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Demonstration of circulating immune complexes in oral and cervical cancer patients

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Sera from 68 patients with oral cancer and 59 patients with cervical cancer were studied for the presence of circulating immune complexes (CIC) by the polyethylene glycol precipitation method and compared with the CIC levels from 50 healthy blood donors. 81% of the samples from oral cancer patients and 88% of the samples from cervical cancer patients showed highly elevated CIC levels ($P < 0.001$) in comparison to the controls. There was no significant difference between oral and cervical cancer patients CIC levels. Oral cancer patients belonging to 71-80 years age group had the highest immune complex levels, whereas in cervical cancer patients, the highest CIC levels was found to be between 41-50 years age group. The CIC levels were studied in oral and cervical cancer patients at various stages of the disease and different time intervals after radiotherapy.

Key Words: CIC, Oral cancer, Cervical cancer

The two most common cancers seen in this region are those affecting oral cavity and uterine cervix which constitute 27% and 14% respectively (13). Extensive data has been accumulated during the past two decades showing the existence of tumour associated antigens in various types of human cancers including lung cancer (17), oral cancer (1) and breast cancer (11). Lymphoid cells from tumour bearing hosts were shown to be specifically cytotoxic *in vitro* (15). This specific cytotoxicity is shown to be inhibited by serum factors. Increasing evidence suggests that tumours synthesise and release antigenic membrane-associated proteins which circulate in a free state or complexed with host immunoglobulin (7). The serum blocking factors are shown to be the antigens or antigen-antibody complexes in circulation (9). The elevated levels of immune complexes are shown to be correlated with increasing tumour burden, metastasis and poor prognosis in breast cancer (3). The immune complexes are consistently shown to be present in Hodgkin's disease (2), Burkitt's lymphoma (10) and various types of leukemias (4). The present study has been undertaken to find out the existence of circulating immune complexes (CIC) in oral and cervical cancer patients.

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Material and Methods

Patients

Sixty-eight patients with oral cancer and fifty-nine patients with cervical cancer, both histologically diagnosed as squamous cell carcinoma, were included in the present study. The study was carried out before commencing any anti-cancer therapy. For comparison, sera of fifty healthy blood donors of both sexes were included.

Sera

Five ml of venous blood was collected, sera separated and stored at $-70^{0}C$ without thawing until further use.

The precipitation of immune complexes was done by polyethylene glycol (PEG) (6) with slight

modification. 0.2 ml of the serum was diluted with 4.8 ml of borate buffered saline (pH 8.4) and an equal volume of cold 7% PEG (mol. wt. 6000), dissolved in borate buffer (pH 8.4) was added to make the final concentration of PEG at 3.5%. The mixture was kept overnight at 4°C, then centrifuged at 3,200 g for 30 minutes at 4°C. The precipitate was washed twice with 3 ml of cold 3.5% PEG, and centrifuged again at 3,200 g at 4°C for 20 minutes. The precipitate was redissolved in 2 ml of phosphate buffered saline (pH 7.4). The protein content was estimated spectrophotometrically at 280 nm., the value of which was expressed as milligram of protein per 100 ml of the original serum sample.

Results

The mean immune complex levels in cancer patients and in normal controls are shown in Table I. Since the oral cancer patients included both male and female, the results with 69 oral cancer patients were compared with 50 normal controls of both male and female. Similarly, results obtained from 59 cervical cancer patients were compared with 25 female normal controls. The level in normal females was higher in comparison to the normal males but there was no significant difference between oral and cervical cancer patients immune complex levels. However the results clearly showed a highly significant elevation of circulating immune complex levels in both oral and cervical cancer patients in comparison to normals. Using the 95th percentile (Mean + 2 S.D.) of the circulating immune complex levels in normal subjects as the cut-off limits, as many as 81% of the samples from oral cancer patients and 88% of the samples from cervical cancer patients showed circulating immune complex levels above normal levels. Further, oral cancer is more prevalent in males (76%) in comparison to females (24%) and the immune complex levels was slightly higher in females, than males with oral cancer (Table I).

Table II shows the distribution of oral cancer cases according to the site of primary lesions. In our study, the maximum number of oral cancer cases was found in the tongue region (37%), followed by cheek (35%) region. The immune complex levels under each site were highly significant in comparison to the mean normal values. Patients with cancer in cheek had the highest immune complex levels, followed by patients with cancer in tongue, alveolus and palate.

Maximum number of oral cancer cases were found to be between 41 to 50 years age group (31%) while maximum cases in cervical cancer were found to be between 61 to 70 years age group (31%) (Table III). The difference in the mean circulating immune complex levels between the normal subjects and each age group of oral and cervical cancer patients were found to be statistically significant ($P < 0.001$). Further, oral cancer patients belonging to 71-80 years age group had the highest immune complex levels, whereas in cervical cancer cases, the highest level was found to be between 41-50 years age group.

Stage wise, maximum number of oral cancer cases was found to be in stage III (40%) whereas in cervical cancer cases, more than

Table I - Circulating immune complex levels in cancer patients

Group of subjects	Sex	Number of subjects	Number of sera Positive[1]	Percentage positive	Immune complex levels in mg% (Mean ± SD)
Normal	Male	25	—	—	126.34 ± 28.75
Normal	Female	25	—	—	151.40 ± 26.56
Normal	Both	50	—	—	133.86 ± 30.16
Oral cancer	Male	52	43	83	299.21 ± 100.15**
Oral cancer	Female	16	12	75	328.44 ± 157.01**
Oral cancer	Both	68	55	81	306.09 ± 115.37**
Cervical cancer	Female	59	52	88	319.27 ± 117.30**

** = $P < 0.001$ highly significant
1 positive = > Mean + 2 SD of controls

Table II - Circulating immune complex levels in oral cancer in relation to site of primary lesions

Site	Number of subjects	Immune complex levels in mg% (Mean ± SD)
Cheek	24	316.87 ± 134.00**
Tongue	25	312.76 ± 111.66**
Palate	6	262.67 ± 88.82**
Alveolus	9	297.11 ± 76.40**
Lip	2	317.50 ± 235.40(NS)
Floor of mouth	2	252.50 ± 126.57(NS)
Normals	50	133.86 ± 30.16

** = $P < 0.001$ highly significant
NS = not significant

Table III - Circulating immune complex levels in relation to different age groups both in oral and cervical cancers

Age group in years	Number of subjects		Immune complex level in mg%	
	oral cancer	cervical cancer	oral cancer (Mean ± SD)	cervical cancer (Mean ± SD)
21-30	—	2		295.50 ± 214.25(NS)
31-40	2	4	275.00 ± 76.37**	300.50 ± 91.89**
41-50	21	11	264.19 ± 108.62**	357.82 ± 124.21**
51-60	15	15	311.73 ± 111.79**	306.13 ± 126.59**
61-70	17	23	302.35 ± 120.68**	315.87 ± 118.03**
71-80	12	4	395.50 ± 87.08**	303.75 ± 81.72**
81-90	1	—	154.00 (NS)	
Total	68	59		

** = $P < 0.001$ highly significant
NS = not significant

Table IV - Circulating immune complex levels in relation to clinical staging both in oral and cervical cancer

Clinical Stage	Number of subjects		Immune complex level in mg%	
	Oral cancer	Cervical cancer	Oral cancer (Mean ± SD)	Cervical cancer (Mean ± SD)
I	5	4	328.60 ± 103.54**	323.00 ± 116.01**
II	11	30	284.09 ± 132.09**	295.73 ± 112.60**
III	27	24	301.56 ± 116.21**	351.33 ± 122.02**
IV	25	1	316.16 ± 114.08**	241.00 (NS)

** = $P < 0.001$ highly significant
NS = not significant

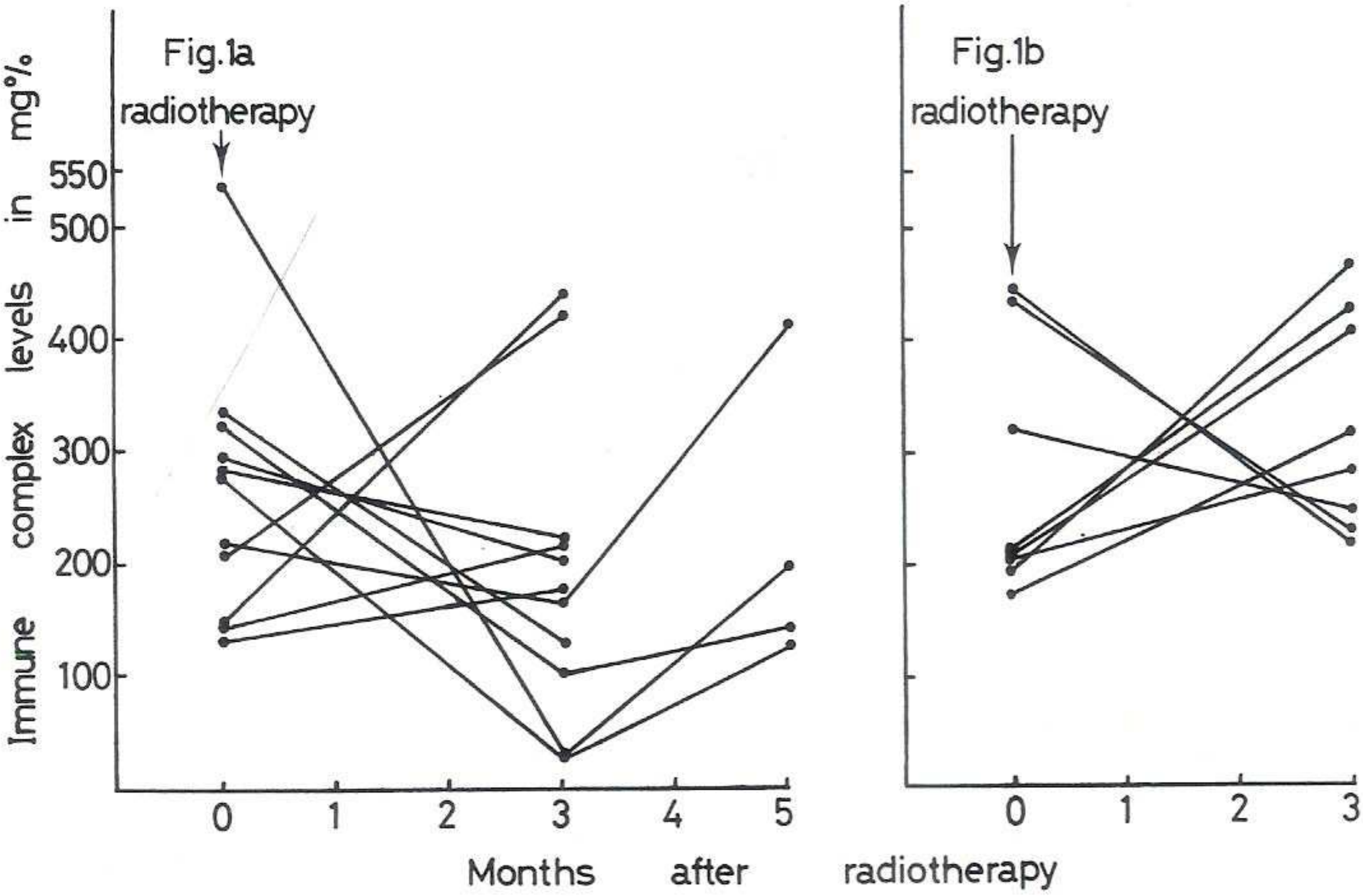

Fig. 1 — Immune complex levels in follow-up cases of oral cancer (1a) and cervical cancer (1b).

50% of the cases were found to be in stage II of the disease (Table IV). The mean immune complex levels in each stages were found to be highly significant in comparison to the normals. There is no direct relationship between the elevation of immune complex levels and the advancement of disease both in oral and cervical cancers.

Eleven oral cancer patients and eight cervical cancer patients were involved in the follow-up study. Out of this, 7 (64%) oral cancer cases (Fig. 1a) and 3 (38%) cervical cancer cases (Fig. 1b) had their immune complex levels decreased after radiation therapy. This decline in immune complex levels after radiation therapy were found to be only a temporary decrease, as further serum sample analysis two months after radiation therapy in four of the oral cancer cases showed a rise again in these levels, indicating perhaps that all cancer cells may not be destroyed or predicting an early relapse. Though the immune complex levels decreased in some of the patients after radiation therapy, the decreased levels were far above the normal range.

Discussion

In the present study, the circulating immune complex levels were found to be increased to about two-fold both in oral and cervical cancer patients, when compared with the respective normal controls. This is in general agreement with other published results, (14) where 40 to 80% of breast cancer cases had increased immune complex levels. Further, the immune complex levels were shown to be elevated in 33% of carcinoma of head and neck, 63% of bronchogenic carcinoma and 66% of squamous cell carcinoma of cervix (12). Recently, it is reported that 70% of Hodgkin's disease cases had increased levels of precipitable immune complexes as compared to normals (5).

Many methods are available for the detection and estimation of circulating immune complexes in serum samples. Each method depends on one or the other limited characteristics of the complexes and the correlation between the results obtained by different assays is often very poor (6). In the method used

by us, circulating immune complexes were precipitated at 3.5% PEG, a concentration at which complexed Igs are precipitated with increasing specificity than monomeric Ig's (8).

In both types of cancer, the maximum number of cases were from stage II onwards. The number of early malignancy was very small. Most of the patients being from lower or middle socio-economic group, were unaware of early manifestation of cancer. Lack of cancer detection clinic may be the another cause of this. There is no direct relationship between the elevation of immune complex levels and as the clinical stage is advanced in both oral and cervical cancers.

In the follow-up study, though the circulating immune complex levels were reduced in certain patients during remission, the values were still significantly higher than normals. Circulating immune complexes were often detectable early in their course but could not always be correlated with either exacerbation or remission induced by intensive radiation therapy. Therefore levels of circulating immune complexes or presence or absence of complexes did not prove helpful in estimating overall prognosis. Similarly, Williams et al (16) reported that in acute lymphoblastic leukemia there was no apparent relationship between the presence or amount of circulating immune complexes and the clinical course. Further studies are in progress to isolate and purify the tumour associated antigen(s) from these immune complexes, so that a more specific and sensitive test for detecting these cancers at an early stage may be developed.

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