Sawa Medical Journal

Reprint from

Vol.1 Issue.2 2025

Sawa Medical Journal

Serological and Immunological Investigation of Epstein Barr Virus Disease in Diabetic Patients

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Abstract

Background and aims: Epstein-Barr virus (EBV) is one of the most common human viruses in the world. The potential role of EBV in increasing the risk of autoimmune diseases as diabetes mellitus (DM) has greatly interested in recent years. This study aims to detection serological prevalence of EBV in diabetic and non-diabetic individuals with measurment the levels of some immune markers in study population.

Materials and methods: A total of 90 individuals; 30 healthy and 60 diabetic patients, were selected and subjected for blood sampling. Qualitative ELISA was used to detect EBV IgG-antibodies; whereas, quantitative ELISA's kits were served to measurement of immune markers.

Results: In this study, the total positive rate of EBV was 58.89% involving 53.33% in the non-diabetic and 61.67% in the diabetic patients. Additionally, 0.425 ± 0.019 was the titer of positivity in positive study population; however, significant higher value was seen in diabetic than non-diabetics. Concerning immune markers, significant elevation in IL-1 was showed in positive EBV-diabetic and positive EBV-non-diabetic patients when compared to negative EBV-non-diabetic and negative diabetic individuals. For IL-6, insignificant differences were detected between positive EBV-non-diabetic, negative EBV-diabetic and positive EBV-diabetic patients; however, values of these groups were higher than values of negative EBV-non-diabetic individuals. Significantly, elevation in values of TNF-α was identified in positive EBV-diabetic patients while lowering was seen in negative EBV-non-diabetic individuals when compared to positive EBV-non-diabetic and negative EBV-diabetic patients. The INF-γ was elevated significantly in positive EBV-diabetic patients when compared to those of negative EBV-non-diabetic, positive EBV-non-diabetic and negative EBV-diabetic groups that showed insignificant variation between their values.

Conclusion: This study suggests the possible relative association between EBV and DM. The findings of immune markers showed that the IL-1 and IL-6 were related to EBV and DM, respectively; while, TNF-α and INF-γ were related to existence of both EBV and DM infections.

Keywords: EBV, Diabetes mellitus, Interleukin, Tumor-necrosis factor, Interferon, Iraq

Introduction

Epstein-Barr virus (EBV) is one of the most common human viruses in the world, which belongs to the Herpes family, a double stranded DNA virus, and considers as the first identified oncogenic virus which establish permanent infection in humans [1, 2]. The virus spreads primarily by saliva and body fluids throughout kissing, coughing and sneezing, sexual contact, sharing of different objectives such as cup, utensils and toothbrushes [3, 4]. Post entry the body, EBV replicates within the B-cells and epithelial cells either by interacts with the cellular integrins or undergoes the lytic cycle [5]. Although, most infections don't cause symptoms, certain cases especially in adolescents and young adults can trigger EBV to result in different illnesses such as weakness, sore muscles, rash, inappetence, fever and fatigue [6-8]. Therefore, it can be challenging to diagnose the virus based on symptoms, and the best choice for confirming diagnosis is the testing of blood to detect specific EBV-antibodies [9]. Also, the viral protein could activate the genes related to autoimmunity or autoimmune diseases like celiac disease, multiple sclerosis, systemic lupus, rheumatoid arthritis, juvenile idiopathic arthritis, inflammatory bowel disease and diabetes mellitus [2, 10].

Diabetes mellitus (DM) is a heterogeneous multifactorial disease with a definite link to modern lifestyle, which typified by an elevation of blood glucose levels and cascading systemic complications [11]. Etiologically, DM is classified mainly to Type-1 and Type-2 with increasing prevalence of the pre-diabetic state due to impairment in glucose tolerance and fasting glucose [12-14]. Further, DM increases the risk of several diseases of diverse origin as a result of a compromised immune response, with a definite predisposition to intercurrent infection and activation of latent infections secondary to altered immune function [15, 16].

In last decade, many Iraqi studies were done to identify the possible association of EBV to different diseases such as arthritis [17], thalasemia [18], DM [19, 20], breast cancer [21], nasopharyngeal carcinoma [22], lymphocytopenia [23], lymphoma [24], sclerosis [25], lupus erythematosus and hemodialysis cases [26]. However, no available online data were found about the possible relationship of EBV to immune status among the DM patients. Hence, this study aims for serological detection of EBV in diabetic patients and estimate the levels of interleukins (IL-1 and IL-6) tumor-necrosis factor-alpha (TNF-α), and interferon-gamma (INF-γ) in study population.

Materials and methods

Ethical approval

This study was licensed by the Scientific Committees of the College of Basic Education (Al-Muthanna University) and the College of Health and Medical Technology (Sawa University).

Samples

A total of 90 individuals; 30 healthy and 60 diabetic patients, attended to the private laboratories in Samawah city (Al-Muthanna province, Iraq) during February-May (2023) were selected. Under aseptic conditions, 5 ml of venous blood was drained using a disposable syringe into free-anticoagulant glass-gel tubes that centrifuged at 5000 rpm for 3 minutes, and the obtained serum of each individual was kept into labeled Eppendorf tube and kept frozen (4°C) until be tested.

Serological detection of EBV

Following the manufacturer instructions (SunLong Biotech, China) of Human EBV-IgG ELISA Kit (Cat. No. SL0674Hu), the sera, negative and positive controls were prepared, diluted, added to their specific wells in the plate and incubated at 37°C for 30 minutes. After washing and drying for 5 times, HRP-Conjugate was added to all wells except Blank and the plate was incubated at 37°C for 30 minutes to be washed and dried later for 5 times. After adding both Chromogen A and B to all wells except Blank, the plate was incubated at 37°C for 15 minutes. Finally, the Stop solution was added to all wells of the plate, and the optical density (OD) was read using the Microplate Photometer Reader (BioTek, USA) at an absorbance of 450 nm. After setting the OD value of the Blank well to zero, the test

effectiveness was determined, and the critical value (CUT OFF) was calculated to estimate the negative and positive judgments. The positive ODs were recorded at ≥234, and the OD values of positive EBV individuals were considered as a titer of positivity.

Immunological examination

Based on the manufacturer instructions (SunLong Biotech, China) of the IL-1 (Cat. No. SL0965Hu), IL-6 (Cat. No. SL1001Hu_1), TNF-α (Cat. No. SL1761Hu) and INF-γ (Cat. No. SL0960Hu) Quantitative ELISA Kits, the sera and Standard of each kit was prepared, processed and read at an absorbance of 450 nm. The concentrations of each marker were calculated in the study samples by plotting the ODs of sera as well as the ODs and concentrations of Standards in the standard curve.

Statistical analysis

The Chi-square (x^2) and One-Way ANOVA in the GraphPad Prism Software *version* 6.0.1 (GraphPad Software Inc, USA) were used to estimate significant differences between values (Mean ± Standard Errors) of study groups at P<0.05 [27].

Results

In this study, the total positive rate of EBV was 58.89% (53 / 90) involving 53.33% (16/30) in the non-diabetic and 61.67% (37.67%) in the diabetic patients (Figure 1).

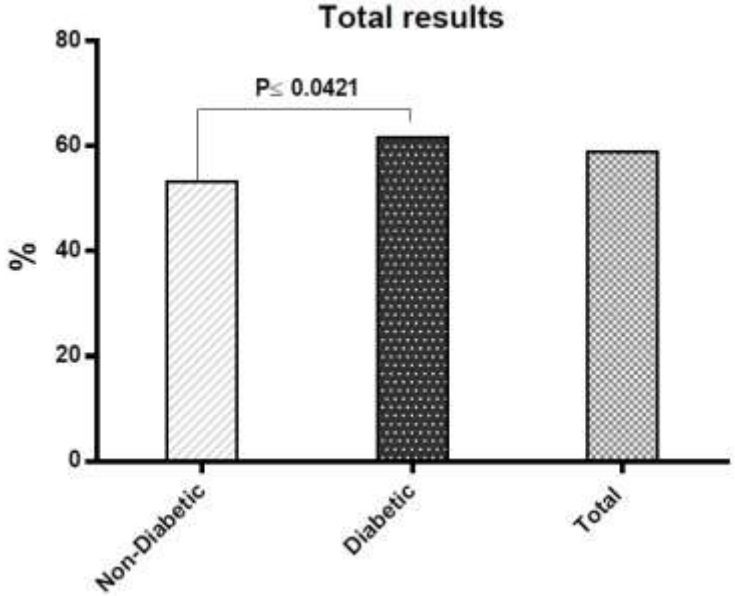

Figure (1): Prevalence rate of EBV-IgG antibodies among study population (Total No: 90)

Additionally, 0.425 ± 0.019 was the titer of positivity reported among the positive study population; however, significant higher value was detected in diabetic (0.453 ± 0.024) than non-diabetic (0.358 ± 0.019) individuals (Figure 2).

Concerning immune markers, the findings were varied significantly (P<0.05) between values of study groups. Significantly (P<0.0403), the higher values of IL-1 was showed in positive EBV-diabetic (43.58 ± 1.9 pg/ml) and positive EBV-non-diabetic (40.36 ± 1.45 pg/ml) patients than those of negative EBV-non-diabetic (28.14 ± 0.42 pg/ml) and negative diabetic (35.87 ± 2.24 pg/ml) individuals (Figure 3).

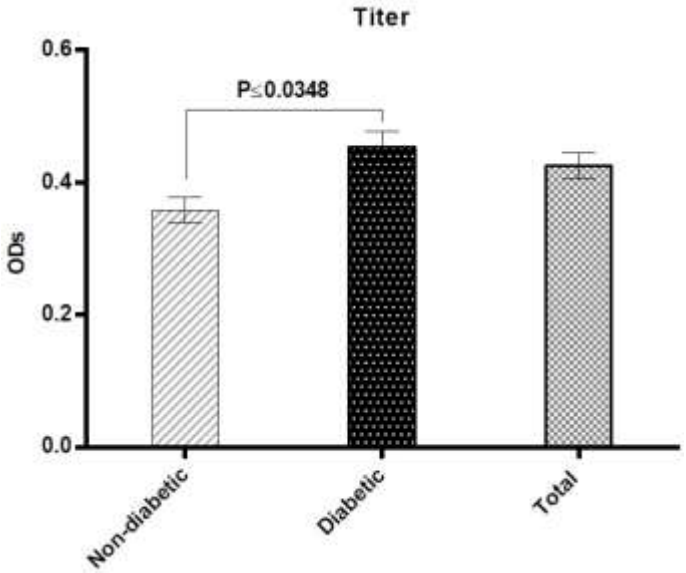

Figure (2): Titer of positivity of EBV-IgG antibodies among study population (Total No: 90)

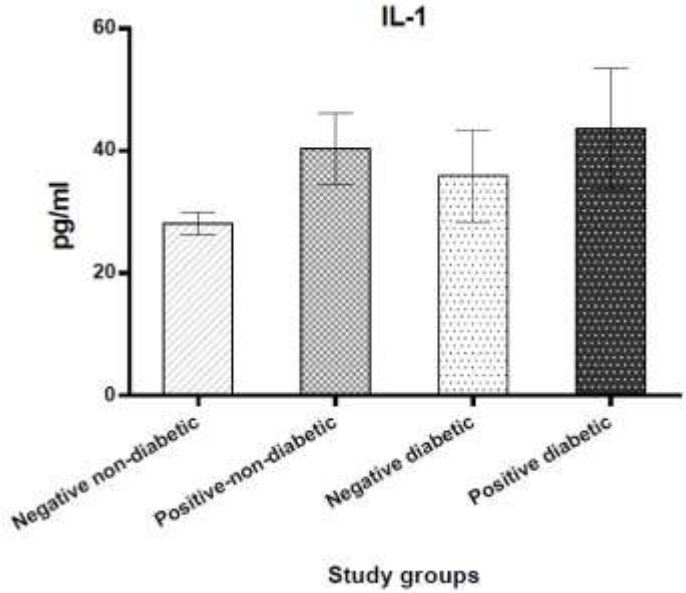

Figure (3): Concentration of IL-1 among the study groups; negative EBV-non-diabetes, positive EBV-non-diabetes, negative EBV-diabetes and positive EBV-diabetic patients

For IL-6, though insignificant difference (P>0.05) was seen between positive EBV-non-diabetic (70.71 ± 3.02 pg/ml), negative EBV-diabetic (77.73 ± 2.31 pg/ml) and positive EBV-diabetic (76.52 ± 2.31 pg/ml) patients, the findings of these groups were significantly (P<0.033) higher than detected in negative EBV-non-diabetic individuals (61.42 ± 0.83 pg/ml), (Figure 4).

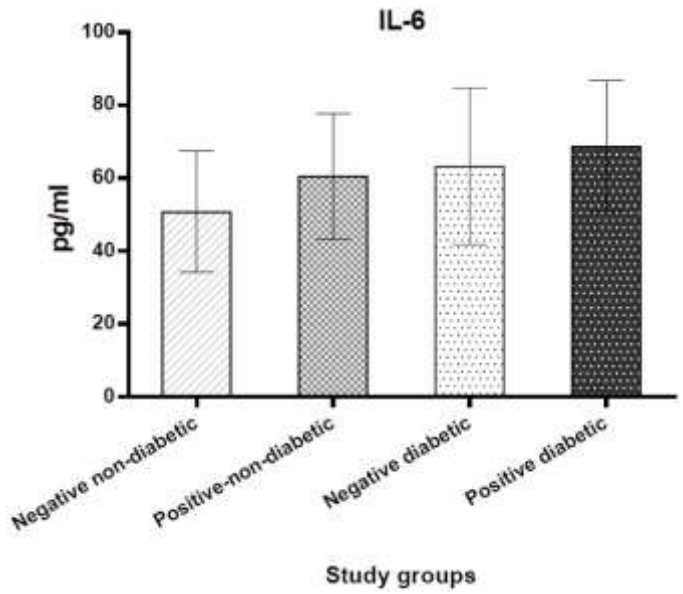

Figure (4): Concentration of IL-6 among the study groups; negative EBV-non-diabetes, positive EBV-non-diabetes, negative EBV-diabetes and positive EBV-diabetic patients

For INF-γ, significant increases (P<0.0332) were reported in positive EBV-diabetic patients (47.79 ± 2.64 pg/ml) when compared to values of negative EBV-non-diabetic (33.54 ± 1.65 pg/ml), positive EBV-non-diabetic (35.32 ± 1.36 pg/ml) and negative EBV-diabetic (35.82 ± 1.85 pg/ml) groups that showed insignificant variation (P>0.05) between their values (Figure 5).

Significant elevation in concentration of TNF-α (P<0.0395) was observed in positive EBV-diabetic patients (78.69 ± 1.57 pg/ml) while significant lowering was seen in negative EBV-non-diabetic individuals (52.64 ± 0.48 pg/ml) in comparison with those of positive EBV-non-diabetic (64.81 ± 2.12 pg/ml) and negative EBV-diabetic (70.49 ± 3.83 pg/ml) patients (Figure 6).

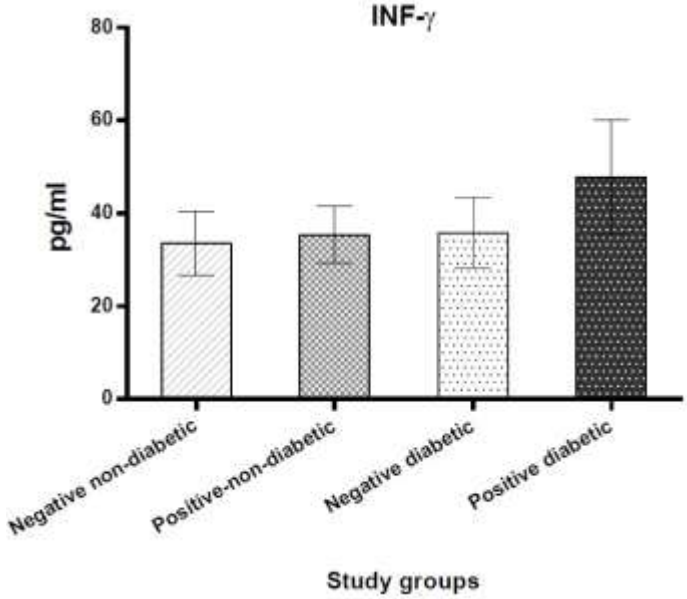

Figure (5): Concentration of INF-γ among the study groups; negative EBV-non-diabetes, positive EBV-non-diabetes, negative EBV-diabetes and positive EBV-diabetic patients

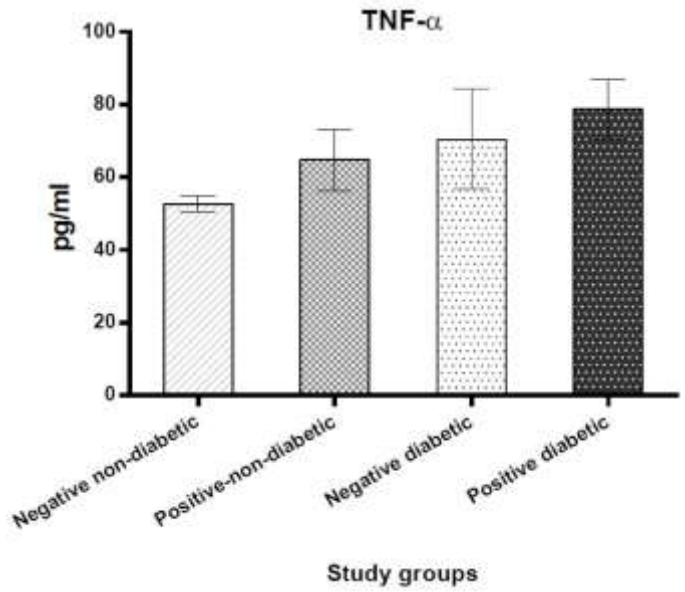

Figure (6): Concentration of TNF-α among the study groups; negative EBV-non-diabetes, positive EBV-non-diabetes, negative EBV-diabetes and positive EBV-diabetic patients

Discussion

In last decade, advances in diagnostic assays have critically expanded our understanding of global patterns of EBV since the virus have a worldwide distribution with a remarkable geographic variation between countries concerning the incidence and prevalence rates. In this study, though EBV-IgG antibodies were detected in both non-diabetic and diabetic individuals, the EBV-IgG antibodies were more prevalent in diabetic patients than non-diabetics. These findings were agreed those detected by other researchers; 71.1% and 30% [28], 22.3% and 2.5% [29], and 90% and 10% [20], The EBV prevalence rates in patients with diabetes and diabetes-related complications have not been studied as thoroughly as those located within the general population. The findings of this research appear to differ from previous studies. For example, Mohammed et al. reported a prevalence rate of 42.9% among diabetic patients and 57.1% for non-diabetic patients, respectively. Similarly, various prevalence rates for different regions have been documented: 18.3% in Mosel, 92.73% in Anbar, 51.74% in Kirkuk, 70.6% in Diyala, 92.5-100% in Basrah, and 12.38% in Erbil. While international prevalence rates have also varied, the largest population-wide study conducted to date found 12.5% among Swedes, 45% among Egyptians, 47.1% among Ghanaians, 97.4% among Spaniards, 97.9% among Qataris, 70.3% among Bahrainis, 82% among Iranians, 41.7% among Syrians, 71% among Saudis and 21.4-9% in China. Additionally, the EBV rate is also increased among patients with the following conditions: lymphoblastic leukemia (83% vs 95% among controls) [46]; leukemic patients (32.3% vs 2.3% among controls) [47]; breast cancer (28% to 45%) [37]; lymphomas (86% to 97%) [48]; periodontitis (52.73%) [49]; renal transplant recipients (33%) [50]; thalassemia (12.33%) [18]; laryngeal carcinoma (85.7%) [32]; multiple myeloma (48.37% vs 5.16% in controls) [51]; other forms of malignant lymph node tumors (11.66% to 100%) [34]; inflammatory bowel disease (96.67%) [52]; and lymphomas (57.5%). [53].

The results of immune markers measured in our study revealed that studied markers were elevated significantly in positive EBV-diabetic patients with existence of remarkable elevation between study groups as IL-1 was related to EBV, and IL-6 to DM; while, TNF-α and INF-γ were associated with both EBV and DM infections. Yao et al. [54] mentioned that the EBV latent membrane protein 1 is capable to regulate the secretion of IL-1. Han et al. [55] mentioned that EBV was shown to bind to the neutrophil surface and subsequently stimulate yielding of IL-1, chemokines and macrophage inflammatory protein. Skinner et al. [56] demonstrated that EBV down regulates IL-1 receptors, and then, altering response to

IL-1 stimuli with changing the levels of cytokine expression within infected cells.
IL-6 is a major proinflammatory cytokine which produced by endothelial cells, adipocyte and activated leukocytes. In comparison to other researchers, Chehboun et al. [57] observed that EBV can activate and induce the proliferation of IL-6; while, Lehner et al. [58] reported a significant correlation between EBV variemia and IL-6 level in COVID-19 patient but not in non-COVID-19 patients. However, cross-sectional data suggest that IL-6 is physiologically sensitive marker [26, 59, 60], and is related to overt type-2 DM, insulin resistance and hyperglycemia [61-63]. In addition, IL-6 has an immunoregulatory action to influence hemostasis and metabolism of glucose through its activity on neuroendocrine cells, pancreatic β-cells and skeletal muscle cells [64-66].
INF-γ was detected as an antiviral factor which preceded the more complexity by which other cytokines were discovered [67]. Akay et al. [68] determine the association of IL28B gene, encodes INF-γ, and development of variemia in EBV, and concluded the role of INF-γ in better control of EBV in particular during lymphoproliferation. In a previous study, Jiang et al. [69] reported that the pancreas taken from the patients with recent-onset type-1 DM contain significantly more INF that the samples taken from non-diabetic patients. Dettmer et al. [70] referred to the role of INF-γ in destruction of β-cells and development of insulin-dependent DM. Other studies showed that mice lacking INF-γ were unable to develop DM [71], and immune response to viral infection was included the higher production of INF-γ and IL-7 which exacerbated the disease [72].
TNF is a critical cytokine which contributes to both physiological and pathological processes [73, 74]. The impaired EBV-specific neutralizing antibody response during acute infections was showed to coincident with global B-cell dysfunction [75]. Cohen et al. [76] demonstrated that the levels of TNF-α and INF-γ were higher in chronically active EBV patients than in healthy controls suggesting that certain genetic disorders are associated with severe EBV disease but not with other infections. Cai et al. [77] found that the EBV envelope glycoprotein 110 plays an essential role in viral fusion by inhibiting the TNF-α mediated NF-kB promoter activity and antagonizing the host's innate immune response. The high levels of TNF-α might act a key component for development of insulin resistance and pathogenesis of type-2 DM [78]. Also, the possible role of TNF-α alone or with IL-1 in development of fatty liver, diabetic nephropathy and polyneuropathy has been recorded by several authors [79-81].

Conclusion

This study suggests the possible relative association between EBV and DM, and speculates that EBV acts as an additional inflammatory agents trigger in critically ill DM patients. The findings of immune markers showed that the IL-1 and IL-6 were related to EBV and DM, respectively; while, TNF-α and INF-γ were related to existence of both EBV and DM infections. However, it remains to elucidate whether EBV represents an epiphenomenon in DM or plays a pathogenetics role as additional trigger of a systemic inflammatory response in this setting.

Author's contribution

TSA: Collection of blood samples, serology to detect the prevalence rate of EBV-IgG antibodies, and statistical analysis of obtained data. ZHM: Measurement of immune markers; IL-1, IL-6, TNF-α and INF-γ. Both authors were completed and approved the final copy of the manuscript.

Acknowledgement

Special thanks to the specialists who contributed in collection of blood samples and providing of the required data.

Funding

No external funds were received (private funding).

Competing interest

The authors declare that they have no competing interests.

Data availability
All obtained data were involved in this study.

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