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SEROTYPING IN HERPES SIMPLEX VIRUS INFECTION

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The study comprised of 50 patients, 45 males and 5 females between 20-58 years of age with genital herpes infection. The duration of the herpetic lesions varied between 1-10 days. The swabs taken from lesions were put up for virus culture in African green monkey kidney 'Vero' cells.

Sixteen out of 50 patients (32%) yielded virus on culture. On serotyping using enzyme linked immunosorbent assay (ELISA), 14 (87.5%) showed herpes simplex virus type 2 (HSV-2) and 2 (12.5%) were herpes simplex virus type 1 (HSV-1). The study confirmed the preponderance of HSV-2 in herpes genitalis.

Key Words : Herpes genitalis, Herpes simplex virus

Introduction

Herpes genitalis is one of the commonest sexually transmitted diseases, where most of the infections are silent.[1,2] Herpes simplex virus type 1 (HSV-1) chiefly causes oral and perioral infections whereas simplex virus type 2 (HSV-2) generally causes genital infections.[3]

The two previous studies carried out at this centre yielded contradictory and divergent results. One study showed HSV-1 and other HSV-2 alone from the genital lesions.[4,5]

Some other workers in the past have also shown a rising incidence of HSV-1 infection in genital herpes.[6] The present study was therefore undertaken to study the pattern of virus causing infection in our patients.

Meterials and Methods

Patients of either sex with a clinical diagnosis of herpes genitalis of not more than 10 days duration attending sexually transmitted diseases clinic of this centre were included in this study. Serological test for syphilis (VDRL) was done in each patient to exclude any concomitant or latent syphilis infection.

Dry cotton swabs were used to collect material from the floor of the ulcers and ruptured vesicles (unruptured vesicles were slit open). The specimen was transferred in the transport medium consisting of Eagles balanced salt solution, lactalbumin hydrolysate, yeast extract, sodium bicarbonate, bovine calf serum (BCS), penicillin, streptomycin and mycostatin. Swabs were stored at -70°C for further processing.

A continuous line of African green monkey kidney 'Vero' cells, maintained in growth medium containing Eagle's minimum essential medium (MEM), BCS, sodium bicarbonate and antibiotics, was used to grow the virus. The confluent cells were maintained in the maintenance medium which had a similar composition to growth medium except the concentration of BCS used here was 2% and sodium bicarbonate 1%. AC strain of HSV-1 and HV-219 strain of HSV-2 were used as prototype virus strains for HSV-1 and HSV-2 respectively.[7,8]

Antigens were prepared in 'Vero' cells according to the method described by Seth et al, 1978.[9] The virus stocks of HSV-1 and HV

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219 strain of HSV-2 were raised in 'Vero' cells. Confluent monolayers of 'Vero' cells were propagated in 32 ounce Roux bottles. The bottles were infected with AC and HV-219 strains at a multiplicity of infection of 0.1-0.2 plaque forming unit per cell. Cells were incubated at 35ºC till a complete cytopathic effect (CPE) appeared. When CPE appeared the cells were scrapped from the glass into maintenance medium. The suspension was frozen and thawed 3 times. It was centrifuged and supernatant collected and stored at -70ºC in 1 ml aliquots.

The viral isolates were grown similarly, giving 2 to 3 passages in 'Vero' cell monolayers. When complete CPE appeared, the virus was harvested and stored in the same manner as AC and HV-219 strains.

Enzyme linked immunosorbent assay (ELISA) plates were coated with HSV antigen (100 ug/well). The antigen coated plates were kept at 4ºC for 12 hours. Then the plates were washed once with phosphate buffer saline - Tween 20 (PBS-T), pH7.2. One percent bovine serum albumin (BSA) was added to each well (100 ul/well) to block nonspecific antigenic sites and kept at room temperature for half an hour. The plates were washed twice with PBS-T for 5 min. Unknown HSV type specific (1 and 2 separately) monoclonal antibodies in a test tube and incubated at 37ºC for one hour. The mixture of antibodies and HSV isolates was added to ELISA plates (100 ul/well).

The plates were then incubated at 37ºC for 2 hours. Thereafter they were washed with PBS-T and the conjugate, antimouse antibody conjugated to horse radish perosidase (HSP Dakopatts) was added. The plates were kept at 37ºC for 2 hours and washed with PBS-T thrice for 3 minutes each time. Then substrate (O-phenylamine dichloride plus H_2O_2) was added in 0.1M acetate buffer (pH5.0). The plates were kept in dark for half an hour for the reaction to take place. The reaction was stopped with 2.5M H_2SO_4. The plates were then read in a ELISA reader (Flow Laboratories, USA) at 492nm. The test was considered to be positive when the optical density of the test was twice the optical density of antigen control.

Results

The present study comprises of 50 patients, 45 males and 5 females, between 20-58 years of age, with herpetic lesions on the genitals of upto 10 days duration. All the patients had a history of unprotected sexual contact 2 days to 4 months prior to the appearance of lesions. They denied any history of orogenital or homosexual sex. There was no past history of any sexually transmitted disease, genital ulcer, or urethral discharge. Serological test for syphilis (VDRL) was non reactive in all the patients. HSV isolates were obtained from 16 out of 50 (32%) patients, of which 14 (87.5%) were HSV-2 and 2 (12.5%) were HSV-1 (Table I). Fifteen of the 16 patients were males and 1 was a female. Thirteen male and the solitary female patient yielded HSV-2 while 2 males yielded HSV-1 on culture.

Table I. Isolation rate of HSV-1 and 2

	No. of patients	HSV-1	HSV-2
Male	45	2	13
Female	5	-	1
	50	2 (12.5%)	14 (87.5%)

Successful isolation varied with the duration of the lesions before culture was attempted. The isolation rate, for instance, was found to be relatively high (8/16 or 50%)

when isolation was attempted from lesions less than 3 days old; it was variable but lower from patients with older lesions (Table II).

Table II. Duration of the lesions and isolation rate

No. of patients	Duration of lesions (in days)	Isolates (%)
16	Less than 3	8 (50%)
34	More than 3	8 (23.5%)
50		16

Eighty percent of the patients had history of recurrence and had 2 or more recurrences lasting for 5-10 days whereas 20% patients reported with the first episode. In patients with recurrences, the period of remissions varied from 2-8 weeks in 57.5%, 2-3 months in 30% and of upto one year in 12.5%. The 5 female patients denied history of premarital or extramarital sexual contact and all of them had recurrence with remissions lasting between 1 month to 1 year.

Three of the 5 female patients had a relapse with the onset of menstruation. One male patient each attributed intake of alcohol, masturbation, or emotional stress as a precipitating factor. No precipitation factors were noted in other patients.

Comments

Herpes genitalis is one of the commonest sexually transmitted diseases the world over and its incidence is continuing to increase.[1,2,9] It is the fourth commonest sexually transmitted disease seen in our clinic; the first 3 being chancroid, gonorrhoea, and genital warts; genital herpes accounts for 22.4% of the new patients. In the United States of America it is commoner than gonorrhoea and has increased 9 folds in the last 2 decades.[10,11] Whether this increase is a true reflection of increased incidence of this disease or a part of overall increase in sexually transmitted diseases, is uncertain.[12] Genital herpes infection is caused mainly by HSV-2 and is transmitted through sexual contact; occasionally it is caused by HSV-1. The course of the disease caused by HSV-2 is not in any way different from that caused by HSV-1 and 50% or more infections may be silent.[13]

Several investigators from different parts of the world in the past have isolated the virus with a success rate varying from 12.5% to 100%, the isolates were chiefly HSV-2.[1,4,5,14]

In sharp contrast to the studies mentioned earlier, some investigators have reported HSV-1 isolation varying from 50% to 100% from genital herpes.[4,6,19] According to some investigators the higher isolation rate of HSV-1 was due to increased orogenital sexual contact whereas others did not offer any convincing explanation. A 100% HSV-1 isolates from an Indian study[4] is not explainable on the basis of orogenital sexual contacts since in our experience such sexual practice is not common. The present study yielded 16 isolates from 50 patients out of which 14 (87.5%) were HSV-2 and isolation rate was 32% which is rather low compared to studies done by Seth et al,[5] Smith et al[16] and Wolonties et al[18] where they have selectively picked up the patients with vesicular lesions and the swabs were directly cultured from the lesions.

The poor isolation rate in our study may have been because the patients with lesions of 1-10 days, were taken up at random. Isolation rate from patients with early (<3 days) lesions was 50%. The present study was planned to look for type of the virus causing the disease and a period of 10 days was arbitrarily selected.

Our results with a preponderance (87.5%) of HSV-2 isolates and a small

isolation (12.5%) of HSV-1 are in conformity with most reports in the literature. HSV-2 is regarded as the virus most often responsible for genital herpes; HSV-1 is an infrequent isolate.[15-17]

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