

Occurrence of HSV with other microorganisms in female genital infection

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الخلاصة

الحلأ التناسلي هو واحد من الأمراض الأغلب شيوعا والتي تنتقل تناسليا. تقرحات الحلأ التناسلي الابتدائي هي أكثر شيوعا عند النساء، وهذه تقرحات ربما تكون قابلة للخمج الثانوي بالبكتيريا أو الفطريات أو حتى الفيروسات. هذه الدراسة وجهت صوب التحري عن ورود الحلأ البسيط والأمراض الأخرى في النساء الذين يشكين من إفرازات مهبلية و/أو عدم ارتياح تناسلي. كما وجهت رعاية خاصة اتجاه احتمال مصادفة أو حدوث الحلأ البسيط مع أمراض أخرى في نفس المريضة. اثنان من الماسحات المهبلية تم جمعها من 80 مريضة ومن 20 امرأة طبيعية ظاهريا والتي اعتبرت كمجموعة ضابطة. واحد من هذه الماسحات فحصت بواسطة ELISA للكشف المباشر لمستضد الحلأ البسيط (HSV 1 & 2). أما الماسحة الأخرى فقد خضعت للفحص الأصيل للكشف عن وجود البكتيريا والفطريات، وكذلك للفحص الميكروسكوبي. تم ملاحظة مستضد الحلأ البسيط في 21 (26.25%) مريضة و 4 (20%) من المجموعة الضابطة. كما أن تشخيص الـ *Candida* رصد في 15% من المريضات و 10% من المجموعة الضابطة على التوالي. أما *T. vaginalis* فقد لوحظ بواسطة التحضير المبطل في 7 (8.75%) مرضى وفي 5% من المجموعة الضابطة. أما بالفحص الميكروسكوبي المباشر فقد تم ملاحظة *N. gonorrhoeae* في 2.5% من المرضى. كذلك تم تشخيص بعض من الممرضات في عدد آخر من المرضى. من نتائج هذه الدراسة يمكن الاستنتاج على أن الحلأ التناسلي مرض مشاع لدى النساء العراقيات فضلا على ذلك انه أحيانا يحدث مع واحد أو أكثر من الأمراض الأخرى.

ABSTRACT

Genital herpes is one of the most common sexually transmitted diseases. Ulcerations of primary genital herpes may be susceptible to secondary infection with bacteria, fungus or even viruses.

This study was directed to investigate the incidence of HSV and other pathogens in female patients presented with vaginal discharge and/or genital discomfort. Special attention was directed toward the possible occurrence of HSV with other pathogens in the same individual.

Two vaginal swabs were collected from 80 female patients, and from 20 healthy women which were represented as age match control group. One swab was tested with commercial enhanced enzyme immunoassay for the direct detection of HSV 1 & 2 antigen. The other swab was used for formal bacteriological and mycological culture, and for microscopy.

HSV antigen was detected from the genital area in 21 of 80 patients (26.25%), and in 4 of 20 control group (20%). The diagnosis of candida spp. *Trichomonus vaginalis*, and *Neisseria gonorrhoeae* was established in 15%, 8.75%, and 2.5% of the patients respectively. Other microorganisms were also diagnosed in some patients.

Of the 21 patients proved to be infected with HSV, combined coexistence with other pathogens was proved to be in 11 patients (52.4%), with a highest percentage (28.6%) was found with candida spp. Followed by combined HSV with different species of bacteria (14.3%), also this coexistence was found in 9.5% (2), and 4.8% (1) of patients infected with *T.vaginalis* and *N.gonorrhoeae* respectively. One HSV infected patient (4.8%) was found to be infected with both *T.vaginalis* and *Staph. aureus*. While this concomitance infection was found in only one (25%) of the 4 normal females proved to be infected with *T. vaginalis* beside HSV.

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From the results of this study we can conclude that herpes genitalis is a common genital tract infection among Iraqi females. Furthermore, genital herpes often occur in association with one or more other pathogens.

INTRODUCTION:

Herpes, from the ancient Greek meaning to creep or crawl, is the name of a family of viruses of herpes simplex virus 1, and herpes simplex virus 2 (HSV 1 and HSV 2) are the most serious human pathogens⁽¹⁾. Both viruses establish latent infections in sensory neurons and, upon reactivation cause lesions at or near point of entry into the body, this latency increases the pathogenicity of HSV⁽²⁾.

Genital herpes is one of the most common sexually transmitted diseases^(3,4). Most genital HSV infections are caused by HSV-2, however, an increasing proportion is attributed to HSV-1^(5,6).

The first exposure to the virus results in primary infection which is frequently inapparent, though, recurrent infection can be either symptomatic or asymptomatic⁽²⁾. Wald and colleagues⁽⁷⁾ reported, a higher frequency of viral shedding in genital secretions between, rather than during, clinical recurrences.

Ulcerations of primary genital herpes are more common in women^(8,9). It is relatively uncommon for the patient to seek medical care before the vesicles rupture, and the usual presentation is vaginal discharge and discomfort⁽¹⁰⁾. Herpetic lesion may be susceptible to secondary infection with other pathogens⁽¹¹⁾.

So, in order to do some correlation between some of these genital infections, this study was directed toward the investigation of such a combined existence in women with genital tract infection. Furthermore, the incidence of HSV and other microorganisms was investigated.

MATERIALS AND METHODS:

Patients:

This study was conducted on 80 female patients presented with vaginal discharge or/and genital discomfort, who were attending Obstetric and Gynecological Dept. of Al-Kadhimiya Teaching Hospital outpatient clinic, during period from the beginning of November 1999 till the end of August 2000. Their ages range 19-42 years. Twenty healthy women were also included in this study, they were indicated as age match control group.

Specimens and Methods:

Two vaginal swabs were collected from each subject included in this study. One swab was placed in plastic sleeve of the swab holder and kept at -20C till tested for direct detection of HSV (HSV 1 & HSV 2) antigen. An Enhanced Enzyme Immuno Assay was used for this purpose. This kit was obtained from Wellcozyme (WZO 2).

The other swab was inoculated immediately on blood agar, MacConkey agar, and Sabourouds agar plates, for bacteriological and mycological investigations. After 24hr. incubation at 37C the isolated pathogens were identified by colonial morphology, and by routine biochemical tests⁽¹²⁾. *N. gonorrhoeae* was identified by direct examination of Gram's stained smears of vaginal swabs as gram negative diplococci inside polymorphnuclear cells. Wet preparation of vaginal swabs was also done for the demonstration of *T. vaginalis*, pus cells, and candida spp.

RESULTS:

Table 1 demonstrate that, HSV antigen was observed to be positive in 21(26.25%) vaginal swabs from women presented with genital discomfort and/or vaginal discharge. Compared to 4 cases (20%) in healthy control group.

The diagnosis of candida was established in 15%⁽¹²⁾ of the above patients, and in 10%⁽²⁾ of control, while *T.vaginalis* was observed in 7(8.75%), and 1(5%) swabs obtained from patients and control group respectively as diagnosed by wet preparation. *N.gonorrhoeae* was detected by direct examination in 2(2.5%) cases.

The results have also shown that, other microorganisms were isolated and identified from those cases, and are also shown in table 1.

Table 1 . Incidence of infectious agents detected in patients and controls.

Type of infectious agents	Patients No = 80		Control No = 20	
	No.+ve	(%)	No.+ve	(%)
HSV	21	(26.25)	4	(20)
Candida spp.	12	(15)	2	(10)
# Trichomonus vaginalis	7	(8.75)	1	(5)
* Neisseria gonorrhoeae	2	(2.5)	-	-
E. coli	1	(1.25)	-	-
Klebseilla aerogenosa	1	(1.25)	-	-
Staph. aureus	5	(6.25)	-	-
Proteaus spp.	1	(1.25)	-	-
Gardenella vaginalis	5	(6.25)	-	-

Identified by wet preparation.

* Identified by direct microscopic examination.

A combined HSV antigen with other pathogens were observed in 11(52.4%) women with genital tract infection and in one female among control group. Table 2 shows that HSV and candida together were detected in 6 out of 21(28.6%) patients. *N.gonorrhoeae* was observed in one (4.8%) patient from which HSV Ag was detected. Two cases proved to be infected with combined HSV and *T.vaginalis*, compared to one case in control group. From 21 women with genital tract infection 9(42.9%) were found to be infected with HSV only. Concomitant infection of HSV, *Staph. aureus* and *T. vaginalis* together was detected in one patient (4.8%). HSV with different species of bacteria together was observed in 3 patients (14.3%).

Table 2 . Combined infection in patients and controls infected with HSV and other pathogens.

Infectious agents	Patients No = 21		Control No = 4	
	No.+ve	(%)	No.+ve	(%)
HSV + Candida spp.	6	(28.6)	-	-
HSV + N. gonorrhoeae	1	(4.8)	-	-
HSV + T. vaginalis	2	(9.5)	1	(25)
HSV + *Other agents	3	(14.3)	-	-
HSV + Staph. aureus + T. vaginalis	1	(4.8)	-	-
HSV alone	9	(42.9)	3	(75)

*Different species of bacteria.

DISCUSSION:

Genital infections fall into two main categories 1- primary infections due to sexually transmitted pathogenic microorganisms (*N. gonorrhoeae*, *T. pallidum*, *T. vaginalis*, HSV, papilloma virus, and *C. trachomatis*); 2- infections due to members of the resident flora (*C. albicans*, and *Bacteriodes fragilis*)⁽¹³⁾.

HSV infection has risen nationwide sharply during the past decade despite a barrage of warnings aimed at preventing the spread of AIDS and other sexually transmitted diseases⁽¹⁰⁾.

In this study 26.25%⁽²¹⁾ of the patients were confirmed to be infected with HSV, which confirm the results of other previous studies^(14,15).

This indicates that herpes genitalis is a common genital infection in Iraqi females. On the other hand, the result of this study showed that 20%⁽⁴⁾ of the control women were found to have HSV infection. This result is in association with a recently reported findings⁽¹⁴⁾. This explained by that primary unnoticed infection, or asymptomatic recurrent infection. It has been shown that most recurrences of genital herpes are either unrecognized by the patient or are entirely asymptomatic⁽⁷⁾. However, we found that the percentage of HSV proved cases were nearly the same in both patients and control group (26.25% ,20% respectively), this could be due to asymptomatic shedding of the virus. Most individuals acquire the infection asymptotically and probably remain asymptomatic for the rest of their lives, and most of these individuals will shed the virus asymptotically from time to time, although some may have minor symptoms that they do not recognize as being due to herpes⁽¹⁶⁾. So it is recommended that the subjects should be followed with dealy investigation to evaluate the frequency and the site of viral shedding in genital area⁽¹⁾.

Vaginal candidiasis is one of the most common infection seen in the general practice. Up to three quarters of all women will suffer at least one episode of this condition during their lifetime, around half of them suffering a further episode⁽¹⁷⁾. Our results showed that candida spp. were isolated from 15%⁽¹²⁾ of studied patients, this percentage is slightly lower than that obtained from previous study⁽¹⁸⁾. This may reflect the low number of studied cases and their socioeconomic class, or may be due to the method and site of sample collection, and the method used for identification. It is recommended that, swabs should be placed in transport media before being plated out, to overcome the environmental effect on the survival of the organism⁽¹⁹⁾.

In control group, which were represented by healthy normal females, results showed a higher percentage rate than the previous study⁽¹⁸⁾, eventhough, the number of the healthy females in this study were ¼ the number of the patients. In fact, this percentage is still high and this can be explained by many reasons, the most important of all was neglection from the part of the female to herself, hence giving no clinical history of the disease, or the infection it self was of low grade, and it gave only a subclinical degree of changes. It has been concluded that the high incidence of vaginal candidiasis was asymptomatic^(17, 20).

As presented in the results, other microorganisms were identified as a cause of female genital infection, any more, this study aimed to confirm the coexistence of some or different microorganisms with HSV in the same patient. It is not uncommon to see other sexually transmitted agents associated with HSV genital infection, so a greater than expected incidence of trichomoniasis, syphilis, condyloma accuminatum, vaginal candidiasis and gonorrhea infections have been observed among women with herpes genitalis⁽¹³⁾.

The occurrence of both HSV and candida in the same individual at the same time was found in 6 out of the 21 HSV positive cases (28.6%), where as, no such combined infection in the control healthy group was observed. This result is nearly compatible to other previous results^(13,18). Herpetic lesions of female genital tract are particularly susceptible to secondary infection with candida⁽¹¹⁾. The incidence of such infection could be higher in cases with past history of vaginal candidiasis or the patient was taking contraceptive or was pregnant⁽¹⁷⁾.

The data presented in this study shows that only one case (4.8%) of genital HSV infection associated with gonorrhoea as observed by direct smear examination. Higher incidence of this coexistence was observed by other investigators^(21,22). This may be due to the low number of cases tested, so that one can not exclude such combined infection, or the method of the diagnosis used. So, for the diagnosis of gonorrhoea, it is recommended to use selective medium for the isolation and biochemical tests for the identification of this organism.

The combined HSV genital infection with other microorganism may be due to secondary infection. As we know herpetic infection characterized by the presence of vesicle in the infected area, this soon may rupture and it becomes susceptible for secondary bacterial or mycotic or even viral infection. Our data confirm the findings of other workers⁽¹³⁾, in that herpes genitalis was associated with one or more other infections. This is an important point for the gynecologist to bear in mind, as these associated infections can be treated easily.

Border study needed to evaluate the association of genital herpes with *N. gonorrhoeae* using specific and sensitive methods for diagnosis. Furthermore, there is a need to measure the coexistence of herpes genitalis with *C. trachomatis*, *N. gonorrhoeae*, and with human papilloma virus, since recent reports observed that this coexistence may associated with an increased risk for invasive cervical cancer⁽²³⁾.

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