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Original Article

Sero-immunological Diagnosis of Epstein-Barr Virus Infection in Type 2 Diabetic Patients

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ABSTRACT

Background and aim: Epstein-Barr virus (EBV) is a human virus that is commonly found around the world. In recent years, it has been a topic of great discussion in relation to an increased risk of autoimmune diseases, such as diabetes. The purpose of this study was to find out the seroprevalence of EBV in diabetic and healthy people by quantifying some immunological markers of the study subjects.

Materials and Methods: A total of 90 people were chosen (of whom 30 were non-diabetic and 60 were diabetic) and blood samples were taken. Immunological markers were measured by quantitative ELISA kits and EBV IgG antibody was detected by a qualitative ELISA test.

Results: Immunological markers were quantified by quantitative ELISA. The prevalence of Epstein-Barr virus (EBV) infection in this study was 58.89% (53/90) of which 53.33% (16/30) of healthy individuals were positive and 61.67% (37/60) of type 2 diabetic patients were positive. In the infected group the positivity rate was 0.425 ± 0.019; however, a significantly higher positivity rate was found in type 2 diabetic individuals than healthy individuals. As far as immunological markers are concerned, a significant rise in interleukin-1 level was noted in the type 2 diabetic patients infected with EBV (viral infection) and also in healthy people infected with EBV as compared to healthy people who were not infected and type 2 diabetic patients who were not infected with EBV. Regarding interleukin-6, no statistically significant difference was found between healthy individuals infected by EBV and type 2 diabetic patients without EBV as well as between those infected and type 2 diabetic patients infected with EBV, but all these values were higher than those of healthy patients without EBV. A significant increase in tumor necrosis factor-alpha (TNF-α) levels was observed in type 2 diabetes patients infected with Epstein-Barr virus (EBV-T2DM), while these levels were decreased in healthy individuals without EBV, compared to healthy individuals infected with EBV and individuals with type 2 diabetes without EBV. A significant increase in interferon-gamma (INF-γ) levels was also observed in type 2 diabetes patients infected with EBV, compared to healthy individuals without EBV, healthy individuals infected with EBV, and individuals with type 2 diabetes without EBV, where no statistically significant difference was shown between these groups.

Conclusion: the present study gives evidence in favor of potential association between the infection with Epstein–Barr virus and type 2 diabetes mellitus. The immunological results suggest that IL-1 might be mainly linked to immune activation related to EBV, whereas IL-6 might be mainly linked to the inflammatory profile typical of type 2 diabetes mellitus. Moreover, the cytokines, TNF-α and IFN-γ were found to be linked with both EBV infection and type 2 diabetes, indicating that there may be similar inflammatory pathways associated with viral persistence, immune deregulation, and metabolic dysfunction.

Keywords: Epstein–Barr virus, interferon, interleukin, tumor necrosis factor, chronic diseases, Iraq.

Introduction

Epstein–Barr virus (EBV) is a very common virus in humans found all over the world and infects most people during their lifetime. It is a double-stranded DNA virus (Herpesviridae). EBV is now historically proven as the first human virus that can cause an oncogenic disease and, as with other herpes viruses, is capable of establishing a latent infection in the host after primary infection. Transmission is mainly via saliva but other body fluids can be involved in viral transmission. The most frequent routes are intimate contact between people, respiratory droplets, and using contaminated personal items (e.g., cups, eating utensils, toothbrushes) [3,4].

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EBV infects mainly oropharyngeal epithelial cells and B lymphocytes after entry into the host. The virus can replicate in epithelial cells and then infect circulating B cells, which may then become latent. Interactions between virus envelope glycoproteins and host cell integrins and complement receptors are involved in viral entry and replication. EBV may remain latent for long periods and become reactivated into the lytic cycle in certain immunological/physiological circumstances [5]. Most EBV infections are asymptomatic, particularly in children, however, symptoms are more common during adolescence/young adulthood. Clinical symptoms may include fever, fatigue, myalgia, skin rash, anorexia, lymphadenopathy and generalized weakness [6–8]. Some of these symptoms are non-specific and overlap with other viral and inflammatory diseases and so laboratory diagnosis is essential. Serological seropositivity for EBV-specific antibodies (viral capsid antigen antibody and nuclear antigen antibody) is commonly used for the diagnosis of past exposure, current infection, and/or potential viral reactivation [9,82].

EBV has been a subject of great scientific interest due to its capability to modulate host immune responses. Various EBV-encoded proteins and non-coding RNAs are able to affect cellular signaling pathways, gene expression and immune surveillance mechanisms. The immunomodulatory properties have led to the speculation that EBV might be a contributing factor in the initiation and/or progression of various immune-mediated and autoimmune disorders. Some diseases that have been studied with regards to EBV include inflammatory bowel disease, diabetes mellitus, multiple sclerosis, systemic lupus erythematosus, celiac disease, rheumatoid arthritis, and juvenile idiopathic arthritis [2,10].

Diabetes mellitus is a metabolically diverse disease in which hyperglycemia is a hallmark due to a defect in the action of insulin, insulin secretion, or both. The disease may be subdivided into type 1 diabetes mellitus, which is mainly of autoimmune origin, and type 2 diabetes mellitus, which is clearly linked to insulin resistance and metabolic dysfunction [83]. Moreover, prediabetes and impaired glucose tolerance are considered intermediate metabolic states, and they are emerging as a significant public health problem. Long-term hyperglycemia causes many systemic complications, such as vascular complications, neuropathy, nephropathy, retinopathy, and impaired immune function.

Notably, diabetes can affect both innate and adaptive immune responses. Hyperglycemia can decrease neutrophil chemotaxis and phagocytosis, modify cytokine production, deplete the activity of lymphocytes and increase oxidative stress. Such immune disorders can lead to heightened susceptibility to infections and can also allow reactivation of latent pathogens such as herpesviruses like EBV [11]. Hence, a study of the presence of EBV infection or reactivation in diabetic patients may yield valuable information regarding the interplay of metabolic dysregulation, immune status, and chronic viral infection.

In Iraq, and internationally, EBV has been well studied in many pathological conditions such as arthritis, thalassemia, diabetes mellitus, breast cancer, nasopharyngeal carcinoma, lymphocytopenia, lymphoma, multiple sclerosis, systemic lupus erythematosus, and hemodialysis associated complications [12-14]. In recent decade, various Iraqi studies have been conducted regarding the possible role of EBV in a range of diseases including arthritis [17], thalassemia [18], diabetes mellitus [19, 20], breast cancer [21], nasopharyngeal carcinoma [22], lymphocytopenia [23] and lymphoma [24] and multiple sclerosis [25]. EBV serological status, however, has not been fully studied in relation to immune profiles in diabetic patients although there are some clues regarding the association of the two. Cytokines are important regulators of inflammation, immune activation and host defense against viral infections. Interleukin-1 and interleukins 6 are pro-inflammatory cytokines that are responsible for the activation and recruitment of immune cells and may play a role in the chronic low-grade inflammation seen in diabetes. Tumor necrosis factor alpha is also highly correlated with insulin resistance, inflammatory signaling and metabolic dysfunction. A critical Th1-type cytokine, interferon gamma (IFN-g) plays a role in antiviral immunity and activation of macrophages. The change in these cytokines can be associated with immune dysregulation, viral reactivation or inflammatory burden of diabetic patients [15,16].

Hence, the current study was aimed at exploring the serological evidence of EBV infection in diabetic patients and to assess certain immune-inflammatory markers in the study population. The purpose of this study was to identify serological markers for EBV, and to quantify serum levels of IL-1, IL-6, TNFa, and interferon gamma. This study may contribute to understanding the possible link between EBV infection and immune changes in diabetes mellitus patients, especially with serological results as well as cytokine profiles in Iraqi diabetic patients which has been limited in publications.

Materials and methods

Study design

The aim of the present study was to compare serological evidence for the presence of EBV infection, in addition to some immune-inflammatory markers, among type 2 DM patients in a laboratory setting. The overall procedure of the study is presented in Figure 1 and was divided into five consecutive steps: **1) ethical approval, 2) recruitment of participants and blood sample collection, 3) serological evaluation of EBV antibody levels, 4) immunological evaluation of cytokine levels, and 5) statistical analyses**.

The study subjects were divided into two groups: the subjects that appeared healthy and had not previously been diagnosed with type 2 diabetes mellitus (control group) and the patients already diagnosed with type 2 diabetes mellitus. All participants had serum samples collected for testing of EBV serological markers and cytokine quantification.

Ethical Approval

This study was approved by the scientific Committee of the Faculty of Veterinary Medicine, University of Wasit, Iraq.

Ethical principles were followed in all procedures involving human subjects. Participants were briefed on the objective of the study before the collection of the sample, and the sample collection was done under sterile conditions by Well-trained personnel.

Sampling

The study population consisted of the residents of the area.

The participants were 90 individuals recruited from private laboratories in Wasit Governorate, Iraq, from February to May 2023. The study subjects were 60 patients with Type 2 Diabetes Mellitus, 30 apparently healthy individuals who were taken as controls.

Venous blood was drawn from each participant with a sterile disposable syringe about 5 mL. Blood samples were placed in sterile plain gel tubes (no anti-coagulant) the scientific clotting and serum separation. All the collected samples were handled with great care to prevent hemolysis, contamination, or repeated freeze–thaw cycles that could affect serological and immunological measurements.

The blood clotted and was then centrifuged at 5,000 rpm for three minutes. The separated serum was then gently aspirated with sterile pipette tips and removed to appropriately labeled Eppendorf tubes. All serum specimens were frozen at −20°C until tested in the laboratory.

Serology for the identification of Epstein–Barr Virus (EBV)

Serological markers of EBV infection were determined by measuring serum levels of EBV specific IgG antibodies By using a commercial human enzyme-linked immunosorbent assay (ELISA) kit (SunLong Biotech, China). Human EBV IgG ELISA Kit was used. No. SL0674Hu. This assay was conducted following the manufacturer's directions.

Briefly, the serum samples, positive controls and negative controls were prepared and put into the corresponding wells of the ELISA microplate. The plate was then incubated for 30 minutes at 37°C to ensure antigen–antibody interaction. After incubation, the wells were washed to remove unbound components. All the wells except the blank well were then filled with horseradish peroxidase-conjugated reagent, and the plate was again incubated at 37°C for 30 minutes.

The second washing step was followed by the introduction of the chromogen solutions A and B in the wells (other than the blank). The plate was incubated for 15 minutes at 37°C for the development of colour. Then, the reaction was terminated by adding stop solution. The OD was determined for each well at 450 nm with a BioTek microplate reader. The blank well was used to calibrate the instrument to zero.

To determine if the samples were EBV IgG-positive or EBV IgG-negative, a manufacturer-recommended cut-off value was calculated. Samples with OD values equal to or higher than the calculated cut-off were considered positive and represented previous exposure or serological evidence of EBV infection. Positive serum samples were used to measure the EBV IgG level to calculate the antibody titer or positivity value.

Immunological Examination

Levels of certain cytokines in serum were measured to assess the immunological and inflammatory state of the study participants. The cytokines measured were interferon gamma, tumour necrosis factor alpha, interleukin 1 and interleukin 6.

The following commercially available human ELISA kits from SunLong Biotech, China were used:

Interleukin-1, IL-1: Cat. No. SL0965Hu, Interleukin-6, IL-6: Cat. No. SL1001Hu_1, Tumour necrosis factor alpha (TNF-α). No. SL1761Hu, Interferon-gamma, IFN-γ: Cat. No. SL0960Hu.

Immunological assays were carried out as per the manufacturer's instructions. To summarize, the following procedure was performed: standards, serum samples and controls were placed in the specific wells of ELISA plates previously coated with specific antibodies against each cytokine. The wells were washed to remove unbound materials after incubation. Enzyme-linked detection reagents were then added, and further incubation and washing steps followed.

Chromogenic substrate solution was then added to each well, and the color developed in proportion to the amount of the target cytokine present in the sample. The reaction was stopped by adding stop solution and absorbance was measured at 450 nm with microplate reader. A standard curve was made for each cytokine using known concentrations of the standards supplied. The concentrations of IL-1, IL-6, TNF-α and IFN-γ in serum samples were determined by the comparison of optical density of the sample and the standard curve. The final cytokine concentrations were expressed according to the units recommended by the manufacturer.

Statistical Analysis

Collected data were statistically analyzed with GraphPad Prism software, version 6.0.1. The Chi-square test was used for categorical variables such as study groups with EBV IgG seropositivity or v. Differences among groups of quantitative data, such as serum cytokine concentrations were analyzed by one-way analysis of variance. All data are presented as means ± SEM. P values <0.05 were defined as statistically significant. To see if any significant differences could be found between diabetic patients and non-diabetic controls in terms of EBV serological status and cytokine levels, statistical analysis was carried out [27].

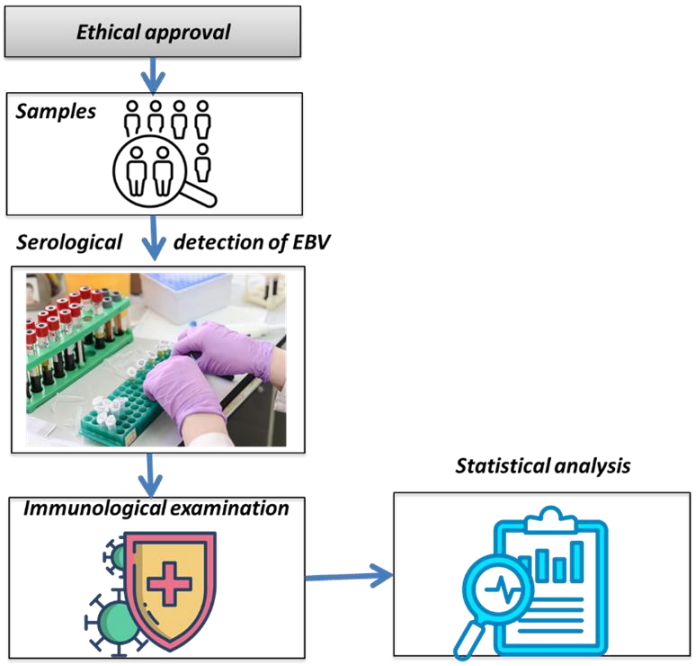

Figure 1: Study workflow schematic. The study involved five major steps: Ethical Approval, Recruitment of Diabetic patients and Healthy control, Collection and Processing of serum samples, Serological detection of serological antibody of EBV (IgG), Measurement of serum Cytokines by ELISA, Statistical analysis of the results obtained.

Results

Table 1: Shows the distribution of the demographic characteristics of the research population.

Variable	Healthy (n = 30)	T2DM (n = 60)	P-value
Age range (years)	38–70	38–70	>0.05
Male, n (%)	15 (50%)	30 (50%)	>0.05
Female, n (%)	15 (50%)	30 (50%)	>0.05

The overall Epstein-Barr virus positivity rate observed in this work was 58.89% (53 / 90) involving 53.33% (16/30) in the healthy population and 61.67% (37 / 60) in the T2DM patients (Figure 2).

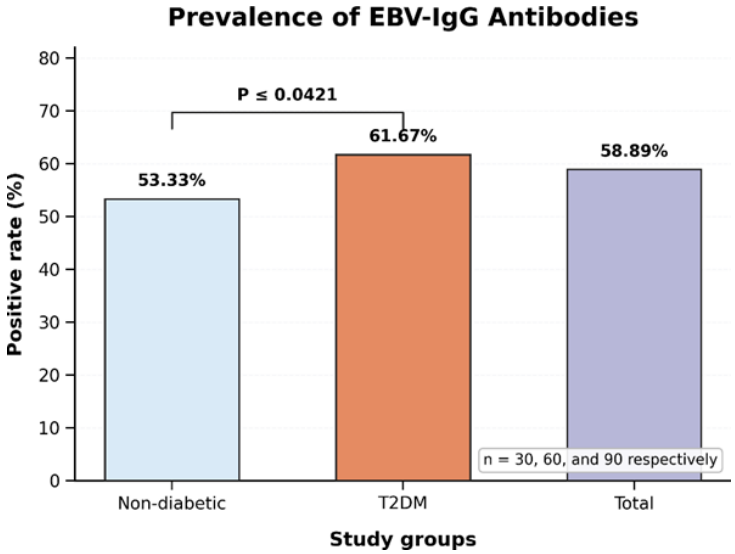

Figure 2: Shows the prevalence range of the EBV-IgG antibodies.

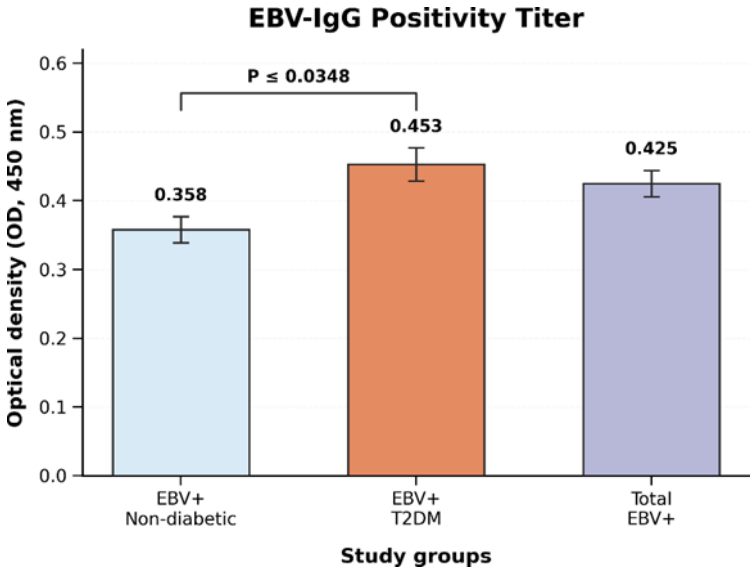

Figure 3: Illustrates the titer of positivity of the EBV-IgG antibodies of this study.

The two age and sex groups of the study were similar. There were participants of both the healthy group and the T2DM group ranging in age from 38 to 70 years. The male and female ratio were equal across the two groups and no significant differences were found between the two groups with respect to the demographic variables.

The presence of EBV seropositivity (IgG antibody) Seropositive was defined as 58.89% (53/90) of the total EBV IgG seropositive. In healthy people, 53.33% (16/30) were EBV-positive and 61.67% (37/60) were EBV IgG-positive in T2DM patients. The mean overall positivity titer of EBV-positive subjects was 0.425 ± 0.019. There was a significantly higher mean positivity titer among the T2DM patient group (0.453 ± 0.024) than among the healthy group (0.358 ± 0.019).

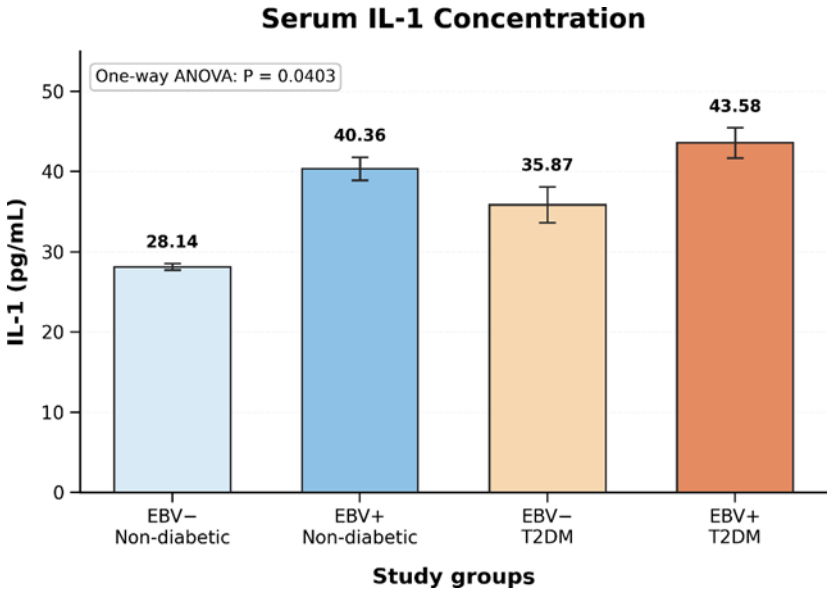

Figure 4: Present the concentration of IL-1 of this study groups.

Immune marker concentrations

Immune markers levels were significantly different from group to group. The levels of IL-1 were significantly elevated in the EBV-positive T2DM patients (43.58 ± 1.90 pg/mL) and EBV-positive healthy participants (40.36 ± 1.45 pg/mL) than in EBV-negative healthy participants (28.14 ± 0.42 pg/mL) and EBV-negative T2DM patients (35.87 ± 2.24 pg/mL).

There was no significant difference between EBV positive healthy participants (70.71 ± 3.02 pg/mL), EBV negative T2DM patients (77.73 ± 2.31 pg/mL) and EBV-positive T2DM patients (76.52 ± 2.31 pg/mL) in the case of IL-6. All 3 groups, however, had significantly higher IL-6 levels than healthy EBV-negative subjects (61.42 ± 0.83 pg/mL).

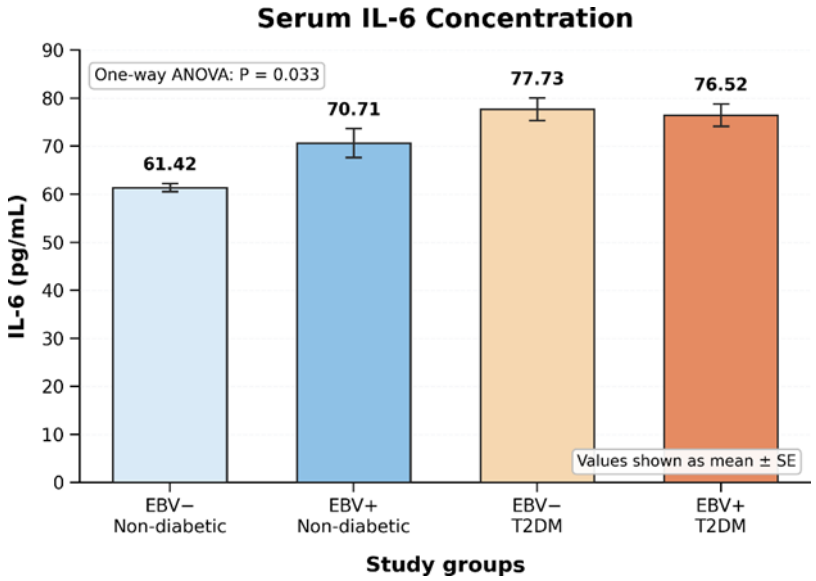

Figure 5: Explain the concentration of IL-6 in the study.

The levels of interferon gamma (IFN-γ) were significantly higher in T2DM patients with EBV compared with healthy individuals without EBV (35.82 ± 1.85 pg/mL) and those with EBV (35.32 ± 1.36 pg/mL) and healthy individuals without EBV (33.54 ± 1.65 pg/mL). The latter three groups did not differ significantly from each other.

EBV-positive T2DM patients had the highest TNF-α concentration (78.69 ± 1.57 pg/mL), while healthy EBV-negative subjects had the lowest (52.64 ± 0.48 pg/mL) TNF-α concentration. EBV-positive healthy participants had intermediate levels (64.81 ± 2.12 pg/mL) while EBV-negative T2DM patients had even higher levels (70.49 ± 3.83 pg/mL).

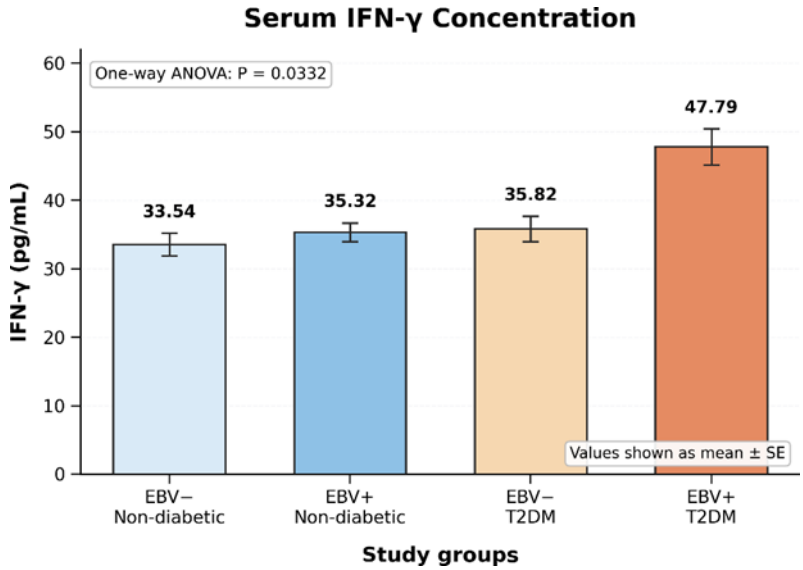

Figure 6: Comparison of serum interferon-gamma (IFN-γ) levels among the four study groups: non-diabetic individuals with negative EBV serology, non-diabetic individuals with positive EBV serology, patients with type 2 diabetes mellitus and negative EBV serology, and patients with type 2 diabetes mellitus and positive EBV serology.

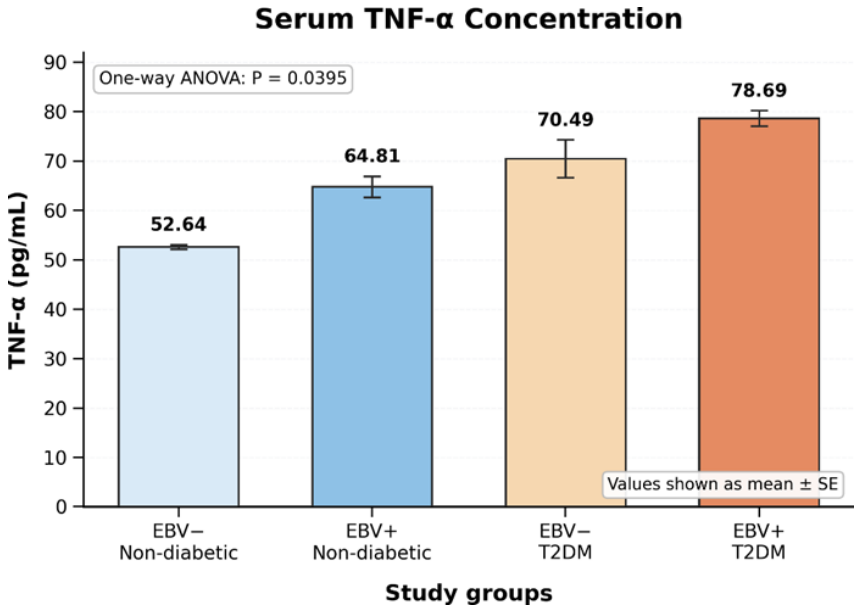

Figure 7: Serum tumor necrosis factor alpha (TNF-α) of four study groups (non-diabetic with negative EBV serology, non-diabetic with positive EBV serology, type 2 diabetes mellitus with negative EBV serology, type 2 diabetes mellitus with positive EBV serology).

Discussion

Over the past few years, there have been huge advances towards identifying Epstein-Barr virus (EBV), which is highly contagious. However, the incidence of infection is very differential by geographical region. The participants of this study were diabetic patients and healthy individuals, and the presence of IgG antibodies against EBV was seen in both groups, though more prevalent in the diabetic group. These findings are consistent with other studies, where researchers reported prevalence rates of 71.1% vs. 30% [28], 22.3% vs. 2.5% [29], and 90% vs. 10% [20]. However, Mohammed and Sabr [19] reported that the numbers of healthy people (57.1%) infected was higher than that of type 2 diabetic people (42.9%).

EBV has been reported as a common virus in Iraq with a marked variation in prevalence. Reported rates include 92.73% in Iraq-Anbar [31], 18.3% in Iraq-Mosul [30], 70.6% in Iraq-Diyala [33], 51.74% in Iraq-Kirkuk [32], 12.38% in Iraq-Erbil [35], and 92.5%–100% in Iraq-Basra [34]. Globally, prevalence rates varied considerably: 45% in Egypt [37], 97.4% in Spain [39], 12.5% in Sweden [36], 47.1% in Ghana [38], 71% in Saudi Arabia [44], 97.9% in Qatar [40], 82% in the Republic of Iran [42], 41.7% in the Republic of Syria [43], 70.3% in **the Kingdom of Bahrain** [41], and 21.4–9% in the Republic of China [45].

Epstein-Barr virus (EBV) has been implicated in a number of diseases. For instance, it has been detected in 83% of lymphocytic leukemia patients and 95% of healthy individuals [46,82] and in 32.3% of the leukemia patients and 28-45% of healthy individuals with breast cancer [37]. The prevalence of EBV infection has ranged from 86% to 97% in cases of lymphoma [48]; 52.73% in periodontitis [49]; 33% in kidney transplant recipients [50]; 12.33% in thalassemia [18]; 85.7% in laryngeal cancer [32]; 48.37% in myeloma compared to 5.16% in the control group [51]; and between 11.66% and 100% in malignant solid lymphomas [34]. EBV infection has been detected in 96.67% of patients with inflammatory bowel disease (IBD) and 57.5% of patients with lymphoma [52,53].

The mean levels of the immune markers were significantly higher in type 2 diabetic patients infected with EBV than in other patient groups, with a statistically significant difference revealed by the current study. In particular, EBV infection correlated with levels of interleukin-1 (IL-1), diabetes with levels of interleukin-6 (IL-6), and EBV infection and diabetes with levels of interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α). Yao et al. [54] identified that latent membrane protein 1 of EBV (EMP1) is able to regulate EBV secretion. It has been reported, however, that Epstein-Barr virus (EBV) binds to the surface of neutrophils and activates macrophages to produce EBV-1, chemokines and inflammatory proteins by Han et al. [55]. In a model of EBV infection in B lymphocytes, Skinner et al. [56] showed that the virus affects the expression of its receptors, which in turn affects the response of infected cells to the EBV-1 genotype and, consequently, the expression of cytokines in the cells. Interleukin-6 (IL-6) is a big inflammatory cytokine produced by endothelial cells or adipocytes or activated leukocytes. While other researchers were unable to activate or stimulate IL-6 proliferation, Shahboun et al. [57] observed that the BV is capable of doing so. Lehner et al. [58] observed the existence of the EBV in the blood of COVID-19 patients, and a strong correlation between the presence of the virus and the presence of IL-6 in the blood of COVID-19 patients, but not in non-COVID-19 patients. However, cross-sectional data have shown that the cytokine, interleukin-6 (IL-6), is physiologically sensitive and is associated with the development of type 2 diabetes, insulin resistance, and hyperglycemia [61, 62, 63]. Moreover, IL-6 also has an immunomodulatory function that works on neuroendocrine cells,

pancreatic beta cells and skeletal muscle cells, thus influencing hematologic homeostasis and glucose metabolism [64-66].

Interferon-gamma (INF-γ) is an antiviral protein that was discovered even before the discovery of more complex cytokines [67]. Concerning Epstein-Barr virus infection, Akai et al. [68] reported their discovery of the IL28B gene (encoding INF-γ) and its association with Epstein-Barr virus, concluding that INF-γ is involved in better control of Epstein-Barr virus, particularly during the proliferation of lymphocytes. In an earlier study, Jiang et al. [69] have reported that pancreas of newly diagnosed type 1 diabetic patients displayed significantly elevated levels of interferon-gamma (INF) as compared with healthy individuals. Interferon-gamma (INF-γ) has been shown to be involved in the destruction of beta cells and the development of type 1 diabetes by Dittmer et al. [70]. In other studies, mice lacking INF-γ did not develop diabetes [71] and immune responses to viral infection such as production of INF-γ and interleukin-7 (IL-7), exacerbated the disease [72].

Tumor necrosis factor (TNF) is an important cytokine involved in both physiological and pathological functions [73, 74]. Impaired neutralizing antibody responses to acute Epstein-Barr virus (EBV) infection have been shown to be associated with generalized beta-cell dysfunction [75]. Additionally, patients suffering from chronic active Epstein-Barr virus (EBV) infection have been reported to have high levels of tumor necrosis factor-alpha (TNF-α) and interferon gamma (INF-γ) compared to patients with alternative types of infection, indicating a genetic predisposition towards more severe EBV disease. Kay et al. have demonstrated that glycoprotein 110 on the EBV envelope is necessary for viral fusion and is able to inhibit activation of TNF-γ [77]. Moreover, some researchers [s] have reported the potential role of TNF-α, either singly or in association with IL-1, in the progression of FLD, diabetic nephropathy, and polyneuropathy.

Overall, the results suggest that EBV seropositivity might be related to increased inflammatory responses in T2DM patients. EBV seropositivity may not differentiate between latent infection and reactivation of the virus, however, further studies are necessary to clarify the clinical significance of seropositivity in diabetic patients.

Conclusion

Based on the results of this study, there is a potential relationship between infection with Epstein-Barr virus (EBV) and diabetes, which indicates that EBV may be another cause of inflammation in severely ill diabetic patients. By immunological analysis, the two cytokines were found to be linked with EBV infection (interleukin-1, IL-1) and type 2 diabetes (interleukin-6, IL-6). In addition, the level of both tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) are increased in the presence of both EBV infection and type 2 diabetes. The pattern underscores the intricate relationship between viral infections and chronic metabolic diseases, such as the potential for shared inflammatory pathways that can affect disease course and severity.

A pathophysiological view is that the production of pro-inflammatory cytokines by type 2 diabetic patients with Epstein-Barr virus (EBV) infection may worsen systemic inflammation which can result in poor glycemic control, accelerated beta cell damage and heightened risk for diabetes-associated complications. These results are corroborated by earlier studies showing that chronic viral infections can lead to chronic immune activation that is responsible for other health challenges and metabolic disturbances. However, known if EBV infection is just a consequence of immune system deficiencies in diabetic patients, or if it actually causes or contributes to general inflammation. To clarify this, future long-term, mechanistic studies should be conducted to extensively explore the time course differences in EBV viral load, immune cell characteristics and cytokine network differences in diabetic patients. The importance of Epstein-Barr virus (EBV) in the diabetic inflammatory milieu could have significant clinical implications and would inform the development of anti-viral or immunomodulatory therapies to decrease the inflammation-related complications of diabetes. To conclude, this study suggests a potential association between EBV and type 2 diabetes, but further, large-scale, multicenter studies are required to clarify the exact mechanisms and implications of this association. Longitudinal and mechanistic studies could be performed to assess EBV viral load, EBV IgM, EBNA-1 antibodies, immune-cell phenotypes and changes in the cytokine network over time. Additionally, larger multicenter studies are warranted to validate these findings and to see if there is any diagnostic, prognostic, or therapeutic relevance of EBV-related immune activation in T2DM.

The EBV IgG immunoglobulin was the only measurement used in our study and it indicates only past exposure, and not whether the active virus is a latent infection or reactivation. Other markers, including IgM and EBNA-1, might be better indicators of characterization. In addition, this study is limited because it did not use ROC analysis.

Author Contributions

BAM: Study design, blood sample collection and statistical analysis.

IME: Serological testing of the samples collected for the presence of EBV IgG antibodies.

HAJG: measured the level of the IL-1, IL-6, TNF-α, and IFN-γ. The manuscript was read and approved by all the authors.

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Competing Interest

The authors declare that they have no competing interests.

Data Availability

There are no data that have been generated and/or analyzed during this study not included in the manuscript.

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