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MOLECULAR AND IMMUNOLOGICAL IDENTIFICATION OF BOTH *HSV*-1 AND *HSV*-2 IN RECURRENT HERPETIC INFECTED PATIENTS AMONG GROUPS OF PEOPLE IN BABYLON PROVINCE, IRAQ

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ABSTRACT

Human herpesvirus includes eight species with distinct biological and clinical characteristics; *HSV* a universal human pathogen, is a neurotropic virus that has a linear double-stranded DNA and measured about 200 *nm* in diameter. *HSV*-1 and *HSV*-2 are generally responsible for recurrent herpetic infection (RHI). Interleukin - 17D is a pro-inflammatory cytokine, which is produced by a group of T helper cell known as T helper 17 cells. Interleukin-2 is a kind of cytokine signaling molecule within the immune system. It is protein that regulates the white blood cells activities (leukocytes, often lymphocytes) which are responsible for immunity. Molecular detection of *HSV*-1 and *HSV*-2 in lesion swab by real time PCR as well as immunological detection and comparison of IL-2 and IL-17D in saliva of healthy control subjects and recurrent herpetic infected patients. Sixty participant subjects participated in this clinical trial study include thirty patients infected with *Herpes Simplex Virus* with age range (6-48years) and thirty healthy control subjects; Thirty lesion swabs and (60) saliva samples were collected from those participant subjects. Real time PCR technique were used for detection of *HSV*-1, *HSV*-2 and ELISA test for IL-2 and IL-17D assessment. Both *HSV*-1 and *HSV*-2 was detected in lesion swabs from recurrent herpetic lesions, all patients were infected with *HSV*-1(100%), while (90%) of patients were infected with *HSV*-2. Interleukin 2 and IL-17D also detected in saliva samples of both *HSV* infected patients and control subjects. Mean interleukin-17D and IL-2 of patients with *HSV* infection was significantly higher than mean of these interleukins of healthy control subjects. Main age group with recurrent herpetic infection was (20-29 years) about 36.6% in the present study with male more than female patients (male to female ratio as **1.5:1**), no significant differences were observed between patients with ***HSV*** infection and healthy controls regarding age (p=0.5) and gender (p=0.1). Patients with recurrent herpetic infection mostly infected with both *HSV*-1 and *HSV*-2 and only 10% of them were non-infected with *HSV*-2 with predominance of male than female. Saliva samples of recurrent herpetic infected patients revealed significant increase in IL-2 and IL-17D levels in comparison with healthy controls as assisted by ELISA test.

***Keywords*:** Recurrent herpetic infection, real time PCR, *HSV*-1, *HSV*-2, IL-2 and IL-17D

Introduction

The family of *Herpes viridae* is separated into the "alpha sub-family" which includes: *Herpes simplex virus*-1 (oral-facial type), *Herpes simplex virus*-2 (genital type), and *Varicella - zoster virus*. The "beta subfamily" includes (*Human cytomegalovirus*, *Human Herpesvirus*-6, and *Human Herpesvirus*-7), and the "gamma subfamily" include (*Epstein–Barr virus* and *Human herpesvirus*-8) (Slots, 2009). The *HSV*-1 and *HSV*-2 are generally responsible for recurrent herpetic infection. *Herpes simplex virus* type 1 (*HSV*-1), a global human pathogen, is a neurotropic virus that has a longitudinal double-stranded DNA genome which contains more than 80 open reading frames (ORFs). Upon primary infection, the viral replication occurs in the epithelial cells and undergoes its typical lytic life cycle with a cascade of immediate-early (IE), early (E), and late (L) genes. Then, it enters the sensory neurons by axon terminals and is backward transported to the corresponding sensory ganglia, usually trigeminal ganglia (TG), where a latent infection is established. In response to the variation of stressors, the latent virus can be reactivated periodically to begin again productive replication followed by the formation of "infectious virus", which is transported back to peripheral tissues or else infects further host neuronal cells (Sancakli *et al.,* 2015).

Herpes simplex virus-1 is usually acquired during early childhood, mainly through oral secretions; while sexual transmission is an increasingly common cause of infection in several countries (Bernstein *et al*., 2013). It should be noticed that, currently, there is no therapy to absolutely eliminate *HSV*-1 once an individual is infected. Reactivation of latently infected *HSV*-1 can cause recurrent lesions and is the main cause for herpes viral encephalitis (Whitley, 2006; Steiner *et al*., 2007; Perlejewski *et al*., 2015). *Herpes simplex virus*-1evades the host immune response by targeting components such as complement proteins, natural killer cells, major histocompatibility complex class I or II molecules and antibodies (Hook *et al.,* 2006).

Herpes simplex virus type 1 (*HSV*-1) and type 2 (*HSV*-2) are closely related. Infections with *HSV*-2 typically affect anogenital sites and are transmitted sexually or from a mother's genital infection to the newborn. By contrast, *HSV*-1 commonly causesorolabial infection. Transmission most frequently occurs via nonsexual routes, although sexual and vertical transmissions also occur. Both viruses are capable of causing either anogenital ororolabial infection and can produce primary and recurrent lesions that are clinically indistinguishable (Xu *et al*., 2002).

Herpes labials are commonly known as cold sores which are *HSV* infection that most commonly cause recurrent oral-facial lesion and often is signed by prodromal symptoms which including tingling, burning, itching, or pain before the development of the herpetic lesions, which typically appear on the vermilion border of the lip, while lesions sometimes occur on the nose, cheek, chin or oral mucosa (Stanberry & Belshe, 2012). The rash usually heals within 10 days, but the virus remains dormant within the trigeminal ganglion, the virus may periodically reactivate to make another outbreak of sores within the mouth or lip, the cause is usually Herpes Simplex Virus type 1 (*HSV*-1) and occasionally Herpes Simplex Virus type 2 (*HSV*-2) (Opstelten *et al*., 2008).

The infection is typically spread between people by direct non-sexual contact; Attacks can be triggered by sunlight, fever, psychological stress, or a menstrual period (Stoopler and Sollecito, 2014). Worldwide, approximately 90% are the global prevalence of *HSV*-1 infection, and in some of the rural areas, the seroprevalence is up to 100% higher, (Levett, 2005; Shen *et al*., 2015). In 2012, an estimated 3.7 billion people under the age of 50 (67%) of the population, had *HSV*-1 infection and an estimated 417 million people aged 15-49 (11%) worldwide have *HSV*-2 infection (WHO, 2017).

Firestein *et al*. (2017) stated that cytokines are peptides that have a fundamental role in communication within the immune system and in allowing the immune system and host tissue cells to exchange information. Interleukin 17 is a pro-inflammatory cytokine, the production of this cytokine is arise from a group of T helper cell known as T helper 17 cells in response to their stimulation with IL-22. Originally, IL17 was identified by Rouvierin 1993 who isolated IL17 transcript from a rodent T-cell hybridoma.

Interleukin 17D does not appear to have the ability to stimulate the proliferation of immune cells on its own, but it does have the ability to stimulate the production of other cytokines from target tissues such as endothelial cells. This is similar to other members of the IL-17 family, which are thought to indirectly modulate the immune response by regulating cytokine production. The cytokines induced by IL-17D, such as IL-6, IL-8 and GM-CSF, are similar to those induced by other IL-17 family members (Aggarwal and Gurney, 2002).

Arenas and woytschak, (2015) show that interleukin-2 (Il-2) is a kind of cytokine signaling molecule within the immune system. It is about 15.5 - 16 kda protein that regulates the white blood cells activities (leukocytes, often lymphocytes) which are responsible for immunity. Interleukin-2is part from the body's natural response to microbial infection, and in discriminating between foreign ("non-self") and "self". Interleukin-2 mediates its effects by binding to il-2 receptors, which are expressed by lymphocytes. The main sources of Il-2 are activated CD4+ T cells and activated CD8+ T cells (Liao *et al*., 2011).The IL-2 furthermore stimulates the differentiation of T cells into both effectors T cells and into memory T cells when the initial T cell is also stimulated by an antigen, so it helping the body fight off infections (Liao *et al*., 2011).

Traditional techniques for *HSV* diagnosis have relied on virus isolation in cell culture, followed by immunofluorescence detection. These methods lack of sensitivity, difficult, highly dependent on the maintenance of cell culture lines and virus viability, and provide only qualitative results (Rose, Herra and Crowley, 2008). The development of nucleic acid amplification tests, such as real-time polymerase chain reaction (rT- PCR), for the detection of *HSV* offers an automated diagnostic method with improved sensitivity and specificity (Doornum *et al.*, 2003).

Quantitative real-time polymerase chain reaction, also can called quantitative polymerase chain reaction and real-time polymerase chain reaction, is a technique based on the conventional polymerase chain reaction (PCR) which is used to amplify and simultaneously quantify a target DNA. By convention of the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines (Bustin *et al.*, 2009) and for uniformity, the abbreviation q-PCR is henceforth used in this research designate quantitative real-time PCR. Also, important to note that it is advised not to use the designation RT-PCR for quantitative/real-time PCR to avoid confusion with reverse transcription PCR. As opposed to the conventional PCR in which quantification, if performed, is based on "end-point" analysis of the amount of the amplicon, q-PCR allows for the product quantification as the process takes place in real time.

In an optimized q-PCR reaction, the concentration of initial template usually determines the number of cycles that necessary before the fluorescence rises. The initial exponential amplification not observable when concentrations are below the detection limits. This is followed by a growth phase, and finally a plateau phase. The point of maximum acceleration of this growth curve are correlates with the concentration of the initial template and the fractional cycle number is inversely proportional to the log of the initial template concentration (Burns *et al.*, 2005).

The advantage of a q-PCR assay is the excellent sensitivity (limited only by stochastic variation), a large dynamic range with a precision of 5–10 %. Specificity is typically dependent on the PCR quality and detection method. For absolute quantification standards can be included to provide an exact copy number; however, for most practical purposes a relative quantification (mRNA or diploid DNA) usually provides the necessary information. A "housekeeping gene" (e.g., β-actin, cyclophilin, or glyceraldehyde-3-phosphate dehydrogenase) is often used as a biological reference to normalize results between different experiments (Huggett *et al.*, 2005).Therefore,q-PCR reaction was used in present study for detection of *Herpes simplex virus*, and ELISA test for assessment of interleukins level.

Materials and Methods

Sixty samples were collected from, (30) participant patients complain from recurrent herpetic infection (after differential diagnoses are ruled out) including (30) lesion swabs beside (30) saliva samples and (30) saliva samples

from healthy control subjects (non-infected with *HSV*) with the same ages and genders as those infected with *HSV*. They were attended to the teaching clinics, collage of dentistry/University of Babylon and private clinic in Hilla city through extended period from September 2019 to January 2020.

Patient name, age, sex, occupation, medical and dental history, episode of recurrent herpetic infection per year, family history and triggering factors was taken.

Babylon Faculty of dentistry / University of Babylon gave the ethical approved for this study.

Specimens Collection

A. Lesion Swabs Samples

Specimens were prepared in a proper way to avoid any possible contamination from dental clinics, Swabs were collected from lesions of participant patients complains from recurrent herpetic lesion (lips or peri-oral region) by scraping the lesion with sterile lancet after the application of topical anesthesia to get blood swabs from the lesion site by using of viral transport media (VTM) which contains both cotton swab and medium for preserving the specimen until being taken to the laboratory examinations, these samples were placed in a cool box containing ice bags to maintain its viability then preserved in deep freezing under (-23 C) to make later the identification for *Herpes Simplex Virus* type 1 and 2 by using Real-time polymerase chain reaction.

B. Saliva samples

Saliva samples were also collected properly in a contaminated free method from each participant patient (in addition to the Lesion Swab samples), also 30 saliva sample were collected from control subject (non-infected with *HSV*), The non-stimulated clean saliva was saved in sterile laboratory plain tube and placed in a cool box containing ice bags to maintain its viability then preserved in deep freezing under (-23 C), then each sample was centrifuged at (5000 rpm) for 15 min. to make a separation for the undesired free salivary particles, after that clear salivary solution was aspirated by using (1ml) micropipette tips and save it in (1ml) sterile Eppendorf tube to carry immunological test for assessment of IL-2 and IL-17D by an Enzyme-linked immunosorbent Assay (ELISA).

Detection of IL-2 and IL-17D by ELISA test

ELISA plate provided in this kits has been pre-coated with an antibody specific to IL-2 and IL-17D. Standard and samples are added to the appropriate micro ELISA plate wells and combined with the specific antibody, then a biotinylated detection antibody and Avidin- Horseradish Peroxidaes (HRP) conjugate was added to each microplate well successively and incubated. Free components were washed away; the substrate solution is added to each well. Biotinylated detection antibody and Avidin–HRP conjugate will appear blue in color. The enzyme –substrate reaction is terminated by the addition of a sulphuric acid solution and the color turns yellow.

Viral DNA Extraction technique

Viral DNA Extraction Kit is designed for rapid isolation of DNA from a variety of sample sources including fresh or frozen serum, plasma and other cell-free body fluids and virus-infected cell/tissue, viral DNA Extraction Kituses advanced silica-gel Membrane technology for rapid and effective purification of DNA without organic extraction or ethanol precipitation. Furthermore, the buffering condition are finely adjusted to provide optimum binding of the DNA to the column.

DNA *HSV*-1/*HSV*-2 Real Time PCR

DNA *HSV*-1/*HSV*-2kit is intended for differential detection of *Herpes simplex virus* types 1 and 2 DNA in clinical specimens (swabs of epithelial cells, tissue fluid, erosive-ulcerative skin lesion) using the method of real-time polymerase chain reaction (PCR) with fluorescence detection of amplified product.

Real-time PCR is based on the detection of the fluorescence produced by a reporter molecule, which increases as the reaction proceeds. Reporter molecule is a dual-labeled DNA-probe that specifically binds to the target region of pathogens DNA. Fluorescence signal increases due to the separation of fluorescence dye and quencher by Taq DNA-polymerase exonuclease activity during amplification. PCR consists of repeated cycles: temperature denaturation of DNA, primer annealing and complementary chain synthesis, the device Programming as following: -

1	Hold 1 *(Holding temperature 1)*	50 °C	2 minutes
2	Hold 2 *(Holding temperature 2)*	95 °C	2 minutes
3	Cycling (Process of cycling)	94 °C 60 °C*	10 seconds 40 seconds
Cycle repeats *(repeat the cycle)*		50 times	

Results

This study included 30 patients infected with *Herpes Simplex Virus* (diagnosed recurrent herpetic infection) presented with mean age of 25.7 ± 12.1 years; 6.7% of patients were in age group <10 years, 20% of them were in age group 10-19 years, 36.6% of them were in age group 20-29 years, 16.7% of them were in age group 30-39 years and 20% of them were in age 40 years and more. Male patients with *HSV* were more than female *HSV* patients with male to female ratio as 1.5:1, as shown in Table-1 and figure-1.

Table 1 : Demographic characteristics of patients with recurrent herpetic infection.

Variable	Number of Patients	Percentage
<10 years	2	6.7
10-19 years	6	20.0
20-29 years	11	36.6
30-39 years	5	16.7
≥ 40 years	6	20.0
Total	30	100.0
Gender		
Male	18	60.0
Female	12	40.0

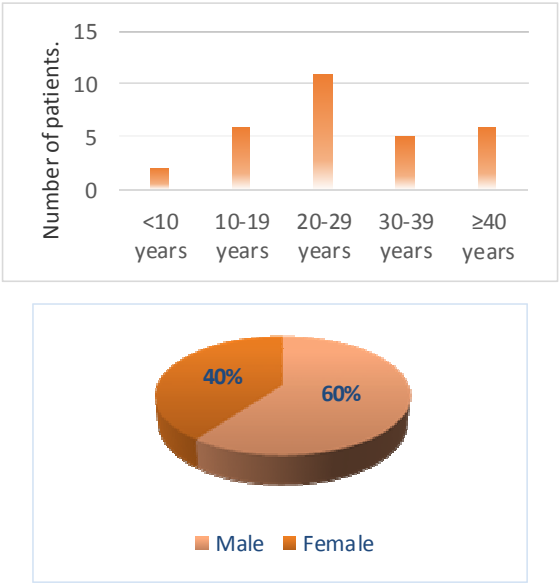

Fig. 1 : Age and gender distribution

No significant differences were observed between patients with *HSV* infection and healthy controls regarding age (p=0.5) and gender (p=0.1), as shown in Table-2.

Table 2: Distribution of Patients' demographic characteristics according to study groups.

Variable	Study groups				Percentage %
	***HSV* cases**		**Healthy Controls**		
	No.	**%**	**No.**	**%**	
Age					
<10 years	2	6.7	3	10.0	**0.5*^{NS}**
10-19 years	6	20.0	7	23.3	
20-29 years	11	36.7	10	33.3	
30-39 years	5	16.7	2	6.7	
≥40 years	6	20.0	8	26.7	
Gender					
Male	18	60.0	17	56.7	**0.1**^{NS}**
Female	12	40.0	13	43.3	

** Fishers exact test, **Chi square test, NS=Not significant.*

Mean appearance duration of RHI was 3.3 ± 1.2 days; 13.3% of RHI were appeared in less than 3 days and 86.7% were appeared in 3 days and more, as shown in Table-3.

Table 3: Appearance durations of patients with recurrent herpetic infection.

Appearance of lesions	**Mean ± SD (3.3±1.2 days)**	
<3 days	4	13.3
≥3days	26	86.7
Total	30	100.0

All recurrent herpetic infected patients were infected with *HSV*-1, while 3 (10%) patients were non infected with *HSV*-2; which are (Age<10 years) group, as shown in Table-4, 5 and figure-2.

Table 4 : *HSV* infection types of studied patients.

Variable	**No. of patients**	**%**
***HSV*-1**		
Positive	30	100.0
Negative	0	-
Total	30	100.0
HSV-2		
Yes	27	90.0
No	3	10.0
Total	30	100.0

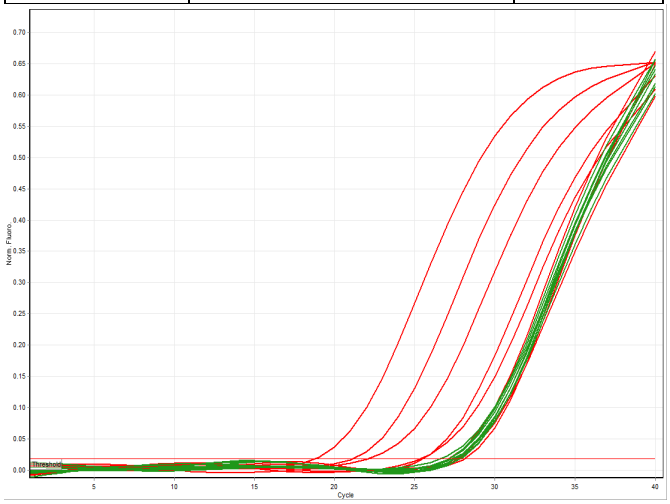

Fig. 2: Amplification plot of *HSV*-1 (red lines) and *HSV*-2 (green lines).

Table 5: Distribution of *HSV* investigations Measures according to different age groups.

Variable	Threshold cycle(CT) Mean ± SD
Age<10 years	
*HSV-1*PCR (CT)	23.1±6.1
*HSV-2*PCR (CT)	0.0±0.0
Age 10-19 years	
*HSV-1*PCR (CT)	22.5±4.6
*HSV-2*PCR (CT)	22.8±11.2
Age 20-29 years	
*HSV-1*PCR (CT)	23.8±4.1
*HSV-2*PCR (CT)	27.2±1.2
Age 30-39 years	
*HSV-1*PCR (CT)	26.3±1.8
*HSV-2*PCR (CT)	28.6±0.7
Age ≥40years	
*HSV-1*PCR (CT)	23.9±4.3
*HSV-2*PCR (CT)	28.1±0.7

Mean interleukin-17D of patients with *HSV* (3.9 ng/L) was significantly higher than mean interleukin-17D (2.8 ng/L) of healthy controls (P=0.04). Whereas mean interleukin-2 of patients with *HSV* (15.8 Pg/ml) which was significantly higher than mean interleukin-2 (14.9 Pg/ml) of healthy controls (P=0.02), as shown in Table-6 and Figures-3

Table 6 : Distribution of interleukins according to study groups.

Variable	**Study groups**		**P**
	***HSV* cases**	**Controls**	
	Mean±SD	**Mean±SD**	
Interleukin-17D (ng/L)	3.9±2.7	2.8±1.1	0.04*^{S}
Interleukin-2 (Pg/ml)	15.8±1.4	14.9±1.4	0.02*^{S}

** Independent sample t-test, S=Significant.*

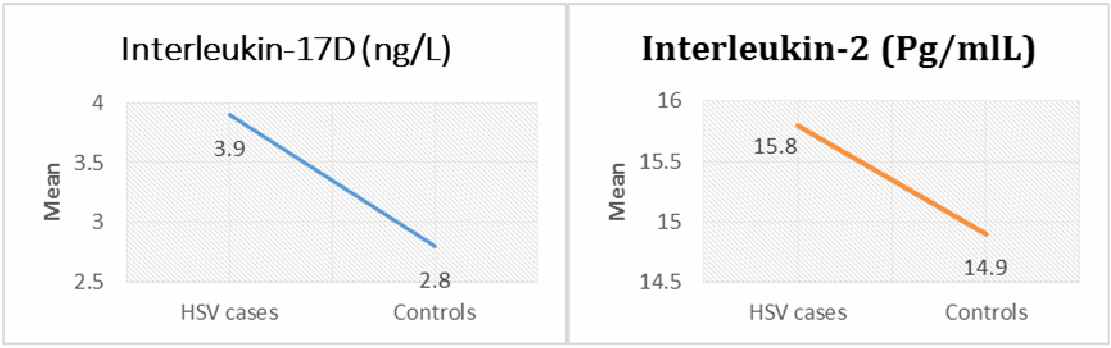

Fig. 3: Interleukin-17D and IL-2 means distribution according to study group.

There was no significant difference in means appearance period of RHI in groups of less than 3 days and these were appeared in 3 days and more regarding IL_17D, IL-2, *HSV1*-PCR (CT) and *HSV2*-PCR (CT) p-value > 0.05, as shown in Table-7.

Table 7 : Distribution of interleukins and *HSV* measures according period of lesion appearance.

Variable	Appearance of lesions		P
	<3 days	≥3days	
	Mean±SD	Mean±SD	
Interleukin-17D (ng/L)	3.09±1.5	4.1±3.1	0.516*^{NS}
Interleukin-2 (Pg/ml)	16.3±0.4	15.7±1.6	0.452*^{NS}
HSV1-PCR (CT)	21.7±2.8	23.9±4.3	0.353*^{NS}
HSV2-PCR (CT)	26.6±1.3	23.8±10	0.591*^{NS}

** Fishers exact test, NS=Not significant.*

On the other hand, there was no significant difference in male and female groups in relation to IL_17D, IL-2, *HSV-1*PCR (CT) and *HSV-2*PCR (CT) p-value > 0.05, as shown in Table-8.

Table 8: Distribution of interleukins and *HSV* measures according to gender.

Variable	Study groups		P
	Male	Female	
	Mean±SD	Mean±SD	
Interleukin-17D (ng/L)	4.7±3.6	3.04±1.2	0.154*^{NS}
Interleukin-2 (Pg/ml)	15.6±1.6	16.1±1.2	0.435*^{NS}
*HSV-1*PCR (CT)	23.2±4.5	24.0±3.8	0.633*^{NS}
*HSV-2*PCR (CT)	21.8±11.8	27.3±1.31	0.141*^{NS}

** Fishers exact test, NS=Not significant.*

Discussion

Recurrent herpes infection is an infectious disease caused by herpes simplex virus (*HSV*). Although both virus subtypes *HSV*-1 and *HSV*-2 affect skin and mucous membranes, the or facial region is most commonly affected by *HSV*-1, lesion last for approximately 12 days and are accompanied by unspecific symptoms as malaise, fever and dehydration (Lotufo *et al*., 2020).

This study presented with mean age of 25.7 ± 12.1 years; 6.7% of patients were <10 years, 20% of them (10-19) years, 36.6% of patients with herpes labials were in 20-29 years, then 16.7% of them were in 30-39 years and finally 20% of them were in age 40 years and more. Male were more than female *HSV* patients with ratio as 1.5:1.

(Bradley *et al*., 2014) study showed that the seroprevalence of *HSV*-1 and *HSV*-2 increase with increasing age. From 2005-2010, the seroprevalence of *HSV*-1 among patients aged 14-19 years was 30.1% and 63.6% among patients aged 40-49 years, Similar trends exist for *HSV*-2 seroprevalence, with 1.2% of persons aged 14-19 years affected and 25.6% of those aged 40-49 years affected, this finding agree with the present study, also the frequency of *HSV*-1 antibodies is slightly higher in females than in males (33.2% vs 27.1%), these disagree with the current study.

McQuillan *et al.* (2018) showed, Data from the NationalHealth and NutritionExamination Survey During 2015–2016 in united states, prevalence of herpes simplex virus type 1 (*HSV*-1) was47.8%, and prevalence of herpes simplex virus type 2(*HSV*-2) was 11.9%. Prevalence of both *HSV*-1and *HSV*-2 increased with age and this in agreement with the current study.

Also the results of this study showed that all recurrent herpetic infected patients were infected with *HSV*-1, while 3 (10%) patients were non infected with *HSV*-2; which are (Age<10 years) group, this in compact with **(Xu *et al*., 2002)** study which presented(n=13,904) 27.1% of persons aged >12 years were seronegative for *HSV*-1 and *HSV*-2; 51.0% were seropositive for *HSV*-1 only, 5.3% for *HSV*-2 only, and 16.6% for both *HSV*-1 and *HSV*-2 **(n=2861)**. The seroprevalence of *HSV*-2 was higher in persons with *HSV*-1 antibody. Approximately 76% of persons who had *HSV*-2 antibody also had *HSV*-1 antibody. Persons seropositive for *HSV*-2 only reported a history of genital herpes more frequently (16.2%) than persons seropositive for both *HSV*-1 and *HSV*-2 (5.9%). The seroprevalence of *HSV*-1 and age at infection may influence the epidemiology of clinical genital herpes, even if prior *HSV*-1 infection does not prevent *HSV*-2 infection. Significantly more male (31.4%) than female (23.2%) subjects were seronegative for *HSV* infection. Although the seroprevalence of *HSV*-1 only was similar in male and female subjects, the seroprevalence of confection was significantly higher in female than in male subjects, this variation in present study and Xu *et al*., may be related to variation in number of participants in two studies.

Susloparov *et al*. (2006) study harmonizing this study which presented; in examined group of patients with genital herpes the virus of type 2 and type 1 was detected in 63% and 26% cases, respectively. The mixed infection of both types is revealed in 11% of the patients.

The results also showed that mean interleukin-17D of patients with *HSV* (3.9 ng/L) was significantly higher than the mean of interleukin-17D (2.8 ng/L) of healthy controls (p=0.04). Also IL-2 of patients with *HSV* infection (15.8 Pg/ml) was significantly higher than that the mean of interleukin-2 (14.9 Pg/ml) of healthy subjects (p=0.02) and this due to the viral infection and immune system stimulation. This study agreed with the results of Kristensen

et al. (2002) that showed the infections with lymphocytic choriomeningitis virus (*LCMV*) and vesicular stomatitis virus in mice as model systems, that investigated the ability of antigen-primed CD8M T cells generated in the context of viral infections to produce IL-2, there results indicated that acute immunizing infection normally leads to generation of high numbers of IL-2-producing antigen-specific CD8M T cells, and these finding indicate that phenotyping of T cell populations based on capacity to produce cytokines, and especially IL-2, can provide important information as to the functional status of the analyzed cell subset. Specifically, combined analysis of the capacity to produce IL-2 and IFN-γ can be used as a predictor for loss of function within the CD8M T cell compartment.

Keenan *et al*. (2013) showed that IL-2 level elevation in patients with herpetic infections that suggested the role of stress in the development of a disease concluded that in stressful condition, the immune system was altering and there was a cascade leading to stimulation of T-lymphocytes which in turn releases IL-2 and IL-12. Hence stress is a potential risk factor for the herpes infection and this fit with the facts that stress one of the most predisposing factors that induce recurrent herpetic infection and this harmonize with the present study.

On the other hand, Lee showed that the lack of IL-17D expression confers protection against Listeria infection. A deficiency in IL-17D also resulted in less weight loss with reduced pathogen burden during influenza- A virus infection. During infection, the loss of IL-17D resulted in compromised CD8 T cell activity so this harmonizing the significant increasing in IL-17D in saliva during *HSV*-1 infection and sensitizing of body immune response.

Whereas Saddawi-Konefka *et al*. (2016) have shown that mice deficient in IL-17D exhibit signs of more severe infection after infection by vaccinia virus (*VV*) or murine cytomegalovirus (*MCMV*) as compared to wild-type mice, so as part of normal body immune response there will be an increase in IL-17D level as protective mechanism. This come in accordance with the significant increasing of IL-17D level in *HSV* infected patient in the current study.

While Lee found that IL-17D from the non-hematopoietic compartment regulates protective immunity during infection. Together, the study data led to the identification of IL-17D as a critical cytokine during intracellular bacterial and viral infection that suppresses the activity of CD8T cells by regulating dendritic cells, also the result of these study showed that IL-17D is a novel cytokine that was harmful to the host in the context of acute infection by suppressing the adaptive immune response and enhancing inflammation.

In addition to that Lee showed that recruitment and activation of the immune system in response to virus infections result in the release of potent cytokines and cytotoxic molecules. While these molecules are important in clearing the virus, they can also cause tissue damage when released at inappropriate levels, as shown in influenza virus infections.

From the best of our knowledge; there was no previous data or articles related with the measuring of IL-2, IL-17D in saliva and *HSV*-1 \ *HSV*-2 detection in lesion swabs of recurrent herpetic infected patient and healthy control subjects.

Conclusions

Real Time PCR technique are highly dependable, reliable, and rapid technique for accurate diagnosis of *HSV*-1 and *HSV*-2 in recurrent herpetic infected patient; Both virus typeis generally responsible for RHI as indicated by 90% infected with *HSV*-2 and100%by *HSV*-1. However, *HSV*-2 was not detected inpatients of age group <10 years. There was significant increase in IL-17D and IL-2 in saliva samples of recurrent herpetic infected patients in comparison with healthy control subjects due to immune system sensitization.

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