Molecular Diagnosis of Human Epstein Barr virus and Varicella Zoster Virus with Type 1 Diabetes Mellitus

Zainab Hassan darweesh[1], Fadyia Mahdi Muslim Alameedy[2]

Abstract

Human samples from type one Diabetes Miletus patients were collected during 15, September 2022 to 19 of July 2023. Type one Diabetes mellitus was including (1 – 20 years). The RT-qPCR, Method was detected Epstein Barr virus and Varicella Zoster virus of all sample; the results showed thirty Positive cases for Epstein Barr virus and seven Positive cases while for Varicella Zoster virus. The populations studied representative samples There were four study groups, with participants in (1-5 ,6-10, 11-15, 16-20) years, changed age to gender. The third group (11-15) were high cases of Human infected (46%) for EBV in compare of aged group (1-5) was (10%), (6-10) was (13%) and the age group (16-20) was (30%). while VZV Age group (1-5) was higher positive cases than other (57%), (6-10) was (28%), (11-15) was (14%) and in the later age group (16-20) no positive case. the samples were isolated from the hospitals including (Al-sadr /diabetes and endocrine gland center , Al-Zahraa hospital) . The first study in Iraq to diagnose Epstein Barr virus and Varicella Zoster virus in patients with type 1 Diabetes by molecular technique.

Keywords: *type one Diabetes mellitus, viral infection with DM, Epstein Barr virus, Varicella Zoster virus, qPCR.*

Introduction

In all populations, diabetes mellitus was a serious endocrine condition. Diabetes develops when the amount of insulin produced was insufficient to meet the metabolic needs of certain organs, such as the liver, fat, and muscle. Type-I Diabetes mellitus (T1DM) was caused by an autoimmune reaction that results in the death of insulin-producing beta cells. (Jansari et al., 2014) , A number of variables, including genetics, age, environment, and viral infections, had been related to autoimmune reactions. Viruses were thought to be important environmental variables that caused autoimmune disorders in people with certain genetic predispositions . In the past, it was thought that viruses carried antigens that were structurally similar to self-antigens, which prompted a reaction against self- and non-self-antigens due to B- and T-cell activation, "Bystander activation," wherein an overly generalised antiviral immune response causes the release of self-antigens from wounded tissue and localised proinflammatory conditions, was another hypothesised mechanism. Autoimmunity was triggered when self-antigens were taken up by antigen-presenting cells (APC) and displayed to surrounding autoreactive T cells. Similar to how a viral infection can lead to the development of new self-antigens and the de novo

---

[1] Department of Pathological Analysis, faculty of Science, University of Kufa, Najaf, Iraq, qtra9789@gmail.com

[2] Department of Pathological Analysis, faculty of Science, University of Kufa, Najaf, Iraq, fadyiam.alameedy@uokufa.edu.iq

activation of autoreactive cells, the latter can then spread to target other self-epitopes via the mechanism known as epitope spreading. (Smatti et al., 2019)

Most people had been exposed to Epstein Barr virus (EBV), which was a member of the Herpesviridae family. Type 1 diabetes (T1D) was one of many autoimmune illnesses that was more likely to occur in people who had previously been infected with Epstein Barr virus. The EBV-produced protein EBNA2 binds in several places on the human genome known to be involved in the development of autoimmunity.(lynne schnieder et al., 2018), The molecular mimicry was through which EBV might contribute to the pathogenesis of T1D.(Mohammed & Sabr, 2019), Stimulation of viral DNA led to the production of interferons and other cytokines, including pro-inflammatory cytokines like IL-10, which could change the host immune system and contribute to the development of disease.Infections that lingered, as well as their secondary infections and other disorders, played a crucial role in this process.(Jakub Dworzański et al., 2019)

Varicella-Zoster virus (VZV) was a member of the family Herpesviridae, the genus alphaherpesviruses, and the subfamily Alphaherpesvirinae, and it has a quick life cycle, a wide host range, causes extensive cell death, and persists in the sensory ganglia. (Zell et al., 2012)

Among Varicella Zoster virus patients, the prevalence of undiagnosed diabetes mellitus (DM) was high; this might be because humoral immunity was still intact but CMI, phagocytosis, and opsonization are impaired. (Odhaib & Mansour, 2020), While the skin and oral mucosa were the main sites of varicella zoster virus infection, the virus could also persist latently in nerve sensory ganglia. Patients with diabetes mellitus might have significantly less cell-mediated immunity (CMI) to VZV, according to some research. It was believed that this decline in VZV-specific CMI might be responsible for the elevated risk of herpes zoster in diabetics, detection of viral infection in patients of diabetes type one by used RTqPCR (Rodriguez-Calvo et al., 2016)

Materials and methods

Collect affected specimens of human type one diabetes patients

Samples were collected of type one Diabetes mellitus patients from September 2022 to July 2023, thirty were positive cases with Epstein Barr Virus ,including fourteen cases were females and sixteen cases were males , while Varicella Zoster Virus positive cases were seven cases, four cases were females and three cases were males , that from infected human patients of age ranged 1 years up to twenty years of specimens..

Real time qPCR Technique

RT-qPCR technique was used to diagnose Human Epstein Barr virus and Varicella Zoster virus (there primer was designed based on the NCBI of human Epstein Barr virus EBNA1gene and Varicella Zoster virus ORF31gene ,GoTaq qPCR Master mix (cat. Number: 023484574400, abm, Canada) viral DNA was extracted by using viral Nucleic Acid Extraction Kit (gSYNC TM DNA Extraction Kit) (Geneaid, Lot No. FA30411-GS, USA). This procedure was carried out at Alamin's Centre for Advanced Research and Biotechnology, by used (Analytik Jena/Qtower3G) device and public health laboratory by using (BIO RAD /CFX96) device.

Table 1: Primers of viruses used , design depended to NCBI

Virus Name	Sequences	Bases	Product size
Epstein Barr virus-F	GCTGACGTGAGAGGATGGTT	20	171bp
Epstein Barr virus-R	GGACCCCATACCTCCCTTTA	20	
Varicella Zoster virus -F	GTTCAGGCAACCGTTTTGAT	20	188bp
Varicella Zoster virus-R	AATGTTTGACGCCAGGTTTC	20	

Results

Molecular method for diagnosis of Human Epstein Barr virus and Varicella Zoster virus by RT- qPCR technique.

Thirty cases was positive with Epstein Barr Virus and seven positive cases with Varicella Zoster Virus from 100 samples of collected serum from patients with type 1 diabetes mellitus of different Areas were diagnosis by real-time qPCR, while 70 cases were negative with Epstein Barr virus and 93 cases were negative with Varicella Zoster virus as show in figure (1) , sixteen cases of male infected consider the higher than fourteen cases of females with Epstein Barr virus as in figure (2), and Varicella Zoster virus four positive cases were females and three cases were males as in figure (3). The age group (11-15) years was highest compared to other age groups for Epstein Barr virus in fig.(4),in Varicella Zoster virus(1-5)years was highest in compared to other totals in terms of age in fig. (4)
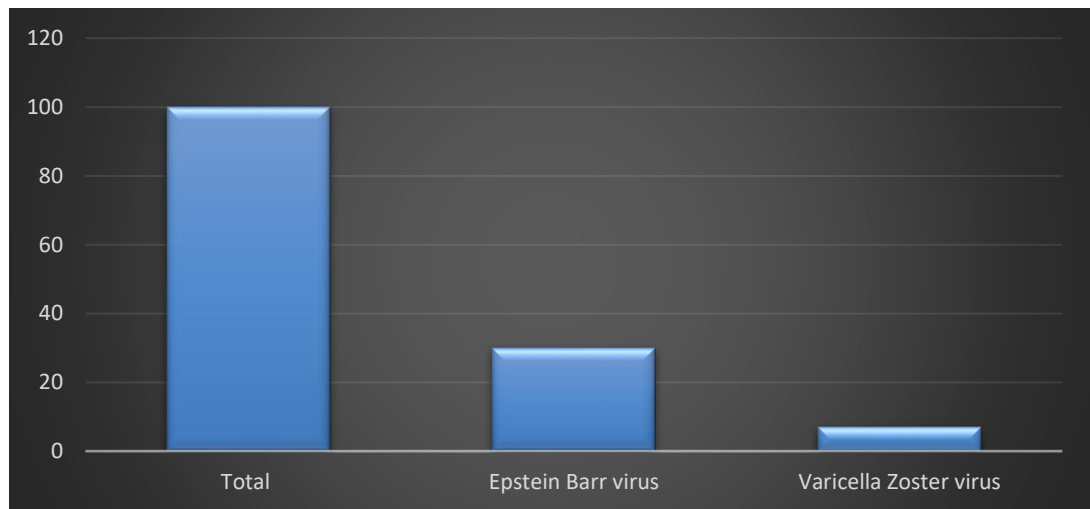

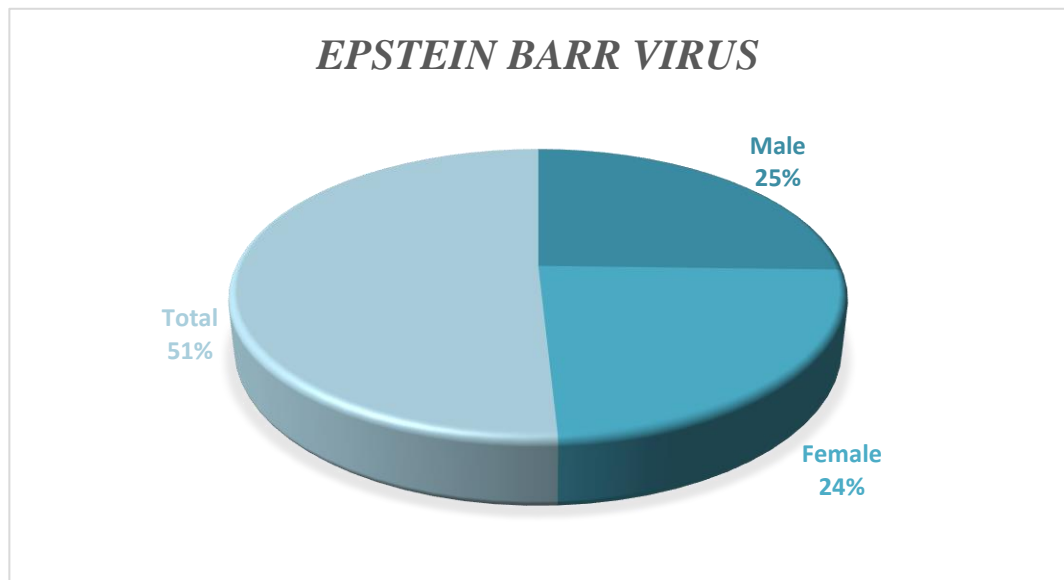Figure (1): positive cases for each viruses with total sample account

Figure 2: male and female positive cases with Human Epstein Barr Virus
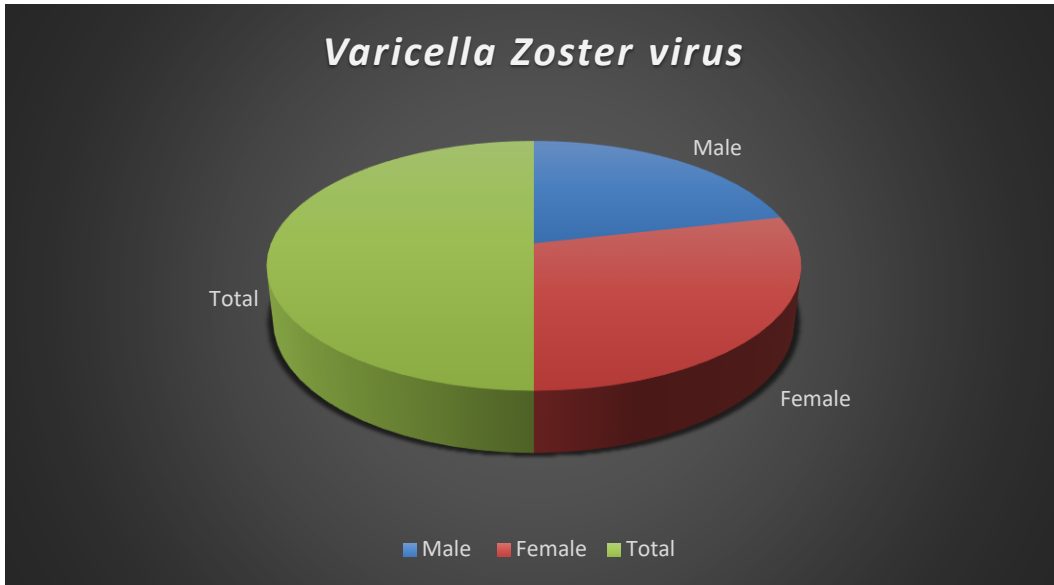
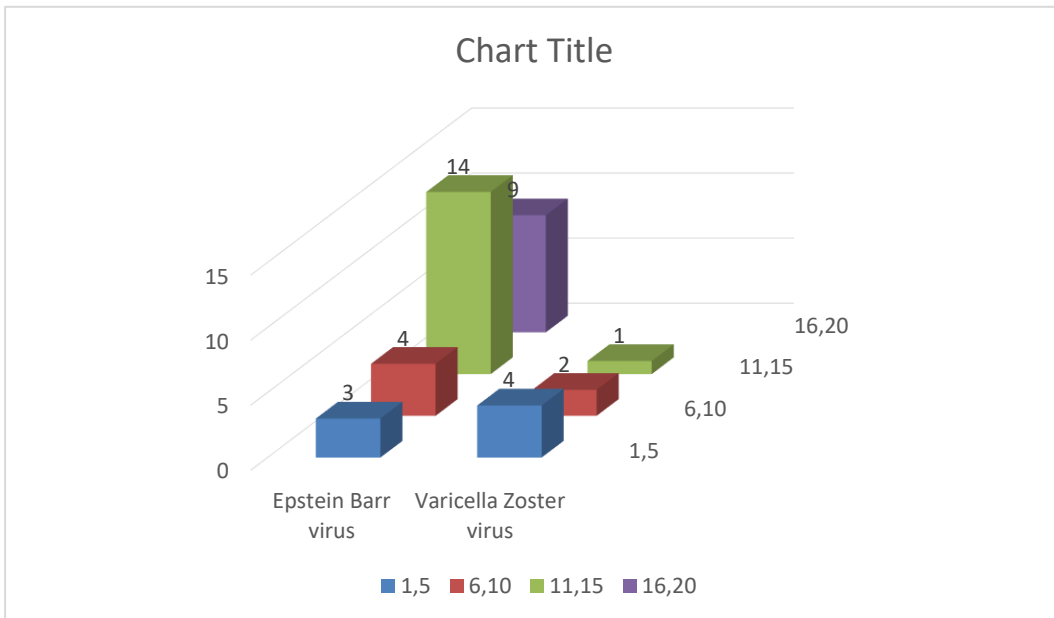Figure 3: male and female positive cases with Human Varicella Zoster Virus

Figure 4: distribution of positive cases on age groups for Human Epstein Barr Virus and Human Varicella Zoster virus

Table 2: Detection of Human viral infection in patients with type one Diabetes

CT	Epstein Barr Virus	Varicella Zoster virus
13-18	2	0
19-24	13	2
25-39	15	5

Discussion

Diagnosis of Human Epstein Barr virus and Varicella Zoster virus with type one Diabetes mellitus the study is considered in Najaf Governorate and at the level of Iraq as well by

RT-qPCR technicality which Resembled with the (Wang et al., 2022) In our study show that many viral infection cause diabetes mellites type 1 especially Epstein Barr virus and Varicella Zoster virus and this study is agreement with study (Houen & Trier, 2021) and (lynne k schnieder et al., 2018).
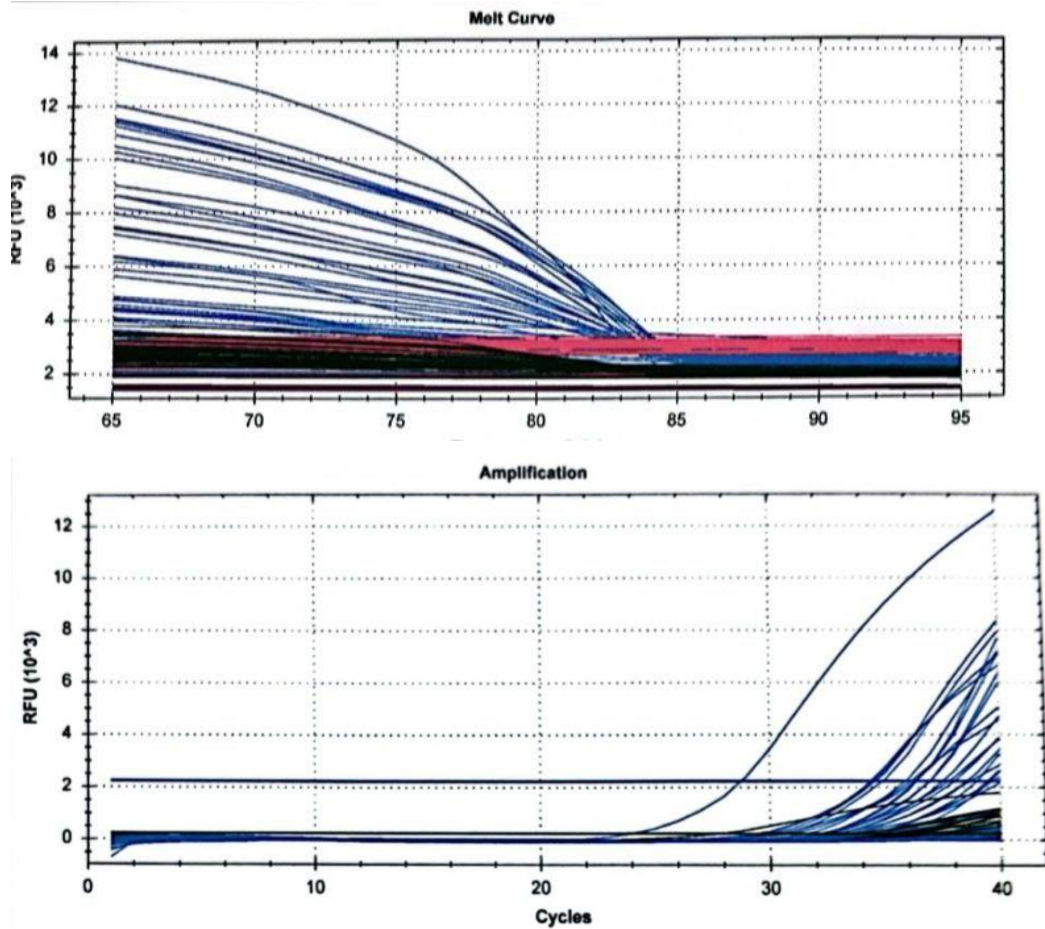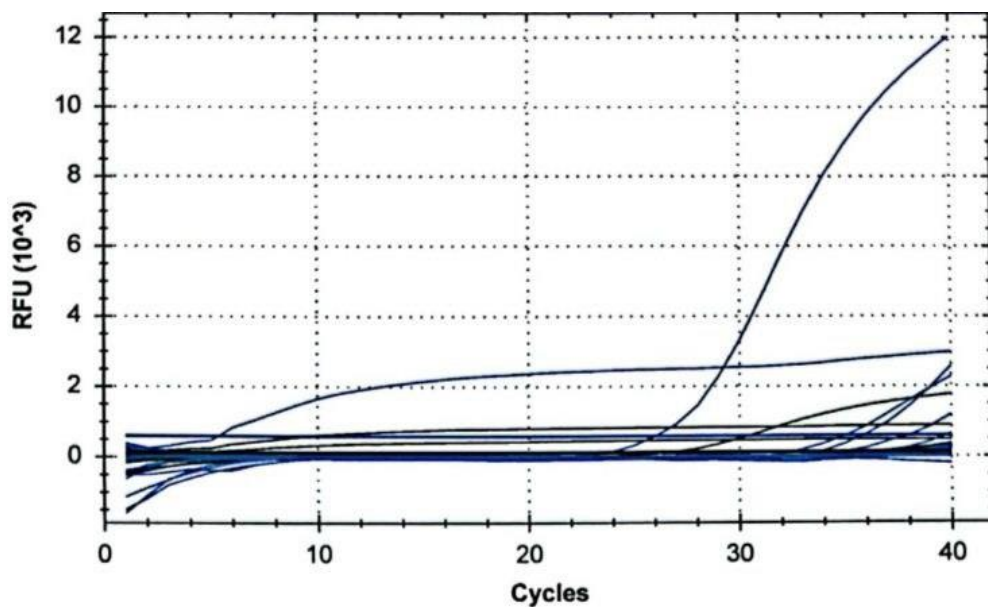

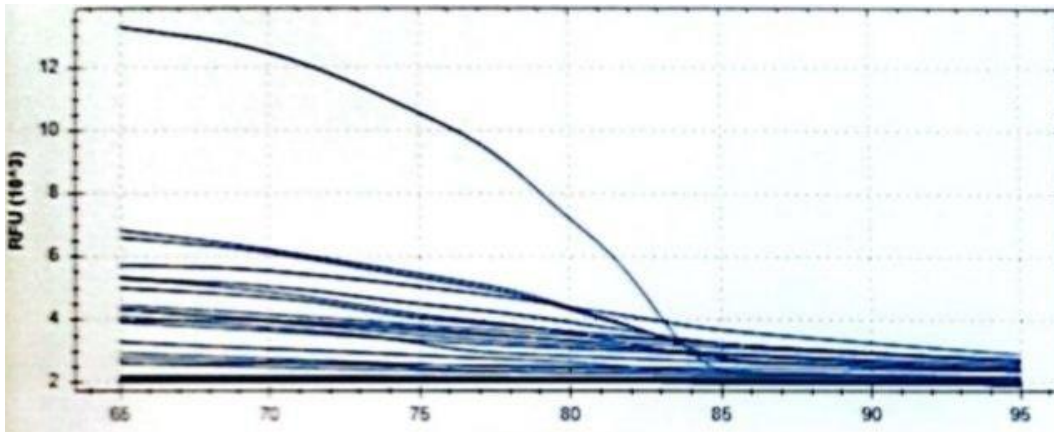Figure 5: Detection of Epstein Barr virus by RT-qPCR

Figure 6: Detection of Varicella Zoster virus by RT-qPCR

Conclusion

1.   The age group (11-15) were more infected with Epstein Barr virus while (1-5) were more infected with Varicella Zoster Virus.

2.   Males were more infected with Epstein Barr Virus than females, and Varicella Zoster Virus patients were females more than males.

References

Houen, G., & Trier, N. H. (2021). Epstein-Barr Virus and Systemic Autoimmune Diseases. Frontiers in Immunology, 11, 3334. https://doi.org/10.3389/FIMMU.2020.587380/BIBTEX

Jakub Dworzański , Bartłomiej Drop , Ewa Kliszczewska, M. S.-, DudziakID, & Polz-Dacewicz, M. (2019). Prevalence of Epstein-Barr virus, human papillomavirus, cytomegalovirus and herpes simplex virus type 1 in patients with diabetes mellitus type 2 in south-eastern Poland. PLOS ONE.

Jansari, S., Devashri, N., Dhanani, A., & Chauhan, N. (2014). DIFFERENTIATION OF STEM CELLS INTO PANCREATIC β-CELLS: REGENERATIVE MEDICINE FOR DIABETES. International Journal of Biological & Pharmaceutical Research.

lynne k schnieder, Harley, J., & Davis, G. (2018). Diabetes Risk Increases with Exposure to Epstein-Barr Virus. Endocrineweb.

Mohammed, A., & Sabr, A. A. (2019). THE POSSIBLE ASSOCIATION BETWEEN EPSTEIN-BARR VIRUS AND TYPE 1 DIABETES MELLITUS. Iraqi Journal of Medical Sciences, 17(3–4), 175–182. https://doi.org/10.22578/IJMS.17.3-4.3

Smatti, M. K., Farhan S. Cyprian , Gheyath K. Nasrallah, A. A. A. T., & Ruba O. Almishal, and H. M. Y. (2019). Viruses and Autoimmunity: A Review on the Potential Interaction and Molecular Mechanisms. MDPI.

Wang, S.-C. ;, Liao, J.-Y., Wang, S.-C., & Liao, J.-Y. (2022). Epidemiologic Implication of the Association between Herpes Simplex Virus Infection and the Risk of Type 1 Diabetes Mellitus: A Nationwide Case-Control Study in Taiwan. International Journal of Environmental Research and Public Health 2022, Vol. 19, Page 7832, 19(13), 7832. https://doi.org/10.3390/IJERPH19137832