as a negative control. Both HSV and adenoviruses infection are very commonly seen in this region, as shown by the values of the control population (*Tables I & II*). But while HSV antibodies are shown to be significantly increased in oral and cervical cancer patients (*Table I*), adenoviral antibodies did not show such a trend (*Table II*).

Table III shows HSV antibody titres of 808 oral cancer patients and 300 normal control individuals. Percentage positivity at each titre is more in oral cancer patients than in normal controls. The difference is more marked at the highest titre of 512 (Table III).

TABLE IV

STAGE OF CARCINOMA WITH VARIATION IN HERPES SIMPLEX VIRAL ANTIBODY DISTRIBUTION

Clinical Stage	Total number of sera tested	Number of sera positive	Percentage positive
T_1	6	2	—
T_2	222	158	71.1
T_3	399	287	71.9
T_4	181	123	67.9

Antibodies against HSV were seen to have a moderate to high increase in cancer patients, compared to normal controls of that age group. Statistically significant increases were seen in the 41-50 and 51-60 age group. No significant difference was seen in HSV antibodies in healthy controls with advancing age. In oral cancer patients, anti-herpes antibodies were almost similar in all age groups except 41-50, supporting the hypothesis of a causal relationship between HSV and oral cancer.

The clinical stage has very little relationship with the percentage positivity of HSV antibodies in oral cancer patients (*Table IV*). Even though at the T_1 stage, the percentage positivity of antibody against HSV is found to be decreased, it cannot be taken into account as very few patients are reported at this stage.

HSV antibodies in 20 patients who came for followup at regular intervals were also studied. Seven of them (35%) showed increasing titres, nine (45%) had decreasing titres and the rest showed no definite pattern following therapy.

DISCUSSION

There is much evidence to show that the Herpes group of viruses is associated with certain human cancers, such as Burkittts' lymphoma (Epstein, 1978), nasopharyngeal carcinoma (Adams, 1979) and cervical carcinoma (Rawls, 1973; Roizmann & Kieff, 1975; Seth, 1980). In the present study, HSV antibodies found to be significantly more in oral cancer patients than in the normal controls. This agrees with the results of Hollinshead *et al* (1973), though the number of cases reported in that study are very few. But Shillitoe *et al* (1979) could not show any significant relation between HSV and oral cancer. This may be due to the difference in co-variables associated with oral cancer actiology and the sensitivity of the serological techniques used in different laboratories of the world. In our region, oral cancer constituted 27% of all cancers observed, the peak incidence being in the 40-50 age group (Padmanabhan & Vasudevan, 1982).

Though there are many reports associating adenoviruses with various types of cancers in animals (Yabe *et al*, 1964 & Freeman *et al*, 1973), similar reports are few in the case of human cancers (Gurkripal Singh *et al*, 1974). Singh and co-workers (1974) found no significant difference in neutralising adenovirus antibody titres between oral cancer patients and healthy controls. Our previous results (Kumari *et al*, 1982) were also in agreement with those of Singh and co-workers. As there are a large number of reports showing the association of HSV and cervical cancer, cervical cancer patients were included in the present study as positive controls. The increased incidence of HSV antibodies in oral and cervical cancer cases may be due to recurrent herpetic infections which can produce higher antibody titres (Jose C. Costa, 1976). In agreement with our results, Shillitoe *et al* (1982) have also found a higher neutralising antibody titre to HSV in oral cancer patients, compared to normal healthy controls matched for age and sex.

Epidemiological evidence indicates that tobacco chewing (Wahi, 1976), excessive smoking (Silverman & Griffitch, 1972) and alcohol consumption (Wynder, 1971) are major risk factors in head and neck cancers, probably serving as initiators of carcinogenesis. Previous reports (Shillitoe et al, 1982) as well as the present study provide further evidence to support a link between HSV and oral cancer.

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a. 5/13/6/80—CAR—I.

b. 3/1/2/37 (159)/82—CAR—II.

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Detection of Antibodies Against Herpes Simplex Virus in Patients with Oral Cancer

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SUMMARY

Sera samples were collected from 808 oral cancer patients, 120 cervical cancer patients, 300 patients with other malignancies and 300 normal, healthly, age and sex-matched individuals. The presence of antibodies against Herpes Simplex Virus type I (HSV-I) was determined using the Indirect Haemagglutination test and confirmed by the indirect Haemagglutination Inhibition test. As many as 507 of the 808 oral cancer patients studied (70.50%), and only 156, of the 300 normal healthy control subjects (52%) showed the presence of HSV antibodies. But no such significant difference was found in the case of adenoviral antibodies. In oral cancer patients, statistically significant increases of antibodies against HSV were seen in the 44-50 and 51-60 age groups. The size of the tumour has very little relationship with the percentage positivity of HSV antibodies in oral cancer patients.

Certain viruses are capable of inducing malignancy in experimental animals. The association between Herpes Simplex Virus type 2 (HSV-2) and human uterine cervical cancer has been widely accepted in published reports (Rawls, 1973; Roizmann & Kieff, 1975; Seth, 1980). The oral cavity, too, is a major site of recurrent herpetic infection. Cancer of the mouth resembles cancer of cervix in that both are predominantly squamous cell tumours, and frequently arise in a premalignant lesion (Shillitoe & Silverman, 1979). Patients with oral leukoplakia have been found to have an increased cell mediated immunity response to HSV-I (Lehner et al, *1973 a) which was depressed during malignant transformation (Lehner* et al, *1973 b). Oral cancer constitutes around 27 per cent of the total cancer cases attending the Regional Cancer Centre, Trivandrum (Padmanabhan & Vasudevan, 1982). This is one of the highest prevalence rates seen anywhere in the world. It has also been reported that HSV-I may play a role as promoter or co-factor in the aetiology of head and neck cancers (Sabin & Tarro, 1973; Hollinshead* et al *1973, 1974).*

The present study describes the presence of HSV antibodies in oral cancer patients in comparison with normal healthy control subjects.

MATERIALS AND METHODS

SERA samples from 808 oral cancer patients, 120 cervical cancer patients and 300 with other cancers were collected from those attending the clinics of the Regional Cancer Centre, Trivandrum. Another 300 healthy individuals were also included in the study as normal controls, matched for age and sex. All sera were kept at —70°C, without the addition of any preservative, until tested.

The presence of antibodies against Herpes Simplex Virus was determined using the Indirect Haemagglutination (IHA) test (Seth *et al*, 1978). IHA is comparable in accuracy and sensitivity with the micro-neutralisation test (Fucillo *et al*, 1970). The viral antigen was prepared using the technique described by Seth *et al*, 1978. An A.C. strain of HSV-I was infected at a multiplicity in 0.1 to 0.2 PFU/cell on Vero cells propagated in Roux bottles. When a complete cytopathic effect appeared, the cells were rinsed once with Phosphate Buffered Saline (PBS) and then scraped into 15 ml of PBS with intermittent freezing and thawing. The antigen was clarified by centrifugation at 5000 g for 20 mts at 4°C and stored at —70°C in small aliquots. Uninfected Vero cells treated in the same manner were used as control antigen. The single batch HSV-I antigen was used for the IHA tests throughout the study. Antibody titres were expressed as log 10 of the reciprocal of the dilution of serum, which gave 50% agglutination with HSV-I antigen-coated tanned sheep erythrocytes.

RESULT

The prevalence of HSV antibodies in different study groups is given in Table I. Out of the 808 oral cancer patients 570 (70.5%) showed the presence of HSV antibodies, as against 156 (52%) in the 300 normal healthy control subjects studied. This variation was found to be statistically significant (P less than 0.01). Since HSV-2 is known to be associated with cancer of the uterine cervix, 120 such cases were studied as positive controls. As expected from previous reports, here also we found a higher percentage (92/120-76%) of positivity of HSV-2 antibodies in cervical cancer patients when compared with normal control individual ($P < 0.01$).

It may be agreed that viruses could non-specifically invade the cancer cells, leading to these results. To rule out this possibility, antibodies against adenoviruses were also tested in 200 oral cancer cases and 151 control individuals

TABLE I

SERUM ANTIBODIES AGAINST HERPES SIMPLEX VIRUS IN CANCER PATIENTS

Groups studied	Total number of sera tested	Number of sera positive	Percentage positive	P Value
Oral Cancer	808	570	70.5	<0.01
Other Malignancies	300	168	56.0	N.S.
Normal Controls	300	156	52.0	—

TABLE II

SERUM ANTIBODIES AGAINST ADENOVIRUS IN CANCER PATIENTS

Groups studied	Total number of sera tested	Number of sera positive	Percentage positive	P Value
Oral Cancer	200	91	45.5	N.S.
Cervical Cancer	110	53	48.0	N.S.
Normal Controls	151	71	47.0	—

TABLE III

SERUM ANTIBODY TITRES AGAINST HERPES SIMPLEX VIRUS

studied Groups	Total number of sera tested		TIRE VALUES 8	16	32	64	128	256	512
Oral Cancer	808	Number positive	35	50	66	80	112	105	122
		Percentage positive	4.3	6.2	8.1	9.9	13.9	13	15.1
Normal Controls	300	Number positive	18	21	37	31	20	17	12
		Percentage positive	6	7	12.3	10.3	6.6	5.6	4