A Study of Role of Free Radical Injury in Genital Malignancy

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Summary:Twenty one cases of cancer cervix, 8 of ovarian cancer and 7 of cancer corpus and 22 normal matching control cases were included in the study. Malonaldehydle MDA which is a by-product produced during lipid peroxidation was estimated in blood and cervical, ovarian and endometrial tissue of cancer and control cases to determine the free radical injury in genital cancers in comparison to normal cases. MDA level were elevated to 0.47 ± 0.47 in cervical cancer to 0.6 ± 0.39 in carcinoma endometrium and to 0.86 ± 0.31 in ovarian carcinoma in comparison to 0.1 ± 0.2, 0.1 ± 0.18 and 0.9 ± 0.12 in normal cervix endometrium and ovary respectively. Same changes were observed in the serum. Levels were elevated in cancer cases with low haemoglobin, low socio-economic status, multiparae and cases with low body weight indicating co-relation of nutritional status to free radical injury.

Introduction:

The role of free radicals can be traced back to the origin of life on earth, when 3-5 billion years ago the basic chemical components of life were produced by free radical reaction with the help of solar radiation.

The recognition of organic free radical by Gomberg in 1900 led inexitably to the speculation that free radicals may be involved in living system.

Recently, free radicals have been implicated in most of human diseases including myocardial infarction, renal failure, septicaemia and carcinogenesis.

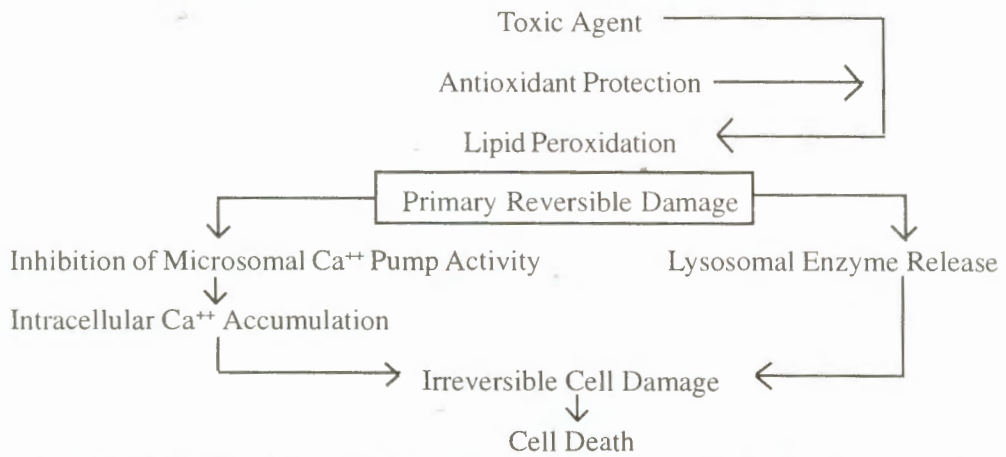
Free Radicals are found to be involved in both initiation and promotion of multistage carcinogenesis. These highly reactive compounds can act as initiater and/or promoter, cause DNA damage, activate procarcinogens and alter cellular antioxidant defense system.

They also stimulate lipid peroxidation by attacking the fatty acid side chain membrane phospholipids specially at areas of several bonds as in arachidonic acid. (Fig. 1).

Fig 1 shows proposed sequence of events leading to cellular death after lipid peroxidation (Young and Segers 1999).

There have been reports on study of free radical injury in relation to oral cancer and colon cancer but literature is scanty on study of free radical injury in relation to genital cancers. Cervical cancer is one of the most common of all genital cancers. It is believed that the natural history of the cervical cancer starts with development of mild dysplasia referred as CIN I and passes through sta;

Fig. 1: Connection between LPO and Tissue Damage



moderate and severe dysplasia referred to as CIN II and III and then carcinoma in situ. Up to this stage changes are considered reversible with the degree of reversion decreasing or increasing with severity of the condition. Further progression to invasive carcinoma is irreversible.

The current hypothesis of natural history of cervical cancer postulates that unknown carcinogenous as well as exogenous stimuli act over extended period of time frame. If action of these stimuli can be prevented, progression of neoplasia can be stopped.

With this aim, the study has been planned to critically evaluate indirect evidence of free radical injury to epithelial tissue in cervical, endometrial and ovarian cancer by estimating (MDA) malonaldehyde.

Material and Method

Study included 21 cases of cancer cervix, 8 cases of ovarian cancer, 7 cases of cancer corpus and 22 normal matching controls. The control cases were those of dysfunctional uterine bleeding for cancer cervix and ovarian cancer and prolapse uterus for endometrial cancer. Samples of cervical tissue, ovarian tissue and endometrial tissue for matching control with carcinoma cervix, ovarian cancer and endometrial cancer was removed from healthy cervix of DUB case, ovary of women recovered for benign condition after abdominal hysterectomy and endometrium from cases of prolapse uterus before hysterectomy

A detailed history was taken and systemic, abdominal and vaginal examination were done. Cervical biopsy was done for cancer cervix cases in OPD, endometrial curettings for carcinoma corpus and ovarian biopsy were done in O.T. during diagnostic D & C and laparotomy. Part of biopsied tissue was sent for histopathology and part along with blood sample was sent to laboratory at 4°C temperature for MDA estimations.

MDA Estimation

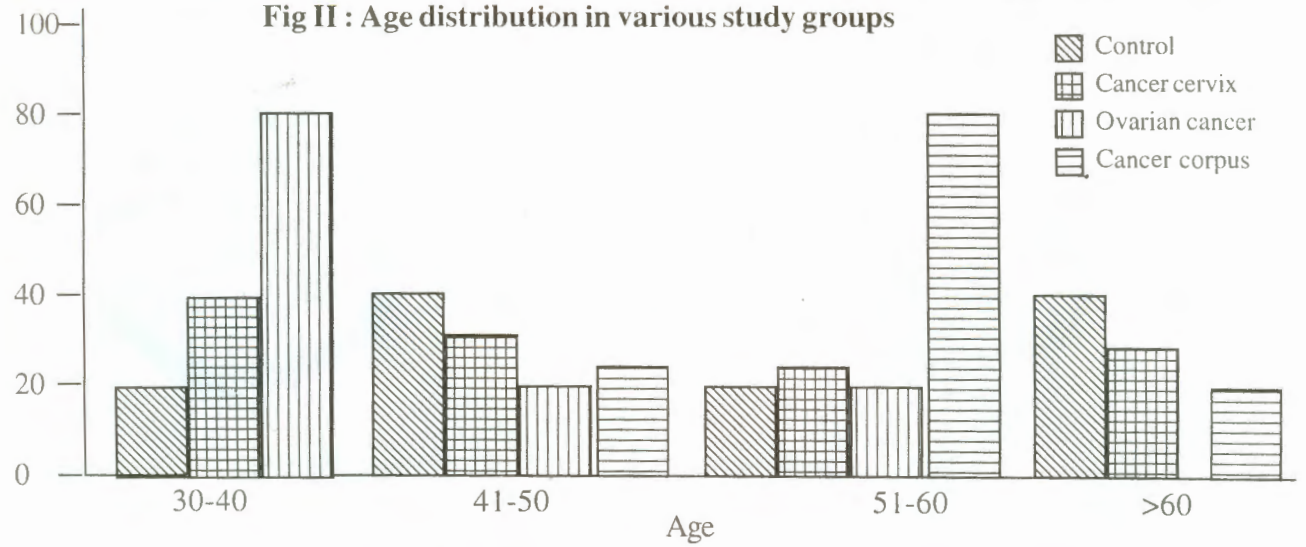
It was measured as MDA/TBA reactive material photometrically. The basis of the test is that TBA reacts with MDA (Thiobarbituric acid), (Gutteridge & Halliwell, 1990) a secondary oxidation product of polyunsaturated

Table 1:
Age distribution in various study groups

Age	Control	%age	Cancer cervix	%	Ovarian cancer	%	Cancer corpus	%
30-40	4	18.18	7	33.33	6	75	-	-
41-50	9	40.91	6	28.57	1	12.5	1	14.29
51-60	3	13.64	4	19.05	1	12.5	5	71.43
>60	6	27.27	5	19.05	-	-	1	14.29

≤0.001

Fig II : Age distribution in various study groups



acid, having 3 or more double bonds. MDA forms a stable pink chromatophore with TBA with an absorption maxima at 535 nm by PMQ II spectrophotometer. Five percent homogenate of the tissue was made by mixing it with 0.1 N acetate buffer and centrifuging it. Then 0.2 ml of supernatent was taken and incubated in a water bath at 37°C for 90 minutes. The sample was then mixed with 2 ml of 67% of TBA and heated on boiling water bath for 10 minutes. The pink colour that developed was read at 535 nm in PNI II spectrophotometer and optical densities were noted. Extinction coefficient of E1 cm = 1.56 x 105 cm2 mol-1 was used to measure the amount of MDA produced.

Observation and Results

Table I shows age distribution in various study groups. It shows that malignancies were more common in 5th decade. (Fig.2)

Table II shows that majority of cases were from low income group 91.5%. (Fig.3)

Table II
Percentage distribution of socioeconomic status in the study groups

Socioeconomic group	Cases	%age
Low Income group	33	91.67
Middle Income group	3	8.33

$p < 0.001$

Table III shows percentage distribution of parity in the study group.

Maximum number of patients were from rural population and were with parity more than 3. (Fig.4)

Table IV shows Hb% taken as representative of nutritional status and respective MDA levels as representative of free radical damage. It shows that MDA levels were higher in patients with Hb% less than 8 gms in comparison to non-anaemic patients with cancer.

Table V shows comparative profile of mean MDA levels in relation to nutritional status. The finding in Table V coordinate well with Table IV observations. It shows that in cases with lower than prescribed normal weight according to Life Insurance Corporation Table, had 13 times higher oxidant stress in cancer tissue as compared to normal tissue while those with overweight showed lower values.

Table VI shows comparative profile of mean MDA levels in various study groups of cancer cervix, cancer uterus and cancer ovary.

Table VI shows that average MDA levels taken as representative of free radical damage are seen to be much higher in cases with carcinoma than in controls in all types of genital cancers and the levels are statistically significant when compared with controls in both serum and cancerous tissues. (Fig. 5, 6, 7)

Fig 3 : Percentage distribution of socioeconomic status in the study groups

Table III

Percentage distribution of parity in the study group

Parity	Number	Urban		Rural	
		No.	%	Number	%
P0	3	2	66.6	1	33.3
P 1-2	7	4	57.1	3	42.9
P ≥ 3	26	8	30.7	18	69.3

Table IV

Correlation of Hb% to free radical levels

Hb%	Average MDA levels incases
<8%	0.98 ± 0.12
8.1 – 9 g%	0.65 ± 0.42
9.1 – 10 g%	0.56 ± 0.41
10.1 – 11 g%	0.31 ± 0.38
>11 g%	0.60 ± 0.56

Fig 4 Percentage distribution of parity in the study groups

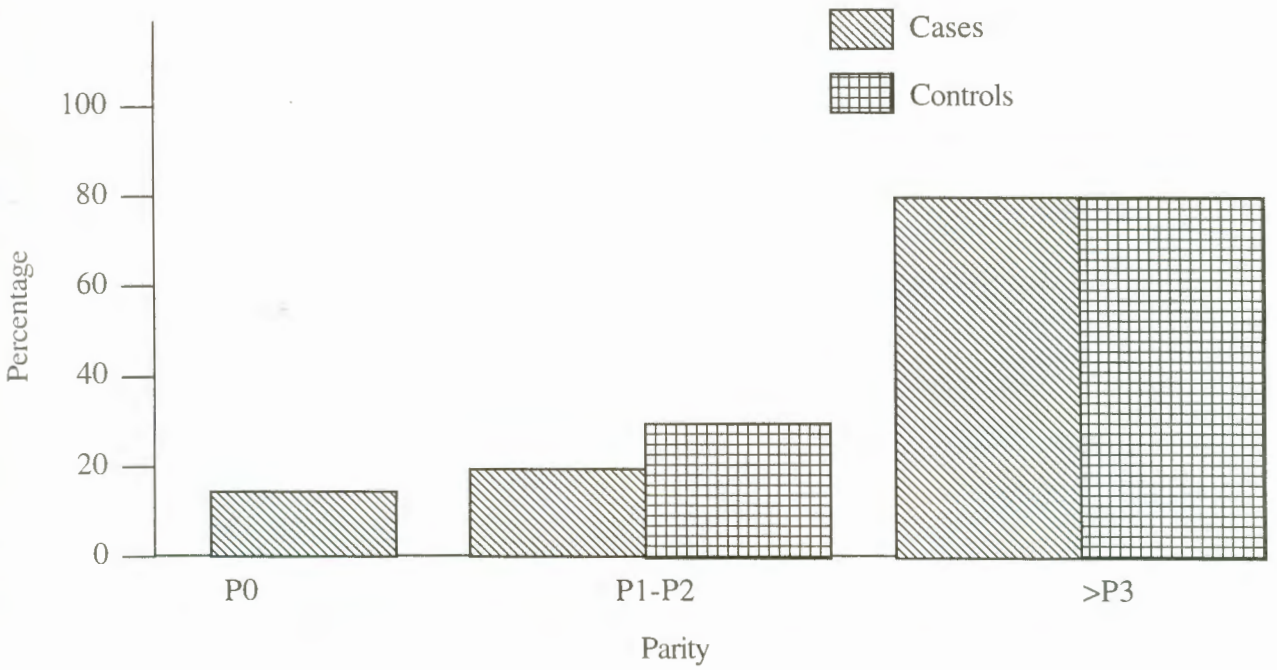


Table V Comparative profile of nutritional status and free radical levels in the study groups

	Tissue MDA levels		Serum MDA levels	
	Control	Cancerous tissue	Control	Cancerous tissue
Over weight group	0.11 ± 0.22	0.54 ± 0.43	0.11 ± 0.22	0.48 ± 0.40
Under weight group	0.05 ± 0.01	0.64 ± 0.46	0.13 ± 0.18	0.60 ± 0.44

$p < 0.05$; $p < 0.001$

Table VI Comparative profile of mean MDA levels in various study groups

	Study Group	No. of Cases	Average MDA level	
	Cases		**Serum**	**Tissue**
1a.	CA Cx	21	0.45 ± 0.45	0.47 ± 0.47
2a.	CA Ut. Corpus	7	0.53 ± 0.35	0.6 ± 0.39
3a.	Ovarian CA	8	0.75 ± 0.30	0.86 ± 0.31
	Controls			
1b.	Cervical tissue	22	0.12 ± 0.21	0.1 ± 0.2
2b.	Endometrial tissue	22	0.12 ± 0.21	0.1 ± 0.18
3b.	Ovarian tissue	9	0.24 ± 0.29	0.09 ± 0.12

Fig 5 Comparitive profile of mean MDA levels (Cervical tissue)

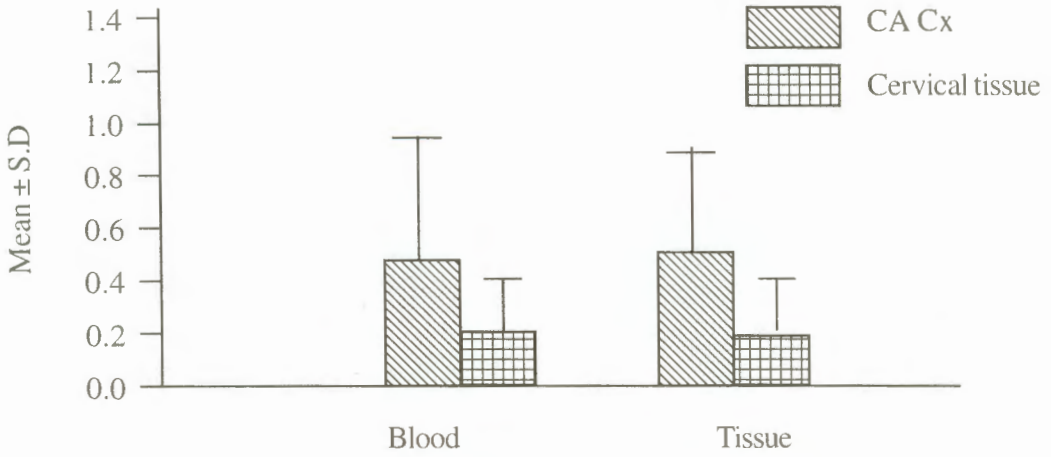


Fig 6 Comparitive profile of mean MDA levels (Endometrial tissue)

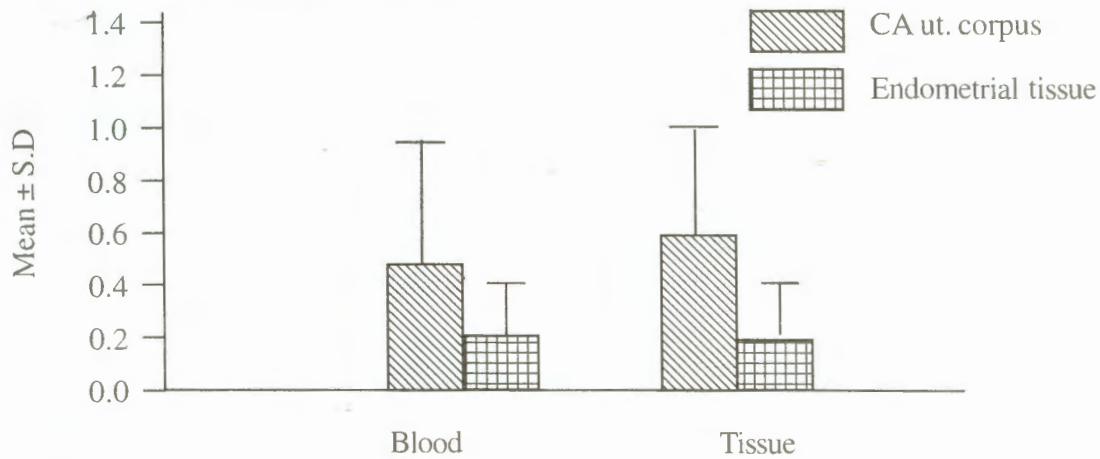


Fig 7 Comparitive profile of mean MDA levels (Ovarian tissue)

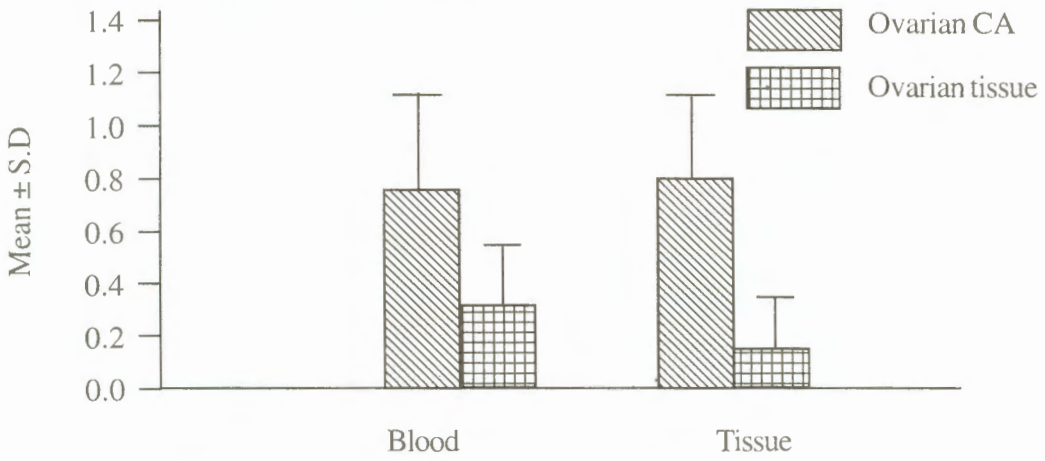


Table VII Comparative profile of mean MDA levels in various stages of cancer cervix

Clinical Stage	Average MDA levels	
	Blood	Tissue
IB	0.963 ± 0.487	0.11 ± 1.06
II A	0.075	0.074
II B	0.89 ± 0.39	0.89 ± 0.39
4. III A	0.42 ± 0.4	0.54 ± 0.51
5. III B	0.58 ± 0.053	0.60 ± 0.55
6. IV A	1.035	1.034

Table VIII

Comparative profile of average MDA levels and histopathological grading in cancer cervix

	HPE	Average MDA levels
1.	Non Keratinizing squamous cell cancer	0.89 ± 0.39
2.	Keratinizing squamous cell cancer	0.33 ± 0.40

$p < 0.05$

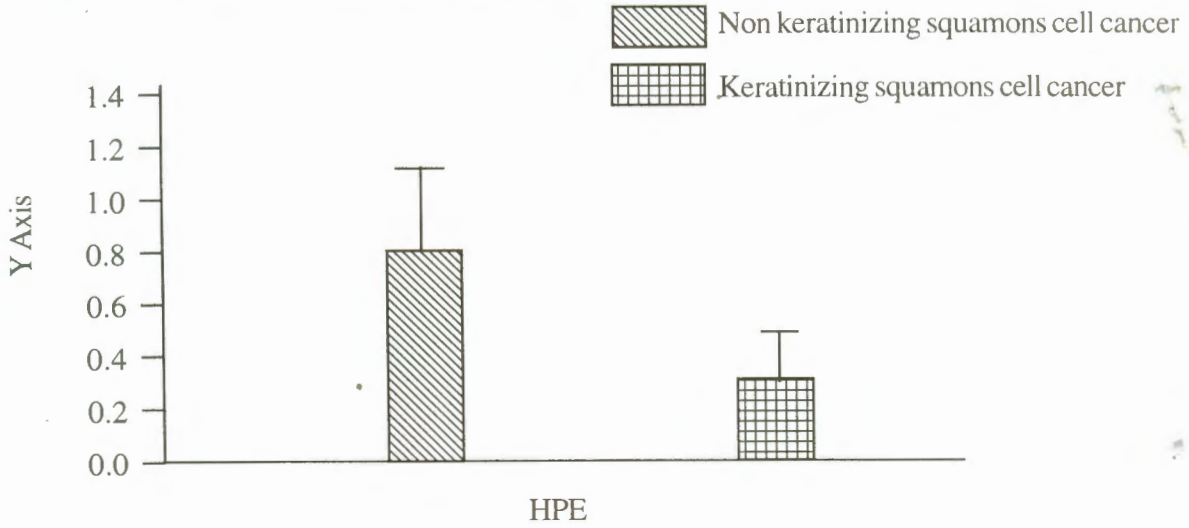
Table VII shows that MDA levels were higher in advanced cancer stages than in early stages but the difference was not statistically significant.

Table VIII shows that MDA levels were found to be higher in poorly differentiated non-keratinizing squamous cell cancer than in well differentiated tumour of cervix (Fig. 8) whereas same findings were not observed in other cancers. This could be due to small number of cases in this group.

Discussion

As early as 450 BC. Hippocratus formulated a concept that cancer resulted from disturbance and unbalance of the "4 humors" and an excess of black bile or "melenchole". He also appreciated the importance of diet in health. Shakespeare saying that "Man begins to die the day he is born", stands true in case of free radicals. Free radicals which are our inseparable partners since evolution of life but paradoxically, also relate intricately to degeneration and death. Though they are needed for proper functioning of certain essential system of organism their role in carcinogenesis is strongly proposed in both initiation and promotion. Already various studies on role of free radicals in cancer stomach (Salim 1992), Colon (Salim 1992), breast (Palen et al 1994) and oral cavity (Garewal and Schantz, 1996) are available in literature.

Fig VIII Comparitive profile of mean MDA levels & Histopathological grading in cancer cervix



Studies on role of female genital cancer are sparse and mostly pertain to cervical dysplasia (Singh and Gaby, 1991). So the study to generate on ground information on role of free radical in etiology of gynaecological cancers cervix, ovary and endometrium was undertaken by measuring serum malonaldehyde, a stable end product of free radical damage to cell lipids.

The results show that all cancers were more common in women with low socio-economic status viz. 91.67% and in multiparous women. These factors further confirm the role of poor nutrition, local trauma due to early sexual activity and STDs in genital cancer specially that of cervix. Higher MDA levels in anaemic group provide clinical evidence in favour of distributed oxidant/antioxidant balance in gynaecological cancer. This observation is consistent with the observation, that MDA level were found to be 13 times higher in cancer tissue obtained from women having lower weight than normal with cancer than in overweight women with cancer according to Life Insurance tables. These facts confirm the observation that nutrition has a great role and its deficiency may aggravate free radical damage.

Role of micronutrients like carotenoids, vitamin C and vitamin E in cancer prevention in human is less clear but diet studies by Dorgan and Schatzkin (1991) suggested protective effect of fruits and vegetable on risk of cancer at several sites, as B carotene in lung cancer and Vitamin C in oral and oesophagal cancer in both diet and plasma studies.

Palan et al (1994) showed inverse association between the plasma levels of B carotene and tocoferrol and increasingly severe graded cervical histology and emphasized role of these micronutrients in pathogenesis of intraepithelial lesions and cancer of cervix. Palan et al (1994) further stated that these antioxidants appear to be essential nutritional requirements implicated in pathophysiology and carcinogenesis of these human organs by showing lower level of these micronutrients in cervical and endometrial carcinoma tissue than in adjacent nonneoplastic tissue. Similarly Charmuszko et al (1990) reported significantly low level of serum retinol in ovarian carcinoma patients viz 31.1 ± 7.5 against 52.8 ± 11.0 in control group.

MDA levels were found to be significantly higher in patients with malignancies than in those without (Fig 2, 3 and 4). Both blood and tissue MDA levels were reported to be higher in tissue taken from endometrium in carcinoma than in normal endometrium and showed a direct co-relation between oestrogen and progesterone receptor levels in tumour tissue and that lipid peroxidation rate variation in endometrial carcinoma could be due to involvement of free radicals in hormonal carcinogenesis mechanism (Bershein et al. 1996). Balasubramaniyam at al. (1994) studied lipid peroxides, glutathione content and activities of antioxidant enzymes in patients with carcinoma of cervix and compared it with those in normal. Their observation suggested that there is impaired antioxidant status in carcinoma of uterine cervix. All these data further suggest that free radial injury is increased in malignancy.

The cases with cancer exhibited similar and higher levels of MDA both in tissue and serum. But samples from noncancerous tissue did not always exhibit similar uniformity of MDA levels in tissue and serum. It may be reasonable to presume, a greater exchange of end products of lipid peroxidation due to broken barriers between cancerous tissue and general circulation.

The comparison of MDA levels in various study groups at different stages hints at this answer. MDA level do not show significant difference according to the stages. This means that despite progression of disease and its spread to nearby tissue the free radical levels have remained elevated to similar magnitudes. The elevated levels of MDA are therefore more reasonably causative rather than the outcome of the cancer.

Increased MDA levels in poorly differentiated tumour than in keratinizing well differentiated tumour of cervix may be explained by local disturbance in homeostasis in case of poorly differentiated cancer though the same results were not found in Ca body, it may be due to less number of cases. Huda et al. (1993) estimated plasma level of antioxidant nutrients retinol and tocopherol in cases with different grades of cervical carcinoma and found that the levels of vitamin A and E were low and constant in all histological grading of cervical cancer. This needs to be further evaluated and confirmed by more studies.

Conclusion

This study gives a convincing support in favour of etiological role of free radical injury in genital cancers. The role of nutrition, poor in antioxidant factors, may further prone to malignant changes in patients who are already more at a risk of endogenous and exogenous factors causing free radicals stress and injury as in cervical cancers. Outcome of present study have significant appeal for validation through repeated and enlarged studies which includes scope of chemoprevention by antioxidation supplements to high risk patients showing dysplasia.

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