http://dx.doi.org/10.15218/zjms.2014.0035

Cytological versus serological diagnosis of Herpes Simplex Virus type 2 infection among women with cervicitis without external genital ulcer or blistering lesions in Hawler Maternity Teaching Hospital

Received: 18/6/2013 Accepted: 26/11/2013

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Abstract

Background and objective: Herpes simplex virus type 2 linked to the genital tract infection may produce significant acute or chronic cervicitis. Identification of the virus is important due to its association with genital tract disease and sexual transmission. The virus establishes lifelong latency with periodic reactivation. Therefore, it causes significant physical and psychological morbidity. The aim of this study was to assess and compare cytological examination with serological test in the diagnosis of genital herpes simplex virus type 2.

Methods: This study included 104 women. Pap smear was collected from 24 healthy women as a control group (group A), 40 patients with severe cervicitis on cytological examination (group B) and 40 patients with atypical cervicitis (group C). Blood sample was obtained from the patients and the control group and tested for Herpes simplex virus type 2 specific serology. The ages of the studied groups ranged from 21-53 years. They were referred to the Maternity Teaching Hospital and private laboratories in Erbil city, Kurdistan region, Iraq during the period from December 2011 to December 2012.

Results: Herpes simplex virus type 2 IgG antibodies were found in 10 sera from patients with nonspecific cervicitis (group-B) and only in three patients with atypical cervicitis (group-C). No positive serological test was identified in the control group. All Pap smear results showed features suggestive of cervicitis but without viral cytopathic herpetic changes.

Conclusion: The serological test was superior to cytology for the diagnosis of Herpes simplex virus type 2 infection in women presented with cervicitis with no clinically apparent genital ulcer or blisters.

Keywords: Pap smear, Herpes simplex virus type 2.

Introduction

Herpes simplex virus type 2 (HSV-2) is one of the most common sexually transmitted infections and is the primary cause of genital ulcer worldwide.[1] Infection with HSV may produce significant acute and chronic cervicitis.[2] It may result in significant physical and psychological morbidity as the virus establishes lifelong latency with periodic reactivations that can be frequent.[3] The seroprevalence of HSV-2 infection in Turkey ranges from 30 to 50% in most sexually transmitted disease clinics and from 20 to 30% in most family practices, obstetric and general medicine clinics.[4] The prevalence of HSV-2 rises with initiation of sexual activity in adolescence and steadily increases through adulthood.[5,6] Women are about twice as likely as men to have HSV-2 with rates of 21% in women versus 11.5% in men. Herpes simplex virus type 2 seroprevalence also increase with number of sex partners.[7] Herpes simplex type-1 primarily infects oropharyngeal regions, and HSV-2 primarily infects genital area.[8] Transmission of HSV may occur during both the active and latent phases (subclinical virus shedding). The virus spreads by close person to person contact with lesions or mucosal secretions, cervical

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http://dx.doi.org/10.15218/zjms.2014.0035

discharge and by sexual contact during asymptomatic viral shedding.[9] The virus can also be transmitted from an infected mother to her infant.[10] Incubation period takes from two to 10 days. Recurrent viral reactivations can be less severe than primary genital herpes, or entirely asymptomatic. The virus is reactivated by various stimuli such as ultraviolet radiation, mental or physical stress or menstruation causing symptomatic or asymptomatic recurrent infection.[11,12] Many patients presented with initial genital HSV infections have sexual partners without recent signs or symptoms of infection.[13] These contacts usually have serum antibody to HSV, but no clinically detectable HSV infections are present when they are examined.[7] It was documented that nearly 40% of newly acquired HSV-2 and two thirds of HSV-1 are asymptomatic.[14] Herpes simplex virus type specific antibody is usually detected within eight weeks of onset of symptoms[15]. Thus, serum obtained from a patient during a true primary episode is very likely to be type specific antibody negative, and type specific antibody of either type in a patient during a first episode implies a high likelihood of previous infection with HSV. Such an episode is classified as initial rather than primary disease.[16] In patients with current genital ulceration from which viral culture has not been obtained, the presence of HSV-2 antibody adds strength to the diagnosis of recurrent HSV-2 disease.[17] Serologic studies yielded conflicting results on the role of HSV-2 in cervical cancer. Several groups have emphasized recently the importance of re-evaluating the role of HSV-2 as a cofactor to human papilloma virus.[7,18,19] Pap smear is the broadest successful application of clinical cytology in the diagnosis of cervical cancer and its precursors. The test was invented by and named after the prominent Greek doctor George Papanicolaou. Today it is widely used both as a screening test in asymptomatic populations and in the follow up of patients with cervical carcinomas.[20] The diagnostic cytopathic HSV infection in early stage of the disease is characterized by moderate to marked nuclear enlargement with faint basophilic and opaque homogenization of nuclear content and is known as ground-glass nuclei, in addition to nuclear multiplication and molding forming very large cells with large eosinophilic inclusions.[21,22] In the final stage, there is a bizarre hyperchromatic pearl-like structure that may mimic cancer cells.[22] The aim of the study was to compare the cytological with serological test in the diagnosis of genital HSV-2.

Methods

This study included 104 women between 21 and 53 years old attending the Maternity Teaching Hospital and private laboratories in Erbil city, Kurdistan region, Iraq during the period from December 2011 to December 2012. Ethical approval was obtained from the Research Ethics Committee at Hawler Medical University. The Pap smear samples were taken from patients who have been referred by the gynecologist to the Cytopathology Department at the Hawler Maternity Teaching Hospital. Most of these samples were collected by the cytopathologist according to the reference request and some were collected by the gynecologist. The studied patients were presented with one or more of the following: vaginal discharge, mild post coital bleeding, painful sexual intercourse, vaginal pain, cervical erosion and burning in the vaginal area. Patients with an apparent external genital vulval ulcer and heavy vaginal bleeding were excluded from the study. Sample preparation technique for Pap smears included**:**

1. The sample was spread uniformly across the surface of the slide.
2. Immediate fixation was done to prevent any air-drying artifact.
3. Each slide was properly labeled then stained for microscopic evaluation by the cytopathologist.[22]

Blood samples were examined in the serology laboratory at Nana-Kali Hospital in Erbil. The separated sera were stored at

http://dx.doi.org/10.15218/zjms.2014.0035

20C° until the time of analysis for IgG antibody test. The kits (KAPRHHG06 HSV 2 IgG ELISA kit) were used to determine the presence or absence of HSV type-2 antibodies. The procedure was applied according to instruction of manufacture. The absorbance of the solution in the wells was tested within 30 minute using a microplate reader at 450nm. The ratio between the average OD value of the sample and that of the cut off value was calculated. A sample was considered positive if the ratio was >1.1.

Statistical analysis

Statistical analysis was done using the Statistical Package for the Social Sciences (version 18.0). ANOVA test was used for comparing the groups. Chi square test was applied for comparing and finding any correlation between age factors of patients with HSV seropositivity. P value ≤0.05 was considered statistically significant.

Results

The studied women were divided into three groups, 24 healthy women with normal Pap smear cytology as control group (A), 40 patients with severe nonspecific cervicitis on Pap smear (group B) and 40 patients with atypical cervicitis (group C). The mean age (±SD) of group (A) was 34.17±7.800 years, for group (B) was 37.05±8.348 years and for group (C) was 37.65±7.950 years (Table 1). The frequency rate of positive serological test for HSV-2 among the age group 20 to 29, 30 to 39, 40 to 49 and 50 to 59 years were 20%, 13.3%, 12% and 30%, respectively, without statistical significant difference (Table 2). The serological results for the control group (A) showed no detectable antibodies to HSV-2. In group (B), only10 patients were seropositive and the rate was 25% while in group (C) only three patients were seropositive and the rate was 7.5%. The proportion of positive serology are significantly different among the three studied groups (A, B and C) P = 0.04 (Table 3).

Table 1: The age parameters of patients and control group.

Group	No	%	Age range(years)	Age (Mean±SD)
Group A - Control	24	23	21-51	34.17±7.800
Group B - Non-specific cervicitis	40	38.5	22-53	37.05±8.348
(Group C) Atypical cervicitis	40	38.5	24-52	37.65±7.950
Total	104	100	21-53	36.62±8.111

Table 2: The HSV-2 positive serology patients and age factor.

Age group	Positive		Negative		Total		p value
	N	%	N	%	N	%	
20-29	3	20	12	17.9	15	100	0.91
30-39	4	13.3	26	38.8	30	100	
40-49	3	12	22	32.83	25	100	
50-59	3	30	7	10.44	10	100	
Total	13	16.25	67	83.75	80	100	

Table 3: The **result of** cytological /serological correlation **of HSV-2 in studied groups.**

Group	Positive serology		Negative serology		Total		
	No	%	No	%	No	%	Titer (Mean±SD)
Group A - Control	0	0	24	100	24	23.07	0.17±0.179
Group B	10	25	30	75	40	38.46	0.73±0.521
Group C	3	7.5	37	92.5	40	38.46	0.45±0.421
Total	13	12.5	91	87.5	104	100	0.49±0.474

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Discussion

Genital herpes simplex virus infection is common and involves in the order of frequency the cervix, vagina, and vulva.[2] Asymptomatic or unrecognized genital herpes is perhaps the greatest diagnostic challenge for clinicians and laboratories.[23] This study revealed a mild increase in the frequency rate of seropositive patients for HSV-2 among the age group 50 to 59 years (30%) in comparison with other age groups. Different results were observed in different studies regarding the highest seroprevalence rate of HSV infection among different age group.[1] Other studies have suggested that the seroprevalence of HSV-2 start to increase at the onset of sexual activity and then increasing with age.[17] Pap smear results of both group B and C showed no specific cytological features suggestive of herpetic cervicitis. Smears of group B showed severe non specific cervicitis with some reactive changes, while in group C smears showed severe cervicitis associated with mild atypical changes in squamous cells in 37 cases and only three cases with moderate atypical changes (high grade squamous intraepithelial lesion). The likely reason for the cytologist not been able to specify herpetic cervicitis in Pap smear report is due to the absence of the characteristic diagnostic epithelial cell changes. The multinucleated cells with ground glass nuclei or inclusion bodies that seen lining the blisters.[22] Similar observations have been mentioned in other studies that the microscopic appearance at the time of biopsy is usually that of intense nonspecific inflammation and only rarely specific diagnostic multinucleated squamous cells with inclusions encountered[20] and the microscopic appearance of both histological and cytological abnormalities of diagnostic cell for HSV infection are similar.[22] In group-C with atypical cervicitis, the cytological findings could be caused by inflammation, irritation and other infectious agents. Our results goes in parallel with other studies that showed the presence of nuclear enlargement and pyknosis in addition to slight irregularities of nuclear shape could be observed in severe infections such as Trichomonus vaginalis or HSV.[22] The presence of marked cervical inflammation produce non specific abnormal Pap test due to the reactive changes of the epithelium and shedding of atypical appearing squamous cells.[2] The presence of these non specific nuclear features without the presence of specific finding make the cytopathologist hesitant to report Pap smear result as suspicious for HSV, because of the psychological impact of such a diagnosis on the patient as the disease is one of sexually transmitted diseases. The detection rate of specific cytological diagnosis of HSV-2 by cervico-vaginal smear in our study was (0%). Naib et al who found the low rate 0.02% in private hospital or clinic compared to 3.3% in female attending venereal disease clinics.[24] Some other studies agree that Pap smear will not detect latent virus.[25] Several studies showed a relationship between HSV-2 infection and cervical neoplasia.[23] However, other studies failed to show such an association and the serological difference between patients and women with invasive cancer was not significant.[26] The detection rate of HSV-2 in our patients by serology was 16.3%. Other studies showed variable results of 9.2%,[27] 23%[23] and 24%[28]. In addition, studies with type-specific serology showed that 65-80% of selected populations in the United States have been infected with HSV-2, most of which have no history of genital herpes.[23] Comparisons of the serological results of genital HSV-2 infection between countries vary according to the differences in tests, methods and populations sample. It has been reported that HSV-2 seroprevalence is higher in the USA than in Europe. The seroprevalence varies widely among European countries.[29] Some studies noticed that the clinical symptoms and shedding of the virus decreases with time, most of whom were infected for years, are unknown.[15,17] Some sero-epidemiological studies had shown

http://dx.doi.org/10.15218/zjms.2014.0035

that while antibody prevalence to HSV-2 varies with age, race, gravidity, and sexual experience; antibody to HSV-2 is often found in patients without a history of clinical genital disease.[7] This discrepancy represents the variation in incidence and prevalence of genital HSV-2 infections in different geographical areas and several other factors such as study design, sensitivity of virus detection test and sexual behaviors.[1]

Conclusion

The serological test was superior to cytology for the diagnosis of HSV-2 infection in women presented with cervicitis with no clinically apparent vulval ulcer.

Conflicts of interest

The authors report no conflicts of interest.

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