A Study of Associated Factors, Including Genital Herpes, in Black Women with Cervical Carcinoma in Johannesburg

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SUMMARY

Herpesvirus hominis **antibodies were measured by a kinetic neutralisation test. Among Black patients in the Johannesburg area there is a high incidence of carcinoma of the cervix and** *H. hominis* **type 2 infection. A correlation exists between carcinomas of the cervix on the one hand and antibodies to** *H. hominis* **type 2 on the other. In addition, patients with keratinising tumours were more promiscuous (mean of 2,9 consorts each) and had more venereal disease (81% positive fluorescent treponemal antibody tests) than those with non-keratinising tumours mean of 2,1 consorts and 52% positive FTAs). The latter had a poorer antibody response (mean 1,9 times lower than matched controls).**

It is clear that more specific differentiation between the *Herpesvirus* **types is needed before firm conclusions can be reached. These findings confirm those found by other workers elsewhere but suggest in addition that differentiation into tumour types is needed to separate tumours of different aetiological origin.**

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The epidemiology of carcinoma of the cervix was reviewed by Coppleson in 1969.[1] The main contributions in South Africa were made by Oettlé,[2,3] Robertson,[4] Higginson and Oettlé,[5] Sutherland[6] and Schonland and Bradshaw.[7]

Carcinoma of the uterine cervix is an important entity in South African Blacks; as is apparent from the high age-standardised rates for the disease, particularly in urban areas, of up to 38,9/100 000.[7] Oettlé[3] found an incidence of 35,3/100 000 for the Johannesburg urban area, which is the source for this study.

These figures can be compared with those for USA Whites of 22,0/100 000 and USA non-Whites of 48,6/100 000 in 1961.[2] The latter is the highest recorded figure quoted by Oettlé.[3] Available statistics from Africa show a high incidence variation in the geographical distribution of some malignancies. The possible reasons for this are the inaccessibility of sophisticated hospital and laboratory services, prejudice against medical services by the indigent populations and poor case reporting by medical personnel. Yet in some instances, e.g. carcinoma of the oesophagus and hepatoma, the variation is too great to be explained by these factors. The proportional frequency of cervical carcinoma in Africa varies from 5,4% of all cancers in females in Kuluva, Uganda, to 49,8% of all cancers in females in Johannesburg.[8] A more satisfactory explanation is, therefore, the variation in the physical and social environments associated with cultural contact.

The main aim of this study was to determine the relationship of *Herpesvirus hominis* type 2 (HSV 2) to cervical carcinoma. Other factors of possible aetiological and epidemiological significance were also compared. The data were statistically analysed in detail to evaluate their significance more clearly.

PATIENTS AND METHODS

Forty-eight Black patients from the Baragwanath Hospital presenting with invasive carcinoma of the cervix in 1971 were matched according to tribe and age (within ± 10 years) with controls from the medical and surgical wards of the same hospital. Patients with known malignancy were excluded from the control group.

The following parameters were studied: educational and social environments associated with cultural contact, coitus and pregnancy and the number of coital partners. All patients were interviewed blind by one Black social worker (S.N.). Trichomoniasis, syphilis and herpesvirus were included under the heading of venereal factors. The incidence of syphilis was determined with the fluorescent treponemal antibody test (FTA). Bilharzial and cercarial complement fixation tests were also performed. Smears obtained from cervical lesions and the cervices of controls were stained by means of the Papanicolaou technique and examined for evidence of trichomoniasis. Though not as reliable a method as direct culture, the Papanicolaou smear is a simple method of showing a difference between the study and control groups.[9] Evidence of HSV 1 and 2 infection was derived from direct viral culture and serology.

The cervical swabs, transported on ice, were inoculated onto diploid human embryonic lung fibroblast cultures in tubes maintained and passaged in Eagle's basal medium

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with extra glycine and serine and 5 - 10% bovine serum. Immediately before inoculation the serum content was reduced to 2%. The tubes were observed for cytopathic effect for 10 days, since in this system we have found virus growth to occur invariably within 7 days. The isolates were typed by pock formation on the chorio-allantoic membrane of embryonated eggs.

Serum was examined for neutralising antibody against HSV 1 and 2 on human fibroblasts (as above) using Russell's method[10] with 10% pooled human serum instead of an agar overlay. The viruses used were E68/70 (a local HSV 1) and the Savage type 2 virus. The tissue was stained with 1% methyl violet after a short alcohol fixation at 2 - 3 days and plaques were counted by naked eye. K values were developed using the formula

$$K = \frac{\log_{10}V_0 - \log_{10}V_1}{0{,}43\ Ct}$$

where V_0 is the control number of plaques, V_1 the number remaining after adding the test serum, C the concentration of serum and t the time in minutes of virus and serum exposure (60 minutes was used) as described by McBride.[11] Antibodies to HSV 1 and 2 were tested for separately, since the plaques of the 2 viruses were indistinguishable from each other, unlike the Hep 2 system. The results were analysed statistically by two-by-two tables, Wilcoxon's matched pair tests and regression line where appropriate.

Clinically there were 39 ulcerative, 7 hypertrophic and 2 infiltrative tumours. The carcinomata were graded histologically according to the classification of Reagen *et al.*[12] (Table I). Clinical staging is compared with other studies in Table II.

TABLE I. PATHOLOGY OF CARCINOMATA

Gross appearance		Histological appearance	
Ulcerative	39	Keratinising	16
Hypertrophic	7	Large-cell non-keratinising	27
Infiltrative (barrel-shaped cervix)	2	Small cell	4
		Mixed anaplastic adeno-carcinoma	1

TABLE II. ANALYSIS OF CASES ACCORDING TO CLINICAL STAGE

Clinical staging	This study (1971)	Schonland[7]* (1969)	Higginson and Oettlé[5]* (1960)	Gusberg[15] (1970)
I & II	(13) 27%	26,5%	20 - 25%	70,3%
III & IV	(35) 73%	73,5%	75 - 80%	29,3%

* Mass cytological screening was not available for these centres before 1971.

RESULTS

Age Distribution

The mean age for the carcinoma group was 49,1 years, while that for the controls was 46,1 years (Table III). This compares with a figure of 48,37 from a larger series.[7] Two-thirds of our cases occurred in the 30 - 59-year age group. Again this approximates the figures of other South African series. The youngest patient in our series was 22 years old. Neither Schonland and Bradshaw[7] nor Oettlé[5] found cases in the under-20 age group. Oettlé did note, however, that above the age of 20 years the frequency of adenocarcinoma of the cervix increased with age in the same proportion as the squamous variety and postulated a similar aetiological relationship between squamous and adenocarcinoma of the cervix in the over-20 age group.

TABLE III. SUMMARISED RESULTS

	Carcinoma group	Control group
Mean age (years)	49,1	46,1
Schooling	18/48	21/48
Reading and writing	14/48	11/48
Mean years school	2,3	2,1
Parity (mean)	5,3	4,3
Nullipara	1/48	8/48
Mean age of menarche	14,4	14,8
Sexual partners (mean)	2,4	2,6
Mean age of first coitus	16,2	16,2
Bilharzial CFT positives	22,9%	10,9%
Positive FTA	64%	52%

Tribal Differences

The 5 large Bantu-speaking groups[13] of South Africa have distinct language, cultural and social differences. The groups, subgroups (e.g. Zulu, Ndebele, etc. of the Nguni group) and individuals within them, differ in the extent to which they have adopted 'Western' habits and customs. The tribal distribution in our original carcinoma group was: Nguni 25 cases, Sotho 21, Venda 2, Shangaan 1 and Lemba no cases. This is not to be taken as a true reflection of the tribal incidence of the disease, since this is a hospital distribution unrelated to the geographical distribution of the disease in the population. We were unable to find a control for the single Ndebele patient with carcinoma, and she was therefore excluded from the study. In the rural setting particularly, there are self-imposed restrictions and taboos on premarital coitus and pregnancy. Similarly, coitus is forbidden during menstruation, certain periods of pregnancy and before weaning. The application of these practices, however, is not uniform even in rural areas, e.g. while the Mopedi subgroup and Venda adhere rigidly to custom, the Tswana apparently do not.

Educational and Socio-economic Status

Of the 48 cancer cases, 18 had no schooling and only 14 could read and write English. The figures for the control group were 21 and 11 respectively. The mean level reached for the carcinoma group was 2,3 years of schooling, while that for the controls was 2,1. The economic status was considered moderate, i.e. R500 - R700 per

year in 16 of the carcinoma group and 18 of the controls. The remainder earned less than R500 per year. The high incidence of social and economic poverty in the study groups was similar to that of the general Black population for 1971.

Parity

The mean parity for the carcinoma group was 5,3, compared with 4,3 for the controls. When the 8 nulliparas were excluded from the controls, the mean increased to 5,2. There was one nullipara in the carcinoma group. These differences are not statistically significant. Although the frequency of nulliparas for the controls was 16%, South African studies performed at the menopause indicated an infertility rate for both urban and rural Blacks of only 3 - 4%.[5,34] Since our controls are hospital patients, it is possible that the reason for admission of some of them could also be responsible for their infertility, e.g. diabetes and cirrhosis.

Menarche

The mean age of menarche for our cancer cases was 14,4 years compared with 14,8 for the controls. Schonland[1] and Oettlé[2] found similar results.

Coital History

The mean number of sexual partners in the cancer group was 2,58, compared with 2,4 for the controls. For 2 cases each in the control and carcinoma groups information about the number of consorts was not available. The mean age at first coitus was 16,2 years for both groups.

Bilharziasis

The incidence of bilharziasis as determined from the bilharzial and cercarial complement fixation tests was 22,9% for the cancer group and 10,9% for the controls. This difference is not significant at $2P>0,1$.

Syphilis and Trichomoniasis

Fifty-two per cent of the control group and 64% of the carcinoma patients had a positive FTA. Thirteen of the 16 cases with keratinising tumours had positive FTA results, while only 14 of the 27 large-cell non-keratinising tumour cases were positive (Table III). Ten of the patients with keratinising tumours had a positive FTA and more than 2 consorts, while only 5 of the 25 in the large-cell non-keratinising group were in this category ($P = 0,01$). Of the carcinoma group 51% had trichomoniasis on vaginal cytology compared with 4% of the controls ($P<0,0001$).

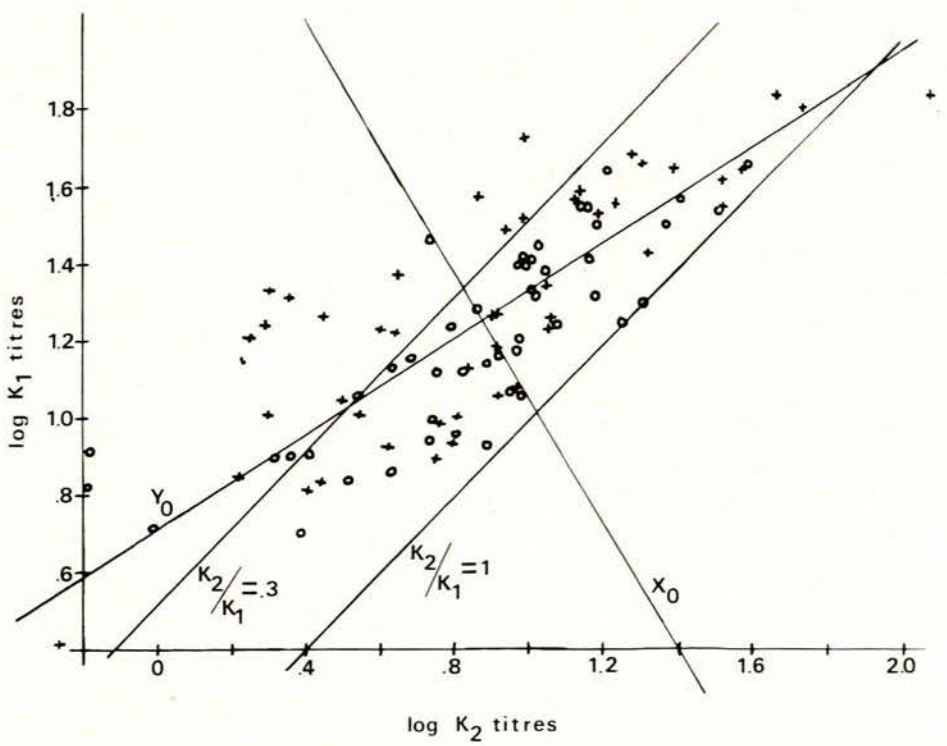

Fig. 1. HSV antibody titre results (+ = control cases; o = carcinoma cases; Y_0 is the regression line for all the results; X_0 is the regression line orthogonal to Y_0; K_2/K_1 is the ratio of HSV 2 to HSV 1 antibody titres).

Herpesvirus hominis

Isolation attempts yielded only one *H. hominis* type 2 strain and one cytomegalovirus, both from the carcinoma group.

The antibody titrations were plotted as the logarithms of the K values to facilitate evaluation of the K ratios as these now appear as parallel straight lines on the graph (Fig. 1).

Using the criteria of Royston *et al.*[15] where a K_2/K_1 ratio between 0,3 and 1,0 indicates a double infection by both viruses, 16 controls and 6 carcinoma patients had only an HSV 1 infection and 1 control had no herpes infection at all (K_1 value 2,6 only). The difference is significant at $P<0,02$.

Since the patients and their controls are matched pairs, a better test is Wilcoxon's test for matched pairs, which is significant at $P = 0,065$ when the perpendicular distance from the 1,0 K ratio line is used as measure.

There is no significant difference in the mean of the HSV 1 and HSV 2 antibody titres ($2P = 0,81$) between the control and carcinoma groups.

However, in our antibody measuring test system, unlike Royston *et al.*[15] we neutralise against HSV 1 and HSV 2 separately. We feel that this could result in a different slope of inactivation by cross-reacting antibodies. If so, the regression line of the results should differ from 45° (K ratio lines). In fact the line is: $Y_0 = 1,609(\log K_1) - 1,15$, with a correlation coefficient of 0,80. The slope of the line is significantly different from 45° at $P<0,001$.

We analysed the results further, using this line rather than the simple K ratios as criteria, for the following reasons: (*i*) that the highest antibody titre result has a K ratio less than 1 and is therefore an HSV 2 infection *only* by this criterion, yet we know that antibody responses to HSV 2 are nearly always less than to HSV 1; (*ii*) because antibody responses to HSV 1 are better than those to HSV 2 on average, we would, if anything, expect the regression line for all points to have a slope greater than 45°, not less; (*iii*) the differences between the groups are maximal when the regression line is used, not the K ratio lines, suggesting strongly that our results are, in fact, measuring neutralisation in a significantly different manner.

Under these circumstances the orthogonal distance from the line towards the K_1 axis indicates a preponderance of HSV 1 antibodies and towards the K_2 axis a preponderance of HSV 2 antibodies. To make this clearer, the results were replotted in Fig. 2 where points to the left of the Y_0 axis have more HSV 1 antibodies than those to the right, which have more HSV 2 antibodies. In addition, distance along the Y_0 axis is a measure of the mean of the HSV 1 and HSV 2 antibody titres. We now find that the carcinoma patients have more HSV 2 antibodies than the matched controls as before, but with $P<0,006$. The mean of the HSV 1 and HSV 2 antibody titres does not differ ($2P = 0,86$) between the controls and the carcinoma patients.

If we now compare the results of the patients with keratinising and non-keratinising tumours, we note that both groups have lower K ratios than their controls but that the non-keratinising group has lower mean antibody

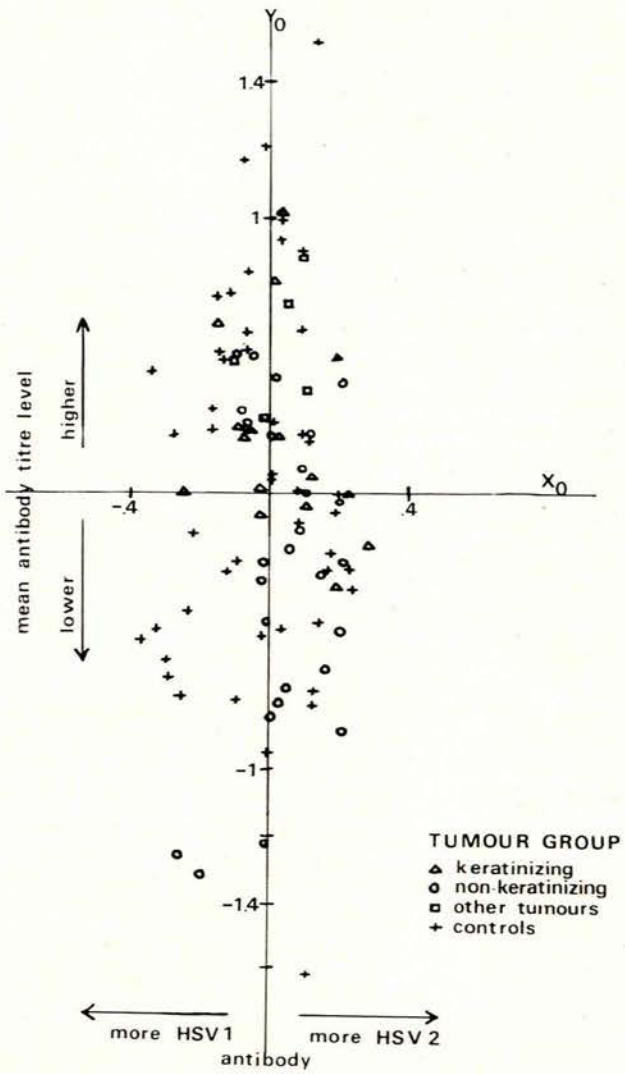

Fig 2. Herpes antibody results according to tumour type. Y_0 and X_0 are the regression lines of Fig. 1. Units are to the same scale as Fig. 1.

levels than the keratinising group ($P<0,01$) or their matched controls ($P = 0,06$). Table IV provides a summary of the above data.

DISCUSSION

Others[16-19] have found a correlation between HSV 2 infection and carcinoma cervix. Rawls *et al.'s*[20] Auckland study did not show any such correlation. The different tumour types were not separated in any of these studies and specific tests for venereal disease or coital history were not done or evaluated consistently. From our own results it is apparent that a clearer answer to the relation of HSV 2 infections to carcinoma cervix would be given if the effect of cross-reacting antigens could be removed and the remainder tested for specifically. Since we were fortu-

TABLE IV. SUMMARY OF HSV ANTIBODY FINDINGS

	Non-keratinising cases	Matched controls	Keratinising cases	Matched controls	All tumours	Controls
Number of cases	27	27	16	16	48	48
Mean log (K_1) titre	1,10	1,32	1,31	1,31	1,21	1,30
Mean log (K_2) titre	0,70	1,02	1,02	0,90	0,86	0,88
Mean Y_o axis value	−0,243	+0,041	+0,156	+0,045	−0,041	+0,028
Mean X_o axis value	+0,007	−0,046	+0,047	−0,029	+0,062	−0,031
% FTA positives	52	55	81	50	65	52
Mean No. consorts	2,1	2,4	2,9	2,7	2,4	2,6

nate in having sufficient information, we could differentiate the carcinoma patients into 2 large groups: keratinising and large-cell non-keratinising tumours (Table IV). A third group of 4 small-cell non-keratinising tumours and 1 adenocarcinoma were not analysed further, since their numbers were too small.

As Rawls *et al.*[27] also found, there was no significant difference in the preponderance of HSV 2 infection between the 2 major tumour groups in our cases (Table IV, mean X_o axis deviation). However, a difference in mean antibody level and promiscuity was found between the tumour groups. The keratinising tumours occurred in patients with K_1 antibody titre like those of the controls (keratinising tumours: Y_o value = + 0,156; controls: Y_o value = + 0,045) but had a greater promiscuity, as evidenced by their more often positive FTAs (81% as against 50% in the controls) and greater number of consorts (2,9 as against 2,4 in the matched controls). This suggests that keratinising tumours are related to an extrinsic coital factor. Conversely, it would appear that the large-cell non-keratinising tumours may not be related to venereal exposure but have an impaired immunological status (Y_o value = − 0,243) or a lesser antigenic exposure to the viruses. The age incidences of the 2 tumour types are not significantly different, the mean being 46,7 and 50,6 years respectively. Seven of the keratinising tumours were stage II carcinomas as against only 2 of the large-cell non-keratinising tumours (P = 0,01). Most of the latter were stage III tumours.

The high level of promiscuity in our carcinoma and control groups explains the high incidence of HSV 2 and syphilis in our patients, both being venereal diseases. Parity, age of menarche, first coitus and first pregnancy are not different in the main tumour, over-all carcinoma or the control groups. Marital and educational status and bilharziasis incidence are also not significantly different. In our study the incidence of nulliparity is 2%. In some studies the incidence of nulliparity in carcinoma cases has been as high as 11%,[21] and 25% of stump carcinomata have occurred in nulliparas.[15] The relationship of parity to carcinoma of the cervix is, therefore, inconclusive.

We feel that the enormous difference between the controls and the carcinoma cases for trichomonas infection as determined by Papanicolaou smear is the result of 2 factors, namely: (*i*) the increased numbers of trichomonads found in any inflammatory condition resulting in a lowered pH of the vaginal secretions. This frequently occurs in carcinoma of the cervix due to secondary infection; and (*ii*) the relative insensitivity of the Papanicolaou smear in detecting trichomonads. Therefore, the controls, who are more likely to be in a quiescent phase of trichomonas infection, will most probably not be diagnosed by this method.

In conclusion, therefore, the significance of the results obtained is open to serious question and our results cannot be used to evaluate the role of trichomoniasis in carcinoma of the cervix.

In Western countries low socio-economic and educational status parallels an increase in promiscuity. In the South African rural areas, as with the Parsis in Bombay,[22] and North American Indians,[23] poverty in terms of Western standards and illiteracy are not necessarily associated with an increase in the incidence of promiscuity or of carcinoma of the cervix; hence the importance of a more critical attitude towards assessing carcinoma cervix risks in terms of socio-economic and educational standards, particularly when applied to more primitive communities.

The incidence of bilharziasis in the carcinoma and control groups is not significantly different. Schonland and Bradshaw[7] found an incidence of 6% in cervical cancer cases in Natal and did not feel this to be significant, while Sutherland[8] in the Northern Transvaal found that 56% of cervical biopsies for various gynaecological conditions had bilharziasis. Gelfand[24] quotes an incidence of 86% for the general population based on the postmortem examination of the female genital tract. Youssef *et al.*[25] recently described 397 cases of genital bilharziasis, of which 30,5% involved the cervix. The incidence of associated cervical malignancy was 13,2%; however, Bland and Gelfand[26] were unable to show such an association in a similar study. It is therefore unlikely that bilharziasis is of any aetiological significance in carcinoma cervix in South Africa.

The results in this article confirm the association between carcinoma cervix and HSV 2 infection and other venereal diseases found by other workers. In addition it was found that a relative immunological deficiency or lesser antigenic stimulation occurred in cases with non-keratinising tumours.

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