Original Research Article

A Study on Seroprevalence of HSV-1 In Clinically Suspected Cases

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Abstract

Background: Herpes Simplex Virus (HSV) type 1 infects 60%–80% of people throughout the world. The prevalence of HSV-2 infection in adults varies markedly from country to country. A high percentage of genital infections are being caused by HSV-1. HSV-1 is misdiagnosed and overdiagnosed as aphthous ulcers and erythema multiforme due to similar manifestations. Patients of acantholytic disorders are also prone to co-infections by HSV.**Objectives:** (1) To correlate the seroprevalence with the age and gender of patients. (2) To detect the antibodies against HSV-1 and HSV-2 in clinically suspected cases using IgM ELISA. (3) To evaluate the significance of IgM ELISA as a diagnostic tool in conjunction with provisional clinical diagnosis.**Methods:** Cross sectional study conducted from January to December 2020. 2-3 ml blood was taken from patients and serum samples were tested for the presence of both HSV-1 and HSV-2 IgM antibodies by ELISA.**Results:** 82 patients were included in our study. 18.2% showed seropositivity for HSV-1 IgM and 8.5% for HSV-2 IgM, while co-infection was seen in 2.4% patients. 44% patients were in the age group of 21-40 years (p value<0.05) which also accounted for maximum number of positive cases. Higher seropositivity for both HSV-1 (22.5%) and HSV-2 (12.2%) was seen among the patients hailing from urban areas belonging to lower socioeconomic strata. 36.5% were provisionally diagnosed as herpes labialis. 30% of these were positive for HSV-1 IgM antibodies. 23.2% were diagnosed and treated as pemphigus vulgaris. 15.8% of these cases and 14.3% of aphthous ulcers had co-infection with HSV-1.**Conclusion:** Serological testing for both types of Herpes viruses is recommended in all suspected cases. Differential diagnoses should be considered in seronegative patients. Laboratory confirmation can avoid misdiagnosis and erroneous treatment modalities.

Keywords: Herpes simplex virus, ELISA, Seroprevalence, Oro-labial herpes, Genital herpes, Pemphigus vulgaris

Introduction

Human herpesvirus 1 and human herpesvirus 2 more commonly known as herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) are both members of the order *Herpesvirales*, family *Herpesviridae* and sub-family *Alphaherpesvirinae*[1]. Herpes simplex virus (HSV) infections are endemic throughout the world[2]. HSV-1 infects 60%– 80% of people throughout the world, whereas the prevalence of HSV-2 infection in adults varies markedly from country to country, from as low as 7% up to 80%, depending on sexual and, perhaps, contraceptive practices[3].

Direct contact with transmission through infected secretions is the principal mode of spread[4]. They cause a wide spectrum of diseases with latent infections and recurrences being fairly common[5].HSV-1 is associated with non-genital disease, usually causing oro-labial manifestations and HSV-2 is related to urogenital disease, although both viruses can cause either clinical syndromes[6].

Latency is central to the success of HSV as a human pathogen. It permits persistence of the virus in the presence of a fully-developed immune response enabling it to cause life- long infections in the host. As a result of periodic reactivation of the latent virus and the production of recurrent infection, virus shedding and transmission of infection to susceptible individuals occurs at intervals throughout life, allowing the virus to persist in populations with high levels of herd immunity.Their frequent reactivation in immunosuppressed patients causes serious health complications.A large percentage of persons who are tested seropositive for HSV-1 or HSV-2 show no clinical manifestation of the disease at all. HSV-1 infection presents in the form of gingivostomatitis, pharyngitis or tonsillitis[7].As the above mentioned symptomatology can be seen in many related and unrelated diseases, viz: impetigo, herpangina, erythema multiforme, pemphigus vulgaris, aphthous stomatitis, acute necrotizing ulcerative gingivitis, hand, foot and mouth disease,[8] candidal infections of the mouth, Stevens- Johnson syndrome (SJS), Epstein Barr virus (EBV) mononucleosis and chemotherapy and radiotherapy induced lesions, [9] HSV-1 infection can be easily misdiagnosed.HSV-2 can present with clinical characteristics similar to those of other conditions such as non-gonococcal urethritis in men, non-chlamydial urethritis and recurrent vaginitis in women, as well as aseptic meningitis. It is estimated that 10 to 15% of all primary genital herpes cases are caused by HSV-1.The data estimates from most studies ignore the contribution of sexually acquired HSV-1 to the epidemic of genital herpes and vice- versa. This may lead to increased incidence of neonatal herpes[10]. Neonatal herpes causes severe infections of the eyes, central nervous system and disseminated infection.Acantholytic disorders, including pemphigus vulgaris, chronic benign familial pemphigus, Darier disease, and Grover transient acantholytic dermatosis, as well as other vesiculo-bullous disorders, including bullous pemphigoid, epidermolysis bullosa, and atopic dermatitis, are prone to florid infections by HSV-1 and HSV-2[11,12].Virus isolation is a definitive diagnostic method [13]. Although, detection of viral DNA by polymerase chain reaction (PCR) has gained increased acceptance even for routine skin infections, replacing culture in most laboratories, the drawback of its non-availability in all laboratories remains[14].

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However, conventional PCR techniques have so far been relatively cumbersome, difficult to interpret, and prone to contamination. Immunofluorescence[15] as well as many other serological techniques such as microneutralization, kinetic neutralization, multiplicity analysis, passive haema-gglutination tests and radio-immunoassay are available. But none of these procedures are able to detect HSV-1 and HSV-2 antibodies separately, particularly in individuals infected with both viruses, due to the extensive antigenic cross-reactivity[16].A number of enzyme-linked immunosorbent assay (ELISA) procedures are available for the rapid detection of HSV. Evaluation of the seroprevalence of HSV-1 and HSV-2 has been markedly enhanced by the development of type-specific serologic assays. These assays allow for detection of HSV-2 in the presence of HSV-1 antibodies and vice versa[13].Both type-specific and non-specific antibodies to herpes simplex virus infection develop during the first several weeks following infection and persist indefinitely. Type- specific serology (TSS) is better than non-specific antibody-dependent serological tests. Specific antibody-based serological tests are of two types - Western blot, and glycoprotein G (gG) assays. Western blot tests are expensive, take 2–5 days to complete, and require expert interpretation. Glycoprotein G assays detect antibodies to the type- specific proteins glycoprotein G-1 (gG-1) and glycoprotein G-2 (gG-2). Very little sequence homology exists between gG-1 and gG-2, allowing differentiation between established infection with HSV-1 and HSV-2 respectively[17,18]. The present study was undertaken to identify the seroprevalence of HSV-1 in patients attending the Dermatology OPD in KIMS, Hubballi using ELISA for detection of IgM antibodies and to determine if ELISA can act as a diagnostic aid to clinical diagnosis for accurate and prompt diagnosis in cases with clinical suspicion.

Aims and Objectives

1. To correlate the seroprevalence with the age and gender of the patients.
2. To detect the antibodies against HSV-1 and HSV-2 in clinically suspected cases using IgM ELISA in serum samples.
3. To evaluate the significance of IgM ELISA as a diagnostic tool in conjunction with provisional clinical diagnosis.

Materials and Methods

Type of study: Cross sectional study.

Study duration: Study was conducted from January 2020 to December 2020.

Place of Study: The Department of Microbiology, Govt Medical College & Hospital, Nalgonda, Telangana, India

Source of data: Patients visiting Dermatology and Venereal Diseases OPD at Govt Medical College & Hospital, Nalgonda.

Sampling: Serum samples from all the patients fulfilling the inclusion criteria, during the study period, were included in the study sample.

Inclusion criteria: Adult and paediatric patients provisionally diagnosed to be having HSV-1 infection.

Methodology

1. Demographic details of the patients were recorded in a proforma.
2. Detailed clinical data was recorded viz: site of lesion, duration of illness, secondary infection if any, immune status of the patient, past history of HSV infection in the partner & history of multiple sexual partners. Definitions of infections as initial or recurrent were based on patients' self-reports regarding presence or absence of previous outbreaks.
3. 2-3 ml of blood sample was collected from all the patients under aseptic precautions in sterile plain test tubes without any anticoagulant.
4. Collected blood samples were subjected to centrifugation to separate the serum from the samples. The serum samples were then tested for the presence of both HSV-1 IgM antibodies and HSV-2 IgM antibodies by ELISA.

Detection of HSV-1 IgM antibodies by ELISA

Kit used: Calbiotech HSV-1 IgM ELISA kit – A rapid test system for detection of IgM antibodies against herpes simplex virus-1.

Detection of HSV-2 IgM antibodies by ELISA

Kit used: Calbiotech HSV-2 IgM ELISA kit – A rapid test system for detection of IgM antibodies against *Herpes Simplex Virus-2*

Results and Observations

The present study was carried out in the Department of Microbiology, Govt Medical College & Hospital, Nalgonda during the period January 2020 to December 2020 to find the seroprevalence of HSV-1 in clinically suspected/provisionally diagnosed cases. A total of 82 patients were included in the study.

Table 6: Age wise distribution of HSV cases (n=82)

Age	Number of patients (%)	HSV-1 IgM (%)	HSV-2 IgM (%)
1-20	16 (19.5%)	4 (25%)	0
21-40	36 (44%)	9 (25%)	6 (16.7%)
41-60	23 (28%)	2 (8.7%)	0
>60	7 (8.5%)	0	1 (14.3%)
Total	82 (100%)	15 (18.3%)	7 (8.5%)

Maximum numbers of suspected HSV-1 cases, 36 (44%) were seen in the age group of 21-40 years. This age group also accounted for maximum number of positive cases for HSV-1, 9 (25%), as well as for HSV-2, 6 (16.7%). The results were statistically significant showing that HSV-1 seropositivity is significantly higher among patients belonging to age group of 21-40 years of age.

Table 7: Correlation of gender with seroprevalence of HSV(n=82)

Gender	Number of patients (%)	HSV-1 IgM (%)	HSV-2 IgM (%)
Male	40 (48.8%)	9 (22.5%)	5 (12.5%)
Female	42 (51.2%)	6 (14.3%)	2 (4.8%)
Total	82 (100%)	15 (18.3%)	7 (8.5%)

A total of 82 cases of the study were divisible into 42 (51.2%) females and 40 (48.8%) males. Male to female ratio was 0.95:1. 9 males (22.5%) and 5 females (12.5%) were seropositive for HSV-1. HSV-2 seropositivity was shown by 5 males (12.5%) and 2 females (4.8%). More number of seropositive cases was seen among the males.

Table 8: Distribution of HSV cases based on area of residence (n=82)

Residence	Number of patients (%)	HSV-1 IgM (%)	HSV-2 IgM (%)
Urban	49 (59.8%)	11 (22.5%)	6 (12.2%)
Rural	33 (40.2%)	4 (12.1%)	1 (3.0%)
Total	82 (100%)	15 (18.3%)	7 (8.5%)

The number of patients hailing from urban areas was 49 (59.8%) as compared to 33(40.2%) belonging to rural areas. Higher seropositivity for both HSV-1 and HSV-2 was seen among the patients hailing from urban areas.

Table 9: Seropositive HSV cases and marital status (n=82)

Marital status	Number of patients (%)	HSV-1 IgM (%)	HSV-2 IgM (%)
Married	62 (75.6%)	10 (16.1%)	4 (6.4%)
Unmarried	20 (24.4%)	5 (25%)	3 (15%)
Total	82 (100%)	15 (18.3%)	7 (8.5%)

Among the cases included in our study, 62 (75.6%) patients were married while 20 (24.4%) were unmarried. Higher seroprevalence of 25% and 15% respectively for HSV-1 and HSV-2 was seen among the unmarried cases as compared to the married people.

Table 10: Patients with HSV and their occupational distribution(n=82)

Occupation	Number of patients (%)	HSV-1 IgM (%)	HSV-2 IgM (%)
Housewife	33 (40.2%)	4 (12.1%)	1 (3.0%)
Student	16 (19.5%)	4 (25%)	1 (6.3%)
Daily wagelabourer	6 (7.3%)	3 (50%)	1 (16.7%)
Driver	5 (6.1%)	1 (20%)	1 (20%)
Unemployed	5 (6.1%)	1 (20%)	2 (40%)
Businessman	4 (4.9%)	0	0
Farmer	4 (4.9%)	0	0
Bank employee	3 (3.7%)	1 (33.3%)	0
Mechanic	3 (3.7%)	0	0
Tailor	3 (3.7%)	1 (33.3%)	1 (33.3%)
Total	82 (100%)	15 (18.3%)	7 (8.5%)

33 (40.2%) patients in our study were housewives, 16 (19.5%) were students, 3 (3.7%) worked as clerks in banks and 5 (6.1%) were unemployed. Rest of the patients were self-employed in a variety of occupations. Maximum seropositivity for both HSV-1 and HSV-2 was seen in patients employed as drivers, daily wage labourers or ones who were unemployed.

Table 11: Correlation of HIV status and seroprevalence of HSV (n=82)

HIV status	Number of patients (%)	HSV-1 IgM (%)	HSV-2 IgM (%)
Reactive	6 (7.3%)	0	2 (33.3%)
Non-reactive	76 (92.7%)	15 (19.7%)	5 (6.6%)
Total	82 (100%)	15 (18.3%)	7 (8.5%)

At the time of sample collection, 6 (7.3%) patients were tested HIV positive while 76 (92.7%) patients tested negative for HIV.

Table 12: Diagnostic division of cases(n=82)

Diagnosis	Number of patients (%)
Herpes labialis	30 (36.5%)
Herpes genitalis	6 (7.3%)
Pemphigus vulgaris	19 (23.1%)
Aphthous ulcers	7 (8.5%)
Oral candidiasis	4 (4.8%)
Chemotherapy induced oral lesions	5 (6.1%)
Stevens-Johnson syndrome	4 (4.8%)
Fixed drug eruptions	3 (3.6%)
Erythema multiforme	4 (4.8%)
Total	82 (100%)

Out of the 82 patients included in our study, 30 (36.5%) were suspected to have Herpes labialis and were treated for the same. Other cases were differentially diagnosed as pemphigus vulgaris (23.1%),aphthous ulcers(8.5%),herpes genitalis (7.3%), chemotherapy induced oral lesions (6.1%), oral candidiasis, Stevens-Johnson syndrome, erythema multiforme (4.8%), and lastly, fixed drug eruptions (3.6%) bearing in mind a differential diagnosis of HSV-1 also.

Table 13: Distribution of cases according to site of lesion at the time of clinicalpresentation (n=82)

Site of the lesion	Number of patients (%)
Perioral lesions	27 (32.9%)
Oral lesions	25 (30.5%)
Perioral + Oral lesions	4 (4.9%)
Oral lesions + skin lesions (torso & extremities)	20 (24.4%)
Ulcerative lesion; labia	2 (2.4%)
Ulcerative lesion; shaft of penis	4 (4.9%)
Total	82 (100%)

A majority of patients, 27 (32.9%) presented with perioral lesions and with oral lesions 25 (30.5%); and 4 (4.9%) had both. The remaining 20 (24.4%) patients had associated skin lesions. 6 patients provisionally diagnosed as Herpes genitalis had ulcerative lesions on the labia/shaft of penis.

Table 14: Results of HSV serology by IgM ELISA (n=82)

Test	Cases showing positive results (%)
HSV-1 IgM	15 (18.3)
HSV-2 IgM	7 (8.5)
Co-infection	2 (2.4)

Of the 82 samples, 15 (18.2%) showed seropositivity for HSV-1 IgM and 7 (8.5%) showed seropositivity for HSV-2 IgM, while co-infection was seen in 2 (2.4%) patients.

Table 15: Correlation of primary clinically diagnosed HSV cases and confirmedHSV IgM ELISA positive samples (n=82)

Diagnosis	Total	HSV-1 IgM (%)	HSV-2 IgM (%)
Herpes labialis	30 (36.5%)	9 (30%)	1 (3.3%)
Herpes genitalis	6 (7.3%)	2 (33.3%)	6 (100%)
Pemphigus vulgaris	19 (23.1%)	3 (15.8%)	0
Aphthous ulcers	7 (8.5%)	1 (14.3%)	0
Oral candidiasis	4 (4.8%)	0	0
Chemotherapy induced oral lesions	5 (6.1%)	0	0
Stevens-Johnson syndrome	4 (4.8%)	0	0
Fixed drug eruptions	3 (3.6%)	0	0
Erythema multiforme	4 (4.8%)	0	0
Total	82 (100%)	15 (18.3%)	7 (8.5%)

Among the 30 patients belonging to herpes labialis, 9 (30%) showed positivity for HSV-1 IgM and 1 (3.3%) for HSV-2 IgM. Two out of 6 herpes genitalis cases were positive for HSV-1 IgM while all 6 (100%) were positive for HSV-2 IgM. Number of cases diagnosed as pemphigus vulgaris were 19 of which 3 (15.8%) were positive for HSV-1 IgM. One case (14.3%) of aphthous ulcers turned out to be positive for HSV-1 IgM.

Table 16: Performance of IgM ELISA for HSV-1 and HSV-2

HSV-1		HSV-2	
Sensitivity	60%	Sensitivity	85.71%
Specificity	68.66%	Specificity	100%
Positive Predictive Value	30%	PPV	100%
Negative Predictive Value	88.46%	NPV	98.68%

Table 17: Distribution of total samples according to respective treatment received after clinical diagnosis (n=82)

Provisional diagnosis	Number (%)	Treatment recieved	HSV 1 IgMpositive (%)	HSV 2 IgMpositive (%)
Herpeslabialis/Herpes genitalis	36(36.5%)	Acyclovir	11 (30.5%)	7 (100%)
Pemphigus vulgaris	17(20.7%)	DCP therapy	2 (11.8%)	0
Oral candidiasis	2 (2.4%)	Fluconazole	0	0
Oral candidiasis	2 (2.4%)	Fluconazole, ART	0	0
Aphthous ulcers	7 (8.5%)	Multivitamins, Lignocaine gel	1 (14.3%)	0
Chemotherapyinduced oral lesions	5 (6.1%)	Chemotherapy	0	0
Stevens-Johnsonsyndrome	4 (4.9%)	Oral and topicalsteroids	0	0
Erythemamultiforme	4 (4.9%)	Topical antibiotics,immolients, steroids	0	0
Fixed drug eruptions	3 (3.7%)	Topical antibiotics,immolients	0	0
Pemphigus vulgaris	2 (2.4%)	Topical steroids,Antibiotics	1 (50%)	0
Total	82(100%)		15 (18.3%)	7 (8.5%)

36 patients who were diagnosed as herpes labialis/herpes genitalis were given acyclovir. 4 patients presented with oral candidiasis and were started on fluconazole, 2 of them were taking Anti-retroviral therapy at the time of presentation. Patients diagnosed as pemphigus vulgaris were given dexamethasone/cyclophosphomide (DCP) therapy.Cancer patients were undergoing chemotherapy and aphthous ulcer patients were given multivitamins and lignocaine gel. Patients who were provisionally diagnosed as Stevens-Johnson syndrome/ fixed drug eruptions/erythema multiforme as well as patients of pemphigus who were not on DCP therapy were started on topical steroids, topical antibiotics and topical immolients

Table 18: Arrangement of cases based on past history of similar infection (n=82)

History of previous infection	Number of patients (%)	HSV-1 IgM (%)	HSV-2 IgM (%)
Yes	12 (14.6%)	1 (8.3%)	2 (16.7%)
No	70 (85.4%)	14 (20%)	5 (7.1%)
Total	82 (100%)	15 (18.3%)	7 (8.5%)

A majority of patients in our study gave no history of past infection. Only 12 (14.6%) patients gave any previous history of similar complaints. Only 1 patient (6.6%) recalled having a similar episode of oro-labial herpes among those showing positivity for HSV-1 IgM while 2 (28.6%) out of 7 HSV-2 positive cases gave a positive history of lesions similar to Herpes genitalis in the past. 93.3% seropositive patients for HSV- 1 and 71.4% positive for HSV-2 could not recall having any similar past infections.

Discussion

Herpes simplex viruses are enveloped, double-stranded DNA viruses with two serotypes classified as HSV-1 and HSV-2. Infections with HSV-1 usually involve the face and skin “above the waist,” although

HSV-1 also can cause genital infection. Infections with HSV-2 usually involve the genitalia and skin "below the waist" in sexually active adolescents and adults. HSV-2 causes most infections in neonates while also causing oral lesions in approximately 25% of the infected population[19]The virus establishes a lifelong latency after primary infection, and periodically can reactivate with shedding of the virus. Most primary infections are mild or asymptomatic in childhood, but they can cause serious complications in fetuses, immunocompromised individuals, or in elderly.Since HSV infections are not reportable diseases, reliable statistics on the incidence and prevalence of infections are not available in our country. This prospective study is an investigation to determine the seroprevalence of herpes simplex virus-1 among the clinically suspected cases and to analyze if ELISA as a serological technique can aid us in reduction of misdiagnosis, over diagnosis or under diagnosis of herpetic oral and genital infections.

Analysis of ELISA results of IgM HSV-1 and IgM HSV-2: In this study, we included 82 patients provisionally diagnosed as a differential for HSV-1 infection, including clinically diagnosed HSV-1 cases. ELISA for the detection of IgM antibodies of both HSV-1 and HSV-2 was run for all the samples. A total of 15 (18.2%) cases showed seropositivity for HSV-1 IgM and 7 (8.5%) cases showed seropositivity for HSV-2 IgM.Interestingly, we found 2 patients who had HSV-1 and HSV-2 coinfection. One patient each of herpes labialis/herpes genitalis was positive for HSV-2 and HSV-1 respectively.Ribes et al. in their study identified that although HSV-2 primarily remains a genital pathogen, HSV-1 is taking on an increasingly important role in causing genital ulcer disease in addition to being a primary non-genital pathogen and about 10-15% cases of genital herpes are now caused by HSV-1[7].While HSV-2 was the predominate cause of genital herpes in Israel in the 1970s, HSV-1 was the principal cause of genital herpes among persons seeking care at a Tel Aviv medical center from 1993 to 2001 identified Samra et al[20].

Comparative analysis of HSV serology with age of the patients:

It has been reported in many studies that the epidemiology of both herpes viruses is changing worldwide and the incidence and seroprevalence is reducing in children and increasing in adolescents and young adults, and this increase has been seen more in the case of HSV-1[20].Majority of infections of HSV-1 occur during childhood while HSV-2 is usually acquired during late adolescence. A study in India, Brazil, Estonia, Morocco and Sri Lanka conducted by FM Cowan et al. positively linked increasing seroprevalence of HSV-1 with age.Findings of AJ Vyse et al. establish that the seroprevalence of HSV-1 and HSV-2 increases with age[21].Our study grouped the most common age to be between 21-40 years in 36 (44%) patients, 23 (28%) cases between 41-60 years, 16 (19.5%) patients between the age of 1 and 20 years and the remaining 7 (8.5%) patients were above 60 years of age amongst the 82 total cases tested.In our study, we found that in the age group of 21-40 years, 9 (25%) patients showed seropositivity for HSV-1 and 6 (16.7%) for HSV-2 IgM antibodies which are the highest. Among the 16 children and adolescents, 25% (4 cases) seropositivity was seen. However, out of the 23 patients belonging to the age group of 41 to 60, only 2 (8.7%) showed seropositive results for HSV-1 IgM. In the geriatric age group, we had 7 patients; none showed seropositive results for HSV-1 IgM and only 1 (14.3%) case was found to be seropositive for HSV-2 IgM without the presence of HSV-1 antibodies. Seroprevalence trends of HSV-2 in our study are in consensus with primary herpes infection by HSV-2 occurring during late adolescence when young adults become sexually active and steadily increasing with the maximum prevalence seen during their third decade of life[22] Another finding in accord with correlation of seroprevalence of HSV-2 and the sexual behavior of subjects is that no HSV-2 IgM positivity was seen among the children and geriatric patients.Our sample demographic comprising of 40 (48.8%) males and 42 (51.2%) females delineated 22.5% seroprevalence among males and 14.3% seroprevalence among females for HSV-1 IgM, different from the male to female ratio of 3.65:1 traced by the study of Shivaswamy et al[23] similar to the study of VK Jain et al[22] with high (84%) male cases and low (16%) female patients.For HSV-2 IgM, 12.5% males and 4.8% females were found to be seropositive by us, in dissent with AJ Vyse et al., whose study announced that the seroprevalence of HSV-1 and HSV-2 was more in females[21].In this study, as many as 22.5% patients showed seropositivity for HSV-1 IgM and 12.2% for HSV-2 IgM from the urban areas and 12.1% and 3% were seropositive for HSV-1 IgM and HSV-2 IgM from the rural areas individually. An increase in urban population, overcrowding, questionable hygiene, sexual promiscuity and unsafe sexual practices could be a few reasons behind a higher HSV prevalence among urban dwellers. In contrast, more numbers of seropositive cases were reported to be from rural areas in South India according to the study done by Chad Hochberg et al[24].

HSV seroprevalence in married and unmarried patients:

Our study population included 62 (75.6%) married and 20 (24.4%) unmarried patients in all. Among the married cohort, 16.1% seropositivity was seen for HSV-1 IgM and 6.4% for HSV-2 IgM while a higher seroprevalence was seen among the unmarried study population – 25% and 15% respectively.

Comparative analysis of patient's occupation, socioeconomic background andHSV seropositivity: The risk of acquiring HSV infection and HSV seroprevalence inversely correlates with the socioeconomic background of the patients.The highest number of patients, 33 (40.2%), in our study were housewives, 16 (19.5%) were students, 6 (7.3%) were daily wage labourers, 3 (3.7%) worked as clerks in banks and 5 (6.1%) were unemployed. Remainders of the patients were self-employed in a variety of occupations. Similar observations were made in a study done in Rohtak by VK Jain et al. in which majority of their patients were housewives, followed by patients who were farmers, students and daily wage labourers[22]. In our study, based on patients' occupation, maximum seropositivity for HSV-1 was seen in students (25%), daily wage labourers (50%), patients employed as drivers (20%), or ones who were unemployed (20%). Unemployed patients and drivers also showed high seropositivity of 40% and 20% for HSV-2 IgM antibodies.

Comparision of HSV serology and HIV reactivity: Seroprevalence of HSV-2 infection is very high among patients with HIV worldwide,[9] with 63–77% in the USA,[25] .In this study, at the time of sample collection, 6 (7.3%) patients tested HIV positive and 76 (92.7%) patients tested negative for HIV. HSV-2 IgMseropositivity among HIV positive cases was 33.3%; none of them showed positive results for HSV-1 IgM. Amid patients with HIV non-reactive status, 19.7% were positive for HSV-1 IgM whereas, only 6.6% were positive for HSV-2 IgM. Studies by S Nag et al. and SJ Reynolds et al. detail the corroborative findings of HSV-2 IgMseropositivity being significantly higher in HIV patients than HSV-1 IgM positivity[26].

Analysis of HSV serology with clinical manifestation at presentation, provisional clinical diagnosis and treatment received by patients: We have also included non-HSV skin lesions in our sampling because the presentations of these other syndromes are similar to the lesions caused by HSV. In this study, we observed 27 (32.9%) cases presenting with only perioral lesions, 25 (30.5%) with only oral lesions and 4 (4.9%) cases presenting with both perioral and oral types of lesions. A set of 20 (24.4%) patients had associated skin lesions. A part of patients – 2 (2.4%) – had ulcerative lesions on the labia and a part of them – 4 (4.9%) – on the shaft of penis of the 6 patients provisionally diagnosed as herpes genitalis.

Herpeslabialis and herpes genitalis: Clinically, 30 (36.5%) patients in this study were diagnosed as Herpes labialis attending the Dermatology OPD, of which, 9 (30%) cases showed viral serology positive for HSV-1 IgM. From the total, 8 (9.8%) cases were treated with acyclovir in whom, 2 serologically tested positive for HSV-1 and 1 tested positive for HSV-2 serology.

Pemphigus vulgaris: We confirmed HSV-1 infection in three (15.8%) patients amongst the 19 (23.1%) diagnosed as having pemphigus vulgaris in our study. As it might be difficult or even impossible to clinically identify herpetic infections in PV patients, Batista et al. suggest that persistent concomitant herpetic co-infections should be considered in the context of PV lesions after confirmation by laboratory tests, particularly since herpes virus infections are often present in PV lesions.In a study involving 63 patients with erythema multiforme, HSV DNA was detected by PCR in skin biopsy specimens in 60% of patients with clinically diagnosed recurrent HAEM and in 50% of patients with recurrent IPEM[27]. Sun Y et al. researched on the genotype of HSV in cutaneous lesions of patients with HAEM and attributed 66.7% of cases to HSV-1, 27.8% to HSV-2, and 5.6% to HSV-1 and HSV-2 co-infection[28]. This suggests that detection of HSV DNA in skin biopsy samples will go a long way in preventing unnecessary treatment modalities as HAEM is a condition that is treatable just with oral acyclovir.

Aphthous ulcers: Aphthous ulcers or recurrent aphthous stomatitis is one of the documented differential diagnosis of herpes labialis. We found only one (14.3%) patient of the seven (8.5%) aphthous ulcer cases reacting serologically against HSV-1. It has also been seen that patients with recurrent aphthous ulcers tend to have more frequent recurrences of Herpes labialis[29].

Stevens-Johnson syndrome/Fixed drug eruptions: Stevens–Johnson syndrome and drug-induced hypersensitivity syndrome/drug rash with eosinophilia and systemic symptoms (DIHS/DRESS) represent contrasting poles of severe drug eruptions characterized by the widespread destruction of the epithelium of the skin and mucous membranes. Although, the view that infectious agents caused SJS had seemed heretical 20 years ago, this view began to change a decade earlier, when some patients with SJS were found to be closely associated with *Mycoplasma pneumonia* infection[30].

Analysis of performance of HSV-1 and HSV-2 IgM ELISA:

The sensitivity of detection of HSV-1 by IgM ELISA according to our study was 60% and specificity for the same test was 68.66%.

The capability of our HSV-2 IgM ELISA test to correctly identify the persons with disease was 85.71% and the ability to recognize patients not having the disease was 100%.The sensitivity of our tests combined, to identify herpes simplex infections (both HSV-1 and HSV-2), was at 80% and its specificity was 67.74%.The positive predictive values for the ELISA of HSV-1, HSV-2 and combined HSV-1 and HSV-2 were 30%, 100% and 44.44% while, the negative predictive values were 88.46%, 98.68% and 91.30% respectively.

Conclusion

To conclude, as evidenced by most studies worldwide, we also concurrently found a statistically significant correlation between the occurrence of HSV-1 infection and age of the patients. However, we were not able to find any such correlation with age and HSV-2 or age and herpes simplex infections in general. Gender discrepancy was evident for the infection as we observed seropositivity in males greater than in females. The urban population seeks medical aid earlier than their rural counterparts hence making the urban folks to be diagnosed with HSV infections more commonly than the rural population which was recorded by our study. HIV positive cases have a higher predisposition to other sexually transmitted infections. Likewise, we observed HSV-2 infections in those seropositive for HIV and no HSV-1 cases. This study also portrays that correct identification of HSV-2 infections in suspected genital infection cases were higher in comparison to diagnosing HSV-1 infections in oral lesions and combined herpes simplex infections. Also the negative predictive values were higher than then the positive predictive values for all the three categories.

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