

DETECTION OF SHINGLES AND CORRELATION WITH GENDER, WEATHER AND RESIDENCY

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ABSTRACT : Herpes zoster (HZ) disease, or shingles, is a viral disease that occurs as a result of recurrent viral infection resulted due to infection of the varicella virus in childhood period between 1-9 years old (Mueller *et al*, 2008). As similar to specific types of herpes viruses such as HSV-1 and HSV-2, herpesviruses remain latent within specific host cells and may subsequently reactivate as a result of immune-suppression. As a result of latent infection, the virus can reactivate and mostly causing symptoms and leading to shedding of virus, and then the viral infection is transmitted among people following direct contact and as an airborne primarily transmitted disease (Weidner-Glunde, Kruminis-Kasziel and Savanagouder, 2020). Varicella-Zoster virus (VZV) is one of the human herpes viruses (HHVs); its official name known as (Human Herpes Virus -3) HHV-3, a member of DNA containing Herpesviridae family. VZV causes varicella (chickenpox) as a primary infection and herpes zoster (HZ) after the reactivation of a latent VZV. Varicella is a childhood illness with highest incidence between 1 and 9 years of age, characterized by fever and a generalized pruritic vesicular rash. Varicella is a worldwide infection more prevalent in temperate climates than tropical ones and often occurs in late winter and spring seasons. Furthermore, varicella is transmitted by respiratory aerosols from infected individuals and by direct contact with skin lesions of individuals affected by VZV (Aubaid *et al*, 2020).

Key words : Human herpes viruses (HHVs), childhood illness, weater, residency.

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INTRODUCTION

A total of 100 subjects, 50 patients and 50 control, males and females with an age range of above than 10 to 80 years then all questionnaire data, such as, age, gender, residency and date of infection are collected.

Through the results of the number of cases infected with herpes zoster, it appeared that there was also an increase in the level of infection among males compared to females. The study demonstrates in a database exceeding 50 cases of shingles standardized excess incidence in males 30 (60%) over females 20 (40%). As for the rate of herpes zoster infection in males and females and its relationship to the age stages, it was according to the total number more in men compared to women in relation to the total number. With regard to the correlation of the level of viral infection with the place of residence among the infected people, it was found

that a higher percentage of shingles infection was found by 72% among people living in cities compared to 28% in those living in rural areas with a statistically significant difference.

Next to the results, it was also noted that there was an increase in the number of infections in the cold weather months began to gradually rise started from November and continued to rise to the highest infection rate in January and stabilized at the same previous level in February and March and reached the lowest Its levels were in the month of April.

Herpes zoster (HZ) infection or Shingles is a skin viral disease that naturally occurred as a result of recurrence or reactivation of latent varicella zoster virus (VZV) hiding in the dorsal root and cranial nerve ganglia. Varicella-zoster virus (VZV) is regarded as a one of the human herpes viruses (HHVs), it is known officially as

human herpes virus type 3 (HHV-III), which is recognized as a member of DNA-containing Herpesviridae family (Head *et al*, 1998). VZV is the primary cause of varicella zoster infection or what is called (chickenpox) as a primary infection, in addition to herpes zoster (HZ) or shingles that is considered as a secondary skin viral infection resulted after the reactivation of a latent VZV (Arvin, 1996). Herpes zoster (HZ) is a skin viral disease that characterized by severe, sharp and lancinating radicular pain accompanied with vesicular rash or eruption on the erythematous base localized in one or more dermatomes next to the affected nerve. A latent HZV then retrograde from its latency in the dorsal or cranial root of ganglion and transmitted through peripheral nerves into specific dermatome on one side of the body during youth or adulthood period. This recurrent viral infection is occurred as a result of various factors, such as immunosuppressant agents like old age, autoimmune diseases, stress factors and other important factors. In addition, HZV or chicken pox is considered as a common epidemic disease of childhood and high prevalence of Varicella zoster infection around the world. VZV is still regarded as a big public health problem with worldwide distributed in different regions around the world (Brooks, *et al*, 2013; Mueller *et al*, 2008 and Awn *et al*, 2007).

Accordingly, herpes zoster or shingles which is mainly occurred among adults with a considerable percentage of infection in all months of year with a higher percentage of infection in winter and spring in compared to that detected in summer in temperate climates. For example, 10–20% of adults older than 50 years, are usually suffered from at least one case of zoster attack during their lifetime, usually after the age of 50 years. Moreover, About 30% of individuals are suppose to be undergoes with HZ in specific period of their lives (Al-Turab and Chehadeh, 2010; Martin and Sergei, 2014). According to what was previously reported data and as a result of the small number of researches related to following up the rate of infection with herpes zoster among the population of Iraq in general and Thiqr Governorate, the main objective of this study was to determine the actual percentage of this disease, as well as to study the direct impact of risk factors for people in general and patients with viral skin diseases in particular and the extent of interdependence between these factors and the rate of herpes zoster infection.

Therefore, this project of study is to identify the incidence of herpes zoster among patients of other skin infections. Also, this study is aimed to investigate any correlation between the level of incidence of herpes zoster infection and risk factors which are detected, such

as gender, geographical areas of infection and effect of weather among patients compared to the control groups of people.

Aims of study

The aim of this study is to identify the incidence of herpes zoster among patients of other skin infections. Also, this study is aimed to investigate any correlation between the level of incidence of herpes zoster infection and risk factors, which are detected, such as gender, location of residency and effect of weather among male and female HZ patients compared to the control groups of people.

Objective of the study

The objective of this study is to determine whether the risk of HZ occurrence would be escalated or affected in patients suffered from different factors, such as gender, location of residency and weather effects.

METHODS

This study is aimed to follow patients with herpes zoster or shingles were primarily diagnosed depending on the clinical symptoms characterized by the appearance of skin vesicles on one side of the body, which were distributed in different areas according to site of the dermatome. In addition, these vesicles fluids samples were used for the purpose of extracting the DNA of the herpes zoster virus using the viral DNA extraction kit and then detecting the presence of the extracted DNA through gel electrophoresis as well as measuring the concentration and purity of the extracted DNA. Then the extracted DNA samples were used to examine the polymerase chain reaction and all these gave Positive results through relay screening test (Gel electrophoresis).

Data collection from patients

This study is primarily targeted and carried out on patients suffering from dermatological diseases who were referred to a dermatologist. Among the many dermatology consultants and dermatology outpatient clinic in hospitals, such as Al-Hussein Teaching Hospital and Al-Habooby Hospital, in addition to the medical clinics which are located in Nasiriyah city during the period extended from October 2020 to April 2021. 50 cases of herpes zoster disease (above 10 years and less than 90 years old males and females) were followed up and recorded. These cases of HZ patients are included 30 males and 20 females, who showed a very characterized and distinctive clinical signs of the disease and were clinically diagnosed directly by the supervising doctors who are specialized in dermatology. In order to determined the incidence of HZ or shingles among patient according to various limitations,

special information are collected directly from the patients as organized in a special questionnaire that includes, gender, place of residence, and date of sample collection according to months of year (extended from October to April).

Collection of samples

As well as taking swabs of fresh vesicles fluid from herpes zoster infected patients from the skin lesion of the vesicles which are localized on a specific side of the body. Specimens are included the base of the vesicle and its content of clear fluid for the purpose of viral detection. The study also included 50 people without shingles disease as a control group. All the mentioned tests were performed on this group except for the swabs and samples taken to isolate the virus from the vesicles.

Viral DNA extraction

Viral DNA was extracted from vesicular fluid and base of vesicles taken from skin lesions scrap samples and these were extracted using special kit of extraction (viral nucleic acid extraction kit III by Geneaid company, USA). All steps followed in this analysis are applied according to the company's guidelines. Then, extracted DNA samples are checked for concentration and purity by using Nanodrop spectrophotometer (Avans, Taiwan), that check and measurement the purity of DNA through reading the absorbance in at (260 /280 nm).

Polymerase Chain Reaction (PCR)

PCR technique was performed on the extracted DNA samples for direct detection of varicella zoster virus (VZV) primarily based totally on amplification of both glycoprotein type B (gp B) and major capsid protein (MCP) gene in VZV from skin lesions scrap samples. This technique was done according to technique defined by Aubaid *et al* (2020) and Al-Khafaji *et al* (2017) with using two kinds of primers as presented in Table 1.

PCR master mix preparation

PCR master mix was prepared by using AccuPower PCR PreMix Kit and this master mix done according to company instructions as mentioned in Table 2.

PCR Thermocycler conditions

PCR thermocycler conditions were done by using conventional PCR thermocycler system as mentioned in Table 3.

Then, the PCR products were analyzed and viewed by agarose gel electrophoresis.

Incidence of HZV among patients

Through the information obtained from the direct questionnaire and the available laboratory tests, the

Table 1 : Primers used in polymerase chain reaction.

Primer	Sequence (5' -3')	Amplicon
gpB-F	CGTTACGTCCGTGAAATCGC	234bp
gpB-R	AATGGCCGTTCCGCTATCAT	
MCP-F	TGACAAATGCTAGCGGGTT	520bp
MCP-R	CGACGCAACGATTCGGTAAC	

Table 2 : PCR Mater mixture used in polymerase chain reaction.

PCR Master mix	Volume
DNA template	5 µl
VZV mcp gene forward primer (10pmol)	1.5 µl
VZV mcp gene reverseprimer (10pmol)	1.5 µl
PCR water	12 µl
2X Green star master mix	5 µl
Total volume	25 µl

incidence of shingles was determined based on each of the following factors: Gender, Residency, date of infection with shingles and season of the year.

Statistical analysis

The data collected from results of this study were statistically analyzed using SPSS version 23 software and the means were compared using T-test and Chi-square under the 0.05 of considered probability level, P value ($P > 0.05$).

RESULTS AND DISCUSSION

This study includes extracting DNA from vesicular fluids of 50 patients infected with shingles. All samples of extracted viral DNA were used by Nanodrop spectrophotometer for measurement the purity and concentration of the extracted DNA.

DNA samples were repeated twice to confirm PCR results. A conventional PCR protocol was used to analyze simultaneously the presence of glycoprotein type B (gpB) gene Also, the presence of major capsid protein (MCP) gene was identified by 520 bp as can seen in Fig. 1.

All the infected cases were diagnosed through the clinical signs characteristic of the herpes zoster virus, represented by skin vesicles located in specific areas on one side of the body, according to the location of the affected dermatome, and according to the conventional methods of diagnosing clinically approved herpes zoster infection or shingles. Shingles is the human herpes type 3 called herpes zoster virus by swabs taken from skin vesicles including vesicular fluid and on a molecular basis by extracting viral DNA from samples and then using the polymerase chain reaction. Where the target gene of the virus was identified and in this study, two genes were targeted, namely the glycoprotein B gene and the major capsid protein, which were targeted in the diagnosis of

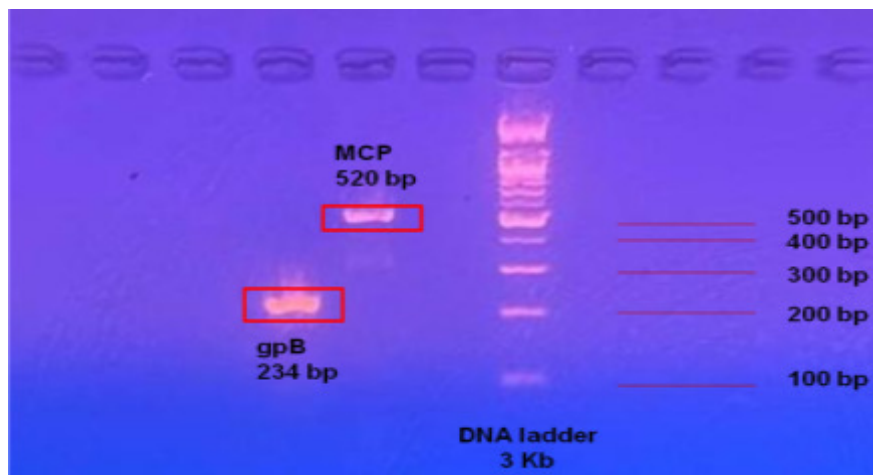


Fig. 1 : Gel electrophoresis of PCR products used a 0.8% of agarose gel. The first left lane shows the predicted PCR products contained the glycoprotein type B (gp B) gene of herpes zoster virus within a band size of 234bp. While the second left lane presents the PCR product representing the major capsid protein of herpes zoster of band size 520bp. In this agarose gel electrophoresis the DNA ladder used is of the whole size 2000bp.

pathological solutions of chicken pox in each of the study conducted by Aubaid *et al* (2020), on the first gp B gene in Al-Diwaniyah Governorate in Iraq. While the major capsid protein (MCP) was used in a similar study on the same disease, chicken pox, which was carried out in Najaf province by Al-Khafaji *et al* (2017), by using specific primers for each of the two genes separately, which were used and showed positive results identical to what appeared in those two previous studies.

Incidence of HZV among patients

According to age and gender

The results of the current study recorded that there were a highest shingles status in the first age groups for male from 21-30 years and 61-70 years of patient with percentage 12%, the second age groups from 31-40 years and 41-50 years of patient with percentage 10% and third age groups from 51-60 years of patient with percentage 8%, while the lowest shingles status in the fourth age groups 11-20 years and 71-80 years with percentage 4%.

On the other hand, the results of this study recorded that there were a highest shingles status in the first age groups for female from 51-60 years of patient with percentage 12%. While, the second age groups from 61-70 years of patient with percentage 10%, and third age groups from 41-50 years of patient with percentage 6% , and fourth age groups 11-20 years and 71-80 years of patient with percentage 4%, while the lowest shingles status in the fifth age groups 21-30 years and 31-40 years with percentage 2% (Fig. 2) showing this result. The result also showed that there is no significant statistical difference at P. value ≥ 0.05 .

Fig. 2 shows the numbers and rates of herpes zoster infection by age and gender. It is clear from this result

that there is an increase in the infection level among males in the age periods from 31 to seventy years, compared to a lower rate in females, which concentrated the highest rates of infection in the age period extending from 40 to 70 years old. In general, there were no statistically significant differences for most of the age periods between males and females.

This study demonstrates in a database exceeding 50 cases of shingles standardized excess incidence in males 30 (60%) over females 20 (40%). This is consistent with the study that was conducted by Al-Kayalii and Numman (2014). As results was obtained by confirmed that the number of males with shingles was 40 (4.9%) and the number of females 24 (3.1%). As for the rate of herpes zoster infection in males and females and its relationship to the age stages, it was according to the total number more in men compared to women in relation to the total number, where the difference appeared fundamentally in the age groups from 21-30 years and 31-40 years, as well as from 41-50 years. The infection rate was higher in men (12%, 10% and 10%) compared to women (2%, 2% and 6%). As for the age group from 51-60 years, it was more in females (12%) compared to males (8%). As for the age groups older than 61-80, they were similar for both males and females. In general, the results of the infection rate were high in the ages of 40-70 years in both women and men alike. While, the results of some studies related to determining the rates of herpes zoster infection and its relationship to the age stages of men and women, there was a noticeable increase in the rates of infection among females in most age groups compared to men, except for the age group from 15-24 years, which appeared in a superiority in the rate of infection among males compared to females, according to what was found

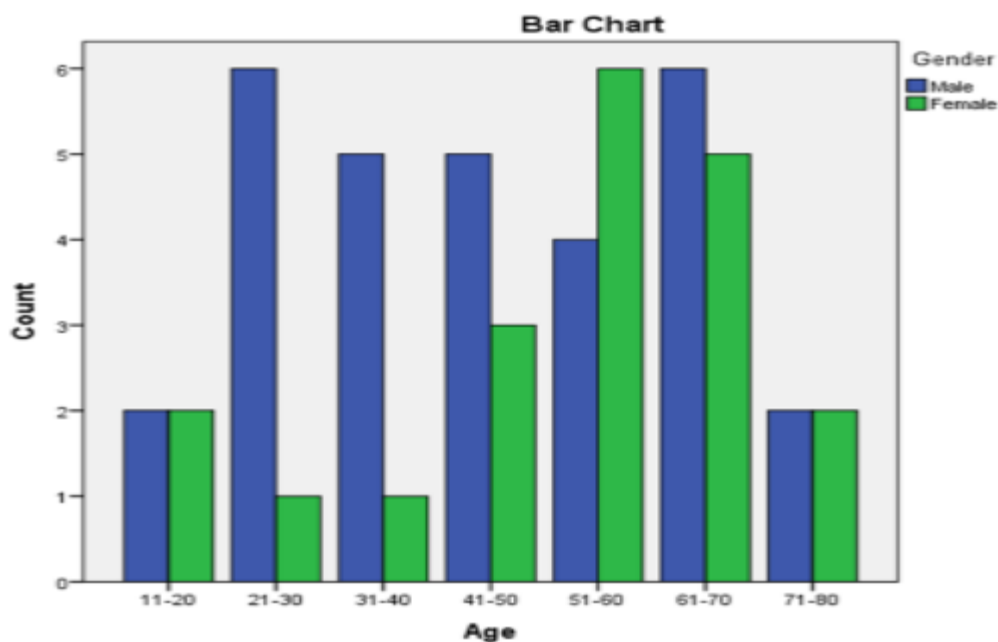


Fig. 2 : Distribution of shingles among patients according age and gender.

by Fleming *et al* (2014). In a similar study, it was found by Li *et al* (2016) that the annual cumulative incidence of shingles increases at the age of fifty years and over. These results was conducted from 2010-2012 showed that the annual incidence of viral disease was 3.43/1,000 in the same previous mentioned group of age. It was also shown through this study that the annual incidence rate rises directly with age, as the percentage increased by more than three times in the age group of eighty years (52.3/1000) and above compared to the age group less than fifty years, in which the recorded percentage of the annual rates of infection was 15.7/1,000. Also, it was found by this study that the cumulative incidence of shingles was higher among females (26.5/1000) in compared to males (18.7/1,000). It was also found that the annual rates of infection were higher in females compared to males.

When comparing male patients with female patients according to age. The results of the current study recorded that there were a highest shingles in male in age groups from 21-30 years of patients with percentage 12%. The results of the current study recorded that there were a highest shingles in female of age groups from 51-60 years of patients with percentage 12%. The current study agreed with previous study of Annual HZ incidence in Europe has been reported as 0.3–0.74/1 000 children < 10 years, 1.6/1 000 adults aged <40 years, 2.5/1 000 adults aged 20–50 years, 7.8/1 000 adults aged 60 years or over and 10/1 000 in elderly adults over 80 years of age (Volpi *et al*, 2005). The correlation between age and HZ incidence may be related to a decreased cellular-

mediated immune response to VZV as a result of immunosuppressive status of patients (Donahue *et al*, 2010 and Gaillat *et al*, 2011).

According to location of residency

The results of the current study illustrated that there were the highest of shingles in urban compared with rural, but there was no significant difference when compared with control group. While, there was a higher percentage of infection with herpes zoster among patients resident in rural areas in compared to control group at P value ≤ 0.05 (Fig. 3).

As presented in Fig. 3, there was highest of shingles in urban compared with rural, but there was no significant difference when compared with control group. While there was a higher percentage of infection with herpes zoster among patients resident in rural areas in compared to control group at P value ≤ 0.05 .

The effect of the location of residence and residence, whether in rural or urban areas and its effect on the increase or decrease in the level of herpes zoster infection, was studied. Also, statistically significant when comparing the number of patients living in rural areas and those infected with the virus and comparing them to the control group of auditors living in the same areas of the city, there was a statistically significant difference in contrast to what was found in the comparison between residents in the city who were infected with the virus and the comparison control group. According to the available references, the results of the study were consistent with the results that appeared in this study, where it was found

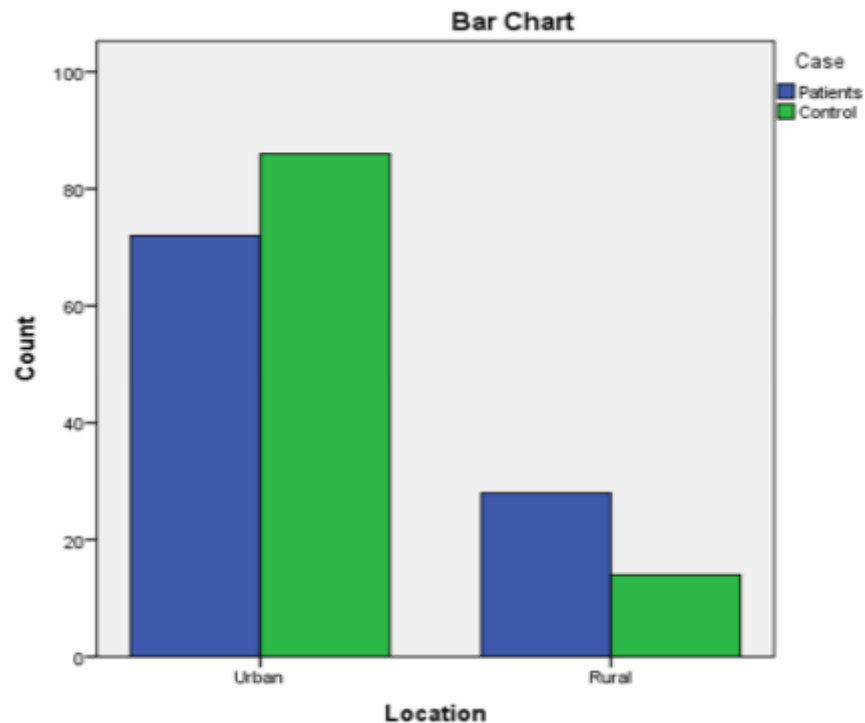


Fig. 3 : Relationship of location of residency and shingles patient and control.

Table 3 : Thermocycler conditions used in polymerase chain reaction.

PCR step	Temp.	Time	Repeat cycle
Initial Denaturation	94°C	5 min	1
Denaturation	94°C	30 sec.	30 cycle
Annealing	58°C	30 sec.	
Extension	72°C	1 min	
Final extension	72°C	5 min	1
Hold	4°C	Forever	-

Table 4 : showing the date of infection in shingles patient.

Date of infection (month)	No.	%
Oct	0	0
Nov	8	16
Dec	9	18
Jan	15	30
Feb	7	14
Mar	8	16
Apr	3	6
Total	50	100
CalX ² = 9.04	DF = 5	P.value = 0.10

by Li *et al* (2016), that the cumulative rate of shingles infection was much higher with a statistically significant difference between urban (39.5/1000) residents compared to what was recorded in rural areas (17.2/1,000).

According to date of infection with shingles

The results of the current study illustrated that the infection of shingles increase from November extended

to January that includes the highest number and percentage of infection in compared to another months of the year (Table 4). However, there was no significantly statistical difference at P value ≥ 0.05 .

According to results obtained from the current study, it was noted that the rate of shingles infection among men and women began to gradually rise from the months of coldness started from November and continued to rise to the highest infection rate in January and stabilized at the same previous level in February and March and reached the lowest Its levels were in the month of April and this confirms that the highest level of infection was in the winter and spring months. The results of the current study were in agreement with most of the sources and studies related to detecting the relationship of infection level with the herpes zoster virus and temperature, as it indicates that the infection level is higher in the winter months compared to the summer months and the reason is that the method of transmission adopted by the herpes zoster virus is through transmission In the air (airborne transmission), in addition to the direct contact way of transmission and through knowledge of the characteristics of the virus, it was found that it is negatively affected by high temperatures and high humidity as which in turn reduces the ability of the virus to transmit in these hot and humid environmental conditions, as found in through a study applied in the USA by Garnett *et al* (2009). The same results were also found in Europe (Bonanni *et al*, 2009; Katsafadou *et al*, 2008),

in South and Southeast Asia (Lee, 1998; Lolekha *et al*, 2001), in Greece (Critselis *et al*, 2012) and in China (Yang, 2015), which confirm the inverse relationship between high temperatures in the summer months and the rate of infection with the herpes zoster virus. The climatic conditions of Iraq are characterized by high temperatures and humidity in the summer season, which negatively affects the vitality and activity of the virus that causes herpes zoster. However, the obtained results from this study were differ little bit from results obtained by Jung *et al* (2015) by a certain percentage from what was found by the one who confirmed through the results of his study that the highest rate recorded for herpes zoster infection was in the summer and spring months compared to what was recorded in the winter months. It was also found by the previous study, that these results differ somewhat in women than in men, as the higher. The rates of repeated infection among women were in the month of March.

CONCLUSION

According to results obtained from this study, it concluded that:

1. The infection rate of shingles among males was higher than that of females
2. The incidence of infection gradually increases with age, especially in the elderly, in both females and males
3. The highest incidence of shingles was detected in urban areas compared to rural areas.
4. The highest number and percentage of infection was detected in cold season (December-January and February to March) in compared to another moths of the year.

Recommendations and future work

In order to reach to the typical way for improvement our public health its recommended:

1. Due to time limitations, there is a need to continue the in-depth and continuous research work on a wide segment of the population and in different governorates, and to shed light on the influencing risk factors that increase the rates of infection with the herpes zoster virus and other diseases that affect and are negatively affected by low immunity.
2. Working on guidance and counseling for citizens in full compliance with health guidelines and to prevent risk factors that increase rates of infection with the virus, especially among the immune target groups.
3. It may be useful and necessary to conduct a vaccination experiment using an honest vaccine to

know the efficiency provided by the vaccine to reduce the chances of exposure to the disease in the target groups.

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Abbreviations: HZ: Herpes Zoster; VZV: Varicella Zoster Virus; HHV-III: Human Herpes Virus type 3; Covid-19: Corona virus disease 19; HHVs: Human Herpes Viruses; DNA: Deoxyribonucleic acid; VTM: Viral Transport Medium; RPMI: Roswell Park Memorial Institute; PCR: Polymerase Chain Reaction; gpB-F: Glycoprotein type B- forward

primer; gpB-R: Glycoprotein type B- reverse primer; MCP-F: Major Capsid Protein-forward primer; MCP-R: Capsid Protein-reverse primer; bp: Base Pair; SPSS: Statistical Package for the Social Sciences.