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Herpes type-2 virus antibody status in groups of patients with neoplasm in Ibadan

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Summary

Various studies have associated Herpes Type-2 (HT-2) virus with carcinoma of the cervix, especially the squamous cell type. In the present study, prevalence of HT-2 virus antibodies was found to be significantly higher in patients with squamous cell carcinoma of the cervix than in cases with squamous cell carcinoma of sites other than the cervix, and other pelvic and extrapelvic malignancies ($P < 0.001$). It was concluded that no association could be found between Herpes Type-2 virus and malignancies of sites other than the cervix.

Introduction

Various seroepidemiologic and experimental studies have associated Herpes viruses with oncogenesis in animals (Epstein, Achong & Bann, 1964; Churchill & Biggs, 1968; Mizell, Tompkin & Isaacs, 1969; Trenton, 1969) and man (Rawls, Tompkins & Melnick, 1969; Nahmias *et al.*, 1970a; Melendez *et al.*, 1972). In particular Herpes Type-2 virus (HT-2) has been associated with carcinoma of the cervix because of the higher prevalence of HT-2 virus antibodies amongst patients with the disease as compared with controls (Nahmias *et al.*, 1970a; Catalano & Johnson, 1971; Adelusi, Osunkoya & Fabiyi, 1975).

In an earlier study undertaken in Ibadan using the indirect immunofluorescence technique (Adelusi *et al.*, 1975), the higher prevalence of HT-2 virus antibodies in patients with squamous cell carcinoma of the cervix uteri was very marked, when compared with other histologic types of cervical growths, as well as with controls. It was then propounded that HT-2 virus might have oncogenic potentiality for squamous cells generally, both of cervical and extra-cervical origin, the squamous cell type no matter where situated, providing an ideal ground for HT-2 virus replication. On the other hand, it might be that this potentiality of the virus is not tissue specific, the virus being associated with various tissues and therefore giving rise to neoplasia in organs of pelvic or extrapelvic origin.

We have found no record in the literature so far of work done on the possible role of HT-2 virus on the pathogenesis of squamous cell carcinoma of sites other than the cervix, although its association with malignancies of sites other than the cervix had been shown (Rawls *et al.*, 1969). This study was initiated to compare the prevalence of HT-2 virus antibodies in patients having squamous cell carcinoma of cervix and other sites, and those with malignancies of non-squamous cell origin.

Materials and Method

Serum samples were collected from blood obtained from the following. (a) Forty patients with histologic diagnosis of squamous cell carcinoma of esophagus (6), larnyx (14), bronchi (1), palate (2), maxilia (4), intra oral tumour (1), nasal tumour (2), eyelid (3), bladder (2) and the skin of mons pubis, back and thigh (5). (b) Thirty patients diagnosed as having malignant trophoblastic diseases (MTD) by histology urinary human chorionic gonadotrophin (HCG) estimation, and pelvic angiogram. (c) Twenty patients with carcinoma of liver on histologic basis as well as serum alpha-feto protein detection. (d) Ten patients diagnosed histologically as having carcinoma of the breast. (e) Six patients with carcinoma of vulva. (f) Twenty patients with histologic diagnosis of squamous cell carcinoma of the cervix uteri. (g) Ten healthy women seen in the

Family Planning Clinic of the hospital, with complaints unassociated with any malignancy. This group constituted the control group.

Patients in the study were generally adults of comparable age groups. The serum samples were coded and tested blind.

Virus stock and methodology

These were as described in the earlier study (Adelusi *et al.*, 1975). Briefly, smears prepared from HT-2 virus infected vero cells (adjusted to $0{\cdot}6 \times 10^6$ cells per ml) were fixed in the cold acetone, washed with PBS (pH 7·2) and covered with test serum for 30 min in a moist chamber. These were stained with FITC-Rabbit antihuman globulin (Microbiological associates, Bethesda, Maryland, U.S.A.), and examined with a Reichert Fluorescent Microscope fitted with HB 200 super-pressure mercury lamp.

Results

Table 1 shows the distribution of antibody titres among all cases and controls. HT-2 virus antibody was present in all sera up to a titre of 20; the distribution varied from 20 up to 2560 titre. The commulative distribution in each group showing positive reactions up to the stated titre is shown in Table 2. When the cummulative distribution for the cervical cancer group was compared with each of the other groups, including the controls, by the use of the Kolmogorov & Simirnov (1956) test, a significant difference ($P < 0{\cdot}05$) was found in each case. Compared with the control group by the use of the same test, the distribution of the extra-cervical tumour groups showed no significant difference.

Using the titre of 640 as the cut-off point (Adelusi *et al.*, 1975), Table 3 shows the prevalence of HT-2 virus antibodies among the various groups. The percentage of the carcinoma of cervix patients showing positive reaction at titre of 640 and above (70%) is significantly higher than among all other carcinoma cases (16·0%) and among the controls (20%). ($\chi^2 = 25{\cdot}17$ on 2 d.f. $P < 0{\cdot}001$). Even when the titre of 320 is used, the difference is still significant ($P < 0{\cdot}005$) whereas the two latter groups exhibit no significant difference ($\chi^2 = 0{\cdot}003$ on 1 d.f. $P > 0{\cdot}05$).

Discussion

Reports of sero-epidemiologic studies in Ibadan (Adelusi *et al.*, 1975) and elsewhere (Nahmias *et al.*, 1970a; Nahmias *et al.*, 1970b; Catalano & Johnson, 1971; Janda *et al.*, 1973) have consistently shown an association between HT-2 virus and cervical cancer, by demonstration of higher HT-2 virus antibody titres as compared with controls. In the earlier study at Ibadan (Adelusi *et al.*, 1975), it was shown that whereas antibodies to HT-2 virus were generally low in the cervicitis, adenocarcinoma, undifferentiated carcinoma of cervix and the control groups on the one hand, it was relatively high in the group with squamous cell carcinoma of the cervix.

TABLE 1. Distribution of HT-2 virus antibody titres in patients with various malignant tumours and control women, as detected by the immunofluorescence technique

Titre	Sq. cell Ca (Cx)	Sq. cell Ca (others)	Carcinoma vulva	MTD	Carcinoma liver	Carcinoma breast	Healthy control
0	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—
20	—	—	—	—	—	1	1
40	1	7	1	4	3	4	1
80	3	16	2	9	7	3	3
160	2	4	1	6	6	1	2
320	—	5	1	6	1	—	1
640	8	4	1	3	2	1	2
1280	4	3	—	2	1	—	—
2560	2	—	—	—	—	—	—
Total	20	40	6	30	20	10	10

(1) Sq. Cell Ca (CX): squamous cell carcinoma of cervix; (2) Sq. Cell Ca (others): squamous cell carcinoma of sites other than cervix; (3) M.T.D.: malignant trophoblastic disease.

TABLE 2. Cummulative distribution of subjects in each group showing positive reaction up to stated titre by the immunofluorescence technique

Titre	Sq Cell Ca (Cx)		Sq Cell Ca (others)		Ca Vulva		M.T.D.		Ca Liver		Ca Breast		Controls	
	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)
10	—	—	—	—	—	—	—	—	—	—	—	—	—	—
20	20	100·0	40	100·0	6	100·0	30	100·0	20	100·0	10	100·0	10	100·0
40	20	100·0	39	97·5	6	100·0	30	100·0	20	100·0	9	90·0	9	90·0
80	19	95·0	32	80·0	5	83·3	26	86·7	17	85·0	5	50·0	8	80·0
160	16	80·0	16	40·0	3	50·0	17	56·7	10	50·0	2	20·0	5	50·0
320	14	70·0	12	30·0	2	33·3	11	36·7	4	20·0	1	10·0	3	30·0
6640	14	70·0	7	17·5	1	16·7	5	16·7	3	15·0	1	10·0	2	20·0
1280	6	30·0	3	7·5	0	0·0	2	6·7	1	5·0	0	0·0	0	0·0
2560	2	10·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0

(1) Sq Cell Ca (Cx): squamous cell carcinoma of cervix; (2) Sq Cell Ca (others); Squamous cell carcinoma of sites other than cervix; (3) M.T.D.: malignant trophoblastic disease.

TABLE 3. Number of subjects showing positive HT-2 virus antibody reaction at titres of 640 and above among % test groups and controls by immunofluorescence technique

	Sq Cell Ca (Cx)	Sq Cell Ca (others)	Ca Vulva	M.T.D.	Ca Liver	Ca Breast	Controls
Positive	14	7	1	5	3	1	2
Negative	6	33	5	25	17	9	8
Total	20	40	6	30	20	10	10
Positive (%)	70·0	17·5	16·7	16·7	15·0	10·0	20·0

(1) Sq Cell Ca (Cx): squamous cell carcinoma of cervix; (2) Sq cell Ca (others): squamous cell carcinoma of sites other than cervix: (3) M.T.D.: malignant trophoblastic disease.

Sq Cell Ca Cx Vs other carcinoma cases+controls $\chi^2 = 25{\cdot}17$ on 2d.f. $P = 0{\cdot}001$.

Other carcinoma cases Vs controls: $\chi^2 = 0{\cdot}003$ on 1s.f. $P = 0{\cdot}5$.

It was suggested that the virus might have a predelection for squamous cells generally as such cells might provide the ideal ground for excessive HT-2 virus replication and hence, the large production of antibodies against the virus, in contrast to the poor growth (and low antibody titre) of the virus in other histologic types of cells.

In the present study where various histologically diagnosed squamous cell carcinoma of sites other than the cervix have been employed, however, the distribution of HT-2 virus antibody titre is very much unlike the distribution in the squamous cell carcinoma of the cervix. It follows more or less the pattern of distribution in the normal control population. As far as we are aware, no similar studies on squamous cell carcinoma of sites other than the cervix was found in the literature with which to compare our results. Hence, this would need confirmation by other workers.

As regards other extra-cervical pelvic and extra-pelvic malignancies, Rawls *et al.* (1969) determined HT-2 virus antibodies in twenty-two patients. Antibodies were detected in two women with carcinoma of the vulva, one case each of carcinoma of the ovary and testis. No antibodies to the virus were found in six cases of carcinoma of the prostate, five cases of carcinoma of the bladder, two cases of carcinoma of the breast and a single case of carcinoma of the gall bladder, tongue, stomach, tonsil and uterus.

Unlike in the study of Rawls *et al.* (1969), however, there was no significant antibody levels in the patients with extra-cervical pelvic and extra-pelvic malignancy in this study, using immunofluorescence method. The possibility of the virus having oncogenic

potentiality for tissues other than the cervical squamous epithelium was not borne out by the results of the present study. Whereas the number of patients with carcinoma of the cervix having antibody titres above 640 was high (70%), this was relatively low in all the other cases of cancer.

The venereal mode of infection of HT-2 virus has been well established (Dowdle *et al.*, 1967; Nahmias & Dowdle, 1968; Adelusi *et al.*, 1976). It is of particular interest to note that the cervix has often been found to be infected in the absence of external genitalia involvement (Nahmias *et al.*, 1969), suggesting that the cervix is often the initial site of genital herpes virus infection in the female. It is possible that the oncogenic potential of the virus is limited to the cervix, although other etiological factors cannot be excluded (Munoz, 1973).

The serologic test employed in this study, as with other serologic methods (kinetics of neutralization and microneutralization test) is not specific for HT-2 virus because of the problems of cross-reactivity between Herpes Type-1 (HT-1) and HT-2 viruses. However, the method of determining titres of antibody in the sera of patients and controls in a blind experiment, and the use of cut-off points at a higher titre for the presence of significant antibodies, were designed to distinguish between two groups of individuals with predominance of HT-1 and HT-2 viruses. This is because genital infection with HT-2 viruses cause the production of antibodies which neutralize HT-2 viruses better than HT-1 viruses (Rawls *et al.*, 1970; Roizman, Keller & Spear, 1970). Furthermore, immunofluorescence is much simpler and more sensitive than the other two methods of serology.

It may be concluded that from the result of the present study, there is no association between HT-2 virus and extra-cervical malignancies. The association already established between the virus and carcinoma of the cervix, especially the squamous cell type (Naib, Nahmias & Josey, 1966; Nahmias *et al.*, 1970a; Nahmias *et al.*, 1970b; Catalano & Johnson, 1971; Janda *et al.*, 1973; Adelusi *et al.*, 1975), may be peculiar to this growth.

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