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Effect of Radiotherapy on Oxidative Stress and Serum Adenosine Deaminase Levels in Carcinoma Cervix Patients

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ABSTRACT

Carcinoma cervix is the most prevalent genital tract cancers in the world including India. Low levels of antioxidants induce the generation of free radicals leading to DNA damage and further mutations. Alterations in enzyme levels like adenosine deaminase (ADA), an enzyme of the purine salvage pathway, has been found to be elevated in various malignancies. The present study was done to evaluate the oxidative stress & adenosine deaminase (ADA) levels in carcinoma cervix(CaCx) patients. Malondialdehyde(MDA), glutathione-s-transferase(GST) & adenosine deaminase(ADA) levels were estimated before and after radiotherapy with Cobalt 60(Co60) in 20 patients. They were compared with age and sex matched healthy controls. Significantly increased levels of MDA ($p<0.001$) was observed, whereas the activity of GST ($p<0.001$) and ADA ($p<0.05$) were significantly decreased in CaCx patients as compared with healthy controls. Upon radiotherapy a significant reduction in MDA levels, with a significant increase in GST levels was observed. Though ADA levels were found to be increased after radiotherapy it is not significant. Thus it may be concluded that oxidative stress is present among CaCx patients, and radiotherapy was found to decrease the oxidative stress in CaCx patients..

Keywords: *Cacinoma cervix (CaCx), Radiotherapy, Malondialdehyde (MDA), Glutathione-S-Transferase (GST), Adenosinedeaminase (ADA), Cobalt 60 (Co60)*

INTRODUCTION

Cervical carcinoma is a global non communicable public health problem, and it is the second most common cancer leading to increased mortality of women worldwide[1]. According to World Health Organization, in India approximately 1,34,420 women are diagnosed with the disease every year, and of them 72, 825 die (WHO 2010) [2]. Cervical carcinoma is a multi factorial disease process with several risk factors which include, early coitus, multiple sex partners, low socioeconomic status & Human papilloma virus infection [3]

During infection free radicals are abundantly produced by which the antioxidant compounds are depleted. A state of oxidative stress develops in which immune cells are continuously activated and tissue damage occurs [4]. These oxygen free radicals which are produced cause lipid peroxidation and generate MDA, Protein alteration, nucleic acid damage, ultimately resulting in cell death[5]. The imbalance between the pro-oxidants and antioxidants in favor of pro-oxidants is called oxidative stress which is assessed by the lipid per-oxidation product MDA[8].

Enzymes such as Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx) are considered to be the primary antioxidant enzymes, as they are involved in the direct elimination of active oxygen species. Secondary antioxidant enzymes Glutathione-S-transferase (GST) help in the detoxification of Reactive oxygen species (ROS) by decreasing peroxide levels or by maintaining a steady supply of metabolic intermediates glutathione reductase(GR) for the primary antioxidant enzymes[6]. Antioxidants have been shown to inhibit initiation and promotion in carcinogenesis, and counteract cell immortalization and transformation [20]. Many studies have shown a link between altered expression of GST in plasma as well as in tissue biopsy samples of CaCx [7].

T lymphocytes contain the enzyme adenosine deaminase (ADA) which is widely distributed in human tissues, a major enzyme in the purine metabolism where it degrades either adenosine or 2-deoxyadenosine producing inosine or 2`-deoxyinosine. The purine nucleoside adenosine is produced at increased levels in cancerous tissue due to local hypoxia. In cancer there is an increased turnover of malignant cells and an increase in nucleotide metabolism leading to an increase in purine metabolizing enzyme ADA [9]. Many studies have demonstrated alterations of ADA activity in the tumor tissue and serum in patients with lung, head and neck, breast and ovarian cancer [23].

Radiotherapy is an important modality of treatment in CaCx. In this study serum MDA, GST and ADA levels were estimated pre and post radiotherapy in CaCx patients.

MATERIALS & METHODS

The present study was conducted on 20 women with CaCx stage-II aged between 35 to 65 years, registered at department of Radiotherapy Sri Venkateswara Ram Narayana Ruya Government General Hospital, and Sri Venkateswara Medical college, Tirupati. The control group comprised of 20 age matched healthy women. The patients were given external radiation using Cobalt 60 at a dosage of 50 GY in 25 sittings for a period of 3

months.Written consent was obtained from each of the participants included in the study.

5ml of fasting (8-12 hrs) venous blood was collected under aseptic condition from the controls and cases (before and after 3 months of radiation treatment), placed into plain tubes & allowed to clot. Serum was separated by centrifugation at 3000 rpm for 10 min. at room temperature. The samples were stored at -4^{o}C until analysis

Lipid per-oxidation products, serum MDA was determined spectrophotometrically as the measure of thio-barbituric acid reactive substances (TBARS) [(10)]

Glutathione-S-transferase activity was measured spectrophotometrically by using l, chloro2, 4 dinitrobenzene as the substrate (CDNB) [(11).]

Adenosine deaminase (ADA) estimation was done by sensitive colorimetric method described by Guisti [(12)].

The bio chemical parameters were compared statistically between the cases & controls by testing the differences in the means by using students't' test (unpaired). The differences in the mean between the cases (pre & post radio therapy) were tested using students t test (paired). A p value < 0.05 was considered as statistically significant.

RESULTS

The results of MDA, GST & ADA in controls and also in CaCx patients before and after radiotherapy (with Co 60) are presented in the Tables 1-3 and Figures 1-3.

Table-1 Mean & S.D. values of various biochemical parameters in cases before radiotherapy when compared to controls

S.No.	Parameters	Mean ± S.D.		p-Value
		Cases (n=20) (before radiotherapy)	Controls (n=20)	
1	MDA(nmol/L)	5.69 ± 1.61	3.34 ± 0.93	<0.001
2	GST (IU/L)	36.84 ± 17.17	72.73 ± 19.33	<0.001
3	ADA(IU/L)	12.18 ± 4.54	17.2 ± 4.84	<0.05

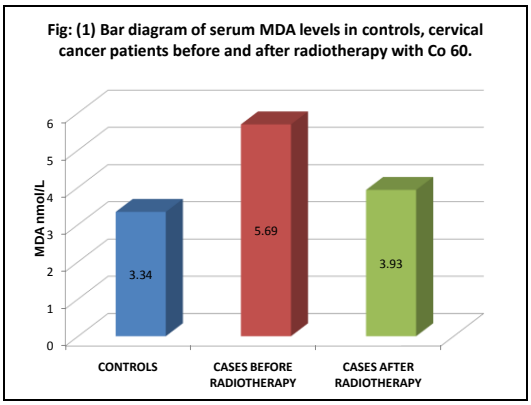
Table- 2 Mean & S.D. values of various biochemical parameters in cases after radiotherapy when compared to controls

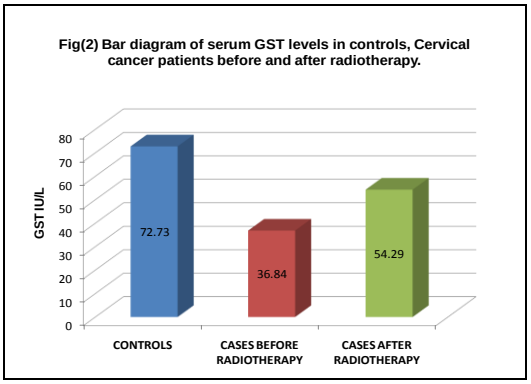
S.No.	Parameters	Mean & S.D.		p- Value
		Cases (n=20) (After Radiotherapy)	Controls (n=20)	
1	MDA(nmol/L)	3.93 ± 1.39	3.34 ± 0.93	<0.01
2	GST (IU/L)	54.29 ± 12.61	72.73 ± 19.33	<0.01
3	ADA(IU/L)	14.59 ± 4.64	17.2 ± 4.84	<0.05

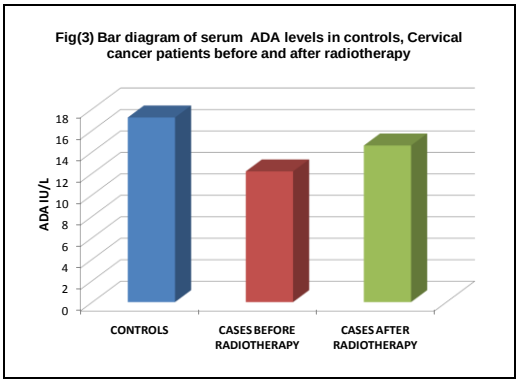
Table-3 Mean & S.D. values of various biochemical parameters in cases before & after radiotherapy

S.No.	Parameters	Mean & S.D.		p-Value
		Cases (n=20) (Before Radiotherapy)	Cases (n=20) (After Radiotherapy)	
1	MDA(nmol/L)	5.69 ± 1.61	3.93 ± 1.39	<0.01
2	GST (IU/L)	36.84 ± 17.17	54.29 ± 12.61	<0.01
3	ADA(IU/L)	12.18 ± 4.54	14.59 ± 4.64	NS

NS- Not Significant







The MDA levels were found to be significantly elevated ($p<0.001$) in CaCx patients when compared with controls. Upon Co60 radiotherapy these levels significantly decreased ($p<0.01$) in the CaCx patients when compared to pre radiotherapy levels. However the post radiotherapy MDA levels were still significantly greater than the healthy controls.

The GST levels was found to be significantly decreased ($p<0.001$) in CaCx patients when compared to controls. Upon radiotherapy these levels increased significantly when compared to ($p<0.01$) that of CaCx patients before radiotherapy.

The ADA levels were decreased ($P<0.05$) in CaCx patients when compared to controls. After radiotherapy though the ADA levels are increased when compared with pre radiotherapy levels the increase in not significant.

DISCUSSION

Oxidative stress caused by increased free radical generation and (or) a decreased antioxidant level in target cells and tissues play an important role in carcinogenesis [(14)].

In our study oxidative stress was evaluated in terms of lipid peroxidation products MDA levels, and antioxidant enzyme activity GST levels.

The mean MDA levels of study group were significantly increased when compared with the controls [(1),(13),(15),(16)] Increased levels of MDA indicate upsurge lipid peroxidation which is a consequence of free radical generation. It causes profound alterations in the function of the cell membrane and also structural organization in the DNA, leading to mutations [(17)].

As radiotherapy reduces the tumor size, the levels of oxidation products were decreased significantly when compared to CaCx subjects [(13)]. Therefore it can be stated that lipid peroxidation is one of the possible causes of CaCx progression.

GST activity was also significantly decreased in the study group when compared with Controls [(7),(18),(19) (22)]. GST uses reduced glutathione as a substrate and plays an important role in protection against xenobiotic compounds. It is involved in the detoxification of electrophilic toxins and carcinogens and is the main enzyme involved in the glutathione redox cycle [(21)]. Insufficient antioxidant potential induce the generation of free radicals leading to DNA damage & further mutations[(1)]. Alterations in circulating antioxidants & free radical scavengers have been linked with various epithelial malignancies[(7)]. Increased lipid peroxidation products, decreased anti-oxidant enzyme activities clearly shows that oxidative stress plays a significant role in the pathogenesis of CaCx.

Mean serum ADA levels are decreased in our study group when compared to controls. After radiotherapy though the mean ADA level is increased when compared with the pre-radiotherapy level, the increase is not significant.

Thus from our study it may be concluded that oxidative stress has a significant role in the pathogenesis of CaCx patients. Radiation therapy increases the level of anti oxidants and protects from oxidative damage and thus increases the immune status of CaCx subjects.

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