Herpesvirus hominis type 2 infection in Ibadan

Problem of non-venereal transmission

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SUMMARY Examination of sera from blood donors, from patients attending a special treatment clinic, a family planning clinic, and an antenatal clinic showed that the prevalence of herpesvirus hominis type 2 antibodies among the adult population in Ibadan is similar to that in other parts of the world.

The possibility of non-venereal transmission of herpesvirus infection was confirmed by the finding that herpesvirus hominis type 2 could survive on cloth samples under humid tropical conditions for long enough to allow transmission of infection via fomites.

Introduction

In a previous paper¹ we reported on the acquisition of antibodies to type 1 and type 2 herpesvirus hominis (HVH-1 and HVH-2) by children and young adults in Ibadan, Nigeria. Antibodies to HVH-1 were acquired rapidly from the age of 1 year onwards, so that by the time the children had reached teenage approximately 80% had developed antibodies to this type of virus. We suggested that the rapid appearance of HVH-1 antibodies reflected the generally poor living conditions and low socioeconomic status of the population studied.²

Antibodies to HVH-2 are uncommon, although not unknown, before the age of puberty,³ but in Ibadan we found that children started to acquire this type of antibody between the ages of 3 and 5 years. From 5-20 years, which was the oldest age group studied, the proportion of children with HVH-2 antibodies remained virtually constant at about 12% of the population. We suggested that non-venereal transmission of infection could be responsible for this early development of HVH-2 antibodies and that the virus might be capable of remaining viable outside the human body for some hours under humid tropical conditions, thus facilitating possible transfer of infection via fomites. The survival of *Neisseria gonorrhoeae* under such conditions had previously been demonstrated in this laboratory⁴ and could possibly account for the relatively common occurrence of gonococcal vulvovaginitis among young girls in Ibadan.⁵

The possible importance of tropical conditions in influencing the spread of infection has also been recently suggested by Leiker,⁶ who showed that *Mycobacterium leprae* could remain viable for prolonged periods in the environment under humid tropical conditions but not in the drier, cooler climate of temperate countries.

Our previous study¹ did not include subjects in older age groups and comprised only school pupils or children who had not yet reached school age. All the subjects were therefore either prepubertal or subject to social constraints which were likely to limit to some extent the chance of acquiring disease by sexual contact.

We now report our findings with regards to older subjects, who were either unemployed, or engaged in civilian jobs or in the armed Forces, or were housewives or small traders. We also report the results of experiments which demonstrate that under suitable circumstances type 2 herpesvirus hominis can remain viable outside the human body for long enough to allow the spread of infection via fomites rather than by direct bodily contact.

Patients and methods

ANTIBODY STUDIES

Four groups of subjects were studied.

Blood donors

Blood donors were all male volunteers and included relatives of patients undergoing surgery in the hospital as well as members of the army, police, and civilian youth organisations. A total of 228 subjects was included in this group.

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A sample of blood was collected into a sterile bottle during the blood donation and after clotting had taken place the serum was separated and stored at -20°C until tested.

Many people did not know their age accurately but from the information given the mean age of this group was 28.5 years with a standard deviation (SD) of 6.9 years.

Antenatal clinic patients

Serum samples were obtained from 283 patients attending the Antenatal Clinic. These were initially collected for routine Venereal Disease Research Laboratory (VDRL) tests but were also examined for antibodies to type 1 and type 2 herpesvirus hominis. All samples were stored at -20°C before examination. Individual ages were not available for this group of patients, who were all young women of child-bearing age.

Family planning clinic patients

Serum samples were obtained from 159 patients attending the Family Planning Clinic. Similarly, the samples were originally collected for routine testing by the VDRL test but were also examined for herpesvirus antibodies. Individual ages were not available but all the patients were young women of child-bearing age.

Special treatment clinic patients

Serum samples collected for routine VDRL tests from 167 consecutive patients attending the Special Treatment Clinic were also examined for antibodies to type 1 and type 2 herpesvirus hominis. Of these patients, 125 were men and 42 were women, a sex distribution which reflects the difficulty experienced in contact-tracing and ensuring the attendance of consorts. Although individual ages are unlikely to be accurate, the calculated mean age for this group was 27.8 years with a standard deviation of 8.1 years.

NEUTRALISATION TESTS

The method used for testing the sera has been described previously¹ and was based on that of Pauls and Dowdle⁷ with the modifications described by Roome *et al.*⁸

Control sera from rabbits initially immunised against HVH-1 and HVH-2 respectively were included in each batch of tests. These control sera were first tested 78 times against each type of virus, the pN values determined, and the pN-type-2:pN-type-1 ratio calculated. This ratio, multiplied by 100, was termed the NpN value. For the type 1 antiserum the mean NpN value was 78.5, whereas for the type 2 antiserum the mean NpN value was 113.2 with standard deviations of 6.5 and 15.2 respectively. In

interpreting the results of test sera those which gave NpN values identical with those of the control sera (± 1 SD) were regarded as containing only a single antibody of the appropriate type. Sera with NpN values falling between the control values (± 1 SD) were considered to contain both types of antibody.

SURVIVAL OF HERPESVIRUS HOMINIS TYPE 2

Fifteen squares of washed and sterilised white cotton cloth were each inoculated with 0.2 ml of a suspension of herpesvirus hominis type 2 virus in baby hamster kidney (BHK) growth medium containing 10% foetal calf serum to simulate the amount of protein that might be present in an exudate from a herpetic lesion. This volume of viral suspension was sufficient to moisten the cloth samples without leaving any excess fluid.

The inoculated pieces of cloth were placed in sterile Petri dishes and left fully exposed to the air in a room without air-conditioning but shielded from direct sunlight. Wet and dry bulb temperatures were recorded.

At the start of the experiment and thereafter hourly for four hours three cloth samples were eluted in 2 ml of BHK growth medium containing 10 mmol HEPES buffer, 10% foetal calf serum, 0.06% sodium bicarbonate, and antibiotics. Elution was performed by thorough mixing on a Vortex Rotamixer followed by squeezing the fluid from the cloth with sterile forceps.

Each eluate was titrated in triplicate using the BHK-HEPES growth medium as diluting fluid; 0.025 ml of each dilution was placed in the appropriate well of a flat-bottomed Microtitre tissue culture plate, to which 0.05 ml of growth medium was added followed by 0.025 ml of cell suspension in growth medium containing 120 million BHK-21 cells per ml of suspension.

The plates were then sealed, incubated at 35°C in air, and observed daily for five days for the development of cytopathic effect. At the end of the observation period viral titres were calculated and expressed as viral content per cloth sample.

EVALUATION OF RESULTS

Where appropriate, χ^2 and P values were calculated using the Hewlett-Packard HP 65 calculator.

Results

ANTIBODY STUDIES

The results of the antibody studies are shown in Table 1. Nearly all these adult subjects had antibodies to either one or both viral types. In the blood donor group (consisting only of men), where individual ages were known, the mean age of those

TABLE I Results of antibody studies to herpesvirus hominis among adults in Ibadan

Patient group	Total tested (A + B + C + D)	Negative (A)*		Type 1 anti- body only (B)		Type 2 anti- body only (C)		Types 1 and 2 antibody (D)		Total with type 1 anti- body (B + D)		Total with type 2 anti- body (C + D)	
		No	%	No	%	No	%	No	%	No	%	No	%
Blood donors	228	6	2.6	175	76.7	24	10.5	23	10.1	198	86.8	47	20.6
Antenatal Clinic patients	283	2	0.7	202	71.4	21	7.4	58	20.5	260	91.9	79	27.9
Family Planning Clinic patients	159	2	1.3	113	71.1	10	6.3	34	21.4	147	92.5	44	27.7
Special Treatment Clinic patients	167	1	0.6	109	65.3	14	8.4	43	25.7	152	91.0	57	34.1

*Sera containing no detectable antibody at lowest serum dilution (1/10) tested

without type 2 antibody was 28.7 years (SD 6.8) whereas the mean age of those with type 2 antibodies was 29.1 years (SD 7.5). Similarly, the prevalence of type 2 antibodies did not appear to be related to either sex or age in the group of patients attending the Special Treatment Clinic. Individual ages were not available for the groups of female subjects attending either the Antenatal Clinic or the Family Planning Clinic.

A high proportion of subjects in each of the groups possessed antibodies against type 1 virus. The proportions do not differ significantly between the groups, with $\chi^2_1 = 3.05$, $P = 0.08$ when blood donors are compared with the Family Planning group, $\chi^2_1 = 3.44$, $P = 0.06$ when compared with the Antenatal Clinic subjects, and $\chi^2_1 = 1.67$, $P = 0.2$ when compared with Special Treatment Clinic patients.

The proportion of blood donors with type 2 antibodies was however smaller than that found in the other groups. Comparison between the proportion of blood donors with type 2 antibodies and those attending the Special Treatment Clinic yields a χ^2_1 value of 9.08, $P = 0.003$. Although the difference between the proportion of blood donors with type 2 antibody and the proportion of positive subjects attending either the Antenatal Clinic ($\chi^2_1 = 3.62$, $P = 0.06$) or the Family Planning Clinic ($\chi^2_1 = 2.60$, $P = 0.11$) is not statistically significant when each of these are considered separately, it does reach the generally accepted level of significance if the numbers are increased by combining the very similar results for the latter two groups ($\chi^2_1 = 4.13$, $P = 0.04$).

SURVIVAL OF HERPESVIRUS HOMINIS TYPE 2

The viral titres are given in Table 2.

At the temperature (31.1°C dry bulb) and relative humidity (83%) at which the experiment was conducted, the cloth samples were still clearly moist after one hour. Definite drying was apparent by the

TABLE II Viral content of cloth samples* after exposure to room conditions† for varying times

Time (hours)	Log ₁₀ titre			
	Sample 1	Sample 2	Sample 3	Mean
0	4.0	4.1	4.1	4.06
1	2.8	2.0	2.6	2.48
2	1.5	1.3	0.0	0.93
3	1.1	0.0	0.0	0.36
4	0.0	0.0	0.0	0.0

*Mean of three titrations for each sample

†Dry bulb temperature 31.1°C, wet bulb temperature 30°C, relative humidity 83%

second hour and the cloth samples were dry to visual inspection by the third hour. At night, when the relative humidity increases to nearly 100%, these drying times could be considerably prolonged.

The viral titre fell by approximately 1.5 log units during each of the first two hours and little virus was detectable by the third hour. No virus could be isolated by the fourth hour, when the cloth samples were apparently completely dry.

Discussion

We have previously reported¹ that by the age of 20 years some 80% of the Ibadan population possesses antibody to herpesvirus hominis type 1, and the present results show that this proportion increases to nearly 90% in subjects who are about 10 years older. We also found that by the age of 20 years some 12% of the population had developed antibodies to type 2 virus; this study shows that this proportion also increases among older subjects.

Approximately 20% of blood donors in Ibadan had antibodies to HVH-2—a prevalence that is almost identical to that reported earlier by Adelusi *et al*⁹ for a group of asymptomatic control subjects in Ibadan. It is also very similar to that reported by Roome *et al*,⁸ who examined blood donors in Bristol

(England) and found that approximately 18% of them possessed antibodies to HVH-2.

In the present study type 2 antibodies were present in a higher proportion of patients attending the Antenatal and Family Planning Clinics but the prevalence is very similar to that found among asymptomatic pregnant women in America.¹⁰ The highest prevalence of type 2 antibodies was found among patients attending the Special Treatment Clinic, and this too agrees with the widely accepted finding that genital herpetic infections occur most frequently among patients attending venereal diseases clinics¹¹ and among those with the greatest sexual exposure.¹²

Our findings relating to adult groups of patients are thus not at variance with those of other workers and would accord with the accepted view that type 2 herpesvirus hominis is generally sexually transmitted—a view that is based not only on sero-epidemiological data but also on the findings that type 2 virus is isolated from most genital herpetic lesions and that genital herpes is often associated with the presence of other infections known to be sexually transmitted.^{13 14}

However, our previous report¹ that antibodies to HVH-2 appear in early childhood is unusual. It seems most unlikely that some 11% of 5-year-old children in Ibadan could have been in close sexual contact with individuals with active genital herpes or even in non-sexual contact since most HVH-2 lesions occur around the genitalia, thighs, and buttocks.¹⁵ Nor would the appearance of HVH-2 antibodies at this age be likely to result from a serological cross-reaction with varicella antibodies, although childhood chicken-pox is common, in view of previous findings by Gerna *et al*^{16 17} that the sensitive immunoperoxidase method failed to detect group cross-reactions at serum dilutions greater than 1/10 to 1/16.

Although herpesvirus transmission is generally considered to require close bodily contact, the possibility of transmission via fomites has to be taken into account. Herpesvirus hominis is regarded as a fairly labile virus,^{18 19} but its rate of inactivation is known to vary considerably under different environmental conditions. When drying is prevented by transport media, the virus can survive for several days at room temperature with little loss of titre,^{20 21} and it has also been found to survive for some weeks at room temperature in dried crusts from herpetic lesions.²²

There have been reports that genital herpes may sometimes be acquired by non-venereal means. Indeed, as early as 1940, when the differences between HVH-1 and HVH-2 were unknown, Sharlit²³ wrote that, “. . . none will be so rash as to

challenge the claim of celibacy or to impugn the professions of chastity or fidelity on the prima facie evidence of herpes progenitalis.”

Childhood genital herpes has been recorded on several occasions, although most reports were published before the two types of herpes viruses had been differentiated. Brain²⁴ described acute herpetic vulvovaginitis of infant girls in 1956 and suggested that the virus might enter through skin lesions, such as napkin rashes, wounds, burns, or abrasions. Krugman²⁵ and Scott *et al*²⁶ had both previously reported cases of genital herpes in children but again the type of virus is not known. Sheward²⁷ later reported perianal herpes in twins aged 2 years. Circumstantial evidence suggests that in these cases the infection was probably due to type 1 virus, possibly transmitted by the zinc and castor oil cream which was applied from the same container to both the infants' napkin rash and their father's "cold sores." Evidence suggesting that at least HVH-1 may be transmitted by fomites has also been supplied by Stern *et al*²⁸ and by Rosato *et al*²⁹, who found herpetic whitlow among medical personnel, in whom many cases may occur without known direct contact with oropharyngeal secretions from patients.

Some years ago, Nahmias *et al*³⁰ described six cases of genital herpes in young children, five of whom were girls and one a boy. Four of the girls were infected with HVH-2 whereas the other children were infected with HVH-1. Two of the girls with HVH-2 infection denied any sexual contact, although the authors of the report considered that the infection must have been sexually acquired.

Amstey and Balduzzi,³¹ however in an account of 15 patients with genital herpes, mentioned one girl who was 15 years old, a virgin, and denied sexual contact. The authors commented that herpesvirus infections of the female genitalia may not be transmitted exclusively by sexual means. This view was reiterated by Kessler³² when reporting the unexpected finding that 58.8% of a group of nuns possessed antibodies to HVH-2 whereas 57.4% of their blood sisters, who were not nuns, also had antibodies to the virus. The latter group however frequently had trichomonads in their vaginal secretions, indicating sexual exposure, whereas no trichomonads were detected among the nuns. Roome *et al*⁸ also suggested that non-venereal spread of HVH-2, possibly by damp towels, might explain the very high incidence of HVH-2 antibodies which they detected among long-term prison inmates.

There are therefore grounds for suggesting that not all HVH-2 infection is sexually acquired, and our present findings indicate that in the tropics the virus could survive outside the human host for sufficient time to allow infection to be transmitted via fomites,

such as shared bed-clothes, towels, or underclothing. If this is so, many of the lesions are likely to be mild and insufficiently troublesome for the child to be brought for medical attention under conditions where medical facilities are scarce and home-treatment of self-limiting conditions common. By the time that children in Ibadan start to develop HVH-2 antibodies, over 50% have already been exposed to HVH-1; the cross-immunity acquired from these infections would substantially reduce the severity of the illness after subsequent exposure to HVH-2.³³

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