Molecular detection of shingles among patients in Thi-Qar province

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Abstract

Herpes Zoster or shingles is still regarded as a significant public health problem worldwide distributed in different regions around the world. Accordingly, herpes zoster or shingles mainly occurred among adults with a considerable percentage of infection in all months of the year, higher in winter and spring than that detected in summer in temperate climates. For example, 10–20% of adults older than 50 years usually suffer from at least one case of zoster attack during their lifetime. Moreover, about 30% of the population are at risk of undergoing and developing shingles during their life at some points. It is estimated that 1 million adults in the United States of America (USA) are affected with shingles annually. According to 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 Thiqar Governorate, this study aimed 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 study pursued to identify the incidence of herpes zoster among patients with other skin infections. Also, to establish possible correlations between the incidence of herpes zoster infection and different factors detected, such as family history, number of family members living as a closed family, and other risk factors among male and female HZ patients compared to the control group.

There were 100 people, 50 patients, and 50 control, males and females with an age range between 10 to 80 years. All data such as age, gender, lesion site, residency, occupation, previous history of coronavirus disease (COVID-19), Blood groups were collected applying a questioner.

Results of the number of cases infected with herpes zoster showed an increase in the level of infection among males compared to females. In addition, the incidence was 30 males (60%) over 20 females (40%). Furthermore, there was a high increase (16-22%) in the incidence of infection in the age groups from 50-70 years, whether in females or males, the age groups most vulnerable to infection. The presence of skin lesions is represented by vesicles in different areas of the body. The highest incidence among patients with shingles was in the sacral region (32%), followed by the lumbar region (26%), then the cervical region (22%), and the thoracic region (16%).

Keywords: Herpes Zoster; Varicella-Zoster Virus; Human Herpes Virus type 3; COVID-19; Human Herpes Viruses

Resumen

El herpes zóster o culebrilla se considera aun un problema de salud pública con distribución mundial en diferentes regiones del mundo. Así, el herpes zoster o culebrilla se presenta principalmente en adultos con un porcentaje considerable de contagio en todos los meses del año, mayor en el invierno y primavera en comparación con el detectado en verano en climas templados. El 10 al 20% de los adultos mayores de 50 años suelen sufrir al menos un caso de ataque de zóster durante su vida. Además, alrededor del 30% de la población está en riesgo de contraer herpes zóster y desarrollar herpes zóster durante su vida en algunos momentos. Se estima que 1 millón de adultos en los Estados Unidos de América (EE. UU.) se ven afectados por el herpes zóster anualmente. Existe poca información en relación a la tasa de infección por herpes zóster entre la población de Irak en general y la gobernación de Thiqar, por ello este estudio tuvo por objetivo determinar la tasa real de infección por herpes zóster, el porcentaje de esta enfermedad, así como estudiar el impacto directo de los factores de riesgo para las personas en general y los pacientes con enfermedades virales de la piel en particular y el grado de interdependencia entre estos factores y la tasa de infección por herpes zóster. Por tanto, se propuso identificar la incidencia de herpes zóster entre pacientes con otras infecciones cutáneas, y establecer la correlación entre el nivel de incidencia de la infección por herpes zóster y los diferentes factores, como antecedentes familiares, número de miembros de la familia que viven como una familia cerrada y otros factores de riesgo entre hombres y mujeres HZ pacientes en comparación con los grupos de control.

Un total de 100 personas, 50 pacientes y 50 de control, hombres y mujeres con un rango de edad de más de 10 a 80 años, luego todos los datos del cuestionario (edad, sexo, lugar de la lesión, residencia, ocupación, antecedentes de enfermedad por coronavirus 19 (COVID-19), grupos sanguíneos, etc.).

Los resultados del número de casos infectados con herpes zoster mostraron un aumento en el nivel de infección entre los hombres en comparación con las mujeres. Además, la incidencia fue de 30 hombres (60%) sobre 20 mujeres (40%). En cuanto a la tasa de infección por herpes zóster en hombres y mujeres y su relación con las etapas de edad, fue mayor en hombres que en mujeres en relación al número total. Además, hubo un alto aumento (16-22%) en la incidencia de infección en los grupos de edad de 50 a 70 años, ya sea en mujeres o en hombres, que son los grupos de edad más vulnerables a la infección. La presencia de lesiones cutáneas estuvo representada por vesículas en diferentes áreas del cuerpo. La mayor incidencia entre los pacientes con herpes zóster fue en la región sacra (32%), seguida de la región lumbar (26%), luego la región cervical (22%) y la región torácica (16%). En cuanto a la relación laboral y su naturaleza con el nivel de infección por herpes zóster entre los diferentes grupos.

Palabras clave: herpes zoster; virus de la varicela zoster; virus del herpes humano tipo 3; COVID-19; virus del herpes humano

Introduction

Herpes zoster (HZ) infection or Shingles is a natural viral skin disease because 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 one of the human herpesviruses (HHVs, it is officially known as human herpesvirus type 3 (HHV-III), which is recognized as a member of the DNA-containing Herpesviridae family[1-3]. 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 considered a secondary skin viral infection resulting after the reactivation of a latent VZV[2-5]. Herpes zoster (HZ) is a viral skin disease characterized by severe, sharp, and lancinating radicular pain accompanied by vesicular rash or eruption on the erythematous base localized in one or more dermatomes next to the affected nerve. Other symptoms are mainly recognized in herpes zoster-affected patients represented by itching and unpleasant sensations or what are called (dysesthesias) that are usually produced by touch (allodynia) occur[6,7]. All these symptoms are gradually developed within a few days and maybe extended to a few weeks with specific vesicular lesions distributed on one side of the body in the corresponding dermatomes, mainly in the thoracic region with a high probability and in the ophthalmic region, cervical and lumbosacral regions[3,8,9]. As herpes zoster infection or shingles is considered a recurrent skin viral disease caused by the varicella-zoster virus (VZV), which mainly infects children younger than ten years of age with herpes zoster infection or chickenpox. A latent HZV then retrogrades from its latency in the dorsal or cranial root of the ganglion and is transmitted through peripheral nerves into a specific dermatome on one side of the body during the youth or adulthood period. This recurrent viral infection occurs because of various factors, such as immunosuppressant agents like old age, autoimmune diseases, stress factors, and other essential factors. In addition, HZV or chickenpox is considered a common epidemic disease of childhood and high prevalence of Varicella-zoster infection around the world[10-12]. VZV is still regarded as an enormous public health problem with worldwide distribution in different regions around the world[3,4,5].

Accordingly, herpes zoster or shingles mainly occurred among adults with a considerable percentage of infection in all months of the year with a higher rate of infec-

tion in winter and spring than that detected in summer in temperate climates. For example, 10–20% of adults older than 50 years usually suffer from at least one case of zoster attack during their lifetime. Moreover, about 30% of individuals are supposed to undergo HZ in a specific period of their lives[6,7,13]. According to 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 Thiqar Governorate, this study aimed 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[8,14,15]. Therefore, this study pursued to identify the incidence of herpes zoster among patients with other skin infections, as well as to study the direct impact of risk factors for people in general and patients with viral skin diseases and the extent of interdependence between these factors and the rate of herpes zoster infection[11,16,17]. Therefore, we studied the incidence of herpes zoster among patients with other skin infections and the possible correlations between the level of incidence of herpes zoster infection and risk factors that are detected, such as gender, age groups, and blood group, and other risk factors among male and female HZ patients compared to the control group. And also, we evaluated the association between HZ infection and these different risk factors.

Methods

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 was distributed in different areas according to the site of the dermatome, as well as by smears taken from these vesicles for all patients identified by a typical laboratory method[10-14]. In addition, these vesicles fluids samples were used to extract the DNA of the herpes zoster virus using the viral DNA extraction kit, which allowed to detect of the presence of the extracted DNA through gel electrophoresis and 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). Also, serum samples were taken for all patients with herpes zoster to determine glucose levels, lipid profile, cholesterol levels, triglycerides, low and high concentration lipoproteins. The results were compared with the control group.

Data collection from patients

This study is primarily targeted and carried out on patients suffering from dermatological diseases referred to a dermatologist. Among the many dermatology consultants and dermatology outpatient clinics in hospitals, such as Al-Hussein Teaching Hospital and Al-Habooby Hospital, in addition to the medical clinics which are in Nasiriyah city, during the period extended from October 2020 to April 2021. 50 cases of herpes zoster disease, aged between 10 and 90 years old, males and females, were followed up and recorded. These cases of HZ patients have included 30 males and 20 females, who showed very characterized and distinctive clinical signs of the disease and were clinically diagnosed directly by the supervising doctors specializing in dermatology. To determine the incidence of HZ or shingles among patients according to various limitations, particular information was collected directly from the patients by applying a special questionnaire that includes age, gender, place of residence, number of family members, any knowledge of the previous history of infection in the same person or his family with chickenpox and shingles, and whether the patient had COVID-19 or not. Moreover, some information is recorded from patients, such as the site of the lesion.

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, the locations of the pathological lesion were also determined whether they were cranial, thoracic, lumbar, sacral and at the foot and the number found within the studied samples was calculated according to its percentage among HZV infected patients. Samples or specimens are included in the base of the vesicle and its content of clear fluid for viral detection. The lesion was scraped and lifted using a sterile single-use blade, in addition to collecting the vesicular fluid using special cotton swabs (Texwipe's Absorbond® Swab) made of non-woven polyester. They were placed in sterile laboratory tubes containing the viral transport medium (VTM) containing viral media (Roswell Park Memorial Institute: RPMI). Then the samples were frozen at -20°C. 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 to isolate the virus from the vesicles.

Viral DNA Extraction

Viral DNA was extracted from vesicular fluid and the base of vesicles taken from skin lesions scrap samples. These were extracted using a 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, the extracted DNA samples were then assessed for concentration and purity using a Nanodrop spectrophotometer (Avans,Taiwan), at a absorbance of 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 the technique defined by Aubaid et al.[8] and Al-Khafaji et al.[9] using two kinds of primers as presented in Table 1.

Table 1. Primers used in polymerase chain reaction

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

PCR master mix preparation

PCR master mix was prepared using (AccuPower PCR Pre-Mix Kit), and this master mix was done according to company instructions as mentioned in Table 2.

Table 2. PCR Mater mixture used in a 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)	1.5µl
PCR water	12 µl
2X Green star master mix	5µl
Total volume	25µl

PCR Thermocycler Conditions

PCR thermocycler conditions were done by using a conventional PCR thermocycler system as mentioned in the following table (Table 3).

Table 3. Thermocycler conditions used in a polymerase chain reaction

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

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 incidence of shingles was determined based on the following factors: age and gender and site of the lesion.

Statistical Analysis

The data collected from the results of this study were statistically analyzed using SPSS version 23 software, and the means were compared using T-test and Chi-square. A value of $p<0.05$ was considered significant.

Results

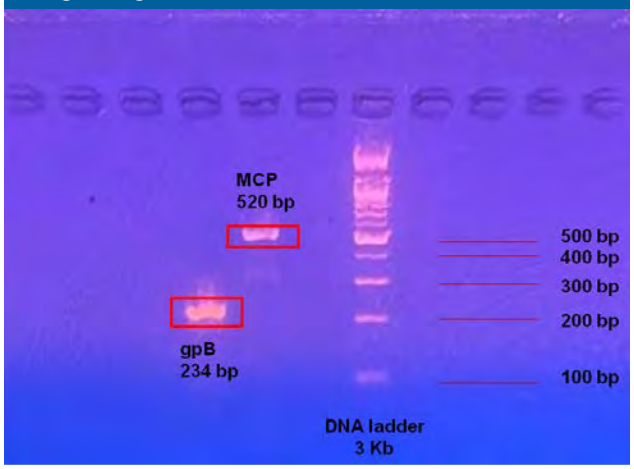
This study includes extracting DNA from vesicular fluids of 50 patients infected with shingles. All samples of extracted viral DNA were used by Nanodrop spectrophotometer for measurement the purity and concentration of the extracted DNA, and the result was as follows:

Table 4. Showing the purity of DNA

Sample Ty	Con(ng/µl)	260/280	Abs280	Abs 260
dsDNA	54	1.78	0.60	1.08

DNA Samples were repeated twice to confirm PCR results. A conventional PCR protocol was used to analyze simultaneously the presence of gpB gene. Also, the presence of the gpB gene was identified by 234 bp, as can be seen in figure 1.

Figure 1. Gel electrophoresis of PCR products used 0.8% of agarose gel.



The first left lane shows the predicted PCR products contained the glycoprotein type B (gp B) gene of the herpes zoster virus within a band size of 234bp. At the same time, 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 full size 2000bp.

All the infected cases were diagnosed through the clinical sign's 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 molecularly 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

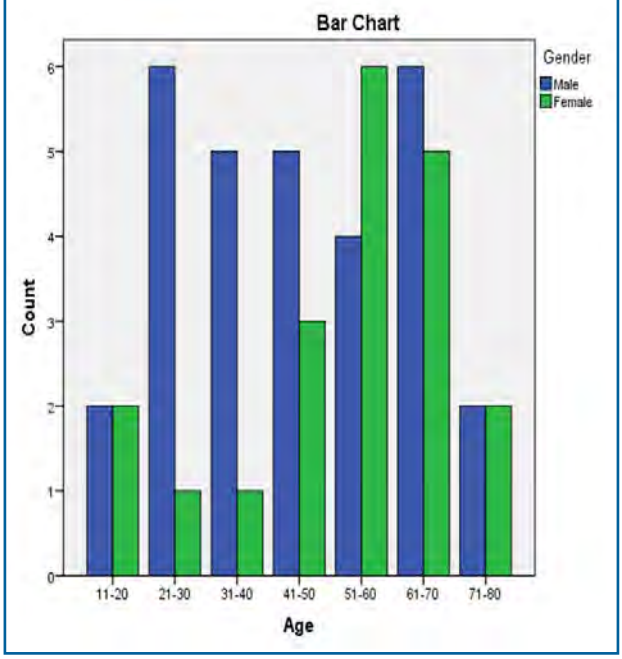
glycoprotein B gene and the major capsid protein, which were targeted in the diagnosis of pathological solutions of chickenpox in each of the studies conducted by Aubaid et al., 2020, on the first gp B gene in Al-Diwaniyah Governorate in Iraq. In contrast, the major capsid protein (MCP) was used in a similar study on the same disease, chickenpox, 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 indicate the highest shingles status in males from 21-30 and 61-70 years of the patient with a percentage of 12%, the second age groups from 31-40 and 41-50 years of the patient with a percentage of 10%, and third age groups from 51-60 years of the patient with a percentage of 8%, while the lowest shingles status in the fourth age groups 11-20 years and 71-80 years with a rate of 4%.

Concerning females, the highest shingles status was at 51-60 years of the patient with a percentage of 12%. While the second age groups from 61-70 years of the patient with a percentage of 10%, and third age groups from 41-50 years of the patient with a percentage of 6%, and fourth age groups 11-20 and 71-80 years of the patient with a percentage of 4%, while the lowest shingles status in the fifth age groups 21-30 years and 31-40 years with percentage 2% (figure 2).

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



This figure shows the numbers and rates of herpes zoster infection by age and gender. It is clear from this result that there is an overall increase in the infection level among males in the age periods from 31 to 70 years, compared to a lower rate in females, which concentrated the highest rates of infection in the age period extending from 41 to 70 years old.

In similar studies[5,8], it was found) that the annual cumulative incidence of shingles increases at the age of fifty years and over. These results were conducted from 2010-2012 showed that the yearly incidence of viral disease was 3.43/1,000 in the same previously 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) compared to males (18.7/1,000). It was also found that the annual rates of infection were higher in females compared to males.

The current study agreed with the 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[11]. The correlation between age and HZ incidence may be related to a decreased cellular-mediated immune response to VZV because of the immuno-suppressive status of patients (Donahue et al., 1995; Gaillat et al., 2011).

According to the site of the lesion

The results of the current study illustrated that there was the highest shingles level of incidence of the vesicular lesion (Figure 3) in the sacral area in the body with a percentage of 32% (1), lumbar 26% (2), and cervical 22% (figure 3.3). While the thoracic region (16%) (figure 3.4) and the lowest status of shingles in the foot with a percentage of 2%. The result also showed that there was a significant statistical difference at P ≤0.05.

Table 5. Distribution of site of lesion among patients with shingle

Site of lesion	No.	%
Cranial	0	0
Cervical	11	22
Thoracic	8	16
Lumbar	13	26
Sacral	16	32
Foot	2	4
Total	50	100
CalX^2=22.80	DF=4	P.value=0.001

Figure 3. Types of vesicles according to the site of lesion

This figure shows samples of skin vesicle samples found in people infected with viral herpes, according to their location in the body and the proportions shown in the above results. These represent each sacral area (1), lumbar (2), cervical 22% (3), and the thoracic region (16%) (4).

Regarding the appearance of the skin lesion represented by the vesicle's characteristic of infection with the herpes zoster virus, it appeared in all areas of the dermatomes on one side of the body, except for the cranial area, where no distinct pathology appeared in this area. The highest percentage of vesicles appeared in the sacral region (32%) and after this in the lumbar region (26%) and then the cervical region by (22%). As for the thoracic region, the percentage was 16%, and the lowest was in the foot region, which was 4%. Many references specialized in herpes zoster infection confirm that there is no significant difference in the occurrence of the pathological lesion, which is the skin vesicles, according to different areas, whether in men or women, in the proportion of its appearance on the right or left side. But, a few references prove that the rate of appearance of the skin lesion is in the thoracic region compared to the rest of the body, as confirmed by Jung, Kang, and Yoo, 2015.

Conclusions

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 shingles level of incidence according to the site of vesicular lesion was in the sacral area, then lumbar, cervical 22%, and the thoracic region.

Recommendations and future work

To reach to the typical way for improving 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 vast portion 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 preventing risk factors that increase infection rates with the virus, especially among the immune target groups.

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Abbreviations: HZ: Herpes Zoster; VZV: Varicella Zoster Virus; HHV-III: Human Herpes Virus type 3; COVID-19: Coronavirus 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.

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