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Circulating immune complexes as a biological marker for solid tumours

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Circulating immune complexes in the sera of 196 patients with carcinoma of the oral cavity, 172 patients with cancer of the cervix and 166 patients with breast cancer were estimated and compared with that of 50 patients with benign lesions of breast and cervix and 100 healthy adult controls. There was a significant rise in the circulating immune complexes in the patients. The CIC were found to be increasing with the progression of the clinical stages. The level of CIC in the sera of patients who had a clinical cure decreased significantly whereas it remained elevated in patients who had residual lesions. The analysis of the CIC revealed significantly higher levels of IgG and IgM in the patients. The incidence of IgA and IgD in the CIC of cancer patients were remarkably high. This study shows the usefulness of circulating immune complex levels in monitoring the prognosis of solid tumours.

Tumour associated antigens may be released from a developing tumour into the extra cellular environment and subsequently be found in free form and/or as immune complexes in serum and other body fluids (17). Soluble tumour antigens are believed to create a protection against the attack of specific antibodies and lymphocytes by blocking antigen receptors (3). Elevated levels of circulating immune complexes (CIC) have been found in studies on lymphomas and leukaemias as well as many types of solid tumours (2).

Celeda et al. (4) reported elevated levels of circulating immune complexes in solid tumours.

The detection and the clinical significance of circulating immune complexes in human neoplasia were reported earlier by Salinas and Wee (18) and Salinas et al. (19).

A correlation between circulating levels of immune complexes and tumour load in patients with breast cancer was reported by Hoffken et al. (9). The levels of serum immune complexes were reported to be elevated in breast cancer and gynaecological tumours (13, 16, 20, 21). In the sera of oral cancer patients, an elevated level of circulating immune complexes was reported by Scully (20).

There is a high incidence of oral cancer (27%), in Kerala. Carcinoma of the cervix constitutes 14% and breast cancer constitutes 7% of the total cancer incidences (15).

No attempts were made by any previous workers to correlate the level of circulating immune complexes with clinical stages of these cases and the level of antibodies in the immune complexes. The present study was undertaken to see whether there is any correlation of the circulating immune complexes with the clinical stages and whether the treatment has any influence on the circulating immune complexes. An attempt is also made to quantitate the amount of antibodies present in the circulating immune complexes.

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Materials and methods

One hundred and ninety six patients with carcinoma of the oral cavity, 172 patients with cancer of the cervix and 166 breast cancer patients were included for the study.

Thirty two women with mild to moderate dysplasia and 18 women with mammary dysplasia were also included in the study.

Sixty normal healthy males and 40 healthy females from the Medical College campus served as controls. Controls were in the age group of 25-50 years and the patients between 30-60 years. All the subjects were screened to exclude any infections and previous history of allergy and autoimmune diseases.

Sixty seven patients who had clinical cure after surgery and/or radiotherapy and 23 patients who had residual lesions were available for follow-up studies six months after therapy.

Five ml of venous blood was collected from all the subjects, and allowed to clot at 37°C for 3 hours which avoided precipitation of cryoglobulins. The serum was separated and stored at —70°C till use. The circulating immune complexes were investigated by precipitation of sera with Poly Ethylene Glycol (PEG 6000) as described by Creighton et al. (5) with minor modifications. In brief, 2 ml of 3.3% PEG (Poly Ethylene Glycol 6000, BDH, England) was added to 0.2 ml serum and the mixture was incubated at room temperature for 2 hours and centrifuged at 2500 x g for 30 minutes at 4°C. The pellet was washed thrice with 3% PEG and dissolved in distilled water (0.2 ml) and diluted to 2.0 ml with 0.1 N NaOH.

Protein concentration was estimated by Lowry's method (12). The immunoglobulin concentration of the circulating immune complexes were determined by radial immuno diffusion technique using Tri-Partigen immuno diffusion plates (M/s-Hoechst Pharmaceuticals Ltd., Division of Behring Institute, Bombay).

Results

The age and sex distribution of all the subjects are given in Table I. The level of CIC in the sera and the immunoglobulin contents of the CIC are given in Table II.

Table I - *Age and sex distribution of subjects.*

	Control		Benign uterine lesions	Benign Breast lesions	Oral cancer		Cervical cancer	Breast cancer
	Males	Females			Males	Females		
No. of subjects	60	40	32	18	123	73	172	166
Age (mean ± SD)	35.4± 6.4	36.8 ± 7.5	40.7± 8.6	43.5± 6.9	47.4± 11.2	49.6± 9.3	44.2± 10.9	46.8± 12.1

Even though there was a rise in CIC in the benign group, the rise was not significant, whereas in all the three types of cancers, the level of CIC was significantly higher ($p < 0.001$). The IgG and IgM content showed a similar tendency. IgA was detected only in 6 percent of the normals and about 10 percent of the benign lesions. In the case of cancer, the incidence of IgA in CIC was 19.9%, 20.5% and 30.8% in carcinoma of oral cavity, breast and cervix respectively. IgD was detected only in 2 oral cancer, 3 breast and 5 cervix cases.

Table III gives the changes in CIC in different clinical stages of the disease. There was a progressive rise in CIC in all types of cancers and the change was significant. The effect of treatment on CIC is given in Table IV. The treatment has resulted in a decrease in the CIC levels in patients who were clinically cured. Those patients who were still having residual lesions had a high level of CIC ($p < 0.001$).

Table II - *Circulating immune complexes and their immunoglobulin concentrations in the sera of patients with solid tumours and control subjects.*

SUBJECTS	Circulating immune complexes (CIC) in mg/dl	concentration of IgG in CIC mg/dl	concentration of IgM in CIC mg/dl	incidence of IgA in CIC	Incidence of igD in CIC
Normal controls (n = 100)	81.5 ± 3.9	25.1 ± 5.2	18.4 ± 4.1	6/100(6%)	Nil
Benign lesions of breast (n = 18)	109.7 ± 8.5	42.3 ± 7.4	23.7 ± 6.6	2/ 18(11.1%)	Nil
Benign lesions of cervix (n=32)	101.4 ± 11.3	47.0 ± 9.5	31.5 ± 7.4	3/32 (9.4%)	Nil
Oral cancer (n= 196)	374.3± 13.2**	131.5 ± 10.2**	99.6 ± 11.5**	39/196(19.9%)	2
Cancer of cervix (n = 172)	481.2 ± 15.3**	201.8 ± 16.4**	149.0 ± 21.2**	53/172(30.8%)	5
Cancer Breast (n = 166)	429.6 ± 11.8**	149.3 ± 13.6**	121.3 ± 14.7**	34/166(20.5%)	3

All values are mean ± SE
n = stands for number of subjects
** = $P < 0.001$
Comparisons of the values are made with that of the normal controls.

Table III - *Changes in the circulating immune complexes in different clinical stages.*

Subjects	Clinical stages I	II	III	IV
Oral cancer	(26) 149.5 ± 11.7 *	(82) 231.5 ± 10.7 **	(51) 347.1 ± 13.3 **	(37) 401.9 ± 17.2 **
Cancer cervix	(12) 169.6 ± 18.9 *	(76) 291.4 ± 14.1 **	(64) 427.6 ± 16.5 **	(20) 509.1 ± 20.4 **
Cancer breast	(8) 133.4 ± 14.7 *	(61) 259.2 ± 13.4 **	(57) 393.3 ± 15.6 **	(40) 459.5 ± 17.2 **

All values are mean ± SE
The figures in parentheses show the number of subjects in each stage.
* = $P < 0.01$
** = $P < 0.001$
Comparisons are made with that of the normal controls.

Table IV - *Effect of treatment on circulating immune complexes.*

Subjects	CIC mg/dl	Concentration of IgG in CIC mg/dl	Concentration of IgM in CIC mg/dl
Normal controls (n = 100)	81.5 ± 3.9	25.1 ± 5.2	18.4 ± 4.1
Patients 6 months after surgery and/or radiotherapy and are clinically cured (n = 67)	97.8 ± 13.4	37.5 ± 6.7	20.5 ± 5.1
Patients still on chemotherapy and are having residual lesions (n = 23)	243.7 ± 17.2**	94.2 ± 12.5 **	71.0 ± 9.2 **

n = stands for number of subjects.
All values are mean ± SE
** = $P < 0.001$

Discussion

The present study clearly shows that there is an elevation in the levels of CIC in the sera of patients suffering from carcinoma of oral cavity, breast and uterine cervix. Our finding is in agreement with that of the previous workers who reported elevated levels of circulating immune complexes in many types of cancers (4, 6, 2, 1). Many methods are employed for the detection and quantitation of CIC (11). The method employing Poly Ethylene Glycol is reported to be a reliable and easy method (4) and hence we utilized a modified PEG technique.

A progressive rise in CIC in different clinical stages observed in the present study is in agreement with that of Baldwin et al. (2) who attributed the rise in CIC to the increased tumour burden and/or tumour dissemination. This is evidenced from our findings that after surgical and/or radiological removal of the tumour, there is a decrease in the CIC levels.

Even though the nature of immune complexes is quite unknown, there is ample evidence to show that besides immunoglobulins, non-immunoglobulin components are present (7). It has been suggested that immune complexes are capable to block cell-mediated immune responses (8). A depression in cell mediated immunity in the patients with solid tumours as evidenced by a decrease in high affinity rosettes forming cells was reported by Vijayakumar and Vasudevan (23). There are contradictory reports on the levels of Igs in the sera of patients suffering from solid tumours (1, 6, 14). None of the above workers tried to quantitate the CIC and the Ig contents in the CIC of the patients. It is not clear whether the rise in serum Igs has got any relation with the rise in CIC. We have earlier observed the changes in the serum Igs in the same group of patients (24). In this study, the Ig content, especially IgG and IgM, was proportional to the quantity of CIC. It is surprising to see that even though IgA was reported to be elevated in almost all types of cancers, its presence was detected only in a very small percentage of the CIC.

The result of the study gives encouragement for the use of immune complexes in the diagnosis and prognosis of cancer. But there is no clear understanding of the host's response leading to elevated levels of CIC in the sera of cancer patients. An elevation in CIC was attributed to the changes in the levels of complement fixing and non-complement fixing of tumour specific antibodies (10). These investigations imply that the tumour growth may be monitored by CIC. Even though in the present study we were able to identify the antibody part of the CIC, we were unable to identify the antigenic counterpart due to the lack of reliable assays for tumour-associated antigens. Efforts are being made to identify the antigenic counterpart of the immune complexes.

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