



Original Research Article

Zinc as an auxiliary marker along with Cancer Antigen 125 in ovarian cancer

Ravindra Maradi^{1,*}, Nisha Abdul Khader², Kishan K³¹Dept. of Biochemistry, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, Karnataka, India²Dept. of Nephrology, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, Karnataka, India³Dept. of Physiology, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, Karnataka, India

ARTICLE INFO

Article history:

Received 07-11-2019

Accepted 08-11-2019

Available online 14-12-2019

Keywords:

Zinc

Ovarian cancer

Marker CA 125

serum CA 125

ABSTRACT

Ovarian cancer is the most common and most lethal gynecologic malignancy. It is one of the most leading cancers in Indian women. The urge for the early detection of ovarian cancer continues to be one of the most important issues in women's health care. The cause for ovarian cancer may be due to increased oxidative stress, heredity, dietary deficiency etc. As zinc is essential for cytotoxic/tumor suppressor effect and it plays very important role in cancer progression. In ovarian cancer the level of zinc in blood is usually decreased. So, the hypothesis of the study is to know the association between the serum zinc levels and the ovarian cancer marker, CA125. Aim of the study is to correlate the serum Zinc levels with serum CA 125 in healthy controls and ovarian cancer subjects.

Totally 90 subjects are enrolled in the study. Out of this 74 were controls and 16 were ovarian cancer subjects. The serum CA 125 was increased in ovarian cancer patients compared to controls. Whereas, serum Zinc was decreased in ovarian cancer patients compared to controls. As well as there was indeed a strong association of serum Zinc and CA 125 in ovarian cancer patients.

Hence measurement of serum Zinc, an inexpensive assay could be used as an auxiliary marker along with CA 125 in screening of ovarian cancer.

© 2019 Published by Innovative Publication. This is an open access article under the CC BY-NC-ND license (<https://creativecommons.org/licenses/by/4.0/>)

1. Introduction

Cancer can be defined as a cluster of diseases which involves the alterations in the expression and status of multiple genes that bring together a survival advantage and undiminished proliferative ability to somatic or germinal cells.¹ Mutations primarily occur in three main classes of genes (proto) oncogenes, tumor suppressor, and DNA repair genes; all these together contribute to the development of cancer genotype and phenotype. These mutations can be caused by physical mutagens like X-rays or ultraviolet rays, chemical mutagens like reactive oxygen species, biological agents like viruses and bacteria. These mutated cells attain the ability to resist the natural and inherent death mechanisms of the cell, namely apoptosis which is coupled with impaired regulation of cell proliferation. Tumor markers are the

biochemical entities which indicate the presence of tumors. In clinical perspective, it is a molecule found in body fluids. These are the measurable entities that are associated with malignancies.¹ They are produced primarily by the malignant cells or they can also be produced by other tissues in response to the tumors. They are then released into circulation and thus can be detected and measured in blood. They are produced at levels which are directly proportional to the degree of the differentiation and mass of the tumor. They are hence not used as the primary modalities for diagnosing the cancer but rather are used in clinical practice to support the diagnosis and prognosis.^{1,2}

Cancer antigen 125 or carbohydrate antigen 125 abbreviated as CA-125 is a glycoprotein of molecular weight 200-2000 kDa.³⁻⁵ It is the largest membrane bound protein expressed on the surface of the cell that is subjected to metaplastic transformation to mullerian- type

* Corresponding author.

E-mail address: dravimaradi@gmail.com (R. Maradi).

epithelium or added into body fluid in soluble form.⁴ The normal level of CA -125 is 0-35 U/ml in blood.⁶ Studies have reported its elevation in malignant conditions, like ovarian cancer, breast cancer, mesothelioma, non-Hodgkin lymphoma, gastric cancer, leiomyoma and leiomyosarcoma of gastrointestinal origin. Elevation is also seen in certain benign conditions such as endometriosis, pregnancy, menstruation, liver diseases and congestive heart failure, in addition to all these it is also raised in infectious disease, tuberculosis.^{4,7} It plays a crucial role in advancement and proliferation of tumor by various mechanisms. It acts by immune system evasion, metastatic invasion, induced motility, chemotherapy resistance. Hence it is the most frequently studied tumor marker for early detection of ovarian carcinoma. It is both used in diagnostics and prognostics. Nevertheless, recently it is used as a tool in diagnosis and prognosis of ovarian cancer. Hence CA-125 testing is not specific because it can give rise to false positive results.⁸ This test is not sensitive too, as every ovarian cancer patient does not show elevated CA-125 level.⁹

Zinc is the multipurpose trace element. It is of utmost importance, required for the normal functioning of the cells. It is required to regulate the immune system as well as to direct the cellular effects resulting in the regulation of gene expression, bioenergetics, metabolic pathways, signal transduction and cell invasion. It plays a key role in cell division, cell growth, wound healing, it also acts as a cofactor for various structural and functional proteins.¹⁰ Unlike adults, infants, children, adolescents, pregnant, and lactating women have increased demand for zinc and thus, these are vulnerable to zinc deficiency.¹¹ It has its unique role in chronic diseases such as cancer, diabetes, depression, Wilson's disease, Alzheimer's disease, and other age-related diseases.¹² Gastrointestinal, epidermal, central nervous, skeletal, reproductive systems are the organs most affected clinically by zinc deficiency.¹³ Hence in ovarian cancer, zinc deficiency is appreciated.¹⁴ Alterations in zinc homeostasis contribute to the severity of the cancer.¹⁰ The prolonged deprivation of zinc renders a person more susceptible to injury induced by oxidative stress. Zinc is known to be a very important component of DNA binding proteins with zinc fingers. It is also a component of copper/zinc superoxide dismutase and several proteins involved in DNA repair. Hence it plays pivotal role in the functions of transcription factor, antioxidant defense system and DNA repair.¹⁰

This study is conducted to know the association of zinc with CA 125. Hence this study may aid in the cancer screening or remission.

As zinc is essential for cytotoxic/tumor suppressor effect and it plays very important role in cancer progression. In ovarian cancer the level of zinc in blood is usually decreased. So, the hypothesis of the study is to know the association between the serum zinc levels and the ovarian

cancer marker, CA125.

Aim of the study is to correlate the serum Zinc levels with serum CA 125 in healthy controls and ovarian cancer subjects.

2. Objective of the Study

1. To compare CA 125 in controls and in ovarian cancer group.
2. To compare serum Zinc in controls and in ovarian cancer group.
3. To correlate serum Zinc with CA 125 in controls.
4. To correlate serum Zinc with CA 125 in ovarian cancer group.

3. Materials and Methods

3.1. Study design

Prospective, cross sectional study.

3.2. Study period

January 2018- August 2018

3.3. Sample size

A sample size of 80 is required to estimate the correlation at 90% power with 5 % of level of significance.

3.4. Study centre

Department of Biochemistry, Kasturba Medical College Manipal, Manipal Academy of Higher Education.

3.5. Ethical statement

The study was carried out after obtaining approval from the Institutional Ethics Committee of Kasturba Hospital, Manipal (IEC: 130/2018).

3.6. For collection of blood sample

Anonymized left over samples of the serum came for the Clinical Biochemistry department for the estimation of CA 125

3.7. Inclusion and exclusion criteria

3.7.1. Inclusion

All patients in the age groups of 20 -60 years for whom CA 125 was requested for testing were included in the study.

3.7.2. Exclusion

Patients with diarrhea, Wilson's disease, sickle cell disease, chronic liver disease, chronic kidney disease and pregnancy.

3.8. Estimation of serum zinc

3.8.1. Method

Zinc Kit (Colorimetric method)

3.8.2. Principle

In the alkaline medium zinc reacts with Nitro-PAPS. A purple colored complex is formed. The amount of zinc present in the sample is directly proportional to the intensity of the color formed by the complex. The absorbance of which is measured at 575 nm.

Zinc + Nitro-PAPS → P Purple colored product.

3.9. Calculations

$$\text{Zinc in } \mu\text{g/dl} = \frac{\text{Abs. T}}{\text{Abs. S}} * 200$$

3.10. Estimation of CA125

3.10.1. Method

The electrochemiluminescence immunoassay “ECLIA” is intended for use on Elecsys and cobase immunoassay analyzers.

3.10.2. Principle

This method is based on Sandwich principle.

3.11. Assay procedure

3.11.1. 1st incubation

A sandwich complex is formed by mixing 20 μL of the test sample, a biotinylated monoclonal CA 125-specific antibody, and a monoclonal CA 125-specific antibody labeled with a ruthenium complex.

3.11.2. 2nd incubation

After addition of streptavidin- coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

The reaction mixture is then aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Results are determined via calibration curve which is instrument- specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

4. Results

In Table 2 CA 125 and serum Zinc showed significant difference across control and ovarian cancer.

As per Table 3, serum Zinc showed a negative correlation with CA 125 in control group. And the correlation is significant.

5. Discussion

In the present study, the research population was made up of 90 participants in total; 74 in control group and 16 in ovarian cancer group. The study found that CA125 was significantly higher in ovarian cancer group when compared with control (Table 2). In previous study, Bastet al. has also shown the elevated levels of CA 125 in ovarian cancer patients.⁴ Some authors suggested the cause for the elevated levels of serum CA 125 in ovarian carcinoma was due to a proteolytic event which catalyzes the shedding of CA 125 from the tumor cell surface, whereupon this shed ectodomain could be measured in the circulating blood. The membrane type-1 metalloproteinase is upregulated in ovarian cancer and this enzyme is responsible for the proteolytic cleavage of CA 125 from the tumor cell surface.¹⁴ Hence the level of serum CA 125 is increased in ovarian cancer.

Whereas, serum Zinc was found to be significantly higher in control group as compared to ovarian cancer group (Table 2).

The present study showed a mild negative correlation between serum zinc and CA 125 in the control group (Table 3), which means as CA 125 increases serum Zinc level decreases. Study by Das et al. revealed that the intra-membrane cleavage of CA125 is by an enzyme site-2 protease.¹⁵ This enzyme cleaves it from the membrane and eventually CA 125 is released into the blood. Site-2 protease is an intra-membrane metalloprotease which is zinc dependent.¹⁵ And hence as level of CA 125 increases, there is increased synthesis of site-2 protease which is zinc dependent and hence intracellular level of zinc decreases.¹⁶ This results in increased uptake of zinc from the blood which decreases the level of zinc in the blood.

In the present study the serum Zinc was significantly decreased in ovarian cancer compared to control [Table 4] and also serum Zinc showed a strong negative correlation with CA 125, which means as serum Zinc decreases the CA 125 level in the serum increases. The reason for decrease in serum Zinc level may be due to SLC39A4 (ZIP4) transporter, which is upregulated in ovarian cancer.¹⁷ ZIP4 is a cell membrane zinc transporter which helps in the uptake of serum Zinc. Hence when ZIP4 is upregulated serum zinc levels may decrease. ZIP4 is also reported to be upregulated in pancreatic cancer. ZIP4, in pancreatic cancer regulates the cancer cell growth by activating IL6-/STAT3 pathway via zinc finger transcription factor CREB.¹⁸ This results in increased levels of IL-6 in the serum. Studies have reported that serum levels of IL-6 are high in patients with ovarian cancer. Hence it is possible that a similar mechanism may be involved in ovarian cancer cell growth. The low levels of zinc may also be due to RSF1 (Remodeling and Spacing Factor 1) which is also upregulated in ovarian cancer.¹⁹ Overexpression of RSF1 gene stimulates cell proliferation and transforms non neoplastic cells to neoplastic cells. RSF1 is also involved in

Table 1: Assay procedure

| Addition sequence | Blank (ml) | Standard (ml) | Test (ml) |
|-------------------|------------|---------------|-----------|
| Working reagent | 1.0 | 1 | 1.0 |
| Distilled water | 0.05 | - | - |
| Zinc standard | - | 0.05 | - |
| Sample | - | - | 0.05 |

Table 2: Comparison of CA 125 and serum Zinc among control, ovarian cancer

| Variable | Control (n =94) Median(Q ₁ , Q ₃) | Ovarian cancer (n =16) Median(Q ₁ , Q ₃) | *p Value |
|----------|--|---|---------------------|
| CA 125 | 15.15(8.82, 46.52) | 371.35(58.55, 974.0) | <0.001 [#] |
| ZINC | 72.00(48.75, 96.0) | 30.00(10.83, 50.0) | <0.001 [#] |

*Mann-Whitney test

Significant

Table 3: Correlation of serum Zinc with CA 125 in control group

| Variables | | r Value | *p Value |
|-----------|--------|---------|---------------------|
| Zinc | CA 125 | -0.484 | <0.001 [#] |

*Spearman's correlation

-significant

Table 4: Correlation of serum Zinc with CA 125 in ovarian cancer group

| Variables | | r Value | *p Value |
|-----------|--------|---------|--------------------|
| Zinc | CA 125 | -0.706 | 0.002 [#] |

*Spearman's correlation

-significant

the development of chemo resistant ovarian cancer by acting as a positive regulator of NF- κ B induced gene expression.²⁰ RSF1 is a Zinc containing protein and the upregulation of this protein leads to increased need for zinc.¹⁹ Hence the serum levels of zinc may decrease due to the uptake of zinc by the cancerous cells. Previous studies have also shown that cytoplasmic copper/zinc superoxide dismutase (Cu/Zn SOD) is also overexpressed in ovarian cancer.^{21,22} Superoxide dismutase is an important enzyme involved in cancer progression since it neutralizes the effect of Reactive oxygen species (ROS), hence preventing ROS induced apoptosis. Overexpression of this enzyme increases the need for zinc and hence the serum levels of zinc decreases.²³

To conclude, though this study was carried out on a small group of ovarian cancer patients has indeed revealed that there exists a difference in the distribution of zinc between control and ovarian cancer group and also there exists a strong association of serum Zinc and the tumor marker CA125. Hence measurement of serum Zinc, an inexpensive assay could be used as an adjunct marker along with CA 125 in screening of ovarian cancer.

6. Source of funding

None.

7. Conflict of interest

None.

References

1. Nagpal M, Singh S, Singh P, Chauhan P, Zaidi MA. Tumor markers: A diagnostic tool. *Natl J Maxillofac Surg.* 2016;7(1):17–20.
2. Virji MA, Mercer DW, Herberman RB. Tumor markers in cancer diagnosis and prognosis. *CA Cancer J Clin.* 1988;38:104–126.
3. Doubeni CA, Doubeni AR, Myers AE. Diagnosis and Management of Ovarian Cancer. *Am Fam Physician.* 2016;93(11):937–944.
4. Scholler N, Urban N. Ca125 in ovarian cancer. *Biomark Med.* 2007;1(4):513–523.
5. Fritsche HA, Bast RC. CA 125 in ovarian cancer: advances and controversy. *Clin Chem.* 1998;44:1379–1380.
6. Gubbels JA, Felder M, Horibata S. MUC16 provides immune protection by inhibiting synapse formation between NK and ovarian tumor cells. *Mol Cancer.* 2010;9:11.
7. Isaksson S, Jönsson P, Monsef N, Brunnström H, Bendahl PO, et al. CA 19-9 and CA 125 as potential predictors of disease recurrence in resectable lung adenocarcinoma. *Public Lib Sci.* 2017;12(10):186284.
8. Nossov V, Amneus M, Su F, Lang J, Janco JM, et al. The early detection of ovarian cancer: from traditional methods to proteomics. Can we really do better than serum CA-125? *Am J Obstet Gynecol.* 2008;199:215–223.
9. Ferrini R. Screening asymptomatic women for ovarian cancer: American College of Preventive Medicine practice policy. *Am J Prev Med.* 1997;13(6):444–446.
10. Dhawan DK, Chadha VD. Zinc: a promising agent in dietary chemoprevention of cancer. *Indian J Med Res.* 2010;132(6):676–682.
11. Roohani N, Hurrell R, Kelishadi R, Schulin R. Zinc and its importance for human health: An integrative review. *J Res Med Sci.* 2013;18(2):144–157.
12. Chasapis CT, Loutsidou AC, Spiliopoulou CA, Stefanidou ME. Zinc and human health: an update. *Arch Toxicol.* 2012;86(4):521–534.

13. Franklin RB, Costello LC. The important role of the apoptotic effects of zinc in the development of cancers. *J Cell Biochem.* 2009;106(5):750–757.
14. Bruney L, Conley KC, Moss NM, Liu Y, Stack MS. Membrane-type I matrix metalloproteinase-dependent ectodomain shedding of mucin16/ CA-125 on ovarian cancer cells modulates adhesion and invasion of peritoneal mesothelium. *Biol Chem.* 2014;395:1221–1231.
15. Das S, Batra SK. Understanding the Unique Attributes of MUC16 (CA125): Potential Implications in Targeted Therapy. *Cancer Res.* 2015;75(22):4669–4674.
16. Feng L, Yan H, Wu Z, Yan N, Wang Z, et al. Structure of a site-2 protease family intramembranemetalloprotease. *Sci.* 2007;318:1608–1612.
17. Fan Q, Cai Q, Li P, Wang W, Wang J, Gerry E. The novel ZIP4 regulation and its role in ovarian cancer. *Oncotarget.* 2017;8:90090–90107.
18. Zhang Y, Bharadwaj U, Logsdon CD, Chen C, Yao Q, Li M. ZIP4 Regulates Pancreatic Cancer Cell Growth by Activating IL-6/STAT3 Pathway via Zinc Finger Transcription Factor CREB. Clinical cancer research : an official. *J Am Assoc Cancer Res.* 2010;16(5):1423–1430.
19. leM S, Sheu JJ, Santillan A, Nakayama K, Yen MJ, Bristow RE. Amplification of chromatin remodeling gene, Rsf-1/HBXAP, in ovarian carcinoma. *Proc Natl Acad Sci.* 2005;102(39):14004–14009.
20. Yang YI, Ahn JH, Lee KT, Shin IM, Choi JH. RSF1 Is a Positive Regulator of NF-B-Induced Gene Expression Required for Ovarian Cancer Chemoresistance. *Cancer Res.* 2014;74(8):2258–2269.
21. Swaminathan S, Gangadaran P, Venkatesh T, Gosh M. Association between serum copper and tumor markers CEA and CA 125. *J Pharm Biomed Sci.* 2011;9(15):2230–7885.
22. Hu Y, Rosen DG, Zhou Y, Feng L, Yang G, Liu J. Mitochondrial manganese-superoxide dismutase expression in ovarian cancer: role in cell proliferation and response to oxidative stress. *J Biol Chem.* 2005;280(47):39485–39492.
23. Hileman EA, Achanta G, Huang P. Superoxide dismutase: An emerging target for cancer therapeutics. *Expert Opin Ther Targets.* 2001;5(6):697–710.

Author biography

Ravindra Maradi Associate Professor

Nisha Abdul Khader Research Scholoar

Kishan K Assistant Professor

Cite this article: Maradi R, Khader NA, Kishan K . Zinc as an auxiliary marker along with Cancer Antigen 125 in ovarian cancer. *Int J Clin Biochem Res* 2019;6(4):601-605.