm 0.01 \mu\text{g}$  per ml respectively, as shown in Table 1.

**Table 1: Selenium Determination in Various Reference Materials.**

Reference Materials	Number of Analysis	Range $\mu\text{g/ml}$	Ave $\pm$ S.D $\mu\text{g/ml}$	Ave $\pm$ S.D $\mu\text{g/ml}$	*C. V
A-2-1974	12	0.41-0.65	0.58 $\pm$ 0.071	0.59 $\pm$ 0.09	12.3
Bovine Liver SRM- 1577	12	0.99-1.34	1.13 $\pm$ 0.11	1.1 $\pm$ 0.1	9.4
Orchard Leaves SRM- 1571	12	0.063-0.107	0.081 $\pm$ 0.09	0.08 $\pm$ 0.01	11.27

\*Coefficient of Variance

The results of blood selenium distribution in various age groups and gender for healthy individuals and cancer patients have been summarized in Table 2.

**Table 2. Selenium Level In The Blood Of Control And Cancer Patients In Various Age Groups**

Age Years	Gender	Number of Analysis	Control Ave $\pm$ S.D $\mu\text{g/ml}$	Number of Analysis	*LCA Ave $\pm$ S.D $\mu\text{g/ml}$	**BCA Ave $\pm$ S.D $\mu\text{g/ml}$
16-30	M	25	0.105 $\pm$ 0.340	20	0.059 $\pm$ 0.019	$\pm$
31-50	M	25	0.115 $\pm$ 0.015	25	0.066 $\pm$ 0.02	$\pm$
51-70	M	30	0.149 $\pm$ 0.029	29	0.052 $\pm$ 0.015	$\pm$
16-30	F	30	0.109 $\pm$ 0.035	10	0.055 $\pm$ 0.018	0.057 $\pm$ 0.018
31-50	F	30	0.114 $\pm$ 0.032	15	0.061 $\pm$ 0.012	0.063 $\pm$ 0.019
51-70	F	30	0.132 $\pm$ 0.035	20	0.047 $\pm$ 0.017	0.050 $\pm$ 0.020

\*LCA: Lung Cancer \*\*BCA: Breast Cancer

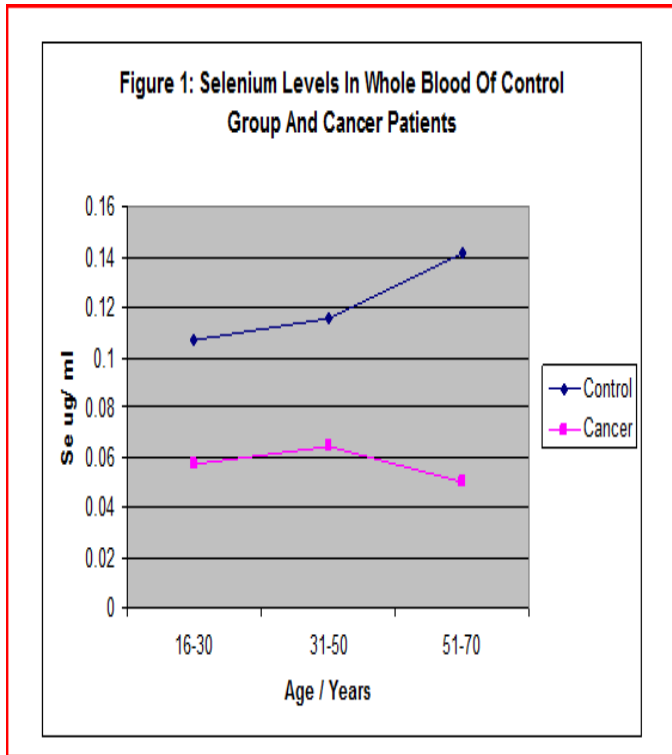
The values of selenium concentrations in the blood of healthy individuals vary between the ranges from 0.105  $\pm$  0.034  $\mu\text{g}$  per ml (n= 25) to 0.149  $\pm$  0.029  $\mu\text{g}$  per ml (n= 30), as compared to the values of selenium level range 0.059  $\pm$  0.019  $\mu\text{g}$  per ml (n= 20) to 0.063  $\pm$  0.019  $\mu\text{g}$  per ml (n=25) in cancer patients. The data shown in the Table 2 suggest that blood selenium concentrations in males are not different from selenium concentrations in females  $P < 0.05$ , and this is true for healthy individuals as for as cancer patients. The difference of 0.005  $\mu\text{g}$  per ml (5ng/ml) in the values of blood selenium concentrations between males and females is  $P < 0.05$  statistically insignificant and may be explained on the basis of lower concentration of red cells in females than in males (20). A comparison of ranges of selenium levels concentration along with weighed averages of these values of clinically healthy and cancer patients has been shown in Table 3.

**Table 3. Selenium Concentration in Blood of Male and Females of Control and Cancer Patient Blood**

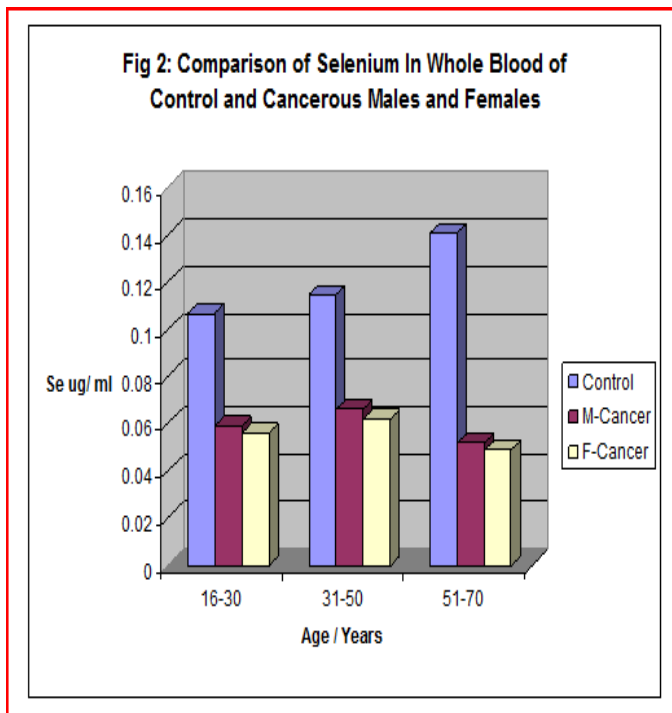
Number of Analysis	Gender	Sample Type	Range $\mu\text{g/ml}$ Min - Max	Ave $\pm$ S.D $\mu\text{g/ml}$	***C.V
3 ( 80)	M	Control	0.051-0.198	0.123 $\pm$ 0.26	21.81
3 ( 90)	F	Control	0.047-0.193	0.118 $\pm$ 0.34	28.77
3 (170)	Combined	Control	0.047-0.198	0.121 $\pm$ 0.37	25.29
3 ( 74)	M	*LCA	0.220-0.101	0.059 $\pm$ 0.018	30.50
3 ( 45)	F	LCA	0.020-0.090	0.054 $\pm$ 0.016	30.26
3 (119)	Combined	LCA	0.020-0.101	0.057 $\pm$ 0.019	30.25
3 ( 79)	F	**BCA	0.021-0.099	0.057 $\pm$ 0.019	34.03
3 (198)	Combined	LCA + BCA	0.020-0.101	0.056 $\pm$ 0.018	31.59

\* LCA: Lung cancer \*\* BCA: Breast cancer \*\*\* C.V. Coefficient of variance

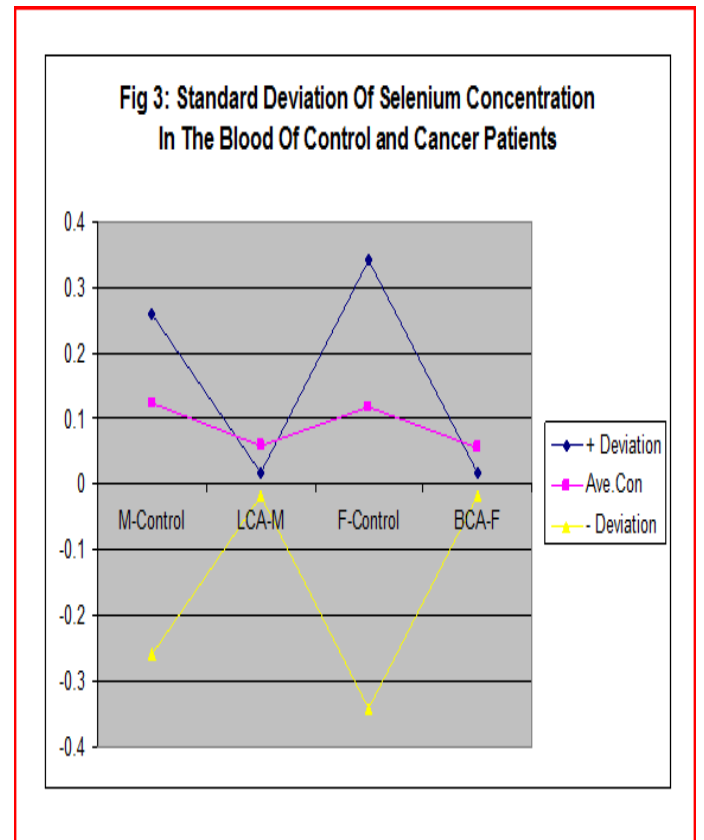
The data shown here suggest that the difference of blood selenium levels are statistically insignificant in males and females  $0.01 < P < 0.05$  (n= 25). The weighed average concentrations of selenium both combined together for males and females are 0.121  $\pm$  0.37  $\mu\text{g}$  per ml, 0.057  $\pm$  0.017  $\mu\text{g}$  per ml and 0.056  $\pm$  0.018  $\mu\text{g}$  per ml for normal individuals, lung and breast cancer patients respectively, Fig 1.



On the basis of results it may be suggested that the value of selenium concentration in the blood of cancer patients is approximately one half as compared to the value of selenium concentration in the blood of the healthy individuals. Overall in both control and cancer patients there is a slight increase in the blood selenium concentration with the increase in age but statistically these values were not significantly different from each other  $P < 0.05$  as shown in Fig 2.



This increase of 0.03  $\mu\text{g}$  per ml in the value of blood selenium concentration of the age group 51- 70 years as compared to these values of the age group 16-30 years may be attributed to the changes in metabolism, life styles , eating habits after the age of 50 years of the healthy individuals. The difference in the values of blood selenium concentration of 0.007  $\mu\text{g}$  per ml between 16-30 and 51- 70 years old cancer patients as compared to the overall difference of 0.065  $\mu\text{g}$  per ml in the values of the selenium concentration in healthy and cancer patients is statistically not significant  $P < 0.05$  as shown in Fig 3.



Therefore it may be suggested that age is not likely to affect the blood selenium level in control as well as in cancer patient. There are different reports [20- 23] in the literature about the age related studies as well as compared to other trace elements with selenium concentrations in the serum of healthy and cancer patients. The blood selenium concentration has similar values in the lungs and breast cancer patients as shown in Table 2. These values between the lungs and breast cancer group do not vary significantly with  $P < 0.05$  and standard deviation index is shown in Fig 1. Results of analysis of variance ANOVA are shown in Table 4, with critical value of  $F = 193$ .



**Table 4: Results Of Analysis Of Variance ANOVA.**

Source	* DF	** SS	*** MS	F- Critical Value		F- Calculated
				Significance level at 0.05	0.01	
Groups	2	0.385	0.193			
Error	365	0.321	0.001	3.02	4.66	193
Total	367	0.706	.....			

The difference of selenium concentration was found 0.064  $\mu\text{g}$  per ml between control group and lungs and breast cancer patients with critical values  $> 0.009$  and  $> 0.01$  respectively. The differences between two types of cancer have been calculated by employing tukey test which shows the value of 0.000 with critical value  $< 0.011$  as motioned in Tables 5. The Kruskal- Wallis test confirms the results of parametric test and gives the value of  $H= 227.339$  with correcting ties the obtained value is  $H=227.3436$ .

**Table 5: Extended Tukey Test Results**

Programs	Difference	Critical Value
C - L	$X_c . X_L = 0.064$	$> 0.009^*$
C - B	$X_c . X_B = 0.064$	$> 0.010^*$
B - L	$X_B . X_L = 0.000$	$> 0.011^{**}$

Where \* indicates a statistically significant difference and \*\* stands for not statistically significant.

C: Control group    L: Lungs Cancer    B: Breast Cancer

This reduction of 0.065  $\mu\text{g}$  per ml in the selenium blood concentration of cancer patients corresponds to 53.7 % decrease in the selenium concentration as compared to the blood selenium level in the control. The depletion of selenium in the blood of cancer patients may suggest its reciprocal accumulation in the malignant tissues in view of the studies [12, 25-28] who have reported higher concentration of selenium in the cancerous and neighboring tissues cells. Availability of excessive selenium in the cancerous cells may be expected to facilitate the synthesis of selenoproteins e.g. Trx, TrxR etc. which are known to provide protection to the tissue cells against the oxidative stress, carcinogens and help to reduce cancerous cell growth. The results of recent studies [29- 31] regarding the diversified multiple functions of selenoproteins has generated tremendous interest in the understanding and elucidation of mechanisms that triggers the role of these proteins from anti-apoptosis in the normal cells to pro-apoptosis in malignant cells. There are numerous studies [32-34], and have proposed different mechanisms to explain the inhibiting effect of selenium on malignant neoplasm; for example, modulation of cellular division rate, decrease in formation of carcinogenic metabolites or cellular protection by an antioxidant system. It is generally believed that due to the anti oxidative characteristics of selenoproteins, these proteins can protect the cells and DNA from oxidative damage; in addition these proteins can react with carcinogens directly to save cells and DNA from their lethal actions. It has been suggested [30] that nitrative inactivation of Trx plays a proapoptotic role if the reactive nitrogen species is increased; and antinitrating treatment may have therapeutic value in those diseases, such as myocardial ischemia/ reperfusion, in which pathological apoptosis is increased. The situation is reversed in malignant tissue cells where apoptosis is beneficial for the inhibition of the cell growth therefore in view of the aforementioned studies it is possible that the pathological conditions in which production of Nitrogen species is increased that may favor the inactivation of Trx and therefore enhance the apoptotic role of this selenoprotein. A kinetic study of the reaction of NO and O<sub>2</sub> in aqueous solutions, based on pH indicator, has been performed by using stopped-flow spectrometry [34]. The results of these studies have shown that at physiological concentrations of O<sub>2</sub> and NO the auto- oxidation of NO does not limit its diffusion from the site of production in endothelial cells to a spatially removed target molecule such as guanylate cyclase in myocytes and platelets. A Trx interacting protein Txnip has been reported [31], which inhibit the antiapoptotic activity of Trx where as NO suppresses the expression of Txnip and enhances the Trx activity, therefore perhaps the oxidative character of Trx in malignant cells as reported in the above mentioned studies may well be interpreted as the inhibition of its antioxidant activity. In different studies [34-35] it has been suggested on the basis of their results that selenite induces apoptosis by producing superoxide ions which activate p53, a well known protein involved in carcinogenesis, which in turn support apoptosis. A key role has been assigned to Trx-2, located in mitochondria [36], in interaction with electron transport chain, determining tumor necrosis ROS generation, NF-  $\kappa$ B activation and apoptosis.

## 7. Conclusion

We report here that there is a 53.7 % reduction in the cancer patient's blood selenium level is observed as compared to the control. Further studies are required to investigate that if selenium depletion in the blood of cancer patients can be stoichiometrically be correlated with the accumulation of selenium in the tissue cells of the cancer patients. Statistically there is no difference in the values of blood selenium level of lungs and breast cancer patients irrespective of age and gender, which makes selenium as a potentials candidate to be used as a diagnostic tool or biomarker for the cancer patients and during the post therapy time period. In view of these studies, we may suggest that in addition to regular tests after surgery or therapy and thereafter intermittent monitoring of blood selenium level, may provide information about the condition of disease which may help to devise a strategy for the diagnosis and treatment of cancer patients.

## References

- [1]. Carstl B., Christopher H. L; and Arne H, "Thiol-based Mechanism Of The Thioredoxin And Glutaredoxin Systems Implications For Disease In The Cardiovascular System." *Am. J. Physiol. Heart Circ. Physiol.*, 2007, H 1227- H 1236.
- [2]. Andoh T. Chock P.B; and Chiueh C. C, "The Role Of Thioredoxine In Protection Against Oxidative Stress Induced Apoptosis In SH-SY5Y Cells." *J. Biol. Chem.* 2001, 10: 1074-1079.
- [3]. Ganther H. E, "Selenium Metabolism, Selenoproteins And Mechanisms Of Cancer Prevention; Complexities With Thioredoxin Reductase." *Carcinogenesis*, 1999, 20 (9): 1657-1666.
- [4]. Jong- Min P., Aeyung K., Jang H. O. and An-sik C, "Methylseleninic Acid Inhibits PMA Stimulated Pro- MMP-2 Activation Mediated By MMP 1- MMP Expression And Further Invasion Through Suppression of Nf-kB Activation." *Carcinogenesis*, 2007, 28: 837-847.
- [5]. Zeng H ., Anderson M. B., Idso J. P., Hunt C. D, "The Selenium Metabolite Methylselenol Inhibits The Migration And Invasion Potential of HT 1080 Tumor Cells." *The J. Nutri.*, 2006, 136 no 6. 1528- 1532.
- [6]. Fomenko D. E., Xing W., Adair B. M., Thomas D. J., and Gladyshev V. N, "High Through Put Identification Of Catalytic Redox Active Cystein Residues." *Science*. 2007, 315(5810): 387- 389.
- [7]. [Van Cauwenbergh R. .](#), [Deelstra H](#), [Picramenos D](#); [Kostakopoulos A](#), "Selenium Concentration In Serum Of Healthy Greek Adults." *J Trace Elem. Electrolytes Health Dis.* 1994 Jun; 8(2):99-109.
- [8]. Liu X. G, "Serum and Tissues Copper, Zinc, And Selenium Levels In Patients With Gastric Carcinoma.," *Zhonghua Zhong Liu Za Zhi*, 1991, 13(2): 93- 6.
- [9]. Brater P. and Schramel P., "Trace Element Analytical Chemistry In Medicine And Biology." 1984, 3: 5-9.
- [10]. V. Valkovic, V" Trace Element Analysis" T. W. Bonner Nuclear Laboratories Rice University Houston. Texas and "Ruder Boskovic" Institute Zagreb, Yugoslavia. Taylor & Francis ISBN 0 85066 072 6. TAYLOR & FRANCIS LTD. 10- 14 Maeklin street London WC2B5SNF. Pp. 102
- [11]. Saito Y. and Takahashi K., " Charecterization Of Selenoprotein P As A Selenium Supply Protein". *Eur. J. Biochem.*, 2002, 269: 5746- 5751.
- [12]. Sibichi A. L., Lee J. J., Taylor R. J., et al, "Selenium Accumulation In Prostate Tissue During A Randomized, Controlled Short Term Trial of L- Selenomethionine: A South West Oncology Group Study". *Clin. Cancer Res*, 2006, 12: 2178.
- [13]. Rayma MP, "Dietary Selenium: Time To Act." *British Medical Journal* 1997. 314, 387- 388.
- [14]. Rayman M.P, "The Importance Of Selenium To Human Health." *Lancet* 2000. 356, 233- 241.
- [15]. Rayman M.P, "The Argument For Increasing Selenium Intake." *Proceedings of the Nutrition Society* 2002. 61, 203- 215.
- [16]. Rayman M.P, "The Use of High Selenium Yeast To Raise Selenium Status: How Does It Measure Up." *British Journal of Nutrition* 2004. 92, 557- 573.
- [17]. Martin R. Broadley et al, "Biofortification of UK Food Crops With Selenium," *Proceedings of the Nutrition Society*. 2006. 65, 169- 181.
- [18]. Naseer K., Rehman. S, and Ahmed S," Determination Of Lead And Cadmium In Milk By Electrothermal Atomic Absorption Spectrophotometry." *Int. J. Environ. Anal. Chem.* 1987. 28, Iss 1-2, 132- 141.
- [19]. Clinton, O. E, "Determination Of Selenium In Blood And Plant Material By Hydride Generation And Atomic Absorption Spectroscopy". *Analyst* 1997, 102: 187- 192.
- [20]. Lyengar G. V., "Reference Values for Trace Elements In Human Clinical Specimens: With Special Reference To Biomonitoring and

- Specimen Suitability". *Biol.Trace element Res.* 1987, 12: 263- 295.
- [21]. Moreno M. A. Marin C., Vinagre F, Ostapczuk P, "Trace Element Levels In The Whole Blood Samples From Residents of The City Badajoz. Spain." *The Sci total Environ*, 1999, 19; 229(3) 209- 15.
- [22]. Morisi G., Patriarca M., Marano G. et Al, "Age and Sex Specific Reference Serum Selenium Levels Estimated for The Italian Population." *Ann. 1st. Super. Sanita.*, 1989. 25(3); 393- 403.
- [23]. Seki H, "Influence of Selenium On Human Health. Part 1" "Sex Difference and Change In Normal Values With Increasing Age." *Nihon Eiseigaku Zasshi.* 1987, 42(5): 947- 53.
- [24]. Wang YX., Qin J.F., Wu SM., Yan L.B, "Study On The Relation of Se, Mn, Fe, Sr, Pb, Zn, Cu, and Ca To Liver Cancer Mortality From Analysis of Scalp Hair." *The Sci. Total Environ*, 1990, 91: 191- 8.
- [25]. Setterdahl A.T. Chivers P.T. Hirasawa M. et al, "Effect of pH On The Oxidation – Reduction Properties of Thioredoxins." *Biochemistry*, 2003, 42(50); 14877- 84.
- [26]. Nonn L., Williams R.R. Erickson RP., Powis G, "The Absence of Mitochondrial Thioredoxin- 2 Causes Massive Apoptosis, Exencephaly and Early Embryonic Lethality in Homozygous Mice. *Mol Cell Biol.*, 2003, 23(3): 916- 22.
- [27]. Powis Gareth and Montfort William R, "Annual Review of Pharmacology and Toxicology." 2001, 41: 261- 295.
- [28]. Patwari P., and Lee R. T, "Thioredoxins, Mitochondria and Hypertension." *Am J Pathol.* 2007, 170(3): 805- 8.
- [29]. Tao L., Jiao X, Gao E, et al, "Nitrate Inactivation of Thioredoxin-1 and Its Role In Postischemic Myocardial Apoptosis." *Circulation*, 2006, 114(13): 1395- 402.
- [30]. Schulze P. C., Liu H., Choe E., Yoshioka J., Shalev A., Bloch K. D., Lee R.T, "Nitric Oxide Dependent Suppression of Thiorexin Interacting Protein." *J Bio Chem*, 2004, 279: 30369- 74.
- [31]. Schrauzer G.N, "Selenium Mechanistic Aspects of Anticarcinogenic Action." *Biol. Trace Elem Res*, 1992, 33: 51- 62.
- [32]. Gilbert H. F, "Thiole/ Disulfide Exchange Equilibria and Disulfid Bond Theory." *Methods Enzymol.*, 1995, 251: 8- 28.
- [33]. kharitonor V. G., Sundquist A. R., and Sharma V. S. "kinetics of Nitric Oxide Autoxidation In Aqueous Solution." *J Biol Chem.* 1994, 269(8) 5881- 5883.
- [34]. Cassidy P. B., Edes K., Nelson C.C., Parsawar K. et al, "Thioredoxin Reductase Is Required for The Inactivation of Tumor Suppressor P53 and For Apoptosis Induced By Endogenous Electrophiles." *Carcinogenesis* 2006, 27(12): 2538- 49.
- [35]. Masaya Ueno., Hiroshi Masutani., Roberto Jun Arai., et al, "Thioredoxin-Dependent Redox Regulation of P53- Mediated P21 Activation." *J Biol Chem.*, 1999, 274(50): 35809-35815.
- [36]. Hansen J. M., Zhang.H., Jones D. P., "Mitochondrial Thioredoxins-2 Has a Key Role In Determining Tumor Necrosis Factor- Alpha-Induced Reactive Oxygen Species Generation, NF- Keppa B activation and Apoptosis" *Toxicol Sci* 2006, 91(2): 643- 50.