

## ANTIBODIES AGAINST HERPES SIMPLEX TYPE 1 AND 2 VIRUSES IN CASES OF CARCINOMA CERVIX

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### Introduction

The search for a causative agent/agents of cancer is the history of oncology itself. Naib *et al* (1966, 1969), Rawls *et al* (1968) and Rapp *et al* (1973, 1974) have considered the association of Herpes simplex virus with cancer cervix significantly mainly on the basis of observations that occasionally tumours developed at site of old herpetic lesions. This contention got support from the serological observations by finding titres of antibodies against herpes simplex type 2 (HSV-2) in cases of cancer, specially of the cervix (Nahmias *et al* 1970; Rays *et al* 1969 and Royston and Aurelian, 1970). It has also been reported that the virus antigen is present in cancer cervix cells whole or in part (Frenkel *et al* 1972, Royston and Aurelian, 1970). However, the interpretation of the seroepidemiological methods, the only ones applicable to large human populations, is complicated due to the cross reactivity of herpes simplex virus type 1 and 2 (Tarro and Sabin 1973) and the widespread occurrence of both the viruses.

The high incidence of cancer cervix in this part of country has prompted a serological study for HSV-2 and HSV-1 in cases and controls using metabolic inhibition test for neutralizing antibody titre. The reciprocal of the dilution are the neutralizing antibody titres, and the titre of activity of HSV-2 and HSV-1 are compared by HSV-2/HSV-1 index.

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### Material and Methods

Neutralizing antibodies were determined by metabolic inhibition test in the sera of 50 cases of histologically proved, carcinoma cervix and 43 age matched controls attending the out-patients department of S.N. Hospital, Agra.

Virus Stock: HSV-1 K 455 and HSV-2 K 456 were obtained from the All India Institute of Medical Sciences, New Delhi, on 23-12-1974. The virus titres were 10-4.5 for HSV-1, and 10-5.5 for HSV-2, and the challenging titres were taken as 100 TCID 50.

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Vero cell lines were obtained from the Virus Research Centre, Poona.

The Metabolic Inhibition Test was standardized by using different dilutions of cells and challenging virus dilution. Equal volume of virus was treated with the 1/10, 1/50, 1/250 dilutions of patients' serum and was incubated at 37°C and at room temperature for 1 hour. To the serum-virus mixture in MEM 1 to 250 dilution of vero cells ( $2 \times 10^6$ ) were added. The tubes were sealed with 0.5 ml. of liquid paraffin. The change of pH was observed each day for 7 days. The test was considered positive if pH remained above 7.4 and negative (cellular nor-

mal metabolism) if pH was lower than 7.2. The same series of tests were carried out with the patients of carcinoma cervix and control using in the same group of patients HSV-1 and HSV-2 viruses.

### Results

Majority of sera from the control and cancer cervix cases gave a positive reaction for both HSV-1 and HSV-2. The dilution varied from 1 in 10 to 1 in 250 and above. The dilution of less than 1 in 10 is considered as negative.

and controls so that the test was positive for HSV-1 in 88% of both and HSV-2 in 100% and 93% respectively. However, when quantitative test is used and titres of 1/250 and above are considered the picture is altered significantly less than 5% of cancer cases and controls sera showed a positive reaction for HSV-1, a 30% showed positive reaction for HSV-2 in the cancer group and only 9.3% in control group. This difference is statistically significant.

To compare the distribution of the antibodies for type 2 and type 1 in cancer and control sera, Neutralizing Index was calculated as follows:

$$\text{Neutralization Index } 2/1 = \frac{\text{Log } 10 \text{ Ab titre to type } 2}{\text{Log } 10 \text{ Ab titre to type } 1} \times 100$$

The distribution of cases and controls according to this Neutralization Index 2/1 is given in graph. It is observed that in 15/50 cases of cancer cervix and 14/43 of controls the index was one. However, 27/50 sera of cancer cases and 15/43 controls showed an index above one. Similarly, index below one was seen in 14/43 controls and 8/50 cancer cervix cases.  $X^2$  test was applied to this data and it was found that the higher number of cancer sera showing index above one was statistically significant ( $p < 0.01$  at  $2^\circ$  of free-

TABLE 1  
Distribution of Cases and Controls Showing Antibodies Against HSV-1 and HSV-2

Group	No. of cases	HSV-1				HSV-2			
		Positive 1/10 or above		Positive 1/250 or above		Positive 1/10 or above		Positive 1/250 or above	
		No.	%	No.	%	No.	%	No.	%
Carcinoma	50	44	88%	2	4%	50	100%	15	30%
Control	43	38	88%	2	4.6%	40	93%	4	9.3%

It is evident from Table I that the antibodies to HSV-1 and HSV-2 have a wide distribution both in cases of cancer cervix

and controls. But the fact remains that similar indices were observed in more than 1/3 controls. The data cannot be interpreted

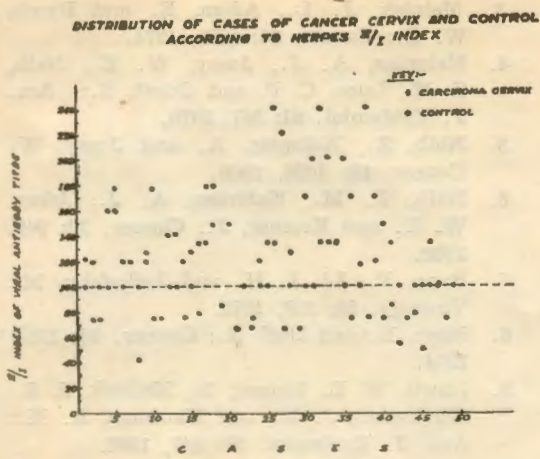


Fig. 1

to indicate direct causal relationship between herpes type 2 virus and cancer cervix. This case is further weakened by the observation that 46 per cent of cancer cervix cases had an index of one or less than one.

#### Discussion

Herpes type 2 (HSV-2) virus has long been said to be associated with the etiology of carcinoma of cervix (Wyburn-Mason 1957; Naib *et al* 1966; Rawls 1919). This feature is based on the epidemiologic studies such as similarity of epidemiologic features of herpes type 2 infections and cervical cancer, occurrence of excessive neutralizing antibodies, higher incidence of cervical abnormalities in women with herpetic infection and antibodies to herpes virus induced non-virion antigens in the sera of patients with cervical cancer (Melnick *et al* 1974).

Royston and Aurelian (1970) detected herpes virus antigen in cells exfoliated from lesion of squamous cell carcinoma of cervix by using immunofluorescent technique and biologically the virus has been shown to have oncogenic potential (Rapp and Duff, 1974).

In the present study all sera from cases of cancer cervix were positive for antibodies against HSV-2 but the controls also showed a positive reaction in 93%, the difference being insignificant. However, it clearly indicates the widespread occurrence of HSV-2 infection. On the other hand, the antibodies were present in higher titres in the sera of cancer cases (30% had above 1/250) while such high titres were observed in only 9.3% of controls. This difference was significant statistically.

The other problem is regarding the specific role of HSV-2 and HSV-1 due to antigenic relationship between the two viruses which cross react biologically due to molecular relationship and common specific antigen reactivity site (Tarro and Sabin 1973). In the present series the neutralization index of HSV-2/HSV-1 was calculated and it was found that in 54% of cancer cervix cases the index was above one. These findings indicate that HSV-2 infection is prevalent in all cases of cancer cervix, though in low titres, the antibodies were also present in the control population, while the antibodies against HSV-1 had similar distribution between cases of cancer cervix and controls. These findings in the light of observation of other workers (Rawls, 1969; 1970; Aurelian *et al* 1973), can be interpreted to mean that Herpes simplex virus 2 cannot be considered to be a specific causative agent for cancer cervix, but may be acting as an oncogenic agent in presence of certain other co-existing factors hitherto unrecognised or it is just a passenger virus which finds a more suitable host in a case of cancer cervix leading to higher titres.

The similar distribution of antibodies against HSV-1 in controls and cancer

cervix cases excludes its role in genesis of cancer cervix.

Summary

The titre of neutralizing antibodies against Herpes Simplex Virus 2 (HSV-2) and HSV-1 in sera from cases of cancer cervix and age matched controls were studied using the metabolic inhibition test. It was considered that antibodies against HSV-2 were prevalent in cases of cancer cervix in high titres while the distribution of HSV-1 was insignificant.

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