Comparative Evaluation of Zinc and Selenium Serum Levels of Cancer Patients in Misurata

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Abstract:
Cancer is one of the main problems facing the quality of human life and health services providers. Despite the causes of cancer are not totally defined, many factors are proposed to be carcinogenic or protectants. Zinc and selenium are considered as protectants because of their antioxidant activities. Therefore, Lower serum levels of zinc and selenium are expected in cancer patients than in healthy population.

This study is designed to compare the serum levels of (Zn, and Se) in cancerous and non-cancerous individuals.

To achieve the aim of the study, serum samples from 19 cancer patients and 23 healthy persons were collected, then samples were prepared and analyzed by Atomic Emission Spectrophotometer. Results were analyzed and graphed using Microsoft Excel and SPSS software, Student t- test was used to compare the means of cancer groups against control group.

Means of Serum zinc level in healthy individuals and cancer patients were 176.23 ppm and 101.75 ppm ($p < 0.05$) respectively, while serum selenium levels were 11 ppm and 3.36 ppm respectively ($p < 0.05$).

In conclusion, a significant difference in serum Zn and Se was found between the cancer patients and healthy individuals.

Key Words: Cancer, Selenium, Zinc.

Acknowledgment:

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Introduction:

I- Cancer.

Cancer is an idiopathic diseased condition of the cell or the tissue. It can be regarded as a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Cancers also can be defined as fetal complex genetic diseases could be caused by environmental factors [(1) (2)].

Cancer is an overwhelming global issue, it affects significantly the quality of life and has its health and financial consequences. For example, in 2008, 12.7 million of new cancer case where diagnosed worldwide [(3)].

II- Free radicals.

Free radical is term used to describe any unbound species (atom, molecule or ion) contains at least one unpaired electron and that is able to exist independently in the outer most orbital, this unpaired electron is said to be paramagnetic as it is attracted weakly to magnetic field [(4) (5)]. Free radical it is highly reactive and can be either accept an electron to or donate an electron from other molecules, therefore behaving as oxidant or reductant [(6)].

Excessive productions of free radical results in a phenomenon of excessive oxidative stress which causes a damage to lipid, protein and, DNA. This damage may contribute to cancer development [(6)]. Oxidative stress induces imbalanced redox regulation which leads to oncogenic stimulation. Moreover, DNA mutation is critical step in carcinogenesis. Elevated level of oxidative stress causes DNA lesion, this action has been noted in various tumor, therefore, mutagenesis through oxidative DNA damage is expected to be frequent in cancer development process [(7)].

III- Antioxidants.

The term antioxidant was used to describe some substances that neutralize free radicals, Antioxidants are also known as free radicals scavengers. The capability of antioxidants to stabilize free radicals -as is shown in figure 1-[(8)] before attack cells is critical to maintain optimal cellular and systemic health well-being. Therefore, many antioxidants are now prescribed for many goals like to decrease the damage to cells, to improve immune function and to lower

risks for infection, cardiovascular disease and cancers [9-11].

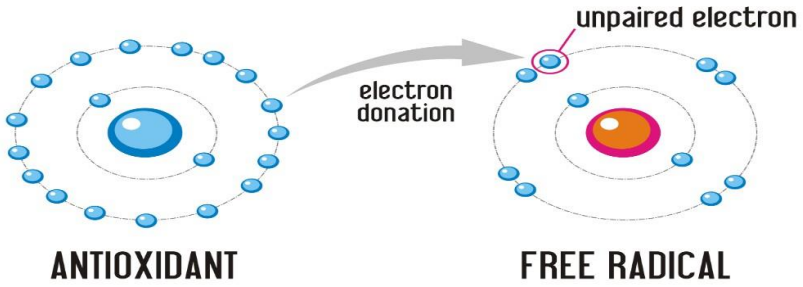

Figure (1): How antioxidants work? [8]

Antioxidants are divided into enzymatic and non-enzymatic. The non-enzymatic antioxidants refer to the substances that have the ability to react directly with radicals and accept the unpaired electrons. The term enzymatic antioxidants are used to describe some in vivo enzymatic systems that play a role in attenuating the oxidative stress and they called antioxidant enzymatic defense systems. As other enzymes, antioxidant enzymatic defense systems need some cofactors (minerals) like selenium, copper, iron and zinc for their activity. [3]. The enzymatic antioxidants can be synthesized in the body from the proteins and mineral presents in the food. Example of enzymatic antioxidant is superoxide dismutase (SOD), it exists in many forms, the form which used copper and zinc (Cu-Zn-SOD) is the most common in eukaryotic, glutathione peroxidase or catalase is another example of enzymatic antioxidants [12].

Trace elements like Se and Zn are useful to slow down the harmful cascade as they are needed as cofactors for the antioxidant enzymatic defense systems. The relation of some of Zn and Se to this topic will be highlighted in this article as they will be our research interest.

IV- Trace elements of interest.

- Zinc.

Zinc is essential trace element, it is present in all organs, tissues and body fluids. Total body zinc in adult human is nearly 1.5 – 2.0 grams and the physiological need of zinc in adult man is 1.4 mg/dL and 1.0 mg/dL in women [13 14]. Endogenous zinc has role in cytotoxic events in the cell, it influences apoptosis by acting on numerous molecular regulators of programed cell death. It is also essential for immune system. Human body has efficient mechanism both on cellular and systemic level to maintain homeostasis over board exposure rang of zinc.

In compare to several other ions with similar chemical properties zinc is relatively less harmful, only exposure to large dose of zinc has toxic effect. Interferes with copper uptake may result in long term and high doses supplementation can explain the toxic effect of high dose of zinc. Nevertheless, efficient regulatory and systemic homeostasis mechanism on cellular level prevents uptake of cytotoxic doses of exogenous zinc [13].

Deficiency in zinc has dramatic implication for immune function, it may associate with carcinogenesis and affect immunological development. Research suggests zinc as antioxidant reduce vision loss and the progression of age-related macular degeneration, by prevent cellular damage [14].

- **Selenium.**

Selenum is stored in body as selenocysteine in selenoproteins and accumulated in hair and nails. Selenium is crucial for glutathione peroxidase which acts with other antioxidants as free radicals scavenger [15, 16].

Daily intake of Se (100-200 mg) inhibit genetic damage and cancer development in human.in contrast, it hypothesized that proper intake of selenium may reduce oxidative damage that cause genomic instability [17].

In the in vitro system, Selenium in it is chemical form has important role in elicited cellular response [15]. Selenium has role in the protection against cancer development by inhibition of covalent DNA adducts formation that is caused by carcinogens that cause oxidative damage to lipid, DNA and protein. Selenium is also important for modulating cellular and molecular events that are critical in cell growth inhibition and in multi stages carcinogenesis process[17]. Moreover, epidemiologic evidence showed that low intake selenium is association with prostate cancer, prospective study of selenium supplementation to demonstrate 42% decrease in cancer disease [16].

From the above reviewed literatures, it is evident that certain metals such as Se and Zn possess potential antioxidant activity. It has been hypothesized that the concentration of beneficial trace element (Se, Zn,) is lower in the serum of cancer patients in comparison to non-cancerous ―normal‖ serum samples. The research question was: is there any significant differences in the serum level of Zn and Se among people with cancer and people having no cancer?

AIM:

This study was designed to compare the serum levels of Zn and Se in cancerous and healthy individuals. The significance of the difference among the tested groups, if found, will be discussed.

Materials and methods:

- **Standard Solution preparation.**

1. (10ppm) of standard stock solution was preparation (Se , Zn) in 50ml of deionized distal water.

2.Standard of (10ppm) dilution to (1ppm, 2ppm, 3ppm, 5ppm, 7 ppm)of (Se, Zn, As) in 25 ml of deionized distal water.

- **Sample collection and analysis.**

Nineteen cancer samples were collected from cancer patients at Misurata oncology institute and twenty-three samples were collected by Ibn Sena Private Labs from non- cancer individuals. For each sample; 3-4 ml of venous blood was collected in a plane tube.

- **Sample Preparation.**

After collecting, samples were centrifuged immediately for 4 minutes at the speed of 4000rps. Serum was separated and transferred into new clean plastic tubes by micropipette, stored in freezer and kept for the digestion step.

Later, the serum was removed from freezer, left about 1hour in room temperature, then some of the serum is added to a test tube and digested with 1.5ml of 1.5 % nitric acid and 10ml of deionized distal water, mixed about a minute by shaker.

The digested sample is filtered through 70mm filter paper then 0.22 micro meter fitter paper under vacuum pressure.

- **Sample Analysis.**

In last step, concentrations of Zn and Se in the prepared filtrate is measured by Atomic emission spectrophotometer and a proper dilution factors were used to point out the samples concentration from the instrument readings.

- **Statistical analysis.**

Excel 2016 was used to summarize and to graph the data, also excel was used to calculate the central tendency and dispersion parameters. Student t-test was used to compare the means of cancer and non-cancer groups; excel was used for descriptive analysis and to perform t-test. SPSS was used to draw boxplots.

The boxplot summarization is used in this research to summarize the results of trace element serum concentration in both cancer and control groups. The X-

axis represents the serum concentration in ppm. The lines inside the boxes represent the median, the borders of the boxes are the third and first quartiles while any value outside the whiskers regarded as outlier.

Results and Discussion:

- **Zinc Results.**

Table (1): Summary of Zinc serum concentration in cancer and control groups

	Control (ppm)	Cancer (PPM)
Mean	176.23	101.75
SD	126.01	121.23
Median	156.53	52.08
Max	631.24	476.67
Min	58.20	14.94
Upper outliers	One sample	Three samples
Lower outliers	None	None
Research hypothesis	Mean $_{control}$ □ Mean $_{cancer}$	
One tailed t-test result	0.029	
Interpretation of t-test results	As the results is smaller than 0.05, the difference between the two groups is significant	

From the above table and following figure, it can be seen that both SD are large in compare to the means of the groups and it was even higher than the mean in cancer group, which denotes for high variation within the both groups. Moreover, in cancer group, the difference between the mean and median is large, from the median of this group it can be concluded that 50% of members have their serum zinc concentration form 52.08 ppm or less, while higher means is obtained because of some extreme values that occur clearly in boxplots diagram., these extreme values might be explained by supplement intake as this information is not available for us.

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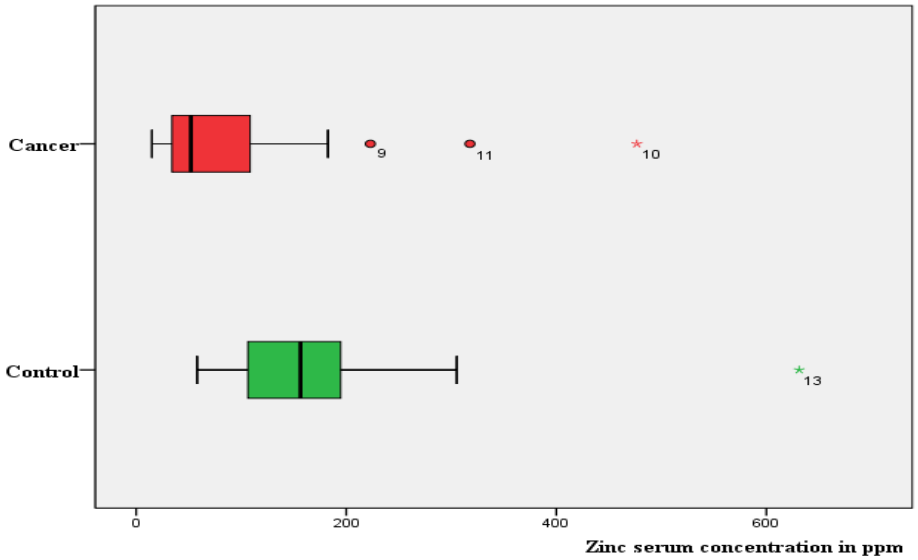

Figure (2): Boxplot summarization of serum zinc results

As hypothesized is this study, the mean of control group was larger than the mean of cancer group, and the difference between both means is regarded significant according to the result of t-test that was done at 95% significant level (p=0.05). However, other small case control studies showing no difference between zinc concentration in both cancer and control groups, in addition ecologic study was conducted in USA observed that countries with higher zinc intake are associated with increased risk for prostate cancer [(18)]. Despite of the above-mentioned studies that found opposite results, the majority of reviewed articles support our hypothesis, for example Elizabeth A and his colleagues observed that patient with reduced zinc level or intake are associated with certain cancer compared with control groups [(18)], moreover, study was done in Turkey found that serum zinc concentration is significantly lower in patients with prostate cancer compared to control. In summary, the results of this research support the hypothesis and are in agreement with most of reviewed article.

Selenium Results:

Table (3): Summary of Selenium serum concentration in cancer and control groups

	Control (ppm)	Cancer (PPM)
Mean	11.42	3.36
SD	13.98	3.45
Median	9.04	3.14
Max	44.04	11.01
Min	0	0
Upper outliers	None	None
Lower outliers	None	None
Research hypothesis	Mean $_{control}$ □ Mean $_{cancer}$	
One tailed t-test result	0.006	
Interpretation of t-test results	As the results is smaller than 0.05, the difference between the two groups is significant	

By revising table (2), it can be noticed that SD is consider high in compare to the means in both groups, so wide variation can be asserted in the both groups. However, the groups seem to be normally distrusted as the differences between mean and median are very small, this is also supported by the absence of extreme values as is seen in the figure (3). In other aspect, the difference between both groups is predominant, for example, 50% of control group members have their serum selenium above 9.04 ppm (the median of the group), in contrast, this was 3.14 ppm in cancer group. Moreover, the difference between the two means is also predominant, mean of the control was more than three folds higher than mean of the cancer group. The obtained results are expected and were hypothesized in this research, the difference between the means was significant according to t-test.

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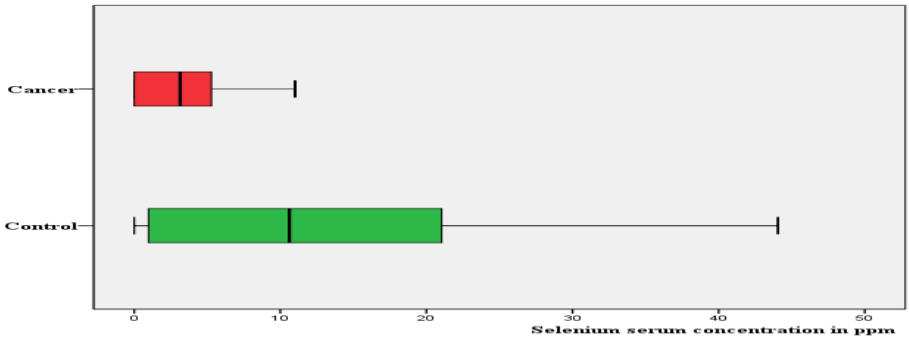

Figure (3): Boxplot summarization of serum selenium results

Although lower selenium level in cancer group was expected and found in this study, some other studies showed that higher selenium level is associated with cancer risk while another study doubted the relation between selenium and risk of cancer [19]. However, the majority of reviewed research consolidate our study hypothesis and findings. For example, several laboratory studies applied on the animal showed that Se has important roles in protection from cancer development [20], moreover, many ecological and prospective epidemiological studies showed a relation between low Se intake and increase cancer risk [20, 21], in addition, many research articles suggested that selenium reduce the risk of prostate cancer and may be inversely associated with colorectal, lung and stomach cancers [22]. In summary, as hypothesized, selenium level was significantly higher in control group and this was in consolidation with the majority of reviewed articles. This finding supports the expected role of selenium in cancer prevention.

Conclusion:

From previous results, we can conclude that selenium and zinc serum levels were lower in cancer patients than in healthy individuals, the difference between two groups was statistically significant ($P < 0.05$). This finding highlights the important role of excessive oxidative stress in cancer generation. More studies are needed to check if zinc and selenium supplements can help to protect from cancer.

تقييم مستويات الزنك والسيلينيوم في الدم لمرضى السرطان بمصراتة

الملخص:

السرطان أحد أكبر المشاكل التي تهدد الحياة البشرية وتشكل تحدي لأنظمة الرعاية الصحية. بالرغم أن أسباب نشوء السرطان غير محددة بدقة إلا أن هناك عديد من العوامل التي تصنف إما مسرطنة أو حامية من السرطان. يصنف عنصري الزنك والسيلينبيوم كمقاومين لنشوء السرطان بسبب دورهما في مضادة الأكسدة. لذلك يتوقع أن يكون تركيز العنصرين في الدم أقل في مرضى السرطان منه في الناس الأصحاء.

هذه الدراسة صممت لتقييم تركيز الزنك والسيلينيوم في دم مرضى السرطان مقارنة بالأصحاء من المرض.

لتحقيق هدف الدراسة جمعت 19 عينة دم من مرضى بالسرطان و23 عينة دم من ناس معافين من المرض. العينات جهزت وحللت بجهاز انبعاثات المطياف الذري، البيانات بوبت وحللت ورسمت باستخدام برنامج الإكسل والبرنامج الإحصائي SPSS، اختبار تي للطالب استخدم لمقارنة المتوسط الحسابي للمجموعتين ولتقييم أهمية الفروق بين المتوسطين.

كان المتوسط الحسابي للزنك للأصحاء 176.23 ppm ولمرضى السرطان 101.75 ppm وكان المتوسط الحسابي لعنصر السلسنيوم 11 ppm و3.36 ppm للمجموعتين على التولي. الفرق في حالة العنصرين كان ذا أهمية إحصائية ($p < 0.05$).

استنتجت الدراسة أن مستويات الزنك والسيلينيوم في الدم عند مرضى والسرطان أقل منها عند الأصحاء، الفرق كان جليًا وذا معنى إحصائي.

الكلمات المفتاحية: سرطان، زنك، سيلينيوم.

References

1- NCCN clinical practice guidelines in oncology: occult primary cancer of unknown primary. 2014; 16(12): 1091–1097.

2- Alison MR. Cancer [internet].London: Encyclopedia of life sciences.2001cited 2016 Jun 20]. Available from: http://web.udl.es/usuaris/e4650869/docencia/segoncicle/ genclin98/ recursos_classe_(pdf)/revisionsPDF/Cancer.pdf

3- American Cancer Society. The History of cancer [internet]. USA: American cancer society. [Cited 2016 Jun 29]. Available from: http://www.cancer.org/acs/groups/cid/ documents/ web content/002048-pdf

4- Caballero B, MD, PHD. Antioxidant nutrient: basic lecture note. Johns Hopkins University, Vic: johns Hopkins Bloomberg School of public health. 2015.

5- Perez JAM and Aguilar TAF. Chemistry of natural antioxidants and studies performed with different plants collected in Mexico. In: Gonzalez JAM. Oxidative stress and chronic degenerative diseases- A role for antioxidants. Mexico: Department of Environmental Systems Engineering at National School of Biological Sciences-National Polytechnic Institute; 2013 May 22. 59-85.

6- Young IS, Woodside JV. Antioxidant in health and disease. US National Library of Medicine National Institutes of Health. 2001 Mar; 54(3):176-86.

7- Dreher D, Junod AF. Role of oxygen free radicals in cancer development. European journal of cancer .1996 Jun; 32 (1): 30-38.

8- How Antioxidants Work On Free Radicals Damage [internet]. India: Indian health blog. 2014 May 23[cited 2016 Jun 23]. Available from: http://www.fitnessvsweightloss.com/ how-antioxidants-work-on-free-radicals-damage/

9- Dining services Factsheet. Antioxidants. US: UND the University of North Dakota Dining services factsheet [internet]. [Cited 2016 Jun 16]. available from: www.und.edu/student- life/dining/_files/docs/fact-sheets/antioxidants.pdf

10- Percival M. Antioxidant. Clinical nutrition insights. 1998; 1(96): 1-4.

11- Young IS, and Woodside JV. Antioxidant in health and disease. J clin pathol. 2001; 54(3): 176 – 186.

12- Qurtex Hawaii. Types of Antioxidants [internet]. US & Canada; Qurtex Hawaii: 2016 [cited 2016 July 20] .available from: http://www.nutrex-hawaii.com/search.aspx? Search=date+of+publication

13- Plum LM, Rink L, and Haase H. The essential toxin: impact of zinc on human health. int. J.Environ. res. Public health.2010; 7(4): 1342 – 1365.

14- Deshpande JD, joshi MM, a giri P. zinc: the trace element of major

importance in human nutrition and health. International Journal of Medical Science and Public Health. 2013 Feb; 2(1): 1-6.
15- Tapiero H, Townsend DM, and Tew KD. The antioxidant role of selenium and seleno- compounds. Biomed pharmacother.2003 May – Jun; 57(3-4):134 -144.
16- Reed A Berger MD. Trace Elements: basic lecture note. university of lowa, Vic: university of lowa.1998.
17- EL- Bayoumy K. The protective role of selenium on genetic damage and on cancer. National Institutes of Health. 2001 April 18; 475(1-2): 123 – 139.
18- Platz AE, Helzlsouer JK.Selenium, Zinc, and Prostate cancer. Epidemiologic reviews.2001; 23(1):93-101.
19- Karaman S, Mansuroglu B, Kizllbey, Derman S, Hazar BA. Selenium status in blood, urine, and hair samples of newly diagnosed of pediatric cancer patient. Turkish Journal of Medical Sciences.20015 April 45 (2):329-334.
20- Zhuo H, Smith HA, Steinmaus Selenium and lung cancer: A Quantitative analysis of heterogeneity in the current epidemiological literature. Cancer epidemiology, Biomarkers and prevention. 2004; 13(5):771-778.
21- Luty-frackiewicz A.The role of selenium in cancer and viral infection prevention. International Journal of Medicine and Environmental Health.2005; 18(4):305-311.
22- Approved public health committee .Cancer council Australia. Position statement: selenium [internet].Australia: Cancer council; 2006 May [2009July; cited 2016 Jun24]. Available from: http://www.cancer.org.au/content/pdf/CancerControlPolicy/PositionStatements/PS_Selenium_%20August_2005_Reviewed_July_2009.