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Serological and molecular comparison study for diagnosis of herpes simplex virus type 1 patients Infection in Mosul/Iraq

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Abstract
Herpes Simplex Virus-1 (HSV-1) have the quiescent nature (dormant and recurrent infections). Therefore it is common to give a false negative results in the diagnosis by using diagnosis methods such as serological methods such as Enzymed-Linked Immunosorbent Assay (ELISA) and molecular methods such as Polymerase Chain Reaction (PCR). The present study is conducted to investigate HSV-1 infections like cold sores, Herpetic Gingivostomatitis (HGS), Herpes Gladiatorum (HG), Eczema Herpeticum (EH), and HSV-Associated Erythema Multiforme (HAEM) from Al-Salam Teaching Hospital, Mosul during the period from 1/2/2021 to 1/10/2021. The tested patients fall within the age category of (1 to 60) years. One hundred swab samples were collected, only 22 and 7 samples (22%, 7%) were indicate positive for HSV-1 for Serological assay (Enzyme-Linked Immuno Sorbent Assay - ELISA) and molecular assay (Polymerase chain reaction - PCR) respectively. ELISA shows high percentage of HSV-1 (8%) in (36-45) year, while PCR shows percentage of HSV-1 (3%) in (11-25) year, (2%) in (26-35) year, and (2%) in (36-45) year. The sensitivity and specificity between ELISA and PCR were (0.93, 0.91) respectively.

Keywords: herpes simplex virus-1, HSV-1, cold sores, herpetic gingivostomatitis, gladiatorum, HSV-associated erythema multiforme, ELISA, PCR

Introduction
The name of this virus comes from the Greek word herpein (to creep) it refers to the quiescent nature, dormant and recurrent infections (Weidner-Glunde *et al.*, 2020; Payne, 2017) [19, 17]. HSV-1 is an effective virus because of two key characteristics. First, it infects nerves, which are immune privileged. Second, it establishes latency in these neurons (Cabrera *et al.*, 2018) [6].
HSV-1 infection risk factors vary based on the kind of HSV-1 infection, from simple yet chronic diseases, through chronic infections, to severe infections, and more serious illnesses and systemic disease which are associated with high mortality (Xu *et al*., 2019; Whitley and Baines, 2018) [21, 20].
HSV-1 is transmitted through close contact with someone who is actively shedding the virus in vesicle fluid, saliva, and even when there are no apparent lesions (Madavaraju *et al.*, 2021; Murray *et al.*, 2020; Ayoub *et al.*, 2019) [11, 14, 3].

Materials and Methods
The study involved the collection of (100) samples of swabs from the infected skin taken from people with Herpes infections like cold sores, HGS, HG, EH, and HAEM from Al-Salam Teaching Hospital in Mosul during one year of study.Swabs of suspected Herpes specimens were transported in plain tubes containing Viral Transport Medium (VTM) according to the procedure code (SOP#:DSR-052-01) of Centers for Disease Control and Prevention (CDC).

HSV-1 immunological Screening:
ELISA technique using Genex, South Korea.

HSV-1 Molecular Screening:
- Extraction of viral DNA/RNA Mini Kit (V2) (Wiz^{TM} Solutions), South Korea
- Genomic amplification for genotyping and primer. use HSV-1 (gD gene) (F = CCATACCGACCACACCGACGA,R=CATACCGGAACGCACCACAC)about (224-243)bp (Franzen-Röhl *et al*., 2007).
- Amplification Program of HSV-1 was (Initial denaturation was 95°C,300 Sec for 1 cycle, denaturation, annealing and extension were (95,60,72°C), (30,45,30 Sec) respectively for 40 cycle while final extension 72°C,600 Sec for 1 cycle and hold was 5°C,300 Sec for 1 cycle.
- DNA Electrophoresis then the gel was exposed to UV-trans-illuminator at 312nm.

Results and Discussion

One hundred swab samples were taken from Herpes infections like cold sores, HGS, HG, and HAEM patients in Mosul city. These samples are placed in VTM medium in plain tubes used for serological test, molecular test, and isolation of viruses.

The prevalence of Herpes infections in our study was higher among females (56%) than males (44%). And that conform with another study where the prevalence was higher among females (50.9%) than males (45.2%) (McQuillan et al., 2018) [12]. While the height of this infection was (28%) in the age (26-35 years) and decrease when we move up and down this age. While another study shows the prevalence increased linearly with age, from 27.0% among those aged 14–19, to 41.3%, 54.1%, and 59.7% among those aged 20–29, 30–39, and 40–49, respectively (McQuillan et al., 2018) [12]. Other study shows the HSV-1 seroprevalence in USA was projected to decline from 61.5% in 1970 to 54.8% in 2018, 48.5% in 2050, and 42.0% in 2100 and though the seroprevalence will decline for several decades, incidence will persist at 3 million new infections every year (Ayoub et al., 2019) [3] and this prove our result. The married people was more than unmarried in the cases and that's also showed in another study for women in Kogi State, Nigeria where 99.2% of the married women were positive for HSV-1, more than unmarried women which was 94.5% (Drisu et al., 2018) [8].

Twenty two samples out of 100 samples-clinically diagnosed were positive by ELISA. The high number of negative results indicated that the majority of these infections were due to other reasons such as HSV-2 as well as HSV-1 (Chayavichitsilp et al., 2009) [7] where the specificity of ELISA is around 61–85% in differentiating HSV types 1 and 2 (Nath et al., 2021) [15], while the sensitivity in detection of HSV-1 antigen is around 78.3% (Morgan and Smith, 1984). In addition to that, some of the samples were taken from old crusted lesions, hence the probability of getting HSV-1 will be lower than getting it from vesicles lesions and may lead to negative results (Murray et al., 2020) [14]. This test uses specific viral proteins, purified or recombinant; the viral proteins used in these tests must be antigenically different, gD for HSV-1 which have proven to be an marker for HSV-1 infection, wherefore if there are any defect in this protein, the result will be negative (Nath et al., 2021; García-Cisneros et al., 2019) [15, 9]. Also we don't ignore that these samples were clinically diagnosed, in a sense the negative results are probable.

The sensitivity of these assays as explained by table 4.2 is around 92–100%, and the specificity was around 61–85% in differentiating both HSV-1 and HSV-2 types. Serological tests with nonspecific binding have poor specificity. Because of this lack of specificity toward whole antigen preparation, the more recent ELISA tests are based on type-specific HSV-1 gD (Aldisi et al., 2018) [2].

PCR technique became a gold standard diagnosis method especially with viruses as on infectious agents that difficulty cultivated which allows the rapid amplification of viral genomes and can be used in clinical laboratories for the detection of HSV-1 (Bamford and Zuckerman, 2021; Nath et al., 2021) [4, 15].

Present results show that seven samples only from all ELISA-positive samples were positive in PCR screening. PCR technique uses viral genome for detected glycoprotein responsible gene as showed in figure 4.1 the amplification products of gD (224-234 base pair) used for detection of HSV-1 and showed the positive results for seven sample. Therefore unless supplying a sufficient amount of HSV-1 DNA due to early replication in the very early infection will giving false negative PCR result even if it was positive in ELISA due to the presence of specific HSV-1 proteins when very early transcription occurs where very late infection can also result in false negative PCR (Niksefat et al., 2020; Schremser et al., 2020; García-Cisneros et al., 2019) [16, 18, 9] The sensitivity and specificity between ELISA and PCR were (0.93, 0.91) respectively by using table 1, that on other hands, viral genetic material concentration was another important reason for negative outcome due to use of PCR technique that need at least 20 ng/ml for achieved of specific amplification (Bergström et al., 2021) [5]. PCR results also being influenced by samples transport, and conditions and period of storage. With that, the presence of PCR inhibitors in the sample is another reason of false negative PCR. Heme factor (if it existence in the sample due to any injury in the site of the infection) inhibits DNA polymerase and lead to false negative PCR result. Also heme products such as hemin, bilirubin and bile salts as well as Lactoferrin and immunoglobulins are also known to inhibit PCR (Niksefat et al., 2020; Schremser et al., 2020) [16, 18].

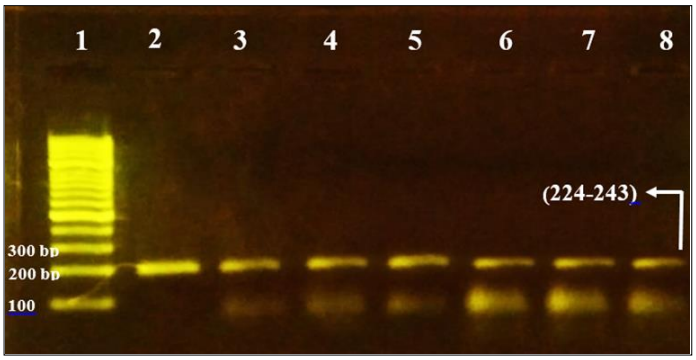

Fig 1: Detection of HSV-1 DNA by Conventional PCR. PCR products were separated on 1% agarose gel electrophoresis and were stained with GreenStar^{TM}Nucleic Acid Staining Solution. Lane (1) DNA Marker 100-1500 base pair (100 bp). Lane (2 to 8) Show positive HSV-1 DNA (224-243 bp).

Table 1: Results of ELISA assay and PCR screening.

Tests		PCR				Total
		Positive		Negative		
		No.	%	No.	%	
ELISA for HSV-1	+ve 22	7	31.8	15	68.2	100
	-ve 78	0	0	78	100	

Other study done by Ahmed Nishat and others were conducted overall the world in this filed for detection HSV-1 at molecular level as the detection of HSV-1in ocular viral infections, were detected of this viruses using RL-2 gene in 170 samples, 13.5% out of them was positive in PCR screening for HSV-1 (Ahmed Nishat *et al*., 2021) [1]. Another study in India was achieved for confirmation the presence of HSV-1 in 50 samples as a causative agent for keratitis, and 78% of the samples were positive for this virus (Guda *et al*., 2019) [10].

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