A STUDY OF SERUM MDA & TAC IN PATIENTS OF CERVICAL CANCER

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ABSTRACT

BACKGROUND

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 Asia¹ (Ferlay J et al 2010). An oxidant-antioxidant imbalance and its involvement in the pathophysiology of Cx Ca and its associated complications can be overviewed.

Aims & objectives- 1. To estimate serum level of total antioxidant capacity and MDA levels in patients with cervical cancer and compare it with healthy subjects. 2. To find correlation between serum total antioxidant capacity and MDA levels in cases of cervical Ca.

MATERIALS & METHODS

The study was conducted in 100 subjects out of which 50 were histopathologically confirmed cases of Cx Ca and 50 were healthy subjects (controls), within the age group of 25-65 years. The quantitative estimation of serum MDA was done by Satoh K method and that of Sr. TAC by Ferric Reducing Ability of Plasma (FRAP) according to the method of Benzie et al. Chi-square/Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups. Pearson correlation between serum TAC & MDA used to find the degree of relationship.

RESULTS

Our study found a significantly ($p < 0.001^{**}$) increased levels of serum MDA, and significantly ($p < 0.001^{**}$) decreased levels of serum TAC in cases as compared to controls. Our study also revealed a significant positive correlation between serum MDA & TAC ($r = 0.026$, $p < 0.001^{**}$) among patients of cervical cancer.

CONCLUSION

The patients of cervical cancer with altered antioxidant capacity and lipid peroxidation may play a contributory role in the pathophysiology of cervical cancer and its associated complications.

KEYWORDS

Cervical Cancer, TAC, MDA, FRAP.

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BACKGROUND

Cervical cancer is the third most common cancer among women in the world, 88% of which occurred in developing countries. Cervical cancer is the leading cause of cancer mortality in India, accounting for 17% of all 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 2025² (Suneeta Krishnan et al 2013). Cx Ca will occur in approximately 1 out of 53 Indian women during their lifetime compared with 1 in 100 women in more developed regions of the world³ (D. Yin, et al 2011). India marks the highest age standardised incidence of cervical cancer with South Asia at 22, Bangladesh 19.2, Sri Lanka 13, and 2.8 in Iran⁴ (Aswathy Sreedevi, et al 2015).

The associated morbidity, mortality and the cost to society due to Cx Ca, has made it important public healthcare challenge.

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The objective of identifying risk factors, early diagnosis and treatment is to reduce the associated morbidity and mortality. 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 virus 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 risks⁵ (Roland KB, et al 2009).

HPV is involved in Cx Ca by damaging DNA, but recent data reveal role of oxidative stress in Cx Ca. The shift of balance towards pro-oxidants in the Prooxidant-antioxidant system is called as Oxidative stress. The decreased levels of antioxidants also induce generation of free radicals, leading to DNA damage, causing dysfunction and disease⁶ (Naidu MSK, et al 2007).

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 carcinogenesis⁷ (Ahmed MI et al, 1999).

The ROS produced by several factors initiate lipid peroxidation and leads to excessive levels of MDA, which alters the cellular function and leads to cancer formation⁸ (Geum Ju Lee et al, 2005).

The rise in MDA concentration could be due to higher generation of ROS due to the increased oxidative damage

generated in uterine cancer patients. The oxygen radical production increases with clinical progression of diseases and involves increased lipid peroxidation. As a result of this, there are cellular membrane degeneration and DNA damage⁹ (Pejic, et al 2006). The rise in the levels of MDA indicate excessive lipid peroxidation as a consequence of the increase in free radical generation. These free radicals may cause marked alterations in the function of the cell membrane and also structural organisation of DNA, hence causing mutations, thus it can be stated that lipid peroxidation product is one of the possible causes of uterine cancer progression⁹ (Pejic, et al 2006). The total antioxidant capacity (TAC) instead of measuring the antioxidant capacity of a single compound, measures the antioxidant capacity of whatever antioxidants in the biological sample. TAC can be used as a reliable biomarker for diagnosis, prognosis and prevention of large number of diseases¹⁰ (C. Cervellati, et al 2014).

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

Type of Study

Case control study, with duration between Jan. 2014-Dec. 2014.

Objectives of the Study

- To estimate serum level of MDA & total antioxidant capacity levels in patients with cervical cancer and compare it with healthy subjects.
- To find the correlation between serum total antioxidant capacity and MDA, in cases of cervical ca.

MATERIALS & METHODS

This study was a duration based case control study for a period of one year (January 2014 – December 2014) 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 checkup and hospital staff members. Total 50 cases of cervical cancer & 50 healthy controls were selected.

An ethical clearance and an informed consent from the study subjects were duly obtained.

Criteria for Selection of Cases

Histopathologically proven cases of cervical cancer visiting the Department of Oncology & Gynaecology, at Vydehi Institute of Medical Sciences & Research Centre, Bangalore. All the cases identified during the period were selected as cases.

Criteria for Selecting Controls

Healthy females within the age group of 25-65 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.

Sample size was calculated using software G* Power. Minimum sample size required was 50 per group with effect size 0.6, level of significance 5% and power 80%.

Blood samples were collected and analysed for serum MDA by Satoh K method¹¹ (Satoh K, 1978) and TAC by FRAP assay¹² (Benzie IF and Strain JJ, 1996).

Statistical Analysis

Student t test (Two-tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups (Intergroup analysis) on metric parameters.

Chi-square/Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups.

Pearson correlation between serum MDA & TAC in controls & cervical cancer cases was performed to find the degree of relationship.

The statistical software namely SPSS 15.0, and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables, etc.

Classification of Correlation Co-efficient (r)

Up to 0.1	: Trivial Correlation.
0.1-0.3	: Small Correlation.
0.3-0.5	: Moderate Correlation.
0.5-0.7	: Large Correlation.
0.7-0.9	: V. Large Correlation.
0.9- 1.0	: Nearly Perfect correlation.
1	: Perfect correlation.

Significant Figures

+ Suggestive significance (P value: 0.05<P<0.10)

* Moderately significant (P value: 0.01<P < 0.05)

** Strongly significant (P value: P<0.01).

RESULTS

Table 1 shows age distribution in the study groups where most of the cases were in the age group of 41-50 years.

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	

Table 1. Age Distribution of Patients Studied

In table 1 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.

Variables	Cases (n=50)		Controls (n=50)		P value
	No	%	No	%	
Total Antioxidant Capacity (μmol/L)					
<875	37	74.0	3	6.0	<0.001**
875-1345	13	26.0	45	90.0	
>1345	0	0.0	2	4.0	
Malondialdehyde (nmol/mL)					
<1.05	2	4.0	21	42.0	<0.001**
1.05-2.00	11	22.0	25	50.0	
>2.00	37	74.0	4	8.0	
Table 2. Comparison of Serum TAC and MDA in Two Groups Studied					

Table 2 shows comparison of serum levels TAC in the study groups where there was a statistically significant ($p<0.001^{**}$) decrease in the mean serum TAC level in cases ($781.36 \pm 228.88 \mu\text{mol/L}$) as compared to controls ($1088.94 \pm 185.07 \mu\text{mol/L}$); and a statistically significant ($p<0.001^{**}$) increase in the mean serum MDA level in cases (2.72 ± 1.01) compared to controls ($1.17 \pm 0.52 \text{ nmol/mL}$).

	Cases		Controls	
	r value	P value	r value	P value
Total Antioxidant Capacity vs. Malondialdehyde	-0.278	0.050*	0.158	0.272
Table 3. Pearson Correlation of Serum TAC & MDA				

Table 3 shows Pearson correlation of serum TAC & MDA in the study groups, moderately significant negative correlation was found between serum TAC with, MDA ($r = -0.278$, p -value: 0.050^{*}) in cases.

DISCUSSION

Cervical cancer is the third most common cancer among women in the world, 88% of which occurred in developing countries. Cervical cancer is the leading cause of cancer mortality in India, accounting for 17% of all cancer deaths among women aged 30 to 69 years. The associated morbidity, mortality and the cost to society due to Cx Ca, has made it important public healthcare challenge. The objective of identifying risk factors, early diagnosis and treatment is to reduce the associated morbidity and mortality.

Our study was designed to evaluate and compare the serum levels of TAC & MDA in histopathologically confirmed cases of cervical cancer and normal healthy female subjects. In the present study, we obtained statistically a significant ($p<0.001^{**}$) decrease in serum total antioxidant capacity 781.36 ± 228.88 in cases as compared to controls (1088.94 ± 185.07).

Similar results were found by Senem et al who found that antioxidant status is altered in patients with cervical cancer compared to healthy controls¹³ (Senem Demirici, et al, 2011). According to Chung et al, the cervical intraepithelial neoplasia was also associated with lower blood antioxidant capacity levels¹⁴ (Chung HW, et al, 2004).

Kim et al also found that cervical intraepithelial neoplasia was also associated with lower blood antioxidant capacity levels⁸ (Geum Ju Lee, et al 2005).

Under physiological conditions, the free radicals are rapidly neutralised by antioxidants. In oxidative stress, these free radicals are not counterbalanced adequately and remain in excess to cause damage by reacting with nucleic acids, proteins, polyunsaturated fatty acids and carbohydrates. Oxidative damage is implicated in the pathogenesis of several medical disorders viz. atherosclerosis, cancer, arthritis and neurodegenerative disorders¹⁵ (KS Rathan Shetty, et al, 2015). DNA damage and anomalous DNA repair mechanisms are key factors in the pathogenesis of cancer. Hence, investigators have evaluated the association between a low antioxidant level and various malignant and premalignant conditions¹⁵ (KS Rathan Shetty, et al 2015).

The other finding of our study was significant increase ($<0.001^{**}$) in Serum MDA levels (2.72 ± 1.01) in cases compared to controls ($1.17 \pm 0.52 \text{ nmol/mL}$).

Similar results were observed by J Grace Nirmala et al, who estimated MDA in patients with cervical cancer to estimate the extent of lipid peroxidation, and found increased circulating levels of MDA in them, and attributed it to increase in oxidative stress due to deficiency of antioxidant mechanism. Grace et al noticed elevated lipid peroxidation and decline in enzymatic antioxidant status in Ca Cx patients¹⁶ (Grace Nirmala, et al, 2011).

Naidu et al also found increased levels of MDA and stated it as a possible cause of Cx Ca progression⁶ (Naidu MSK, et al 2007).

Same results of elevated MDA levels were found in patients with cervical cancer compared to a control group by Senem Demirici et al, who stated oxidative damage leads to the formation of products like MDA and DNA damage may lead to mutagenesis, carcinogenesis and cell death¹³ (Senem Demirici et al, 2011).

These results were also in agreement with other investigations done by Pejic et al, 2006, which showed an increase in concentration of lipid peroxidation products, as markers of oxidative stress whereas the level of antioxidants decreased in uterine cancer patients. The rise in MDA concentration could be due to increased generation of ROS due to the excessive oxidative damage generated in uterine cancer patients, oxygen radical production, which increases with clinical progression of diseases, involves increased lipid peroxidation, as a result of which there are cellular membrane degeneration and DNA damages⁶ (Naidu MSK, et al 2007).

CONCLUSION

The present findings demonstrate the imbalance in levels of serum total antioxidant capacity & malondialdehyde among the patients of cervical cancer in comparison to healthy controls. These changes may play an important role in the pathogenesis and progression of cervical cancer by the involvement of these parameters in exaggerated oxidative stress. Estimation of serum TAC & MDA can be helpful in knowing the oxidative stress excess and decreased antioxidant defence system involvement in the pathophysiology of cervical cancer, prognosis, response to treatment and associated complications.

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