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# Prevalence and Diagnosis of Genital Herpes by Immunological and Molecular Study

**Mariam K. Ali**[1] *MSc,* **Huda D. Hathal**[2] *PhD*, **Hala A. Almoayed**[3] *ABOG*

[1]Dept. of Microbiology, College of Medicnie, University of Baghdad, Iraq, [2]Dept. of Microbiology, [3]Dept. of Gynecology and Obstetrics, College of Medicine, Al-Nahrain University, Baghdad, Iraq

## Abstract

**Background** Genital herpes simplex infection is a viral infection caused by the herpes simplex virus (HSV) type 1 or 2. This disease transmitted during close skin or mucus membranes contact with an infected person who is shedding the virus. Infections that are commonly spread by sex, especially vaginal intercourse, anal sex and oral sex.

**Objective** For detection of HSV by immunological and molecular methods.

**Methods** Two hundred (200) samples were collected from females attending the Gynecology Outpatient Department in the Al-Imamein Al-Kadhimein Medical City and Baghdad Teaching Hospital during the period from May 2014 to April 2015. Based on availability of full clinical information about each patient, high vaginal swabs were taken from females at different ages (15-54 years) representing patients group complaining of abnormal vaginal discharge with or without other symptoms. The Statistical Analysis System- SAS program was used to study the effect of difference factors in study parameters. Chi-square test was used to significant comparison between percentages in this study.

**Results** Each of the vaginal swabs collected were examined, was preserved at -20 °C for DNA extracts were analyzed. In RT-PCR, the rate of infection was in women with HSV, those with age group (25-34) years and (35-44) years were 50%.

**Conclusion** HSV infections were detected in genital tract infection in women; molecular methods are considered the gold standard for diagnosis, given the excellent sensitivities, specificities, rapid and accurate laboratory diagnosis of HSV.

**Keywords** Genital herpes, diagnosis, immunological, molecular study

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**List of abbreviations:** ELISA = Enzyme Linked Immuonosorbent Assay, HSV= Herpes simplex virus, RT-PCR = Real-time polymerase chain reaction, STPs = Sexually transmitted pathogens

## Introduction

Herpes simplex is an enveloped DNA virus (150-200 nm in diameter) belonging to the alpha-herpesviridae. Based on antigenic, biochemical and biological differences it can be divided into two serotypes, HSV-1 and HSV-2. Man is the only known natural host and source of the virus. The types of disease seen in patients depend on route of infection and individual host factors. Infection is relatively common, with seroprevalence approaching 80% for HSV-1 and 20% for HSV-2 in adult populations; however, prevalence can be much higher in certain demographics or in undeveloped countries [1-3]. An important property of both viruses is the ability to establish latency following initial infection, leading to lifelong carriage [4,5]. While there is currently no cure for latent infection, effective therapy exists for alleviating

symptoms, shortening the duration of severe outbreaks, and treating some of the more life-threatening manifestations. Effectiveness of therapy for severe acute HSV infections hinges on rapid administration of appropriate antivirals. This creates the need to establish a prompt diagnosis and necessitates HSV diagnostic testing that is both rapid and sensitive [6,7]. Testing must also be highly specific, since clinical manifestations of HSV are relatively nonspecific and overlap other potentially severe infections. Finally, while many tests are designed for use on mucocutaneous or skin lesions. There is often a need to test patients without such lesions. Physicians may need to establish a serologic diagnosis or detect nucleic acid. Therefore, effective testing should be applicable to a variety of clinical specimens [8,9].

The objective of this study was detection of HSV by immunological and molecular methods.

## Methods

Two hundred (200) samples were collected from females attending the Gynecology outpatient department in the Imamein Al-Kadhimein Medical City and Baghdad Teaching Hospital during the period from May 2014 to April 2015. Based on availability of full clinical information about each patient, high vaginal swabs were taken from females at different ages (15-54 years) representing patients group complaining of abnormal vaginal discharge with or without other symptoms, questionnaire was applied.

Five ml of venous blood sample was collected from each woman, the serum was collected into another sterile tube and was kept in deep freeze at -20 °C for diagnosis of herpes virus antibodies. Each of the vaginal swabs collected was examined, what are the remaining was preserved at -20 °C for DNA extraction and analyzed with the real-time polymerase chain reaction (RT-PCR).

This research underwent to the terms of ethical considerations and in accordance with the form prepared for this purpose by the committee of ethical standards in the Collage of Medicine, University of Al-Nahrain.

### Identification of Herpes simplex virus

### Enzyme-linked Immunosorbent Assay (ELISA tests)

Detection of Herpes Simplex virus 1, 2 IgGby (ELISA; (NovaLisa™, Germany).

These tests were done using human diagnostic ELISA kits, Germany, for the qualitative determination of human antibodies of the IgG against herpes simplex virus in serum or plasma.

### Molecular Study

Singleiplex RT-PCR kit (Sacace ™ Biotecnologies) for the direct, qualitative detection of herpes simplex virus. The principle of (DNA extraction Nano drop, Agarose electrophoresis, and RT-PCR) preparation sample, and interpretation of the results were as same as those in herpes simplex virus determination method.

## Results

### HSV-2

Only 1/200 samples were positive by ELISA test. This test assay showed (1) case of herpes simplex virus (IgG) Ab.

PCR amplification was performed using RT-PCR. The detection of appropriate channels was used as follow: channel (FAM) for detection HSV2, channel (CY3) for internal control of DNA (ICD). Used for no evidence of inhibition of the amplification in any of the samples, with the internal control of the RT-PCR samples as shown in Figure (1).

## Discussion

Only one of the 200 samples was positive by ELISA test. This test assay showed one case of herpes simplex virus (IgG) Ab.

The classical technique can be replaced by ELISA for its simplicity and the limited laboratory tools requirements and also the use of immunological methods has increased lately, the ELISA method chosen due to the reality that, it is rapid, and reliable and particularly is

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useful for the rapid investigation of a large number of blood samples in laboratories [10].

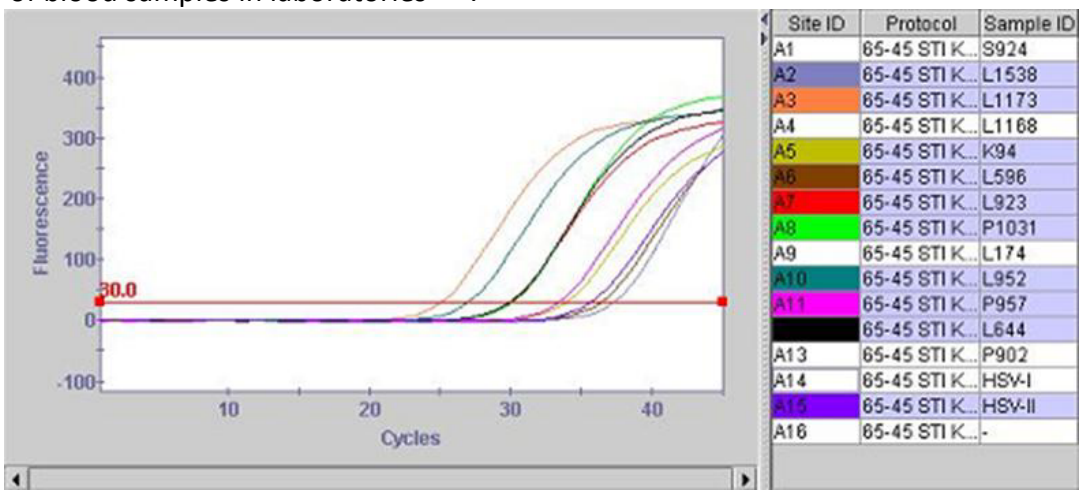


**Figure 1. Showing that from 200 samples, only 2 (1%) cases of Herpes simplex virus infection**

This result was lower than the percentage in other neighboring countries; Syria (52%) [11], KSA (27.1%) [11], Qatar (26.3%) [12], Iran (43.75%) [13], Turkey (63.1%) [14] and with other countries such as Indonesia (9.9%) [15], Tanzania (20.7%) [16], Australia (30%) [17], USA (22%) [2], Canada (17.3%) [19] and in Iraq (Waset), (6.60%) [20].

The HSV -2 IgG seroepidemiology varies among different countries, and between groups of individuals depending on the demographic and clinical characteristics of the population.

The low infectious rate of HSV2 in symptomatic patients may be due to large number of co infection with other causative agent that produce genital tract infections, this study agrees with other studies [21-24].

Among 200 samples, only 2(1%) cases of Herpes simplex virus 2 infection were detected by RT-PCR.

PCR is more accurate to detect the STPs. Molecular methods are considered the gold standard for diagnosis, given the excellent sensitivities and specificities in diagnosis, because it allowed to distinguish between STPs. However, new molecular methods such as PCR, qualitative real time PCR for rapid detection of HSV2 in comparison to serological methods have been used.

Moreover, monitoring of DNA level of a pathogen in body fluids can reveal the status of the disease, its response to medication, and its resistance patterns [24,25].

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## Authors Contribution:

Ali: conducted the sampling, isolation, and staining, the molecular work and writing the manuscript. Dr. Al-Marsome: drafting the article and revising it critically for important intellectual content. Dr. Almoayed: Selection of samples and patients.

## Conflict of interest

The authors declare no conflict of interest

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**Correspondence to Mariam k. Ali**
**E-mail: mariam.kareem 25@yahoo.com**
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