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ORIGINAL ARTICLE

Serological Detection of IgG in Type 2 Diabetic Patients Against EBV, HSV-1, VZV: Evaluating Immunity and Past Infection

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SUMMARY

Background: **The goal was to explore the seroprevalence in order to evaluate past infections and immunity status in type 2 diabetic individuals compared to the seroprevalence of the common members of Herpesviridae family viruses.**

Methods: **One hundred and fifty individuals (50 females, 100 males) were enrolled in this study, all from Taif city. Samples were collected by drawing 3 mL of peripheral blood into the yellow cap tubes for serum collection. The samples were collected between the 3rd and the 8th of February 2025. IgG serostatus was evaluated by using Synergy Neo2 microplate reader at a wavelength of 450 nm. Chi-squared test was applied for statistical analysis purposes.**

Results: **High IgG titer was detected among our study group, which is indicative of recent infection or vaccination. HSV-1 IgG seropositivity was higher in males (90%) than females (76%); VZV IgG seropositivity was lower in males (82%) than females (86%), while EBV IgG seropositivity was higher in males (82%) than females (58%). Different IgG titers were detected among the study groups, and coinfection were detected in 54% for HSV-1/VZV, 28% for EBV/VZV, 15.4% for HSV-1/EBV, and 14% among all three viruses.**

Conclusions: **Our study assessed the seropositivity of VZV, HSV-1, and EBV in T2DM patients. The prevalence among them was lower than other studies. Gender-based differences were detected as most detected cases were males except in VZV females were higher, coinfection is common among two viruses or the three together, indicating the essential importance of targeted regular screening and vaccination of T2DM patients.**

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INTRODUCTION

Type 2 diabetes (T2DM) is a chronic metabolic disorder with several health impacts, the resistance to insulin, hyperglycemia, and several immune system disfunctions leading to mortality and morbidity. In T2DM patients, the dysfunction in immune systems leads to increased infection due to impaired immune cell functions

such as neutrophils [1], imbalance in cytokine functions and profiles, and increased cardiovascular disorders [2]. Viral infections are common in T2DM patients, and HSV-1 [3], EBV [4], and VZV [5] are members of the Herpesviridae family, all three viruses can remain latent inside the host and remain for life. During the occasion when the host-immune system weakens, these viruses reactivate and impact the host [6,7]. EBV targets B-cells and epithelia cells and causes infectious mononucleosis and can also lead to lymphoproliferative diseases [7]. HSV-1 is known to cause several health conditions related to herps diseases, both oral and genital diseases [8, 9]. VZV is known to cause chickenpox and shingles when it reactivates [5,8,10].

Many studies have reported health comorbidities due to these viral infections and the impacts increase during chronic diseases [11-13]. This is further explained in other literature about T2DM [14,15]. In T2DM it was reported that viral shedding and reactivation occurs [16]. Coinfection with these herpesviruses can lead to clinical burden specifically in T2DM patients, and lead to systemic inflammation and complications like neuropathy, cardiovascular diseases, weak wound healing. It can also lead to more concerns in the management of T2DM. In Saudi Arabia, where T2DM prevalence is among the highest globally, and EBV seroprevalence exceeds 90% in adults, the potential public health impact of such coinfections is significant [17]. The routine monitoring of herpesvirus serostatus in diabetic patients, coupled with preventive strategies such as antiviral prophylaxis or vaccination (e.g., for VZV), could be valuable in reducing the morbidity associated with viral coinfections in this vulnerable group.

Our study aims to identify the prevalence and distribution of EBV, VZV, and HSV-1 among T2DM patients in Taif, Saudi Arabia, to contribute to improved clinical decision-making, optimized resource allocation, and better management of T2DM in healthcare settings.

MATERIALS AND METHODS

This was a cross-sectional study.

Study group

A group of 150 individuals (100 males, 50 females) were enrolled in this study, all from Taif city. The inclusions to the study are confirmed diagnosis of T2DM, no known recent history of infections with one of these viruses, no vaccinations or boosters in the past 10 years against any of these viruses, and age > 45 years. Patients were excluded if they failed any of these conditions. Any hemolyzed samples were re-collected from the individuals.

Sample collection and preparation

Samples were collected by drawing 3 mL of peripheral blood into the yellow cap tubes for serum collection. The serum collected and stored in -80℃ freezer, and the samples were collected between the 3rd and 8th of February 2025. Then IgG was detected by using Synergy Neo2 microplate reader at a wavelength of 450 nm. Chi-squared test was applied for statistical analysis purposes. The period of the study was from January 2025 to March 2025.

Sample processing

The process begins with carefully thawing the serum after they were stored in -80℃ freezer. The thawing was done by placing the samples in a water bath at 30℃ to avoid degradation of the protein. Then, they were diluted by 1:101 according to the manufacture protocol, by mixing 10 µL in 1,000 µL (1 mL) PBS, then mixed and loaded into the assigned well and each sample was tested as duplicate. All the immunoenzymatic methods were done according to the manufacture's protocols, first well was blank, 2nd negative control, 3rd positive control, 4th to 6th for cutoff control. The used kits were enzywell VZV IgG ref D91088, enzywell EBV ref D91-056, and Bio-rad HSV-1 IgG ref 72820.

Detection of IgG

After the samples were processed on the 96-well microplates, the reactions were read by using Synergy Neo2 microplate reader at a wavelength of 450 nm. The threshold of the cutoff assisted in data interpretation. Samples were considered positive if the OD is higher than the cutoff of the test. EBV, VZV, and HSV-1 analysis were performed as follows, not-detected < 0.9, equivocal 0.1 - 1.1, and positive > 1.1. Equivocal results were re-analyzed after 3 weeks by using fresh samples.

Statistical analysis

The Shapiro-Wilk test for normality was applied and our data showed no normal distribution. Therefore, to compare the findings of our study, the chi-squared test of independence was applied via Microsoft excel version 2501. A p-value less than 0.05 was considered significant.

Ethical approval

This study obtained ethical approval from the research and ethics committee of Taif University Institutional Review Board (IRB), on the 24th of September 2024 with the approval number HAO-02-T-105.

RESULTS

The serostatus of IgG can be attributed to many factors, in our study we have excluded any participants with recent vaccination or booster dose of the vaccine against VZV, EBV, and HSV-1; therefore, high IgG titer is considered in this case as recent infection. After the IgG-ELISA analysis of our 150 T2DM participants, gender-based differences were detected, HSV-1 and EBV immunoglobins were higher in seropositive males than females, while VZV was higher in females (Table 1). Fe-

Table 1. The prevalence and serostatus in our study groups of the three viruses.

Participants		HSV-1		VZV		EBV	
		Seronegative	Seropositive	Seronegative	Seropositive	Seronegative	Seropositive
Male	100	10	90	18	82	18	82
		10%	90%	18%	82%	18%	82%
Female	50	12	38	7	43	21	29
		24%	76%	14%	86%	42%	58%

Table 2. The IgG titers of the detected cases is illustrated, most compared values have shown significant p-value, except female seropositive cases of EBV.

Total		150			
p-value		0.01		0.09	
EBV IgG Seropositive	triple infection	13	16%	11	38%
	double infection	37	45%	7	24%
	Single infection	32	39%	11	38%
p-value		0.02		0.01	
VZV IgG Seropositive	triple infection	19	23%	8	19%
	double infection	22	27%	14	32%
	Single infection	41	50%	21	49%
p-value		0.04		0.03	
HSV-1 IgG Seropositive	triple infection	31	34%	12	33%
	double infection	26	29%	9	24%
	Single infection	33	37%	16	43%
Participants		Cases	Percentage	Cases	Percentage
		Male		Female	

Table 3. The coinfection analysis of the study group, HSV-1 & VZV were the most prevalent groups, followed by VZV & EBV, then HSV-1 & EBV. In the study group, 14% showed IgG seropositive status for the three viruses.

Viral IgG patterns	Number of cases (%)
HSV-1 & VZV	81 (54%)
HSV-1 & EBV	23 (15.4%)
VZV & EBV	42 (28%)
HSV-1 & VZV & EBV	21 (14%)
p-value	0.001

male HSV-1 and EBV seropositivity were lower than males, that showed gender-related trend which can be due to exposure or susceptibility. Table 1 revealed that HSV-1 was seropositive in 90 (90%) males, 38 (76%) females. VZV was seropositive in 82 (82%) males, 43 (86%) females which was higher in females. Lastly, EBV was seropositive in 82 (82%) males, 29 (58%) females.

Table 2 illustrates the seropositivity levels of HSV-1, VZV, and EBV IgG antibodies. Regarding all three viruses, significant p-values resulted after comparing the detected IgG titers in male T2DM participants. HSV-1, 37% of male had single infections, 29% had double infections, and 34% had triple infections. Female T2DM participants have shown the following IgG titers of three viruses, HSV-1, 43% of females had single infections, 24% had double infections, and 33% had triple infections.

For males VZV, 50% had single infections, 27% had double infections, and 23% had triple infections. For females, VZV, 49% had single infections, 32% had double infections, and 19% had tripple infections. Lastly, for EBV, 39% had single infections, 45% had double infections, and 16 had triple infections. For females, EBV, 38% had single infections, 24% had double infections, and 38 had triple infections. Moreover, only EBV among females showed insignificant p-value, while VZV and HSV-1 showed significant p-values.

Coinfection analysis (Table 3) showed that 54% of our total participants were seropositive for HSV-1 and VZV, while 28% participants were seropositive for EBV and VZV. HSV-1 and EBV coinfection was less common with only 15.4%. However, immunoglobulins against multiple viruses, indicating coinfections, were detected in 14% of all cases.

DISCUSSION

The serostatus of IgG can be due to several factors such as past exposure and/or immunity. IgG is produced by B-cells after the immune system fights and clears any pathogen, high titers of IgG can be due to recent vaccination. Therefore, we excluded any participants with recent vaccination or booster doses of the vaccine in the past 10 years. A high IgG titer in this case is therefore considered to be a recent infection. After the IgG-ELISA analysis of our 150 T2DM participants, gender-based differences were detected, HSV-1 and EBV immunoglobulins seropositivity were higher in males than females, while VZV was higher in females than males [16-22].

Regarding all the three viruses, significant p-values resulted after comparing the detected IgG titers in all of our T2DM participants which indicated high levels of this immunoglobulin, which provides a sign of infection and a protection against these pathogens. Those findings are lower than what has been reported in other studies which were done in the eastern province of Saudi Arabia. These inconsistencies between our study and the other can be due many factors such as weak immune system that leads to reactivation of dormant viruses, hence, our samples were from T2DM patients who already have developed weak immune systems [21], and less than what has been reported in another study, that was performed among blood donors [22], who are under strict conditions of being not diabetic, or must be controlled diabetic patients under 50 years of age. And for VZV which is also lower than what was reported in other studies in Saudi Arabia, the discrepancy can also be attributed to factors, for example they focused on children participants in their research [18]. Lastly, for EBV which was also lower than what the other study reported [17], as this virus is contagious and the transmission can be due to blood transfusion, body secretions, and close contact with an infected person.

Coinfection analysis in our study showed different findings to other studies, when we compared our results to other literature, inconsistency was detected. A study performed in China reported the coinfection of these viruses was 10.2% which is less than ours [23]. Another study among inflammatory bowel disease reported 21.2% which is higher than ours [24]. Those variations can be attributed to many factors such as methodological differences, target population, and the varied inclusion and exclusion criteria. The main factor is our study group was under weak immune system protentional.

CONCLUSION

Our study examined the seropositivity of three common viral infections which can cause severe complications in T2DM patients. Most of our findings were less than what has been reported in other studies. A notable gender-related difference was found, as more male T2DM were seropositive than females in HSV-1 and EBV while VZV was higher in females, which can be related to exposure rate and immunological conditions. Coinfection analysis highlighted some dual and triple seropositivity between the three IgG of the viruses. A high pattern rate was seen between VZV and HSV-1. The coinfection patterns were inconsistent with other studies. Overall, all our findings underscore the important of target screening in diabetic population to evaluate their immunity and control the spread of viral infections.

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Declaration of Interest:
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