ARAŞTIRMA MAKALESİ / Research Article

Potential Contribution to Antidiabetic Treatments of Epstein-Barr Virus Proteins LMP1 and EBNA1

Epstein-Barr Virus Proteinleri LMP1 ve EBNA1'in Antidiyabetik Tedavilere Potensiyel Katkısı

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Geliş Tarihi / Received : **26.09.2018** Kabul Tarihi / Accepted : **14.11.2018**

Şen M., Altındiş M., Şen S., Çelik S., Altunbaş K., Ciğerci İH.
Potential Contribution to Antidiabetic Treatments of Epstein-Barr Virus Proteins LMP1 and EBNA1
J Biotechnol and Strategic Health Res. 2018;2(3):148-155.

Abstract

The prevalence and incidence of diabetes is a global healthcare-management problem that is increasing almost every day. The high numbers of complications make the diagnosis and treatment of diabetes extremely important. Nowadays, it is believed that a large number of diseases, including diabetes, are related to the Endoplasmic Reticulum (ER) stress-induced apoptosis. Proliferative activity of Epstein-Barr Virus (EBV) proteins occurs via two mediators Epstein-Barr Nuclear Antigen-1 (EBNA1) and Latent Membrane Protein-1 (LMP1). In this study, it was aimed to investigate the reversal of pancreatic cell loss by applying recombinant viral EBV proteins. It has also been investigated how related proteins can effect DNA damage, ER stress, and contribute to insulin synthesis-release. For these targets, it has been decided to use doses of 0.1 ppm and 0.5 ppm of commercially obtained recombinant EBNA1 and LMP1 proteins. The possible effects of these proteins on mRNA expression levels of genes known to be responsible for the specified proliferation have been investigated. In the study, DNA damage was analyzed by Comet Assay, and Endoplasmic Reticulum stress was analyzed by Real Time RT-PCR. In RT-PCR studies, mRNA expression levels of cell proliferation markers including PDX 1, WNT 4, FoxO1, TCF7L2 and beta catenin genes in the EBNA1 group were increased by 6.48, 4.25, 2.37, 3.8 and 1.4 times, respectively compared with control group. At the end of the study; EBV recombinant proteins LMP1 and EBNA1 have been shown to increase the expression levels of genes responsible for proliferation. It has also been shown that related proteins may be advantageous in reversing beta cell losses by suppressing the expression levels of ER stress-inducing genes. It has been thought that the use of viro-therapeutic agents in antidiabetic treatments may be appropriate if supported by higher-level studies in this regard.

Keywords Epstein Barr Virus, EBNA1, LMP1, Diabetes, Endoplasmic Reticulum Stress

Özet

Diyabet prevalansı ve insidansı her geçen gün artmaya devam eden global bir sağlık problemidir. Diyabetik komplikasyonların sıklığı diyabet tanı ve tedavisini önemli kılmaktadır. Günümüzde, diyabet de dahil olmak üzere çok sayıda hastalığın Endoplazmik Retikülüm (ER) stres kaynaklı apoptoz ile ilişkili olduğuna inanılmaktadır. Epstein-Barr virusun proliferatif etkinliği; Epstein-Barr Nuclear Antigen-1 (EBNA1) ve Latent Membrane Protein-1 (LMP1) adlı iki mediatör protein aracılığıyla olmaktadır. Bu çalışmada; pankreatik beta hücre kayıplarının bertarafında iligili EBV proteinlerinin kullanılması amaçlanmıştır. Ayrıca ilgili proteinlerin ER stresi, insulin sentez ve salınımı ve DNA hasarı üzerine etkilerinin araştırılması hedeflenmiştir. Bu hedefler için, 0,1 ppm ve 0,5 ppm dozlarında ticari olarak elde edilen rekombinant EBNA1 ve LMP1 proteinleri kullanılmıştır. Bu proteinlerin proliferasyondan sorumlu olduğu bilinen genlerin mRNA ekspresyon seviyeleri üzerindeki olası etkileri araştırılmıştır. Çalışmada DNA hasarı Comet Assay ile, proliferasyon düzeyleri Real Time RT-PCR ile analiz edilmiştir. RT-PCR çalışmasında, hücre proliferasyonundan sorumlu oldukları bilinen PDX 1,WNT 4, FoxO1, TCF7L2 ve beta katenin genlerinin mRNA ekpresyon düzeyleri control grubana nazaran EBNA 1 grubunda sırasıyla 6.48, 4.25, 2.37, 3.8 ve 1.4 kat arttığı tespit edilmiştir. Aynı zamanda ilgili proteinlerin ER stres kaynaklı genlerin ekspresyon seviyelerini bastırarak beta hücre kayıplarını tersine çevirmek açısından avantajlı olabileceği gösterilmiştir. Bu proteinlerin antidiyabetik tedavilerde viro-terapötik ajan olarak kullanılması bu konuda daha üst düzey çalışmalarla desteklenmeye muhtaçtır.

Anahtar Kelimeler Epstein Barr Virus, EBNA1, LMP1, Diyabet, Endoplasmik RetikulumV

Introduction

The prevalence of diabetes in adult (20-79 years) population around the world was calculated as 6.6% by the year of 2010, and this ratio is estimated to be 7.8% by an increase of 18% in 2030[1-3].

In a study carried out in Germany, it was determined that diabetic individuals applied to polyclinics an average of 13.5 times and non-diabetic individuals applied to polyclinics an average of 7.1 times in 2010. While the average duration of hospital stay was 4.8 days for all diabetics, it was calculated as 1.6 days for people without diabetes; and it was reported that the annual expense was 4377 euro for each patient with type 2 diabetes and the annual expenses of all patients with diabetes were 3.3 million euro[1]. In studies carried out, the annual health spending per capita due to diabetes was calculated as 572 US dollar for Turkey, the cost was reported to increase approximately 4-fold in complicated patients with diabetes. In this context, the importance of eliminating the treatment of diabetes and its complications is increasing with each passing day[2-4].

Epstein-Barr Virus (EBV) belongs to Gamma-1 herpesvirus or lymphocryptovirus family. Among the viruses in lymphocryptovirus family, only EBV causes infection in people[5]. EBV leads to infections that may cause lytic, persistent, latent and transformation as the other members of lymphocryptovirus genus to which it belongs[6]. When lymphocryptoviruses cause latent infection in B lymphocytes, and this event results in the production of latent gene products that contribute to the cell proliferation and transformation process[7]. In this study, Epstein Bar Nuclear Antigen 1 (EBNA1) and Latent Membrane Protein 1 (LMP1) were preferred as the EBV recombinant proteins to be applied to the INS1E (832/13) beta cell culture. The reasons of this preference are as follows;

EBNA1 is a DNA-binding protein which is required for the episomal replication, protection and continuity of EBV genome[7,8]. EBNA1 is required for the persistence of the virus in the cell, and all cells infected with EBV necessarily synthesize EBNA19. In a study carried out, it was demonstrated that EBNA1 expression resulted in B-cell proliferation in B-cells in transgenic mice. This gives rise to thought that EBNA1 has a direct role in oncogenesist[10]. EBNA1 plays a key role in the regulation of EBV latent gene expression and is the only viral protein required for EBV plasmid replication[11].

In various studies showing the proliferative activity of LMP1, it was observed that it led to the increase of cell surface adhesion molecules10, it prevented B lymphocytes infected with EBV from the apoptosist by increasing the production of cellular oncogene bcl-27, some of the fibroblast sequences that do not produce tumors normally became tumor cell in the transfer of the latent membrane protein gene into fibroblasts7. In vitro studies show that it is necessary in terms of the continuation of proliferation and immortality in B lymphocytes[12].

II. Materıals and Method

A. Cell Culture

INS-1E (832/13) beta cell culture was used in the study. These cells are the rat beta cells that synthesize insulin when they are stimulated with glucose. INS-1E (832/13) cell line was incubated in an incubator containing 5% CO2 and 95% air at 37°C RPMI 1640 medium. The experimental groups were formed after obtaining a sufficient number of cells. The groups were designed as 11

groups, these are; group C: Control, group DC: DMSO control, group T: treatment by thapsigargin group ET 0,1: treatment by 0,1 ppm EBNA1 protein and thapsigargin, group LT 0,1: treatment by 0,1 ppm LMP1 protein and thapsigargin, group ET 0,5: treatment by 0,5 ppm EBNA1 protein and thapsigargin, group LT 0,5: treatment by 0,5 ppm LMP1 protein and thapsigargin, group E 0,1: treatment by 0,1 ppm EBNA1 protein, group L 0,1: treatment by 0,1 ppm LMP1 protein, group E 0,5: treatment by 0,5 ppm EBNA1 protein and group L 0,5: treatment by 0,5 ppm LMP1 protein.

B. The formation of experimental ER stress model in cell culture

The cells that were proliferated in flask were firstly passaged, and the cells that were confluented again at the end of 4th day were washed with PBS and treated with thapsigargin (in DMSO) (PubChem CID: 446378) for 18 hours to form ER stress. The same amount of solvent (DMSO) was administered to the control group.

C. EBV recombinant proteins application of EBNA1 and LMP1

After passaging made following the proliferations of the cells, EBNA1 and LMP1 recombinant viral proteins were applied to the cell culture in 0.1 ppm and 0.5 ppm concentrations.

D. Glucose Stimulated Insulin Secretion (GSIS) Analysis

The secreted insulin and cellular insulin measurements were made by photometric method using specific ELISA kit (Millipore, Rat/Mouse Insulin ELISA kit Cat. EZRMI-13K).

E. The protein measurement in cell lysates

The cells to which necessary procedures were made according to the test protocols were treated with lysis buffer and centrifuged, and supernatant was obtained. The protein levels that supernatants contained were measured by the Bradford method in which bovine serum albumin was used as standard[13].

F. RNA isolation and Real Time QPCR analyses

The total RNAs to be used in mRNA expression analyses that would be performed by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) method were prepared using ready commercial kit (Norgen). The degree of purity and quantitation of the obtained RNA isolate were determined with the help of Nanodrop, and RNS the OD260/280 ratio of which was in the range of 1.7-2.1 were used in cDNA synthesis. cDNA synthesis was performed in accordance with the kit instruction using First Strand cDNA Synthesis kit (Thermo Scientific). Real Time PCR analysis was performed using a Strategene Mx3005P QPCR system (USA), and the expression level of the target gene was normalized with the control gene (-actin). The gene expression level was determined by Ct method. Each measurement was studied as triplicate. The primer sequences and PCR conditions used in PCR reactions are given in Table 1.

Table 1. Oligonucleotide primer sequences and PCR programs

Genes	Primer sequences	PCR programs	Cy
p53	F-5 cggaggtcgtgagacgctg 3	94o-1 min/59o-1min/72o-1min	40
	R-5 cacatgtacttgtagtggatggtgg 3		
Caspase-3	F-5 gagcactggaatgtcatctcgctctg 3	94o-1 min/57o-1 min/72o-1 min	35
	R-5 tacaggaagtcagcctccaccggtatc 3		
Caspase-12	F-5 tccgacagcacattcctggtc 3	94-1 min/65-1 min/72-1 min	35
	R-5 ccctttgcttgtggataccca 3		
Caspase-8	F-5 tgaaggagctgcttttccat 3	94o-1 min/57o-1 min/72o-1 min	35
	R-5 atcaagcaggctcgagttgt 3		
TNF-α	F-5 agccaggcaggttccgtccctc 3	95o-15 sec/60o-1 min/72o-1 min	40
	R-5 ttactgtgcccaccagccgac 3		
Bcl-2	F-5 cagctgcacctgacgccctt 3	95o-30 sec/58o-30 sec/72o-45 sec	30
	R-5 cccagcctccgttattctgga 3		
IL-1 β	F-5 gctatggcaactgtccctgaa 3	94o-1 min/59o-1 min/72o-1 min	40
	R-5 atcccacgagtcacagaggacg 3		
IL-6	F-5 atgaagtttctctctgcaagagac 3	95o-30 sec/58o-1 min/72o-1 min	35
	R-5 cactaggtttgccgagtagatctc 3		
Fox01	F-5 gtgaacaccatgcctcacac 3	95o-30 sec/58o-1 min/72o-1 min	35
	R-5 cacagtccaagcgctcaata 3		
CHOP	F-5 cactactcttgaccctgcatcc 3	95o-30 sec/58o-1 min/72o-1 min	35
	R-5 cttcatgcgctgtttcccagc 3		
GRP78	F-5 ctgggtacatttgatctgactgg 3	95o-30 sec/59o-1 min/72o-1 min	40
	R-5 gcatcctggtggctttccagccattc 3		
ATF4	F-5 tctgtatgagccctgagtcctacct 3	93o-15 sec/55o-30 sec/68o-1 min	30
	R5 ggtcataaggtttgggtcgagaaccac 3		
ATF6	F-5 ggatttgatgccttgggagtcagac 3	95o-30 sec/58o-1 min/72o-1 min	40
	R-5 atttttttctttggagtcagtccat 3		
$NF_κB$	F-5 tccccaagccagcaccccagc 3	95o-30 sec/58o-1 min/72o-1 min	35
	R-5 ggccccccaagtcttcatcagc 3		
PERK	F-5 aggagtctgaaactcagtgcca 3	95o-30 sec/58o-1 min/72o-1 min	35
	R-5 gctccctgtttgggagacgga 3		
PDX-1	F-5 ggcttaacctaaacgccaca 3	95o-30 sec/58o-1 min/72o-1 min	35
	R-5 gggaccgctcaagtttgtaa 3		
Bcl-xL	F-5 gaactctttcgggatggggta 3	95o-30 sec/59o-1 min/72o-1 min	40
	R-5 cagaactacaccagccacagtc 3		
Wnt-4	F-5 gccacgcactaaaggagaag 3	93o-15 sec/55o-30 sec/68o-1 min	30
	R-5 ggccttagacgtcttgttgc 3		
TCF7L2	F-5 gcctctcatcacgtacagca 3	95o-30 sec/58o-1 min/72o-1 min	40
	R-5 ggatgggggatttgtcctac 3		
IFN-γ	F-5 atctggaggaactggcaaaaggacg 3	93o-1 min/55o-1 min/72o-1 min	40
	R-5 ccttaggctagattctggtgacagc 3		
JNK-1	F-5 tcagacccatgctaagcgagc 3	95o-1 min/58o-1 min/72o-1 min	35
	R-5 aggtgcttgattccacacagca 3		
β-Catenin	F-5 gctaaaatggcagtgcgccta 3	95o-30 sec/58o-1 min/72o-1 min	40
	R-5 gtggtccacaggagcttctcg 3		
β-Actin	F-5 catcgtcaccaactgggacgac 3	93o-15 sec/55o-30 sec/68o-1 min	30
	R-5 cgtggccatctcttgctcgaag 3		
Bax	F-5 agaagctgagcgagtgtctcc 3	95o-30 sec/58o-1 min/72o-1 min	40
	R-5 gccttgagcaccagtttgcta 3		
P35	F-5 aaaggccacactgtttgagga 3	95o-30 sec/58o-1 min/72o-1 min	40
	R-5 cctcttccaaggcagtaccga 3		

G. Comet Assasy

The cells which were placed into agarose (NMA) gel were spread on the lam, and they were fractionated in high salt concentration with lysis solution in the slides prepared. After the release of DNA, electrophoresis was performed in alkaline electrophoresis buffer at 4°C. After the completion of electrophoresis, slides were stained with ethidium bromide, and the assessment was conducted within 4 hours. DNA images that were obtained from the stained slides in fluorescence microscope were assessed; the assessment was conducted as semi-quantitative by visual scoring. DNA images were scored from 1 to 4 according to the degree of resulting damage, those without damage were considered as 0 point, and the results were assessed as arbitrary unit (AU).

H. Determination of intracellular and extracellular insulin levels by ELISA technique

Intracellular insulin was analyzed from cell lysates and extracellular insulin was analyzed from culture medium. Analyses were carried out by ELISA techniques using commercial kit for insulin (EMD Millipore Corporation, USA) determinations by ELISA reader (Trinity Biotech, Wicklow, Ireland).

I. Statistical analysis

The cells obtained from the same passages were used in the study groups. Maximum attention was paid to sterilization at every stage of the research. Each experiment consisted of 3 replication, and the average values obtained were evaluated in the SPSS statistical program using One-Way ANOVA test

III. RESULTS

Intracellular and Secreted Insulin Levels

In insulin synthesis and release, statistically significant increases were determined in L 0.1 group compared to the control group. There was a statistical increase only in insulin secretion (secreted insulin) compared to the control group in E 0.1 group (Table 2).

Table 2. Intracellular (lysate) and extracellular (secreted) insulin concentrations.

Insulin concentrations		
Groups	Intracelular insulin (ng/µg protein)	Extracelular insulin (ng/ml)
T	0,314±0,0787^{c}	0,313±0,106^{b}
DC	0,027±0,013^{a}	1,245±0,215^{c}
C	0,073±0,017^{a,b}	0,026±0,004^{a}
L 0,1	0,58±0,164^{d}	1,293±0,176^{c}
E 0,1	0,108±0,012^{b}	0,407±0,072^{b}
L 0,5	0,042±0,009^{a,b}	0,152±0,046^{a}
E 0,5	0,085±0,016^{a,b}	0,353±0,079^{b}
LT 0,1	0,016±0,003^{a}	0,021±0,004^{a}
ET 0,1	0,031±0,007^{a,b}	0,043±0,008^{a}
LT 0,5	0,0138±0,003^{a}	0,064±0,014^{a}
ET 0,5	0,0184±0,005^{a}	0,307±0,088^{b}

$^{a, b, c:}$ The differences between the averages with different exponential expressions are statistically significant ($P < 0,005$). All experiments were performed in triplicate, and data was expressed as mean±SD for all parameters

The statistically significant increases were determined both in intracellular insulin and secreted insulin concentrations in T group (ER stress group) compared to the C group. ER stress model was formed with thapsigargin, and statistically significant decreases were determined in the insulin synthesis and release in other groups (E 0.1, L 0.1 and L 0.5) administered with EBV recombinant protein except for E 0.5 group compared to T group.

mRNA Expression Levels

The mRNA expression levels of genes responsible for beta cell proliferation

In the mRNA expression levels of (FoxO1, PDX-1, Wnt-4, TCF7L2 and -Catenin) genes which are indicated to be responsible for the beta cell proliferation by the literature data; FoxO1 increased by 2.75-fold, PDX-1 increased by 2.05-fold, Wnt-4 increased by 2.23-fold, TCF7L2 increased by 7.16-fold and -Catenin increased by 15.24 -fold in T group compared to C group. It was understood from the mRNA expression levels of the related genes that 0.1 ppm dose of EBNA1 was the best dose that stimulated the proliferation, from the EBV recombinant proteins EBNA1 and LMP1 proteins. In this regard, the mRNA expression levels of FoxO1, PDX1, Wnt4, TCF7L2 and -Catenin genes increased by 2.37, 6.48, 4.25, 3.8 and 1.4-fold respectively in E 0.1 group compared to C group. Similarly, although 1.31, 2.26, 1.4 and 2.1-fold increases were respectively observed in the mRNA expression levels of the same genes except for -Catenin in ET 0.5 group compared to T group, -Catenin was interestingly suppressed by 4.59 fold. On the other hand, significant results increasing cell proliferation could not be achieved in the groups administered with LMP.

mRNA Expression Levels of Genes which are ER Stress Markers

The mRNA expression levels of GRP78, CHOP, ATF4, ATF6 and PERK genes which are accepted as ER stress markers increased by 6.63 fold, 8.05 fold, 10.62 fold, 19 fold, 15.03 fold respectively in T group compared to DC group. The mRNA expression levels of the same genes except for ATF4 decreased in LT 0.1, LT 0.5, ET 0.1 and ET 0.5 groups compared to T group, the expression level of ATF4 gene increased in these groups.

mRNA Expression Levels of Proapoptotic Genes

When the mRNA expression levels of proapoptotic genes cas3, cas8, cas12, TNF-, p53, JNK, IFN- and bax genes were analyzed, it was determined that there were increases/decreases by 1.00 (no change), 2.69, 1.12, -1.13, 1.13, 1.37, -1.55 and 1.34-fold respectively in L 0.1 group compared to the control group. Similarly, it was determined that the mRNA expression levels of the related genes increased by 12.78, 5.66, 1.31, 2.47, 9.41, 4.5, 2.25 and 10.48-fold in the E 0.1 group compared to the control group. The moderate increases and decreases were observed in the mRNA expression levels of the related proapoptotic genes in L 0.5 and E 0.5 groups. For the same genes, increases/decreases by 3.91, -1.31, -1.01, -1.14, 5.61, 4.02, -2.01 and 2.63-fold were observed in T group compared to DC group. The increases/decreases by -1.09, -63.26, -1.17, -2.14, 1.2, 1.35, 1.72, -1.33-fold were observed respectively in the mRNA expression levels of the related genes in LT 0.5 group compared to T group. The increases/decreases by -1.12, -44.32, 1.05, -3.44, 1.00 (no change), 1.38, 2.32 and -1.42-fold were observed respectively in the mRNA expression levels of the related genes in ET 0.1 group compared to T group. On the other hand, no result that could be significant in terms of the related recombinant proteins that were used in the study was achieved in IL-6 and IFN- mRNA expression levels as proinflammatory markers.

mRNA Expression Levels of Antiapoptotic genes

When the mRNA expression levels of Bcl2, Bcl-xL and p35 genes which were included in the study as antiapoptotic genes were analyzed, the increases by 1.28, 1.25 and 3.78-fold were observed respectively in the mRNA expression levels of the related genes. The increases/decreases by -1.2, -54.56 and 1.24-fold were observed respectively in the mRNA expression levels of the related antiapoptotic genes in LT 0.5 group compared to T group. The increases/decreases by -1.56, -43.11 and 1.12-fold were observed respectively in the mRNA expression levels of the same genes in ET 0.1 group compared to T group. Although the increases/decreases by -1.12, 10.5,-1.13-fold were observed respectively in the mRNA expression levels of the related antiapoptotic genes in L 0.1 group compared to C group; in the same genes, increases by 2.05, 24.02 and 7.39-fold were observed respectively in the mRNA expression levels of the related genes in ET 0.1 group compared to C group.

Comet Assay Results

The DNA damage score occurred in the T group was determined as 69.4±3.6 AU as a result of the thapsigargin administration in cells. The DNA damage that occurred as a result of thapsigargin administration was observed to be more compared to the control group and groups administered with thapsigargin solvent DMSO.

The DNA damage that emerged in the groups administered with thapsigargin was observed to be statistically significantly more than other groups except for ET 0.1 group. When at what level this effect of thapsigargin was changed from recombinant proteins was analyzed, it was determined that the DNA damage statistically significantly decreased in all groups except for ET 0.1 group. In this regard, LT 0.5 and ET 0.5 groups were the two groups that exhibited the best effect in suppressing the DNA damage caused by thapsigargin. The visual comet scores were determined as 13.2±3 and 9.2±1.9 AU respectively in the related groups.

When these results obtained were evaluated, LMP1 and EBNA1 recombinant proteins were determined to be statistically significantly effective in decreasing the DNA damage formed by thapsigargin.

IV. DISCUSSION

The most remarkable findings in terms of pancreatic beta cell proliferation were observed in E 0.1 group. The effect that occurred in 0,1 ppm and 0,5 ppm administrations of LMP1 protein was not found significant (Table 2).

When LMP1 and EBNA1 proteins were evaluated in terms of insulin synthesis and secretion, they were determined to be parallel with the mRNA expression levels of genes responsible for the proliferation. In this context, it was determined that the insulin synthesis and secretion were stimulated to constitute a statistical significance in L 0.1 compared to the control group. Similarly, it was determined that the insulin secretion was increased to give statistical significance in E 0,1 group compared to the control group, the insulin synthesis increased but this increases was not statistically significant. The reasons for the fact that LMP1 and EBNA1 proteins changed insulin synthesis and release in this direction are due to the fact that they suppressed the ER stress and stimulated the cell proliferation. However, although it was indicated that LMP1 and EBNA1

proteins stimulated the insulin synthesis, according to results that emerged in COMET analyses, the related doses of both proteins caused DNA damage. If the reasons of the fact that the related proteins led to the formation of DNA damage is determined and this situation can be brought under control, the way to use them as viro-therapeutic agents in future studies can be opened. In another literature data related to this issue, in the study carried out by Gou Y., et al. (2014), it was demonstrated that there was a correlation between DNA damage and EBV infections in nasofaringial carcinoma cells infected with EBV14. In this regard, it can be said that the emerging results are in compliance with the literature data.

In the study carried out by Ju et al.(2014), it was demonstrated that FoxO1 gene was suppressed in situations where JNK activation was suppressed[15]. In this study, although 0.1 ppm dose of the EBNA 1 protein increased the mRNA expression level of the FoxO1 gene by 2.37-fold compared to C group, its 0.5 ppm dose suppressed it by -1.31-fold . As reason of this difference, although JNK gene increased by 2.37-fold in E 0.1 group compared to the control group, it was suppressed by -2.0-fold in E 0.5 group. In this regard, these results are in compliance with the data of Ju et al.[15], and it is thought that the proliferative difference between two doses resulted from this.

In the study16 carried out by Hayakawa et al. (2003), it was demonstrated by the comet assay that the DNA damage caused JNK activation that led cell to apoptosis17. In our study, the activation of genes responsible for proliferation in E 0.1 group was ensured, however it was also demonstrated that the related protein caused DNA damage. 0.1 ppm dose of EBNA statistically increased DNA damage compared to C group, and this resulted in the increase of JNK activation in E 0.1 group by 4.5-fold compared to the control group. JNK activation in E 0.1 group dramatically increased the mRNA gene expression levels of p53, cas3 and cas 8, which are the proapoptotic genes in the same group, by 9.41, 12.78 and 5.66-fold respectively compared to C group. These results were found remarkable because they were in compliance with the literature data. In prospective studies, the answers of the fact that EBNA1 and LMP1 stimulated the DNA damage and the expression of proapoptotic genes can be explained by analyzing these mechanisms.

In studies carried out by Laherty et al.18 and Rowe et al.[19], it was demonstrated that the expression of LMP1 in lymphocytes resulted in the activation of transcription factor NF-kB. In our study, it was determined that the mRNA expression level of NFKB increased by 10.8-fold in LMP0.1 compared to the control group. However, the expression of NFkB gene was suppressed by -97-fold in the LT 0.1 group, which cannot be explained.

In the study carried out by Tran et al.[20], it was demonstrated that TNF- increased the NFKB expression. Similarly in our study, although TNF- expression level increased by 2.47-fold in E 0.1 group compared to the control group, NFKB mRNA expression level showed a dramatic increase by 154.34-fold compared to C group.

In the study carried out by De Leo et al. (2011), it was demonstrated that the antiproliferative activity was increased when they silenced the gene of LMP1 protein using LMP1 siRNA[21]. So this means that the presence of LMP1 is important for cell proliferation. Similarly in our study, it was determined that there were increases in the expression levels of the genes that were associated with cell proliferation in L 0.5 group. However, it was not possible to mention about these increa-

ses in L 0.1 group except for beta-catenin gene.

V. CONCLUSION

Consequently, it was observed that EBV recombinant proteins LMP1 and EBNA1 increase the expression levels of the genes responsible for the proliferation, and also they could provide advantage in the elimination of ER stress that causes pancreas beta cell losses by suppressing the expression levels of ER stress marker genes. If the results obtained are supported by further studies, these proteins have the potential to be used as virotherapic agent in antidiabetic treatments. The fact that the related proteins can lead to DNA damage is considered to be the only handicap of this study. However, when literature data were analyzed, it was observed that many drugs used in the treatment of many diseases caused DNA damage at certain dosages. The results of the studies related to aspartame[22] and ketamine[23] can be shown as an example for this. Therefore, the fact that LMP1 and EBNA1 cause DNA damage, even a little, compared to the control group seems as an issue that should be evaluated within the profit and loss balance in the future.

Our study is the first research that could be guiding in terms of testing EBNA1 and LMP1 proteins as viro-therapeutic agents in the treatment of diabetes.

Another outcome of this study is that it can be guiding for the development of a new method on decrease in cellular growth rate or difficulties experienced in cell reproduction, one of the most important problems of cell culture researches. Another scientific contribution of the study results is that EBNA1 and LMP1 have the potential to be preparation for therapeutic purpose or to be trigger in planning the clinical, phase and pharmacokinetic studies related to their local administrations to the pancreas.

ACKNOWLEDGMENT

This work was funded by TUBITAK (The Scientific and Technological Research Council of Turkey) under the contract number 113S153.

Journal of BSHR
2018;2(3):148-155

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