10. Association of Herpes Simplex Virus with Human Oral Carcinoma

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Associaton of Herpes Simplex Virus (HSV) type 2 with human uterine cervical cancer has been firmly established (1, 2, 3). Oral cavity, as in the case of uterine cervix, is a major site of recurrent herpetic infection. Cancer of the mouth as well as cervix are predominantly squamous cell tumours. Patients with oral premalignant lesions were shown to be associated with cell mediated immunity against HSV_1 (4). Oral cancer constitutes about 27% of the total cancer cases registered in this region (5). This is one of the highest prevalence rates seen anywhere in the world. Oral cancer has been shown to be associated with chewing tobacco, but other factors, including viruses as etiological agents, are to be carefully evaluated. Previous reports showing association of Herpes virus with oral cancer are scanty (6). We had elswhere reported that the antibodies against HSV_1 are increased in oral cancer patients (7,8) and the oral cancer cells contain HSV related antigens on their cell surface (9). Here we extend the work and also show the presence of HSV gene in the oral cancer cells.

DNA hybridisation studies: a) DNA extraction from homogenised specimens by phenol and chloroform-isoamylalcohol extraction and precipitation by ethyl alcohol: The DNA Strands were disrupted by NaOH treatment at room temperature. The DNA was quantitated by optical density at 260 nm. The DNA was resuspened in Tris-EDTA solution, and 50 uL, containing 10 ug of DNA was added on Nitrocellulose membrance (Schliecher & Schuell, BA-85) applied on Hybri-Dot Manifold (BRL, Bethesda). A series of dots containing DNA was prepared on each filter. Each well was then washed, twice, baked at 80°C for 3 hr; placed in sealed plastic bags with calf thymus DNA at 50°C for 2 hr and removed the pre-hybridization mixture.

b) Viral probe: ECoRI fragments of HSV_1 were cloned in a phage lambda vector. DNA was purified from phage that had been amplified in E. Coli. The cloned HSV_1 was nick translated by employing DNA polymerase I and DNA ease 1 and incubating with p^{32}-d CTP at p_H 7.5 for 60 mints. at 15°C: The reaction was stopped by sodium EDTA at p_H 8; preciptate by 90% ethanol at 20°C and used for hybridisation. Specific activity of about 10^7 cpm/ug was achieved.

c) Prehybridised host cell DNA sample on nitrocelullose was then incubated with 1 ug of nick translated viral probe, and hybridisation was carried out at 50°C in thermally sealed plastic bags for 18 hr. with gentle shaking. Then filters were washed 6 times air dried, and finally autoradiographed with Kodak ,XAR film in cassettes containing image intensifier screens for 6 hr to 48 hr at minus 70°C, and then developed in a Kodak X-omat processor for 4 hr. Unknown dots were read as positive when film density was equal to or greater than that of the internal standard dot of 50 ng of HSV DNA.

The DNA from cancer specimens were extracted, denatured, preciptated on nitrocellulose membrane and hybridised with radioactive nicktranslated cloned HSV probes. The results are given in Table 1. It is seen that HSV probe was hybridised with 56% oral cancer cases and 38% cervical cancer cases. Further Human papilloma virus (HPV_{16}) was seen to be hybridised with 10% oral cancer and 37% of cervical cancer specimen. The normal cells did not hybridise with viral probes, The HSV and HPV genes are generally mutually exclusive, as very few samples contain both viral genes concurrently.

The above experiments strongly show the relationship of HSV with oral cancer. The HSV_1 antibodies are consistently higher in oral cancer patients, the HSV_1 related antigens are shown to be on the oral cancer

cells and the HSV probe could be hybridised with the DNA from oral cancer, specimens. From the studies reported here, we could say that the HSV_1 genes are related with oral cancer. Whether the viral gene is integrated could be established only by restriction analysis of DNA, which we are now attempting. Epidemiological evidences indicate that tobacco chewing and smoking are major risk factors in oral cancers (13). In view of the present study, it can be assumed that the HSV may play a role as promoter or co-factor in the etiology of oral squamous cell cancers

ACKNOWLEDGEMENTS: This work is supported by a grant from the Dept. of Science and Technology, Govt. of India and a grant from the Fogarty International Foundation. USA. The authors are thankful to the technical help from Miss. Mariamma and Mr. Michael.

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TABLE-1

HYBRIDISATION OF DNA FROM CANCER SPECIMENS WITH NICK-TRANSLATED HSV PROBES

	Oral cancer			Cervical cancer			Normal cells		
Probe	No. tested	No. +ve	% +ve	No. tested	No. +ve	% +ve	No. tested	No. +ve	% +ve
HSV 1 & 2	50	28	56%	102	38	38%	12	0	0
HPV_{16}	56	5	10%	69	25	37%	12	0	0
HSV+HPV	50	3	6%	102	4	4%	12	0	0

TABLE - II

SERUM ANTIBODIES AGAINST ADENOVIRUS IN CANCER PATIENTS

Group studied	Total No.	No. +ve	% +ve	P. value
Oral cancer	200	80	40	N.S
Cervical cancer	110	53	48	N.S
Normal controls	151	71	47	

TABLE - III

ANTIBODY TITRES AGAINST HERPES SIMPLEX VIRUS

Groups studied	Total No.		Tire value				
			32	64	128	256	512
Oral cancer	608	No+ve	80	86	84	60	50
		%+ve	13.2	14 1	13 8	9.9	8.2
Normal controls	151	No+ve	6	16	19	12	7
		%+ve	3.9	10.5	12.6	7.9	4.6

TABLE - IV

STAGE OF CARCINOMA WITH VARIATION IN HSV ANTIBODY DISTRIBUTION

Clinical stage	Total No. tested	No.+ve	%(+)ve
T_1	6	2	—
T_2	111	57	51.4
T_3	126	82	65.1
T_4	131	76	58.0

TABLE—V

HSV ANTIBODY TITRES OF ORAL CANCER PATIENTS DURING FOLLOW UP

Patient No.	Months after completion of radical therapy			
	1	2	4	6
1	64	256	256	256
2	512	512	256	32
3	512	32	32	64
4	64	-ve	16	64
5	8	32	64	32
6	512	64	256	32
7	512	256	64	32
8	128	128	128	32
9	-ve	512	256	512
10	8	-ve	16	32
11	256	32	16	64
12	512	32	64	128
13	256	128	32	64
14	64	32	64	128
15	-ve	64	128	128
16	512	16	64	32
17	64	32	64	128
18	256	64	-ve	128
19	8	64	64	32
20	16	88	16	32

TABLE—VI

DETECTION OF HSV_1 ANTIGEN ON CELL SURFACE BY IMMUNOFLUORESCENCE METHOD

Specimen from	No. tested	No.+ve	Percentage of positivity
Normal oral	20	6	30%
Oral cancer	175	135	77%

TABLE—VII

HYBRIDISATION OF DNA FROM CANCER SPECIMENS WITH NICK-TRANSLATED HSV PROBES

Probe	Oral cancer			Cervical cancer			Normal cells		
	No. tested	No. +ve	% +ve	No. tested	No. +ve	% +ve	No. tested	No. +ve	% +ve
HSV_1 & 2	50	28	56%	102	38	38%	12	0	0
HPV_{16}	56	5	10%	69	25	37%	12	0	0
HSV+HPV	50	3	6%	102	4	4%	12	0	0

Figure 2 (See Article No. 7) Autoradiograph of the nitrocellulose membrane in which DNA from oral cancer cells are precipitated and then probed with nick translated Herps virus.

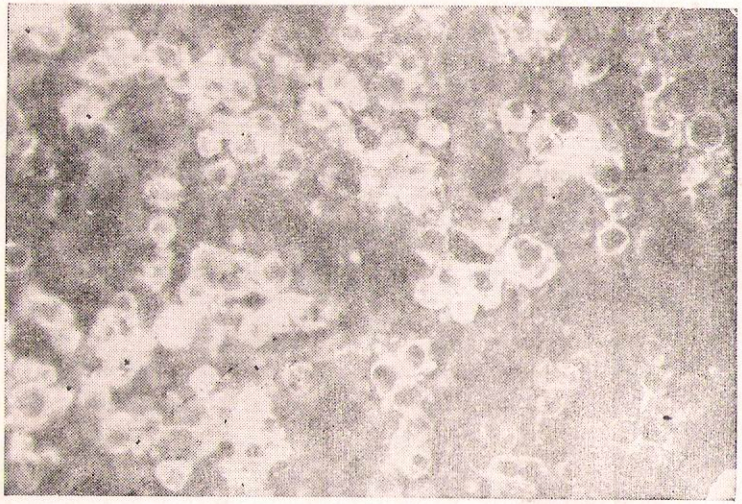

Figure 3. (See Article No. 7) Viro cells infected with HSV_1 were incubated with serum from oral cancer patients. The anti-HSV antibodies will be attached to the cells. Then FITC conjugated antihuman immunoglobulin is added and examined under fluorescent microscope, The fluorescence indicates presence of specific anti-HSV antibody in oral cancer patient's serum.

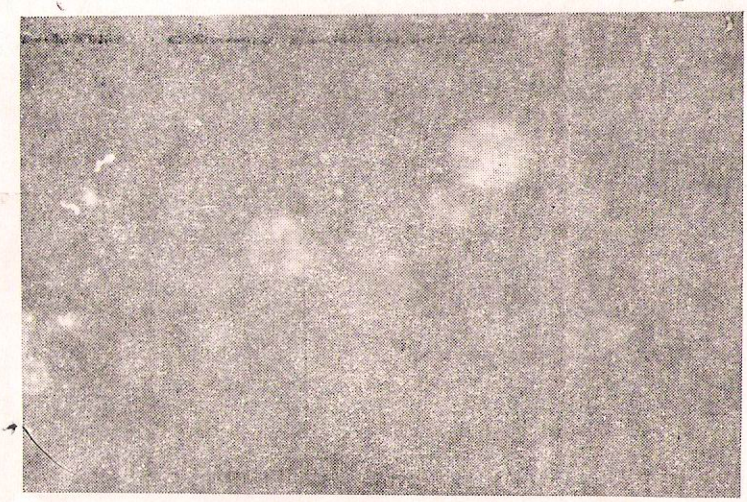

Figure 4. (See Article No. 7) Single cell suspension from oral cancer biopsy is made into a smear, fixed an specific HSV_1 antibody followed by FITC conjugated second antibody is added. Brilliant membrane fluorescence indicate HSV related antigens on the cell surface of oral cancer cells.

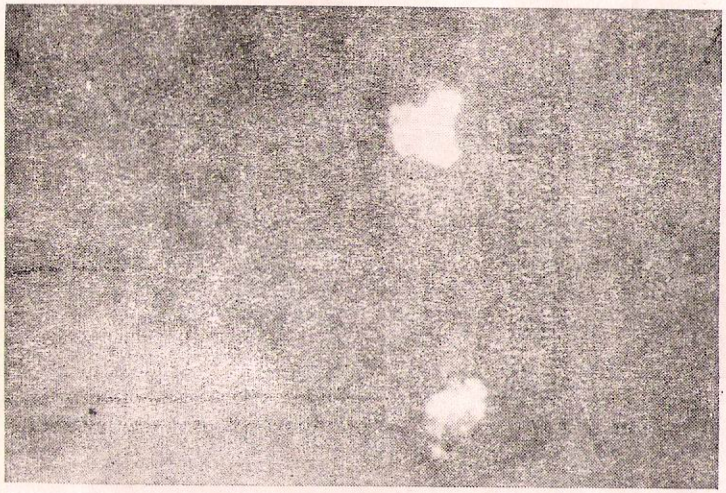

Figure 5 (See Article No. 7)
Similar preparation as in Fig. 4.

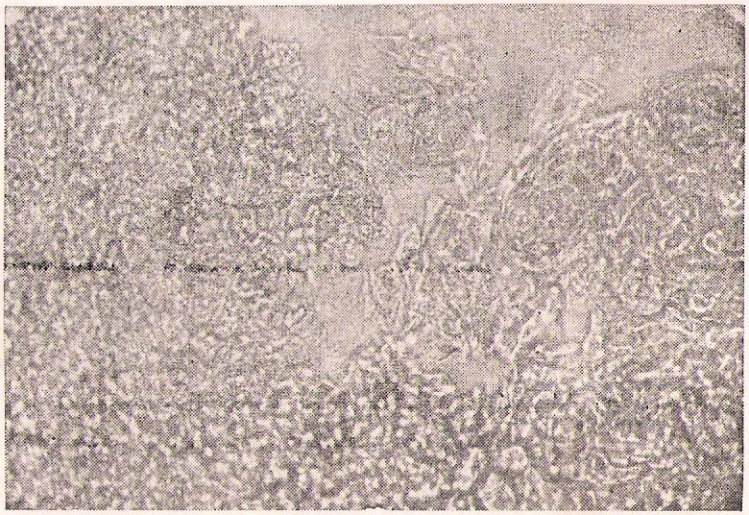

Figure 6 (See Article No 7)
Cell line produced from oral cancer biopsy.
Note the pearl formation.