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DETECTION OF VARICELLA-ZOSTER VIRUS AMONG ADULT PATIENTS SUFFERING FROM SKIN LESION IN HILLA PROVINCE, IRAQ

Jwan Ahmed Ali Ahmed

Department of Microbiology, College of Medicine, University of Babylon, Iraq.
e-mail: jwanalhamawandi@gmail.com

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ABSTRACT : In this study, the virus was identified on 150 specimens by using the varicella-zoster virus Card test, the results revealed that 62(41.3%) specimen were related to virus infection, the sera of patients were showed anti-IgM to varicella-zoster virus antibodies. The results showed that, out of 62 positive viruses, the IFA was detected 56(90.3%) positive for the varicella-zoster virus. DNA was extracted from all suspected isolates that were previously identified as Varicella-Zoster Virus genes, These DNA samples were used to perform PCR for the amplification of specific VZV primers based on the sequences. After that, gel electrophoresis revealed that 52 (90.8 percent) of the 56 samples of Varicella-Zoster Virus detected by IMF developed a 282 bp DNA fragment when particularly in comparison to the allelic ladder. The human cytokines enzyme-linked immunosorbent assay was used in this study for the quantitative determination of IL-6, IL-8 and IL-10 concentrations adult patient's serum samples, the results showed that all 52(100%) serum samples of clinical isolates were detected varicella-zoster virus by PCR technique were found these cytokines.

Key words : Virus, Indirect immunofluorescence antibody, skin lesions, PCR, varicella-zoster.

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INTRODUCTION

Varicella-zoster (VZV) is one of 9 herpes viruses widely referred to as human alphaherpesvirus 3 (HHV 3) (Markus *et al*, 2017). It triggers chickenpox (varicella) in infants, teenagers and young adults, along with shingles (herpes zoster) in adults; shingles in children are uncommon (Gould, 2014). VZV is a human disease that occurs in children such as varicella or chickenpox and then reappears as zosters or shingles (Jarosinski *et al*, 2017). The virus has well suited its human host and infects much of the population. The varicella-zoster virus (VZV) is responsible for chickenpox and herpes zoster (Freer and Pistello, 2018). Chickenpox is a mild, self-limiting childhood disease with a distinctive exanthema that can spread in immunocompromised children (Drago *et al*, 2017). Latent virus reactivation causes the herpes zoster with a dermatomal rash., which is frequently accompanied by pain in the rash's distribution (postherpetic neuralgia) (Parkar *et al*, 2020). VZV induces vesicular dermal lesions that manifest clinically as varicella (primary infection) or zoster (reactivated) diseases (Kennedy and Gershon, 2018). Following reactivation from the dorsal-root ganglia, the varicella-zoster virus (VZV) replicates and ensures full enveloped viruses in the skin, resulting in herpes zoster rash (shingles) (Zhou *et al*, 2020). Varicella (chickenpox) is a contagious disease with a short incubation period. VZV remains latent in the body after the primary infection (in the sensory nerve ganglia) (Gershon and Gershon, 2018). Varicella is caused by a primary VZV infection (Drago *et al*, 2017). Fever and a self-limiting rash on the skin and sometimes the mucosa are symptoms of varicella. There are also headaches, malaise, and a lack of appetite (Preety *et al*, 2020). The rash starts as macules, then progresses to papules, vesicles, and crusting of the lesions. After 1 to 2 weeks, the crusts slough off (Padlipsky and Young, 2018). Skin inflammation, benign brain ataxia, adult encephalitis, and pneumonitis are complications (Ivanovich *et al*, 2019). The risk of serious diseases is always for immunocompromised people, neonates, adults and pregnant women (Hanaliolu *et al*, 2018). Because of the possible exposure to viremia before the production of maternal antibodies, neonates are at greater risk if primary maternal infection occurs four days before to 48 hours after birth (Schwartz *et al*, 2020).

Aim of the study : The objects of this learning to detection of Varicella-zoster viruses in adult patients by indirect immunofluorescence antibody technique and PCR technique, and Identification of some cytokines by Enzyme-Linked Immunosorbent Assay (ELAZA).

MATERIALS AND METHODS

Patients and clinical specimens

The patients in the sample ranged in age from 60 to 75 years old. Between August 2020 and January 2021, patients with skin lesions were seen at Hilla's Allergy and Dermatology Health Center and Clinical Private.

Ethical approval

The required ethical consent from the patients and their supporters' ethical committees must be obtained. Furthermore, before the collection of samples, all participants involved in this research are notified and allowed to provide the required consent for conducting the experiments and publishing the results.

Blood samples

Every patient with a skin lesion had 5ml of blood pulled from a vein, steadily pushed into disposable test tubes with separating gel, after 30 minutes of clotting at room temperature, the sample was centrifuged at 3000g for 3 minutes then sera were securely stored at (-20^{0}C) before they could be analyzed.

Dermal swabs

Skin lesion samples were collected from each patient by 2 to 3 swabs, and placed in a container with a transport medium (Cary–Blair) and taken to the laboratory for DNA extraction for detection virus by direct PCR technique.

Identification of viruses

Viruses were identified using the Varicella-Zoster Card test, approximately 100 mg of skin lesion specimen was transferred by a stick into the skin collection tube with diluent samples, after that, the tube was shacked to ensure proper sample dispersion. After that, four drops of the kit's solution were inserted in the circular window outlined with an arrow, and the effects were read by looking at the coloring bands after 10 minutes:

Negative: Only one green band existed around the central window in the site denoted by the letter C (control line).

Varicella-zoster virus positive : A red band varicella-zoster test line) emerged in addition to the green control band in the site denoted by the letter T.

Detection of viruses by indirect immunofluorescence antibody technique

Indirect immunofluorescent assay (IFA) Kit for detecting IgM antibodies to the major etiological agents of gastrointestinal infectious diseases in human serum. The IFA approach was based on an antibody reaction in the sample that was evaluated with antigen adsorbed on the slide surface. The antigen reacts with the particular antibodies in the sample and the immunoglobulins that are not bound to the antigen are extracted during the washing process. The antigen-antibody complexes then react with fluorescein-labeled antihuman globulin in the next step. An immunofluorescence microscope should be used to study it.

Table 1 : The primers, sequences, and PCR conditions.

Gene name	Primer sequence (5´- 3´)	Size of Bp	Conditions	References
VZV gene	5' -GACAATATCATATACATGGAATGTG-3 5' -GCGGTAGTAACAGAGAAT TTC TT-3	**282**	Step 1: 95ºC, 2 min. Step 2: 95ºC, 30 sec. Step 3: 56.3ºC, 30 sec. Step 4: 72ºC, 30.0 sec. Step 5: Repeat steps 2-4 29 more times Step 6: 72ºC, 5 min. Step 7: 4ºC, forever	Levin *et al* (2018)

DNA extraction of a skin lesion by specific primers for detection Varicella-zoster virus

The skin lesion's genomic DNA was done by a Genomic DNA purification kit and by manufacturer's instructions (Geneaid, USA). The graphic was shown using a UV-trans illuminator. The sequences of primer and conditions of PCR used in the analysis are shown in Table 1.

Identification of IL-6, IL-8 and IL-10 by Enzyme-Linked Immunosorbent assay

The human IL-10, IL-8 and IL-6 ELISA kits (Elabscience, China) were used in this study for the quantitative determination of cytokine concentrations in adult patient serum samples.

RESULTS AND DISCUSSION

Identification of virus by using varicella-zoster virus card test and immunofluorescence antibody technique

The virus was identified on 150 specimens in this study using the varicella-zoster virus Card test and the results revealed that 62 (41.3%) specimens were related to virus infection, with patient sera demonstrating anti-IgM to varicella-zoster virus antibodies. The results showed that, out of 62 positive viruses, the IFA was detected 56 (90.3%) positive for varicella-zoster virus as shown in Table 2, Fig. 1. These data are compatible with those of Wang *et al* (2018), who detected IgM antibodies in 98.2% of herpes zoster cases. Indirect-type antibody assays, in which antigen bound to a solid phase is treated with the test serum first, followed by a marked reagent directed against human immunoglobulin are usually adequate for detecting IgG antibody but are not optimal for detecting IgM because the results can be mistaken by IgG preferential binding or falsely positive by IgM with anti-IgG action (Zunino, 2017; Slater, 2019). The antibody-capture approach largely removes these issues and has the added advantage of calculating the antibody amount, which is proportional to the specific ratio of total IgM, by evaluating a single dilution of serum (Richardson and Page, 2018). In comparison to indirect-type studies, using a negative control antigen is not needed. The immunofluorescence (IF) technique is commonly used for detecting virus infections in clinical specimens by recognizing virus antigens (Dilnessa and Zeleke, 2017). IF staining is typically thought to be very quick (about 1 to 2 hours) and provides responsive and precise viral identification (Ali *et al*, 2020). Herpes zoster (HZ) is caused by the reactivation of latent VZV, which has a 50% lifetime probability of reactivation. HZ causes a vesicular rash that is local and unpleasant, affecting one or more adjacent dermatomes, most typically one or more nearby unilateral thoracic dermatomes (Hayward *et al*, 2019). In immunocompromised hosts, the rash can affect several non-contiguous dermatomes or even the central nervous and pulmonary systems (Nagel *et al*, 2017). A one-to four-day prodrome of pain and paraesthesia in the affected dermatome (s) characterizes HZ (Dofalcon, 2017). Children under the age of 12 are rarely affected by HZ, and the vast majority of cases affect individuals above the age of 40. Herpes zoster becomes more prevalent as people get older, and it is more prevalent in people who are immunocompromised (Chen *et al*, 2017). Serological testing is essential to determine an individual's immunity to VZV (Zhang *et al*, 2020). Serum VZV-specific IgM antibodies indicate recent infection, but due to biological false positives, non-specific reactivity due to other infectious or non-infectious causes, recurrence of IgM during zoster reactivation, and/or protracted identification following recent infection, this alone is not diagnostic of acute varicella infection (Liu *et al*, 2021). Antibodies develop within a few days of infection and to a lesser degree, upon further exposure. Because of the high predictive ability of a varicella record and the relatively high frequency of immunity in those who do not have one, immunity to varicella is an unusual sign for laboratory testing (Huang *et al*, 2020). As the varicella vaccine replaces naturally acquired immunity, this can improve the overall immunity. Because the vaccine provides such a high level of immunity – after two doses of vodka vaccine, 95% of adults seroconvert using high-quality testing–post-immunization serological monitoring for immunity is not commonly recommended (Fishman, 2017). Furthermore, clinical VZV IgG assays are less reliable than those used in vaccine trials, and undetectable IgG after adequate immunization in an immunocompetent person does not always imply vaccine failure (Smith-Norowitz *et al*, 2018). Immunity monitoring is most relevant in high-risk patients, such as pregnant women and the immunocompromised (to aid in healing after an illness occurs), as well as healthcare workers of unknown immune status (Cagol *et al*, 2020). The indirect-immunofluorescence assay (IIFA), which uses a fluorescent-labeled anti-immunoglobulin to detect bound antibodies, is a less common method for detecting VZV antibodies (Ayhan and Charrel, 2020). The antibody titer is measured by determining the maximum dilution of a serum that enables direct measurement of fluorescence, and the stained VZV-infected cells are studied utilizing fluorescent microscopy (Mohammadi *et al*, 2020). Checking for VZV IgG avidity could help to determine if a rash caused by VZV infection is the result of a primary infection or perhaps the reactivation of a latent virus (Jones *et al*, 2019). Individuals that have been infected already have higher affinity IgG antibodies attached to the antigen than people who have recently been infected.

Table 2 : Identification of viruses by using cer test Varicella-Zoster virus and immunofluorescence antibody technique.

No. of samples	cer test varicella-zoster virus	%	Immunofluorescence antibody technique	%
150	62	41.3	56	90.3

PCR-based detection of varicella-zoster virus genes

The PCR technique is focused on the capacity of DNA polymerase to generate a new strand of DNA that is complementary to the provided template strand and at

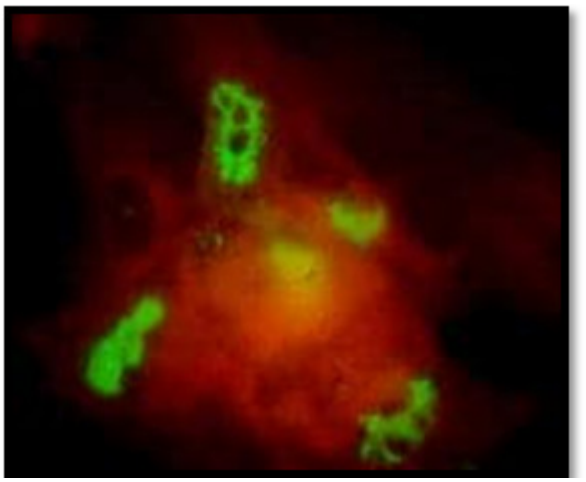

Fig. 1 : The indirect immunofluorescence antibody technique was used to identify Varicella-Zoster Virus.

quantitative identification of VZV, gene-specific primers and FRET (fluorescent resonance energy transfer) labeled probes, or a dsDNA intercalating fluorescent dye followed by melt curve examination, may be used. ORF 29, 62, glycoprotein 19 and the polymerase gene are all potential PCR targets (Yip *et al*, 2019).

Enzyme-Linked Immunosorbent Assay for IL-6, IL-8 and IL-10 detection

The human cytokines enzyme-linked immunosorbent assay was used in this study to determine the quantitative concentrations of IL-6, IL-8 and IL-10 in adult patients' serum samples. The results showed that all 52 (100%) serum samples of clinical isolates were detected varicella-zoster virus by PCR technique, were found these

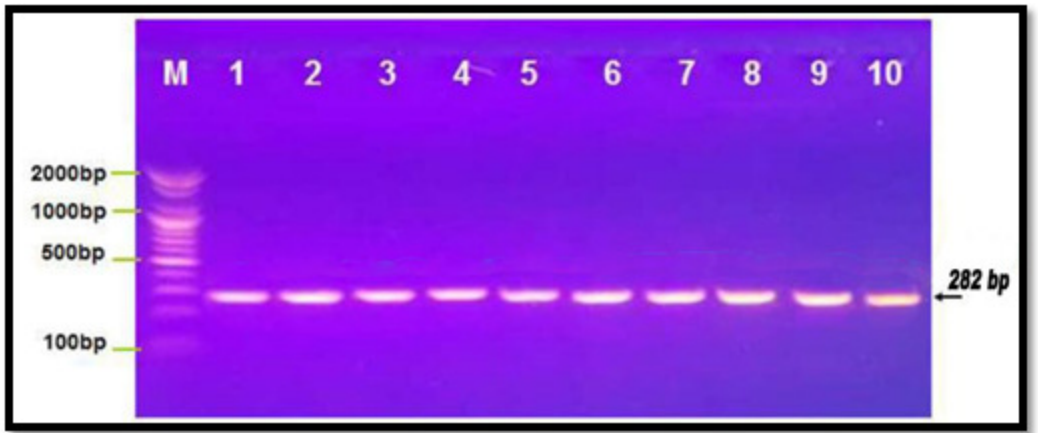

Fig. 2 : RCR amplified of VZV gene (282)bp of this gene was separated by electrophoresis on a 1.5 percent agarose gel for 55 minutes at 70 volts. M: ladder (DNA marker), (1, 2, 3, 4, 5, 6, 7, 8, 9, 10) Amplify the VZV gene in varicella-zoster virus clinical isolates.

the end of the PCR reaction, billions of copies of the same sequence are accumulated (Amplicon). DNA was extracted from all suspected isolates that had previously been identified as containing Varicella-Zoster Virus genes, and PCR was performed using these DNA samples for the amplification of specific VZV primers, according to the sequences and program mentioned in Table 1. Following that, gel electrophoresis revealed that, of the 56 samples of Varicella-Zoster Virus detected by IMF, 52 (90.8 percent) VZV developed a 282 bp DNA fragment when compared to an allelic ladder as shown in Fig. 2. Minkus et al. (2019) discovered that PCR allows for the effective and highly sensitive detection of VZV from clinical specimens in the clinical laboratory. Sequence-specific probes and intercalating dsDNA fluorescent dyes are two other PCR approaches for the amplification and identification of VZV gene targets (Becherer *et al*, 2020). There are manual and automatic platforms available that combine nucleic acid extraction, amplification, and detection into a single instrument (Dunbar and Das, 2019). For both qualitative and

cytokines as a result of Zou *et al* (2018), Bouquet *et al*, (2020); StAn inflammatory response characterized by fever and cytokine development is associated with acute VZV infection (Shaw and Su, 2021). TLR2 is expressed by human monocytes infected with VZV during acute infection. VZV-infected cells were introduced to human monocytes. The introduction of VZV-infected HELF resulted in a dose-dependent increase in the cytokines IL-6 and IL-8, while the addition of control uninfected HELF resulted in a selective increase in IL-6 and IL-8 (Gerada *et al*, 2020). Since, VZV latency is maintained by VZV-specific cell-mediated immune responses, immunocompromised and elderly people are at risk of reactivation and associated neurological disorders (Kennedy and Mogensen, 2020). The cytokines that control VZV in neurons in the immune system, on the other hand are largely unknown (Laing *et al*, 2018). Researchers tested the capacity of interleukin-6, tumor necrosis factor-alpha and type 1 interferon to suppress VZV replication in human neurons in vitro to better understand how the immune system can limit VZV

replication in neurons (Como *et al*, 2018). Interleukin-6, type 1 interferon, and tumor necrosis factor-alpha all greatly decreased VZV transcription and viral propagation. Researchers may be able to help understand how the innate immune system controls viral replication in living neurons as a result of these findings (Bubak *et al*, 2018).

CONCLUSION

Virus isolation using a card test, immunofluorescence-based assays and nucleic acid analysis using molecular techniques have all been useful in detecting and recognizing viruses. The IFA test for IgM antibody was found to be a reliable method of detecting IgM responses in the sera of patients with viral infections. IL-8 and IL-10 are immunomodulatory cytokines that are present in both healthy and diseased tissues.

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