A **Surveillance of *Varicella Zoster Virus* among School Age Children in Al-Diwaniyah Governorate**

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Abstract:

Background: chickenpox is the primary infection of *Varicella-Zoster Virus* (VZV), It is usually a childhood infection providing life long immunity, High-risk group of individuals is more likely to develop serious complications.

Objective: To find out the prevalence rate of chickenpox infection caused by *Varicella-Zoster Virus* among School Age Children in Al-Diwaniyah Governorate**.**

Methods:A total of 4545 children with an age range of 6 to 10 years were enrolled and data about those children obtained form 10 primary schools in Al-Diwaniyah governorate in the Mid-Euphrates region of Iraq, then information about age, gender and residency about each child were introduced into an excel spread sheet.Skin crusted lesion was taken for purpose of analysis..

Results: Infection documented in 800 out of 4545 children making the prevalence rate (17.6 %). No significant difference was obtained of VZV infection with respect to age and gender. The mean age of the 43 children selected was 8.44 ±1.65 years and the age range was 6 – 10 years. The

study sample included 23 male children (53.5 %) and 20 female children (46.5 %). The slight difference in male proportion from that of female proportion was statistically insignificant ($P = 0.67$)

Conclusion: **:** The most prevalent VZV genotype in Diwanyia governorate was B genetic subtype (wild type) than other. So, it recommended giving an attention to this genotype in the further studies.

Keywords: ***Varicella-Zoster virus*; School Age Children;, Surveillance ; Mid-Euphrates;IRAQ.**

Introduction:

Varicella zoster virus (VZV) causes a primary infection known as varicella (chicken pox). The virus then migrates from the skin lesions *via* nerve axons and, probably also by viremic spread, to spinal and cranial sensory ganglia where it becomes dormant. Later in life, in some individuals the virus is reactivated (usually within a single ganglion) to cause a secondary infection known as herpes zoster (HZ; shingles). Individuals with HZ can transmit VZV to their seronegative contacts, who may develop varicella, but not HZ, the household transmission rate of HZ (to cause varicella) is 15%, making it significantly less contagious than varicella but nevertheless of relevance to at-risk contacts (1).

The estimated average overall incidence of HZ is about 3.4–4.82 per 1000 person years which increases to more than 11 per 1000 person years in those aged at least 80 years .HZ-associated mortality is rare, with reported incidence ranging from 0 to 0.47 per 100,000 person year, and the majority of deaths occur in those aged at least 60 years(2).

Varicella zoster virus (VZV), which is a human alphaherpesvirus of the genus *Varicellovirus*, causes varicella (also known as chickenpox) and zoster (also known as shingles) (3).

The primary infection with VZV is varicella, commonly known as chickenpox. Varicella is highly contagious; it is most commonly seen in children under the age of 10 years in countries where live attenuated varicella vaccine is not routinely administered (4).

The infants of women with varicella in the first 20 weeks of pregnancy are at about a 2% risk of developing the congenital varicella syndrome These infants often have a variety of severe abnormalities of their brain, eyes, extremities, and skin and most succumb in infancy or early childhood. They frequently experience recurrent VZV reactivation and may have multiple cases of clinical zoster. Fortunately the syndrome is unusual in that only about 2% of women who develop varicella in pregnancy give birth to an infant with the congenital varicella syndrome (1).The aim of study was to find the occurrence the VZV in primary school children in Al-dewaniyah governorate.

Materials and Methods:

Out of the many primary schools in Al-Diwaniyah governorate, randomly selected 10 schools including rural and urban areas. A total of 4545 children were enrolled with an age range of 6 to 10 years 800 were diagnosed clinically to have VZV infection, then information about age, gender and residency about each child were introduced into an excel spread sheet Skin crusted lesion was taken for purpose of analysis. The samples of crusted obtained by crust lever then transferred into a tube (Texwipe’s Absorbond® Swab) which is made of polyester (hydroentangled) nonwoven material. Then samples transferred into

freeze at -20 C. PCR technique was performed for direct detection of *Varicella Zoster Virus* (VZV) based on amplification of **major capsid protein** gene in VZV from skin crusted lesions samples. This technique was done according to company instructions.(table 1).

Table (1): The PCR detection primers with their sequence and amplicon size

Primers	Sequence (5'-3')		Amplicon
Mcp	F	TGACAAATGCTAGGCGGGTT	520bp
	R	CGACGCAACGATTCGGTAAC	

Viral DNA was extracted from transport media of skin lesions scrap samples were extracted by using Genomic DNA mini kit extraction tissue, and done according to company instructions . PCR master mix was prepared by using (AccuPower PCR PreMix Kit) and this master mix done according to company instructions .

After that, these PCR master mix components that mentioned above were placed in standard AccuPower PCR PreMix Kit that containing all other components which needed to PCR reaction. Then, all the PCR tubes transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes, then placed in PCR Thermocycler (Mygene. Bioneer. Korea).

PCR thermocycler conditions were done by using convential PCR thermocycler system .The PCR products were analyzed by agarose gel electrophoresis .

Statistical analysis:

Data were summarized, analyzed and presented using two software programs; these were the statistical package of social sciences (SPSS

version 23) and Microsoft Office Excel 2010.. The level of significance was considered significant at $P < 0.05$ and highly significant at $P < 0.01$.

Results and Discussion:

In the present study, Infection was documented clinically in 800 out of 4545 children making the prevalence rate (17.6 %) as shown in figure (1). The prevalence rate according to school is shown in table (2).

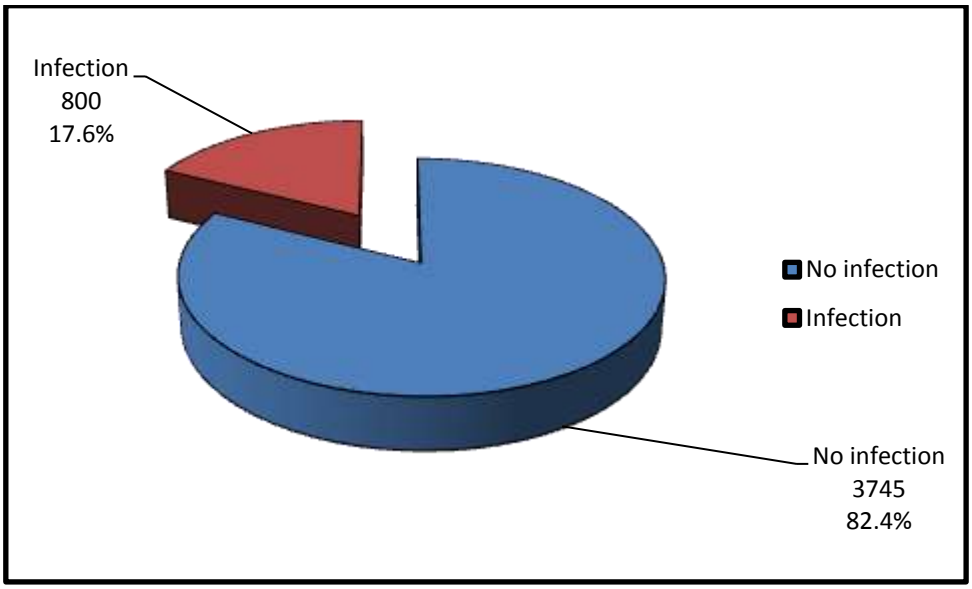

Figure 1: Pie chart showing the prevalence rate of Varicella Zoster Virus (VZV) infection among children in 10 primary schools in Al-Diwaniyah province (Mid-Euphrates region, Iraq)

Schools were arranged according to the prevalence rate so that the highest rate was seen in school number one and the lowest prevalence rate was observed in school number 10. The first school included 147 infected children out of 541 with a prevalence rate of 27.2 %. The second school included 88 infected children out of 369 with a prevalence rate of 23.8 %. The third school included 109 infected children out of 461 with a prevalence rate of 23.6 %. The fourth school included 127 infected children out of 583 with a prevalence rate of 21.8 %. The fifth school

included 83 infected children out of 409 with a prevalence rate of 20.3 %. The sixth school included 63 infected children out of 356 with a prevalence rate of 17.7 %. The seventh school included 66 infected children out of 508 with a prevalence rate of 13.0 %. The eighth school included 53 infected children out of 420 with a prevalence rate of 12.6 %. The ninth school included 21 infected children out of 208 with a prevalence rate of 10.1 %. The tenth school included 43 infected children out of 690 with a prevalence rate of 6.2 %. So far, the prevalence rate of VZV infection rate ranged from 6.2 % to 27.2 %.

Table (2) The prevalence rate of VZV infection according to primary school .

School No.	Clinical observation positive	Total	Total prevalence %
1	147	541	27.2
2	88	369	23.8
3	109	461	23.6
4	127	583	21.8
5	83	409	20.3
6	63	356	17.7
7	66	508	13.0
8	53	420	12.6
9	21	208	10.1
10	43	690	6.2
Total	800	4545	17.6

According to residency, the prevalence rate of VZV infection in urban areas was 16.5 % and that of rural areas was 18.1%. Despite some difference in the prevalence rate between rural and urban areas, the

difference was statistically not significant (P = 0.207), as shown in table (3).

Table (3) Association between child residency and prevalence rate VZV infection

Infection with VZV according to clinical findings	Total *n* = 4545	Urban *n* = 1299	Rural *n* = 3246	χ^2	*P* *
Positive, *n* (%)	800 (17.6 %)	214 (16.5 %)	586 (18.1 %)	1.594	0.207 NS
Negative, *n* (%)	3745 (82.4 %)	1085 (83.5 %)	2660 (81.9 %)		

n: number of cases; *: according to Chi-square test; NS: not significant at $P \leq 0.05$

According to gender, the prevalence rate of VZV infection in male children was 17.4 % and that of female gender was 18.0 %. Despite some difference in the prevalence rate between male and female children, the difference was statistically not significant (P = 0.600), as shown in table (4).

Table 4: Association between child gender and prevalence rate VZV infection

Infection with VZV according to clinical findings	Total *n* = 4545	Male *n* = 2787	Female *n* = 1758	χ^2	*P* *
Positive, *n* (%)	800 (17.6 %)	484 (17.4 %)	316 (18.0 %)	0.275	0.600 NS
Negative, *n* (%)	3745 (82.4 %)	2303 (82.6 %)	1442 (82.0 %)		

n: number of cases; *: according to Chi-square test; NS: not significant at $P \leq 0.05$

Out of 800 children with clinical manifestation of VZV infection, 43 were selected normally for the purpose of molecular diagnosis and identifying genetic strains. Conventional PCR method (figure 2) showed that all the 43 children were positive for VZV. PCR product analysis for major capsid protein (*mcp*) gene in *varicella-zoster virus*

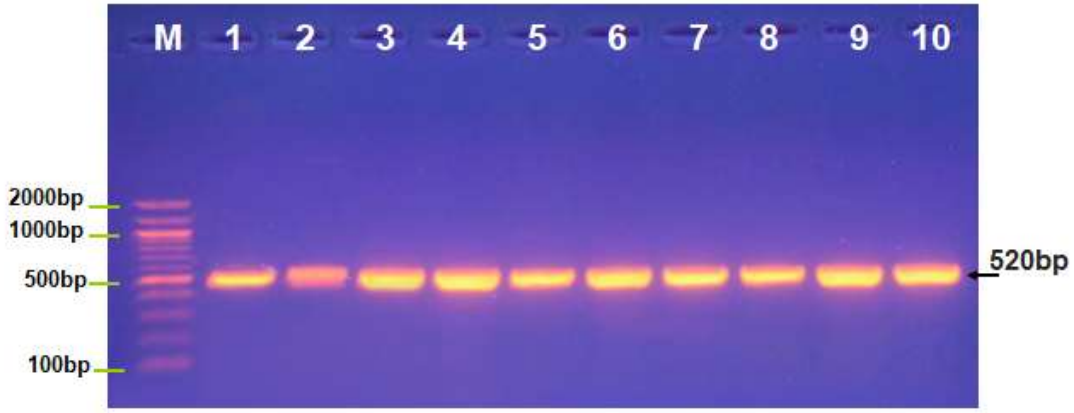

Figure 2: Agarose gel electrophoresis image that showed PCR product analysis for major capsid protein (mcp) gene in varicella-zoster virus. M (Marker ladder 2000-100bp). Lane (1-10) some positive varicella-zoster virus isolates at 520bp PCR product size.

The mean age of the 43 children selected for molecular study was 8.44 ±1.65 years and the age range was 6 – 11 years, as shown in table 4.4. The distribution of children according to one year age interval was as following: children aged 6 years accounted for 7 out of 43 (16.3 %), children aged 7 years accounted for 6 out of 43 (14.0 %), children aged 8 years accounted for 10 out of 43 (23.3 %), children aged 9 years accounted for 7 out of 43 (16.3 %), children aged 10 years accounted for 7 out of 43 (16.3 %) and children aged 11 years accounted for 6 out of 43 (14.0 %), as shown in table (5) and figure(3).

The study sample included 23 male children (53.5 %) and 20 female children (46.5 %), as shown in figure (4). The slight difference in male proportion from that of female proportion was statistically insignificant (P = 0.647).

Table (5) Mean age, age range and distribution of children according to one year age intervals

Age interval	*n*	%
6 years	7	16.3
7 years	6	14.0
8 years	10	23.3
9 years	7	16.3
10 years	7	16.3
11 years	6	14.0
Mean ±SD (years)	8.44 ±1.65	
Range (years)	6 - 11	

n: number of cases; SD: standard deviation

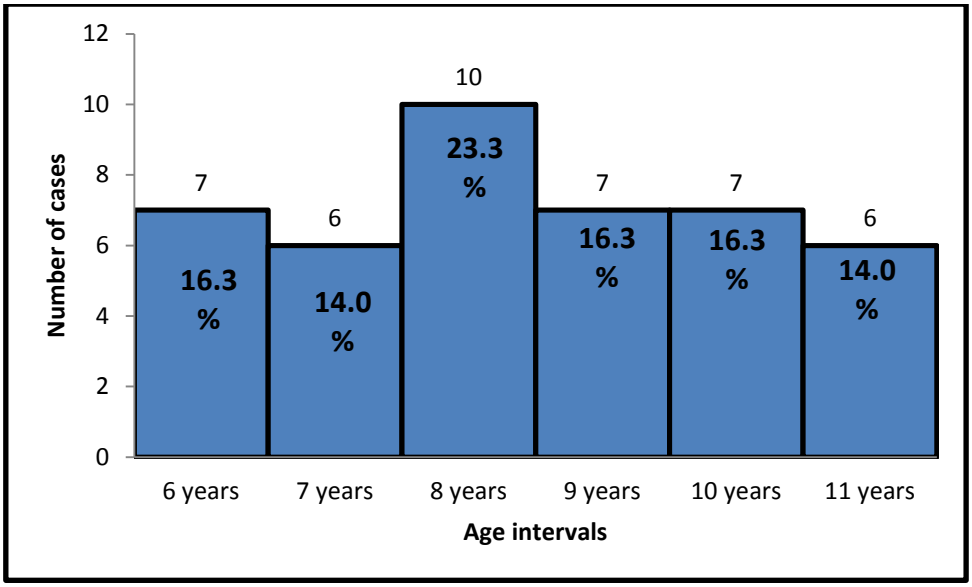

Figure 3: Histogram showing the distribution of children infected with VZV according to one year intervals

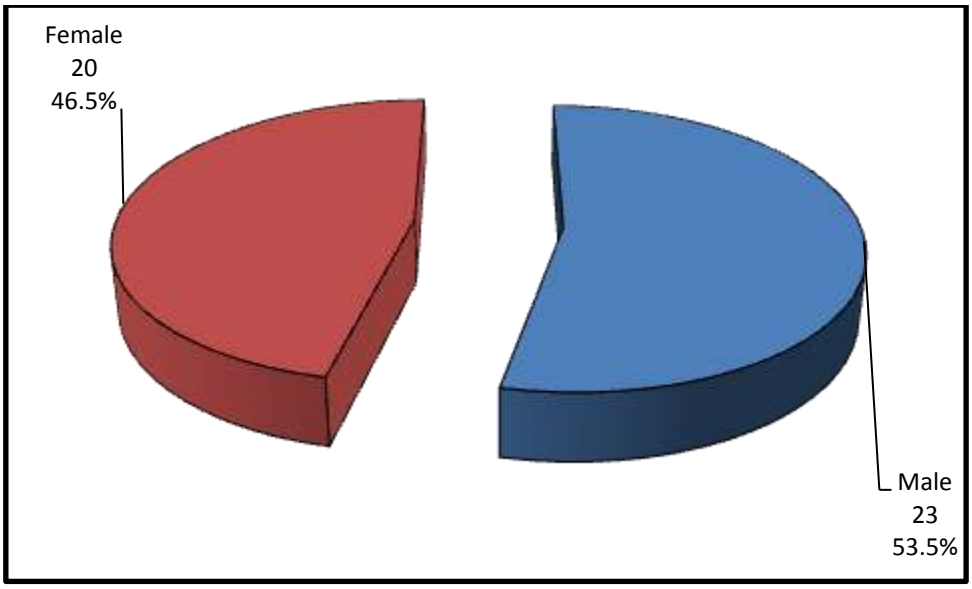

Figure 4: Pie chart showing the distribution of children infected with VZV according to gender.

Varicella-zoster virus (VZV) is a pathogenic human alpha-herpesvirus that causes chickenpox (varicella) as a primary infection, which usually occurs in children in locales where vaccination is not practiced (5). Following the primary infection, this neurotropic virus becomes latent, primarily in neurons in peripheral autonomic ganglia throughout the entire neuroaxis including dorsal root ganglia (DRG), cranial nerve ganglia such as the trigeminal ganglia (TG), and autonomic ganglia including those in the enteric nervous system (6). Up to decades later, latent VZV may reactivate, either spontaneously or following one or more of a variety of triggering factors to cause herpes zoster (shingles), which usually appears as a painful or pruritic cutaneous vesicular eruption that occurs in a characteristic dermatomal distribution (1). This viral reactivation becomes more frequent with the increased age of the human host because of diminished cell-mediated immunity to the virus in such individuals (7). Other specific triggers for viral reactivation

include immunosuppression from disease or drugs, trauma, X-ray irradiation, infection, and malignancy (1). While the main and most important complication of herpes zoster is postherpetic neuralgia (PHN), it has been increasingly recognised over the last decade that VZV reactivation causes a variety of acute, subacute, and chronic neurological syndromes, so its clinical manifestations are protean (7). The reality of subclinical reactivation was demonstrated when it was determined that one-third of astronauts developed reactivation of VZV transiently during space travel. The diagnosis was made by finding VZV DNA in saliva; the astronauts had no symptoms of zoster and the viral DNA disappeared within a few weeks after return to Earth (8). Importantly, it is very rare to isolate infectious VZV from saliva of patients with active or subclinical VZV infections (9). Since the introduction of widespread childhood varicella vaccination, no impact has been observed on the incidence or age distribution of HZ . Other studies have reported ,Takahashi (10) an increasing trend in the general population as well as in immunocompromised populations, but this trend preceded the implementation of childhood varicella vaccination (Long-term surveillance will be necessary to establish if there will be an increased incidence of HZ (11). Further analysis of viruses from around the world confirmed that the *Bgl*1-positive genotype was present in 100% of 100 viruses collected from different countries in Africa, East Asia, and the Indian subcontinent but accounted for <20% of viruses collected in Europe, the United States, and other countries settled by Europeans . A prospective study of >400 patients presenting with clinical zoster allowed us to type VZV from 200 white British-born subjects aged 5–98 years. (12).

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