ORIGINAL RESEARCH PAPER

Biochemistry

KEY WORDS: cervical cancer, copper, oxidative stress.

COPPER IN CERVICAL CANCER : PRO-OXIDANT ROLE.

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ABSTRACT

Background: Cervical cancer, the third most common cancer among women in the world. Copper plays a pivotal role in the oxidant-antioxidant mechanism with imbalance leading to increased susceptibility to oxidative damage and formation of free radicals, can interact directly with the bases of DNA at the sites of G-C causing inactivation or loss of certain tumour suppressor genes thereby commencement and/or progression of carcinogenesis. Hence, its involvement in the pathophysiology of Cx Ca and its associated complications can be overviewed.
Aims & objectives- To estimate serum copper levels in patients with cervical cancer and compare with healthy subjects.
Materials & methods : The study was conducted on 50 histopathologically confirmed cases of Cx Ca and 50 healthy subjects, within the age group of 25-65 years. Serum copper was estimated by modified spectrophotometric micro-method using guanidine hydrochloride.
Results: Significantly ($p<0.001^{**}$) increased levels of serum Copper in cases of cervical cancer as compared to controls.
Conclusion: Cervical cancer patients have a raised serum copper levels, which may play role in the pathophysiology of cervical cancer and its associated complications.

Aim: To study the role of copper in pathogenesis of Cervical cancer.

Objectives: To estimate serum level of serum copper in patients with cervical cancer and compare it with healthy subjects.

Main terms: Copper, oxidative stress & cervical cancer.

METHODOLOGY

Source of data: This study was a duration based case control study for a period of one year conducted on histopathologically proven cases of cervical cancer visiting the department of Oncology & Gynaecology, at Vydehi Institute of Medical Sciences & Research Centre, Bangalore and age, matched healthy women as controls visiting the hospital for routine health check up and hospital staff members.

Sample size: The sample size comprised of 50 cases and 50 controls.

Inclusion criteria:
Controls: Healthy females within the age group of 25-65 years.

Cases: Histopathologically proven cases of cervical cancer patients, in any stage of ca aged between 25 to 65 years.

Exclusion criteria:
- Subjects who had any pathophysiological condition such as severe cardiovascular,respiratory diseases, diabetes mellitus , neurological and psychiatric disorders, and renal disorders
- Subjects on any concomitant medication such as oxidants vitamins, minerals.
- Cigarette smokers and alcoholics.
- Any such concurrent or past history.

Method of sample collection and preparation: Prior to collection of samples, ethical committee clearance was taken and an informed consent was taken from cases and controls and there was no financial liability on them.

- Patients and controls baseline data, clinical findings, basic investigation report was obtained on a pre-structured proforma. 5 mL of venous blood samples was collected from median cubital vein by venipuncture avoiding hemolysis into an evacuated vaccum tube. Samples were centrifuged after 30 minutes at 3000 rpm for 10 minutes.
- The samples were alliquoted and kept at -20° C until analysis was done. All the analysis was carried on serum samples.
- Serum copper was estimated by modified spectrophotometric micro-method using guanidine hydrochloride and bathocuprinedisulphonate disodium salt.

RESULTS

Study Design: A Comparative case-control study with 50 controls and 50 cases was undertaken to study the levels of serum copper among age matched cases and controls .

The age group was between 25-65 years. The mean age in Ca Cx patients was 43.98±6.38 yrs and in controls, it was 31.56±6.84.

Table 1 : Age distribution of patients.

Age in years	Cases		Controls	
	No	%	No	%
20-30	1	2.0	25	50.0
31-40	14	28.0	18	36.0
41-50	25	50.0	7	14.0
51-60	10	20.0	0	0.0
Total	50	100.0	50	100.0
Mean ± SD	43.98±6.38		31.56±6.84	

The above table shows, samples were matched according to their age. Maximum number of cases, 50% were in the age group of 41-50 yrs followed by 28% patients in 31-40 yrs. The mean age in cases was 43.98±6.38 yrs and in controls, it was 31.56±6.84 yrs.

Table 2 : Comparison of Serum-Copper in two groups studied

Copper (micrograms/dl)	Cases (n=50)		Controls (n=50)		P value
	No	%	No	%	
<70	3	6.0	2	4.0	<0.001**
70-140	8	16.0	45	90.0	
>140	39	78.0	3	6.0	

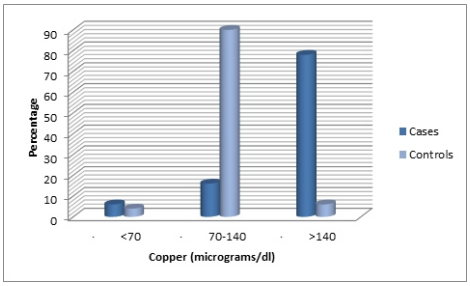

Fig1: Bar diagram showing Sr. Copper in study groups.

Figure 1 and table 2 shows comparison of serum copper levels in both cases and controls. The normal level of serum copper level is 70-140 µg/dl. 78% of cases had copper levels above 140 µg/dl, 6% of the control levels had copper levels above 140 µg/dl. 16% of cases and 90% of controls had copper levels between 70-140 µg/dl.

Copper levels are significantly more associated with cases with P< 0.001**.

Introduction:

Cervical cancer (Cx Ca) the third most common cancer among women in the world, was responsible for 275,000 deaths in 2008, 88% of which occurred in developing countries and 159,800 in Asia[1]. Cx Ca is the leading cause of cancer mortality in India and accounts for 17% of all the cancer deaths among women aged 30 to 69 years. At current incidence rates , the annual load of new cases in India is expected to increase to 225,000 by 2025[2].

Cx Ca will occur in approximately 1 out of 53 Indian women during their lifetime compared with 1in100 women in more developed regions of the world[3]. Cx Ca rates among women in the 30–64 age group decreased by 1.8% per year on average but still accounted for 16% of the total female cancer burden .Over the years from 1983–1987 to 2003–2007 there was a fall in the percentage of all Cx Ca from 43.8% to 37.6%[4]. In 2010, there were nearly 74,000 new cases of Cx Ca in India. Approximately 38% of cases are seen in women in age group of 15 to 49 years , which has adverse social and economic impact of cervical cancer on families and communities[3]. India marks the highest age standardized incidence of cervical cancer with South Asia at 22, Bangladesh 19.2 , 13 in Sri Lanka, and 2.8 in Iran[4]

Cervical cancer is as a result of Human Papilloma Virus (HPV) that is spread through sexual intercourse, in most cases the male is a carrier of the papilloma virus but it infects and generates in females. In spite of the risks of the HPV virus both males and females are unaware of this infection and its accompanying risks[5]. HPV is involved in Cx Ca by damaging DNA, but recent data reveal role of oxidative stress in Cx Ca[6]. The shift of balance towards pro-oxidants in the Prooxidant-antioxidant system is called as Oxidative stress. The decreased levels of antioxidants also induces generation of free radicals, leading to DNA damage, causing dysfunction and disease[6]Severe oxidative stress is not only known to cause DNA damage and mutations of tumour suppressor genes, which account for the initial events in carcinogenesis, but can also play an important role in the promotion of multi-step carcinogenesis[8].

Copper plays a pivotal role in the oxidant-antioxidant mechanism. The imbalance of Cu leads to increased susceptibility to oxidative damage .Copper acts as an pro-oxidant and may be involved in formation of free radicals, catalysed by metal[9].Copper can interact directly with the bases of DNA at the sites of G-C [6]. In vitro, the addition of copper to DNA mediates extensive DNA base damage inducing more mutations[6]. Copper also elaborates other free radical species such as OH, therefore, the inactivation or loss of certain tumour suppressor genes can lead to the commencement and/or progression of carcinogenesis. The elevation in copper levels may be due to movement of copper from tissue to serum [6].

The present study is carried to estimate serum copper levels in patients with cervical cancer and compare it with healthy subjects, in order to have additional information about the role of copper in pathogenesis of disease which can later help in monitoring , treatment & prognosis of cervical cancer.

Discussion:

Copper is an important trace element that is associated with number of metalloproteins. It is present in biological systems in both the 1^+ and 2^+ valence states.

Copper (atomic number 29. relative atomic mass 63.54) has Cu^+ and Cu^{2+} oxidation states in biological systems. The easy exchange between these ions gives the element important redox properties. Because of their high electron affinities these ions are the most strongly bound to organic molecules of all the essential trace metals. For example, copper in biological material is complexed with proteins, peptides, and other organic ligands. An elaborate series of binding and transport proteins inside cells protects the genome from copper generated free radical attack. Copper is both a pro-oxidant and an antioxidant[79].

In our study we obtained a significant (p<0.001**) increase in serum copper level (152.96±32.88) in cases as compared to controls (104.88±24.45).

Our results of high serum copper in cervical cancer patients are also in agreement with the study of Ralph et al who found increased level of serum copper in Ca Cx subjects when compared to the healthy controls[85]. According to Ralph et al , there is increase in serum copper in cancer patients, and the reasons for this increase in serum copper levels among cancer patients are not known. It may result from increased liver production of copper-containing ceruloplasmin as an inflammatory response to the cancer or from a tumour-induced decrease in catabolism of the serum ceruloplasmin[85].

Similarly, Chakarvarthy et al found that serum copper level(SCL) was elevated in presence of tumour, and in patients not responding to treatment and those with relapse, it remained constantly higher[82] . So he stated that SCL may provide an effective means of evaluating the extent of the disease and is of value in estimation of prognosis after therapy.

Our findings also corroborate with the result of Cruzhi et al[84] who also reported increase in copper levels in cervical cancer patients. The increased copper level could be related to the fact that copper is needed to form new blood vessels and because cancer needs new blood vessels in order to grow (American cancer society, 2011). Hence, increase in copper level in cervical cancer patients.

Our results of high serum copper in cervical cancer patients are also in agreement with the study of Naidu et al who found increased level of serum copper in cervical cancer subjects when compared to the normal healthy females in reproductive age group[6].

However, our results are in disagreement with the study of Wong et al, who reported that there was no change in Sr. Cu level in cervical cancer patients[99].

Copper is both a pro-oxidant and an antioxidant[77]. Copper in its free, unbound form catalyses the production of various toxic free radicals. Elevated copper levels have the potential to produce a relatively continuous supply of free radicals ROS formed within cells are highly reactive and they are able to oxidize most of the biomolecules within the cell, leading to tissue injury and cancer. ROS have been associated for many years with oncogenesis, only recently a new role is emerging for ROS as mediators of signalling pathways leading to cell proliferation and tumour initiation and promotion.

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