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Demographic Study of Varicella Zoster Virus in Cerebrospinal Fluid of Stroke Patients in Thi-Qar Province

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***Abstract*- Varicella Zoster Virus (VZV), also known as alpha herpesvirus Virus 3, belongs to the Alpha Herpesviridae family and is a unique virus that can reactivate neurons and root ganglia in the nervous system and causes many neurological diseases, especially stroke. Molecular and genetic studies of viruses are valuable tools for virus development and identifying viral treatments to combat the disease. The study aims to demonstrate the causal roles of Varicella-zoster virus in the progression of stroke by detecting the presence of virus DNA in the patients CSF by real-time PCR technique. The study aimed to identify the relationship between VZV infection and demographic characteristics.**

The current study involved 120 participants collected in Al-Husein Teaching Hospital, of which 90 patients with systemic manifestations of the central nervous system (CNS) with/without rash and 30 participants as a control group, the age of population ranging from 1 to70 years among them 73 males and 47 females VZV DNA was detected using Quantitative real-time PCR (ORF4) from CSF.

The results recorded 47 (39.17%) of both patients and control groups were positive for VZV, among them 34 (37.78%) in patients group and 13 (43.33%) cases in the control group. According to sex of patients, the females have the highest infection percentage. Also, noted that 52.78% of infection in the female group and 47.22% in the male group, regarding age groups, the sixth age group it was scored the high percentage of infection 66.67%, while the first age group scored a low infection percentage 7.69%. According to Thi-Qar distracts, the study investigated the high infection percentage in Gharraf 75%, and the lowest in Nasiriyah distract 13.64%.

Keywords: **Varicella, Stroke, Cerebrospinal Fluids, Chicken Pox, Shingles .**

I. INTRODUCTION

Stroke is a serious public health concern globally, which leads to mortality and severe permanent disabilities. Strokes are the world's second biggest cause of mortality, accounting for 5.5 million deaths annually [1]. The majority (85%) of strokes are ischemic, produced mostly by small vessel arteriolosclerosis, cardio embolism and big artery another thromboembolism. Intracerebral hemorrhage, which can be deep (basal ganglia, brainstem), cerebellar, or lobar, causes around 15% of all strokes globally [2] Virology infection is one of the severe risk factors of stroke; reactivation of the (VZV) causes a unilaterally dispersed vesicular rash that can lead to a variety of problems. VZV not only causes neurological disorders including postherpetic neuralgia and ocular zoster, but it also produces inflammatory vasculopathy, which raises the risk of hemorrhagic or ischemic consequences [3]. An infection with the (VZV) may result in vascular inflammatory alterations that increase the risk of stroke [4]. The virus that replicates in the arteries of the human brain is the VZV virus, which is a member of the Alpha Herpesviridae family within the Herpesviridae subfamily [5].

After VZV infection, the risk of stroke rises. Post-infection vascular inflammatory alterations are common in the middle cerebral artery and its branches, with most patients having a better prognosis and less frequent chronic progression [4] Recent research has found that even low childhood illnesses relate to an elevated risk of AIS. Following herpesvirus infections, post-infectious inflammatory processes can produce focal cerebral arteriopathy (FCA), one of the most prevalent causes of AIS in previously healthy children [6].

Epidemiological research with diverse study designs and heterogeneous populations has revealed that HZ is related to an elevated risk of stroke, MI, and TIA and that the relationship appears to be significant between HZ and stroke [7]. VZV–is a pathogenic human herpes virus that causes varicella (chicken pox) as a primary infection [8]. VZV, which was latent in the cranial nerve or dorsal root ganglia, reactivates to cause the viral shingle [9].

The neurotropic human Alpha herpesvirus (VZV) is endemic to every country worldwide. It creates a permanent latency in neurons after initial infection and periodically reactivates to cause a range of moderate to severe illnesses [10]. It continues to be acknowledged as a significant worldwide public health concern that impacts individuals across several geographic regions. Typically, 10–20% of

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people over 50 have experienced at least one zoster episode in their entire life [11].

Reactivation of the VZV has been associated with a higher risk of stroke [12]. The virus replicates and causes illness in the arteries of the human brain is the VZV virus, which is a member of the Alpha herpesvirinae family within the Herpesviridae subfamily [5].

Several studies were conducted on the varicella Zoster virus in Iraq, two in Thi-Qar province, Rashad, [9] and Nasser et al. [11] Other one in Najaf Governorate [13], one of them in the city of Al-Hilla [14] and the last one in the city of Basra in southern Iraq [15] The study is aimed to demonstrate the causal roles of Varicella-zoster virus in progress of stroke by detecting the presence of virus DNA in patient's CSF by real-time PCR technique and identifying the relationship between VZV infection and demographic characteristics.

II. Materials and Methods

A- Sample Collection

A total of 90 patients with and without symptoms were included in this study their age between 1 to 70 years. Samples were obtained from Al-Nasiriyah Teaching Hospital and private clinical from–September 2023 to January 2024. As a control group, the study included 30 healthy people, from each patient 3 mL of cerebrospinal fluids were collected under sterile conditions and put in an EDTA tube. During transportation we used ice box for specimens transport. The samples were frozen at -80 ℃ and dissolved at room temperature to complete melting when used for viral detection.

B- Nucleic Acid Extraction

From 140 μL of clinical specimen extracted, 60 μL material was obtained by (FAVORGEN-TIWAN). According to the manufacturer's instructions. Both real-time PCR (rt-PCR) and melting curve analysis were performed with identical nucleic acid.

C- *Real-Time PCR and Melting Curve Analysis*

Table 1 provides the gene targets as well as the primer used for amplification. Light Cycler probe design software 2.0 (Roche, Penzberg, Germany) was used to build all primers except two for internal control. 20 μL of the reaction mixture , 3.0 μL of isolated nucleic acid, one Light Cycler FastStart DNA Master SYBR Green I (Roche), 3 mMMgCl2, 0.3 M of HERV-3 primer, and each of the detection primer (1.0 μM of primer) were first denatured for 10 min at 95 °C. After that, the primers were treated with Light Cycler 2.0 (Roche) for 45 cycles (5 sec at 95 °C),(3 sec at 65 °C), and (10 sec at 72 °C). The ttemperature was raised to 99°C after the program for analytical melting. Starting at 65°C and ramping at a rate of 0.1°C/s. The primers was putted into the pTA2 vector using the Target Cloning multiplex Kit Toyoko, Osaka, and Japan.

D- Real-Time PCR

After three transformed colonies were chosen to be cultured in Luria- Bertani medium for one additional night, the plasmid DNA was extracted using the Gene All Expert Plasmid SV Mini Kit (Gene All Biotechnology, Seoul ,and Korea. A plasmid copy number was determined, and transformation was verified using restriction enzymes (Hind III and BamH I) . Using a ten-fold distilled water dilution to create standard curves with 102– 106 copies per reaction, the sensitivity for both (r-t PCR) and melting curve analysis was determine .

E- Primer

Table -1: Primer

Gene target	Primer sequences (5 → 3)	Amplicon (bp)	Reference
ORF4	Forward primer: GCCCATGAATCACCCTC Reverse primer: ACTCGGTACGCCATTTAG	79	16

III. RESULTS

A. Prevalence of Positive and Negative Varicella Zoster in Studies Groups

The present study recorded that among 90 patients with stroke disease, 34 (37.78%) of patients were infected with Varicella zoster virus 19 (30.16%), while 56 (62.22%) where negative results, with significant differences among patient group.

On the other hand, among 30 of the control group the study showed 13 (43.33%) of group were had positive virus result, and 17 (56.67%) was negative with a non-significant difference among control group, the study also recorded a non-significant difference between patients and control at p. value < 0.05, as in Table -2.

Table- 2 : Prevalence of positive and negative Varicella zoster in studies groups

	Positive		Negative		Total		p. value
	No.	%	No.	%	No.	%	
Patients	34	37.78	56	62.22	90	75.0	0.016
Control	13	43.33	17	56.67	30	25.0	0.162
Total	47	39.17	73	60.83	120	100	
CalX^2= 0.519 TabX^2= 3.84 DF= 1 p. value 0.471							

B. Prevalence of Varicella zoster According to Sex

The present study recorded among patients the high positive Varicella zoster was in the female group 19 (52.78%), while in the male group 15 (27.78%), the results was noted a significant difference among the patients group. Also, the highest positive Varicella zoster in control group was in female group 5 (45.45%), while in the male group 8 (42.11%), the results noted a non-significant difference among control group; in addition, the study also recorded a significant difference at p. value < 0.05 between patients and control groups as in Table -3.

Table- 3 Prevalence of positive and negative Varicella zoster according to sex

Sex		Positive		Negative		Total		p. value
		No.	%	No.	%	No.	%	
Patients	Male	15	27.78	39	72.22	54	45.00	<0.001
	Female	19	52.78	17	47.22	36	30.00	
Control	Male	8	42.11	11	57.89	19	15.83	0.669
	Female	5	45.45	6	54.55	11	9.17	
Total		47	39.17	73	60.83	120	100	
$CalX^2$= 12.9 $TabX^2$= 7.81 DF= 3 p. value 0.004								

C. *Prevalence of Varicella zoster According to Age Groups*

The present study showed a significant difference, was noted the high positive Varicella zoster in the second and sixth age groups of control group 100% compared with patient group, with. In contrast the third, fourth, and fifth age groups of patient group scored a significant increase of positive Varicella zoster 46.67%, 56.25%, and 22.22%, respectively. In contrast, a non-significant difference was noted between patients and the control in the fourth age group. For all age groups the study recorded a significant difference at p. value < 0.05 between patients and the control groups, as in Table -4.

Table- 4 Prevalence of positive and negative Varicella zoster according to age groups

Age in years		Positive		Negative		Total		p. value
		No.	%	No.	%	No.	%	
1 – 10	Patient	1	7.69	12	92.31	13	10.83	<0.001
	Control	8	40.0	12	60.0	20	16.67	
11 – 20	Patient	2	13.33	13	86.67	15	12.50	<0.001
	Control	2	100	0	0.00	2	1.67	
21 – 30	Patient	7	46.67	8	53.33	15	12.50	0.043
	Control	1	33.33	2	66.67	3	2.50	
31 – 40	Patient	18	56.25	14	43.75	32	26.66	0.395
	Control	1	50.0	1	50.0	2	1.67	
41 – 50	Patient	2	22.22	7	77.78	9	7.50	<0.001
	Control	0	0.0	2	100	2	1.67	
≥ 51	Patient	4	66.67	2	33.33	6	5.00	<0.001
	Control	1	100	0	0.00	1	0.83	
Total		47	39.17	73	60.83	120	100	
$CalX^2$= 477.3 $TabX^2$= 19.67 DF= 11 p. value <0.001								

D. *Prevalence of Varicella zoster According to Residency*

The current study noted, the high positive Varicella zoster was in Garaf 75% distract for patients group with non-statistically calculated, followed by Suq Al-Shuyukh distract 50% for both patients and the control group with a non-significant difference. The present study recorded a significant difference between patient and the control within Nasiriyah, Refaai, and Shatrah distracts. For all residences the study recorded a significant difference at p. value < 0.05 between patients and control groups, as in Table -5.

Table- 5 Prevalence of positive and negative Varicella zoster according to residency

Residency		Positive		Negative		Total		p. value
		No.	%	No.	%	No.	%	
Nasiriyah	Patient	3	13.64	19	86.36	22	18.33	0.002
	Control	3	33.33	6	66.67	9	7.50	
Refaai	Patient	4	36.36	7	63.64	11	9.17	0.046
	Control	3	50.0	3	50.0	6	5.0	
Shatrah	Patient	5	26.32	14	73.68	19	15.83	0.035
	Control	2	40.0	3	60.0	5	4.17	
Suq Al-Shuyukh	Patient	12	50.0	12	50.0	24	20.0	1.00
	Control	5	50.0	5	50.0	10	8.33	
Fager	Patient	1	50.0	1	50.0	2	1.67	------
	Control	0	0.0	0	0.0	0	0.00	
Graph	Patient	9	75.0	3	25.0	12	10.0	------
	Control	0	0.0	0	0.0	0	0.0	
Total		47	39.17	73	60.83	120	100	
$CalX^2$= 102.5 $TabX^2$= 16.91 DF= 9 p. value <0.001								

IV. Discussion

Varicella Zoster viruses Alphah erpesvirinae are extremely contagious and can induce a variety of clinical manifestations, including cutaneous vesicles and widespread viral infection. Prompt antiviral therapy improves morbidity and mortality. Hence, laboratory detection of these virus infections together with clinical symptoms or signs is very critical for diagnosis. The present study recorded among patients, the high positive Varicella zoster was in female group 52.78% while in the male group 27.78% .The study is consistent with another study in Najaf city in Iraq, which showed that female are more infected with the virus than males Hasan et. al. [17] and agree with other study by (Li et al.,2016) found a higher in year incidence rate in female than in male in China. The current study is in contrast with a study by Rashad-[9] and Nasser et al. [11] The differences in the results may be due to the different methods that are used for viral diagnosis.

The virus infects all ages, but the infection is more concentrated in children, the elderly, immunocompromised patients, AIDS, some other immune diseases, and organ transplants of the patients. VZV is the source of a highly contagious childhood illness that worsens with age and kills adults 10–30 times more frequently than it does children [18] The present study shows that most of the cases within the age gang between (11 – 20) years and agree with previous study in India found that the mean age was 20.28 years Kujur et al. [19] The study disagreed with other studies usually occurred in people between the ages of 5 and 14, according to the age distribution of the registered cases [20] Ather study discovered that Infection rates were highest in the age group of 46–60 years old, and lowest in the age group of 16–30 years old Rashad, [9] as show in Table 4, There were more VZV infections in rural areas than in urban areas, and the difference was significant when compared to the control group. The influence of home location, whether in rural or urban regions, on the growth or reduction in viral infection levels was investigated.

There was a statistically significant difference between the number of patients living in rural areas and those infected with the virus, compared to the control group of auditors living in the same city areas. This contrasts with the comparison between infected city residents and the comparison control group. The present study disagrees with Rashad [9] and Nasser et al. [11] the reason for the

difference in results between studies and their inconsistency may be due to the difference in the samples examined, as well as to the nature of the laboratory tests used in the studies.

V. CONCLUSIONS

The current study found that among ninety patients, 37 % were infected with the Varicella zoster virus. The percentage of females was higher than that of males. Also, people between the ages of 11 and 20 and those less than or equal to 50 were the most infected with the virus, and infections were distributed more in urban areas than in urban areas.

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Conflicts of interest: On behalf of all authors, the corresponding author states that there is no conflict of interest.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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