ISSN: 2755-0125

Journal of Virology Research & Reports

SCIENTIFIC Research and Community

Research Article

Open Access

Production of the Proinflammatory Cytokine TNF-α in Patients with Chronic Epstein-Barr Virus Infection

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ABSTRACT

Aim of the Study: Study of the production of the pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α) in patients with Chronic Epstein-Barr Viral Infection (CEBI).

Material and Methods: 90 patients with CVEBI were examined. The group consisted of 66 women and 24 men. The average age of the patients was 38.37 ± 1.15 years. The duration of the course of CVEBI from the appearance of the patient's first complaints to diagnosis was 3.73 ± 0.22 years. The amount of EBV DNA in saliva samples (PCR method) and the level of production of the cytokine TNF-α ware examined in patients.

Results: The copy number of EBV DNA in saliva samples from patients was 135898.61±23196.21 cop/ml. The level of induced TNF-α in the general group was 3288.73±260.88. All patients were divided into three groups: group 1 (n = 49) - the level of induced TNF-α was reduced - 1624.69±105.53 (pg/ml) (95% CI: 1412.52; 1836.86) ; Group 2 (n =30) - the level of induced TNF-α is normal - 3819.19 ±427.00 (pg/ml) (95% CI: 3045.27; 4701.81); Group 3 (n =11) the level of induced TNF-α is high – 7203.00 ± 627.50 (pg/ml) (95% CI: 6135.37 - 8609.87). Patients did not differ in age, disease duration, or number of EBV DNA copies in saliva samples. The linear and exponential regression method was used to show the effect of disease duration on the level of TNF-α production in the general group of patients (R2 =0.386 F=55.925 p=0.0001 β=0.621 and R2 =0.735 F=246.808 p=0.0001 β=0.857, respectively). TNF-α influences the development of headaches in patients (Pearson correlation R = 0.223 p = 0.040) and the severity of postnasal drip (r = -0.231 p = 0.033 τ = - 0.171 p = 0.039).

Conclusion: The study showed that the duration of the disease affects the level of production of induced TNF-α. TNF-α levels contribute to the development of headaches and postnasal drip in patients in the general group.

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Received: February 19, 2024; **Accepted:** February 22, 2024; **Published:** February 28, 2024

Keywords: Epstein-Barr Virus, Tumor Necrosis Factor-Alpha, DNA Copy Number

Introduction

Epstein-Barr Virus (EBV) is a lymphotropic herpes virus 4 and the causative agent of Infectious Mononucleosis (IM), leading to the development of pronounced changes in the immune system. EBV infection mainly occurs in the oropharynx, where the virus enters through droplets of saliva from an infected person [1]. Epithelial cells and B cells are predominantly infected; monocytes may also be infected [2].

Infection of epithelial cells is part of the normal cycle of EBV persistence in the human host. The literature suggests three possible mechanisms for virus attachment to an epithelial cell.

1. A virus coated with immunoglobulin A specific for gp350/220 can bind to the polymeric immunoglobulin-A receptor, which is more relevant for trans-epithelial transport than direct infection [3].
2. Epithelial cell receptor for the gHgL glycoprotein complex, which may be involved in virus attachment. The molecule of this receptor has not yet been identified, but it is designated gHgLR [4].
3. Interaction between the viral multilayer membrane protein encoded by the open reading frame BMRF2 and integrins [5].
4. Antibodies to integrins and the BMRF2 fusion protein partially block binding to polarized epithelial cells. However, the role of the integrin BMRF2 in the attachment itself or in the mechanism after attachment of the virus to the epithelial cell remains unclear [6]. B cell infection occurs by endocytosis through interaction between gp350/220 and CR2, gp42 with HLA class II in a pH-independent manner. Effective fusion requires the viral proteins gHgL, gB, and gp42. The interaction of gp42 with HLA class II stimulates

the interaction of the nuclear fusion mechanisms, gHgL and gB, with the endosome membrane. When the viral membrane and endosome fusion, the coated capsid penetrates into the cytoplasm [2, 7].

Following infection, EBV replicates in B lymphocytes and epithelial cells of the nasopharynx and surrounding lymphoid tissues, circulating in memory B cells in a quiescent state, which is also invisible to the immune response [8]. The biology of EBV is very similar to normal mature B lymphocytes. The virus uses its transforming abilities to activate newly infected resting B-cells. Viral latency-associated proteins then trigger signals that allow newly activated cells to differentiate through the germinal center into a pool of memory B-cells [9].

A characteristic feature of herpes viruses is persistence in a small number of target tissue cells in the form of a latent infection, as a result of which the virus remains stably at a very low level (1 infected cell per 5 ml of blood) throughout the life of the host [10]. As a result, the virus can evade the host's immune response, remaining in its cells until the end of its life. Acute infection and reactivation of the virus to a new host are hypothesized to result in minimal impact on the host [9]. In peripheral blood, EBV is found only in resting memory B-cells, i.e. in classical memory B-cells, which are the site of long-term persistence of the virus, where it can persist throughout the life of the host, because immunological memory persists for life, as a result the virus becomes non-pathogenic for the host [8]. The virus is also safe for immunological monitoring since it does not express viral proteins that act as targets for the immune system.

Shedding of EBV into saliva occurs continuously, and the number of viral particles is restored in ≤2 minutes, the average time before swallowing. Thus, the oral cavity is a channel through which a continuous flow of virus enters the saliva. Consequently, virus shedding occurs at a much higher rate to be explained by replication in B cells only in the Waldeyer's ring. Virus shedding is relatively stable over short periods of time, which is why variation cannot be explained only by viral replication in B-cells. The process that regulates virus release occurs independently at least 3 times at any time, grows exponentially, and then stops randomly. Apparently, the B cell can sporadically release the virus with further damage from 1 to 5 epithelial cells. As a result, infected plaques form from epithelial cells [9]. Virus from the plaque is continuously released into saliva over several days, providing a continuous flow of virions in saliva. In Waldeyer's ring, about 100 B-cells simultaneously complete the replication cycle [10, 11]. Therefore, the release of the virus is also explained by the increase of epithelial plaques infected with the virus, but over time the spread of these plaques stops. In humans, there is ~1 infected cell in 5 ml of blood. Once infected, the virus becomes vulnerable to extinction due to random changes, but the value only changes by ±25% over many years [9]. This is because the infection cycle ensures that the destroyed B cell population can be quickly repopulated, returning the system to the same equilibrium [8].

EBV infection may be under the control of the immune system and not cause the development of the disease, but the influence of certain factors can lead to the development of diseases such as acute infectious mononucleosis, chronic persistent EBV infection, lymphoproliferative, malignant and autoimmune diseases [12].

The interaction between the virus and cytokines plays an essential role in the immune control of EBV infection. There is evidence indicating changes in the production of pro-inflammatory cytokines interleukin-1β (IL-lβ) and tumor necrosis factor-alpha (TNF-α) in patients [13, 14]. The major envelope glycoprotein of EBV gp350/220 is capable of inducing the production of TNF-α by monocytes, which has a wide range of physiological functions. TNF-α has pronounced antiviral activity against the B-lymphotropic Epstein-Barr virus, which, when interacting closely with cells of the immune system, has a strong inhibitory effect on the production of TNF-α. This effect was demonstrated under experimental conditions in a cell culture infected with EBV, where TNF-α was not detected. The authors concluded that the virus is a potent inhibitor of TNF-α production [15]. Insani Budiningsih et al. demonstrated in their work that EBV-infected cells themselves can produce TNF-α [16]. TNF-α induces the cytotoxic activity of macrophages and lymphocytes, and is involved in the regulation, maturation and proliferation of lymphocytes [17].

The Purpose of our work was to study the production of the pro-inflammatory cytokine TNF-α in patients with CVEB infection.

Material and Methods

Patients

90 patients suffering from chronic Epstein-Barr viral infection were examined. The group consisted of 66 women and 24 men. The mean age of the patients was 38.37 ± 1.15 years. (95% CI: 36.34 - 40.53). The duration of the course of CVEBV from the appearance of the first complaints in the patient to laboratory examination and confirmation of EBV infection and diagnosis was 3.73 ± 0.22 years (95% CI: 3.30 - 4.16).

The diagnosis of "chronic EBV infection" was made on the basis of complaints and anamnesis and was confirmed in the laboratory during examination by relevant specialists at the previous stage. After confirmation of the diagnosis, patients were referred for treatment to an immunologist.

The clinical research carried out in compliance with the WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects, 2013) and the Protocol of Council of Europe Convention on human rights and biomedicine 1999 and articles 20, 22, 23 of the Act "On the basics of healthcare for the Russian Federation citizens" dated November 21, 2011 Fed. Statute №323-FZ (May 26, 2021 edition). The clinical study was conducted in accordance with a procedure approved by the local ethical committee under LLC "Center of Dialysis of St. Petersburg" FRESENIUS MEDICAL CARE. All study participants had signed voluntary informed consent. During initiation of the research, the patients did not indicate any other infections, chronic diseases, or changes of immune status system, which could affect outcomes of the study.

Clinical research methods included

1. Taking anamnesis
2. Presence of concomitant diseases
3. Data on previous therapy

The clinical condition of the patients was assessed using a generally accepted method, including objective data and patient complaints at the time of examination. Registration of patient complaints was carried out using a subjective assessment scale on a 3-point scale (0 – absence of symptoms, 1 – mild severity of symptoms, 2 – moderate severity of symptoms, 3 – significant severity of symptoms).

All patients underwent a differential diagnosis of CVEBV with other viral infections (cytomegalovirus infection, viral hepatitis, human immunodeficiency virus), toxoplasmosis, helminthic infestations, and autoimmune diseases associated with EBV infection.

Patients who received antiviral or immunomodulatory therapy within the last 6 months were not included in the study.

Methods

The patients were tested for the virus DNA by PCR method (in saliva samples via Polymerase Chain Reaction (PCR) with hybridization-fluorescent detection in real-time mode). The employed test-systems "AmpliSence EBV/CMV/HHV6-screen-FL" from the Central Scientific Institute of Epidemiology (Russia). Measurement units, used for assessing the viral load during extraction of DNA from saliva – number of copies of the EBV DNA per 1 ml of the sample (NCDNA). According to the manual, this indicator is calculated by a formula: NCDNA=CDNA x 100, where CDNA—number of copies of virus DNA in the sample. The test-system analytical sensitivity is 400 copies/ml.

Production of tumor necrosis factor-alpha (TNF-α) in the culture environment (spontaneous, induced production) and in blood serum. Whole heparinized blood diluted fivefold with RPMI-1640 nutrient environment with L-glutamine (Biolot, Russia) was cultured in round-bottomed plates in the presence or without an inducer of cytokine synthesis.

Pyrogenal was used as a synthesis inducer (Salmonella typhi lipopolysaccharide 100 μg/ml, N.F. Gamaleya Research Center for Epidemiology and Microbiology, Russia). Cultivation was carried out for 24 hours at 37°C in a CO2 incubator. At the end of incubation, the supernatant was transferred to Eppendorf tubes and frozen for further work. Spontaneous production of cytokines was assessed in the supernatant of blood cell culture without the addition of an inducer, and induced production with the addition of pyrogenal. Determination of the content of cytokines in the serum and in the supernatant liquid of the blood cell culture was carried out by enzyme-linked immunosorbent assay using reagent kits for ELISA (Vector-Best JSC, International Certificate ISO 13485) according to the manufacturer's instructions. The sensitivity of the test kit for TNF-α did not exceed 1.0 pg/ml.

Statistical Treatment

Of acquired data was conducted by means of a software package IBM SPSS Statistics, 26 version (Armonk, NY: IBM Corp.). Group results are presented in form of mean arithmetic M ± Standard Error. For the statistical treatment we employed parametric (Pearson's method) and nonparametric (Spearman's method, tau (τ) Kendall) methods.

To verify compliance with condition of independence of observations we conducted linear regression analysis (with computation of coefficient of determination (R Square) and criterion of Durban-Watson) and dispersion analysis (ANOVA Analysis of Variance) with computation of criterion of Fisher (F) for verification of model significance. Standardized rate ß with 95% confidence intervals was calculated. Critical significance level of difference of indicators was taken to be equal 0.05.

Results

All patients (n=90) underwent EBV DNA copy number testing using PCR in saliva samples. The EBV DNA copy number was 135898.61±23196.21 cop/ml (95% CI: 93489.67 - 189261.78).

Chronic Epstein-Barr viral infection is characterized by a long course and frequent relapses of the clinical and laboratory picture of the viral infection, which manifests itself as mononucleosis-like symptoms [18]. Patients complain of prolonged low-grade fever (37.1-37.3°), which manifests itself only in the daytime, severe weakness and fatigue, increased sweating (especially at night), lymphadenitis (cervical and submandibular lymph nodes, less often axillary), constant feeling discomfort and/or pain in the throat, frequent exacerbations of stomatitis with the appearance of aphthous ulcers, less often manifestations of gingivitis, burning sensation of the mucous membrane of the tongue, severe postnasal drip, development of arthralgia of large and small joints, stiffness of small joints, myalgia, frequent colds.

Rheumatological pathology was not detected in patients. Characteristic neurological disorders are severe headaches, memory impairment, decreased concentration, sleep disturbances, irritability, tearfulness, and the development of depression. Patients note the development of a deterioration in their condition and the appearance of the described complaints against the background of prolonged stressful situations, psycho-emotional and physical overload. Patients consult psychiatrists, receive specific therapy, and visit a psychologist. Based on the above, we analyzed the frequency of occurrence of the described clinical complaints in patients (Table 1).

Table 1: Frequency of Main Clinical Complaints (%) in Patients with CVEB Infection in the General Group

Frequency of clinical complaint (%)	Group of patients (n=90)
Subfebrile temperature	83,33
Lymphadenitis	50,00
Throat pain	86,66
Weakness	75,55
Chills	66,66
Hidrosis	83,33
Postnasal drip	53,33
Stomatitis	42,22
Irritability and tearfulness	60.00
Headache	84,44
Decreased concentration and memory	40,00
Sleep disturbance	44,00

TNF-α Production

The next step was to study the production of the pro-inflammatory cytokine TNF-α in the culture environment (spontaneous, induced production) and in blood serum. The results obtained are presented in Table 2, Figure 1.

Table 2: Production of TNF-α in the Culture Environment in Patients with CVEB Infection in the General Group

Studied Indicator	Level of TNF-α (pg/ml)	Reference Values (pg/ml)
Serum level	6,56±1,86 95% CI: 3,41 – 11,09	0 - 6
Spontaneous TNF-α	47,51±19,78 95% CI: 18,21 – 93,99	7 - 30
Induced TNF-α	3288,73±260,88 95% CI: 2755,95 - 3824,57	2810 - 5700

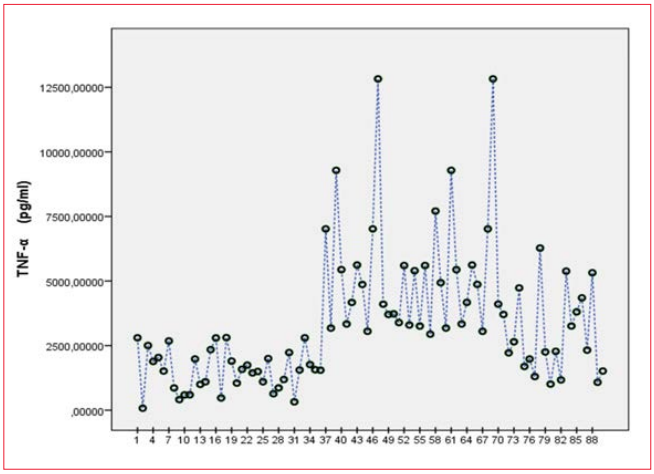

Figure 1: Level of Production of Induced TNF-α in the Culture Environment in Patients with CVEB Infection

When analyzing the level of induced TNF-α in the culture environment in patients in the general group of CVEB infection, it was revealed that these values differed significantly among patients. They were either significantly lower or within the reference values or significantly higher than those of healthy donors (2810–5700 pg/ml, reference values provided by the manufacturer of the test systems).

Therefore, all patients in the general group were divided into three groups according to the initial level of induced TNF-α
- Group 1 (n =49) - the level of induced TNF-α is reduced - 1624.69±105.53 (pg/ml) (95% CI: 1412.52; 1836.86).
- Group 2 (n =30) - the level of induced TNF-α is normal - 3819.19 ±427.00 (pg/ml) (95% CI: 3045.27; 4701.81)
- Group 3 (n = 11) the level of induced TNF-α is high – 7203.00 ± 627.50 (pg/ml) (95% CI: 6135.37 - 8609.87).

When conducting a comparative analysis of these three groups of patients by age, duration of the disease, and number of EBV DNA copies in saliva samples, the following data were obtained, presented in Table 3.

Table 3: Comparative Analysis of the 1st, 2nd and 3rd Groups of Patients with CVEBI

	Group 1 (n =49)	Group 2 (n =30)	Group 3 (n =11)	P
Patient age (years)	35,92±1,59 95% CI: 34,92 - 41,25	38,14±1,57 95% CI: 32,57 – 39,28	41,73±2,89 95% CI: 35,72 – 47,00	P1,2 =0,001 P1,3= 0,001
Duration of the disease (year)	4,32± 0,35 95% CI: 3,68 - 5,02	3,09±0,31 95% CI: 2,52 - 3,71	2,82±0,49 95% CI: 1,91-3,91	P1,2 =0,001 P1,3 =0,001
Number of EBV DNA copies in saliva sample (cop/ml)	192046,19± 39941,07 95% CI: 119028,47 - 269652,04	176140,24± 33819,45 95% CI: 110982,56 - 242150,03	168818,18± 51867,65 95% CI: 74456,85 – 277636,36	P1,2 =0,002 P1,3 =0,002

The table data shows that groups of patients with different levels of induced TNF-α production differed significantly in the age of the patients, the duration of the disease, and the number of EBV DNA copies in saliva samples. That is, patients with a short period of illness have low levels of EBV DNA copy number in saliva samples and high levels of induced TNF-α.

Next, a comparative analysis of the severity of clinical complaints in these groups of patients was carried out (Table 4).

Table 4: Frequency of Occurrence of Main Clinical Complaints (%) in Patients with CVEB Infection in the 1st, 2nd and 3rd Groups

Frequency of Clinical Complaint (%)	Group 1 (n =49)	Group 2 (n =30)	Group 3 (n =11)
Subfebrile temperature	87,75	80,00	72,72
Lymphadenitis	53,06	53,33	54,54
Throat pain	87.75	80,00	81,81
Weakness	73,46	60.00	63,63
Chills	67,34	53,33	45.45
Hidrosis	83,67	60.00	63,63
Postnasal drip	53,06	46,66	54,54
Stomatitis	44,89	40,00	36,36
Irritability and tearfulness	59,18	46,66	45,45
Headache	81,63	76,66	72,72
Decreased concentration and memory	42,85	33,33	27,27
Sleep disturbance	44,89	43.33	63,63

From the presented data it follows that clinical complaints are more expressed in patients in group 1 with a reduced level of production of induced TNF-α.

The next stage of work was to identify the relationship between the clinical complaints of patients and the level of production of induced TNF-α. Correlation analysis in each of the groups did not reveal significant connections. Therefore, further analysis was carried out in the general group of patients.

Prognostic Significance of the Level of Induced TNF-α

To determine the prognostic significance of induced TNF-α in the general group of patients, linear and exponential regression analysis was performed with the calculation of coefficients of determination R2 (R Square) and using the Durban-Watson test with a 95% confidence interval. The results are presented below (Figure 2).

The level of Production of Induced TNF-α Depends on the Duration of the Disease

- Using the linear regression method we obtained: R2 =0.386 F=55.925 p=0.0001 β=0.621.
- Using the exponential regression method, we obtained: R2 = 0.735 F = 246.808 p = 0.0001 β = 0.857.

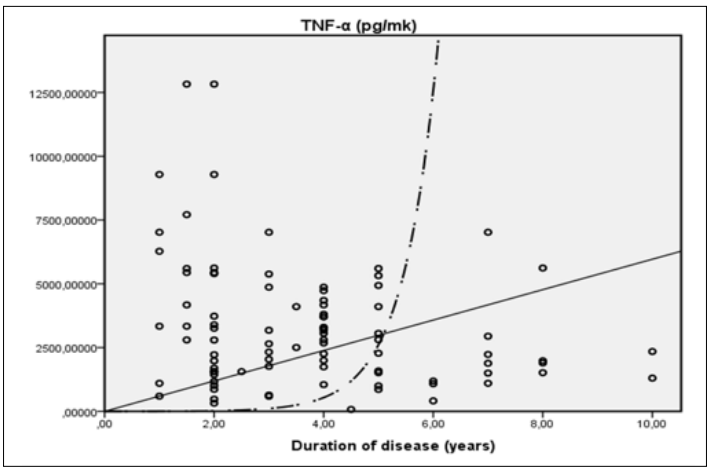

Figure 2: Production of Induced TNF-α in the General Group of Patients with CVEB Infection Depending on the Duration of the Disease («–» - Linear Regression; «• – •» - Exponential Regression).

The Linear Regression Method Showed

1. The level of production of induced TNF-α in the general group of patients depends on the number of EBV DNA copies (R2 = 0.307 F = 34.92 p = 0.0001 β = 0.554) (Figure 3).

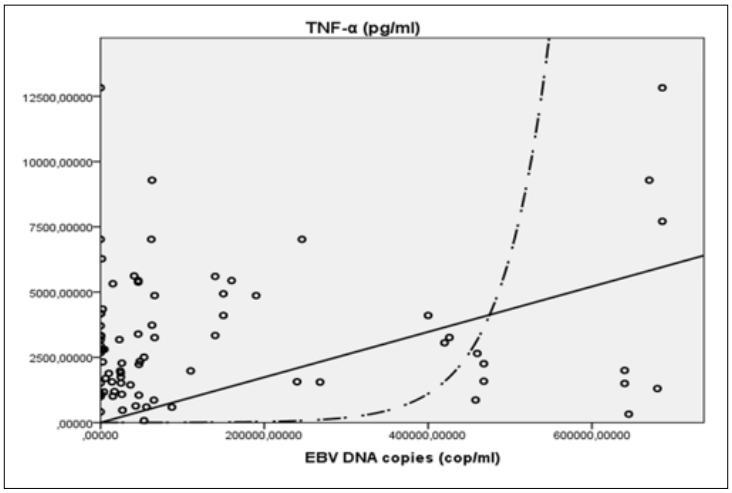

Figure 3: Effect of the Number of EBV DNA Copies on the Production of Induced TNF-α in the General Group of Patients with CVEB Infection («–» - Linear Regression; «• – •» - Exponential Regression).

Correlation analysis of the relationship between the level of induced TNF-α and clinical complaints in patients in the general group showed that the level of induced TNF-α affects

- On the severity of postnasal drip r = -0.231 p = 0.033 τ = - 0.171 p = 0.039.
- On the development of headache - Pearson correlation R=0.223 p=0.040.

No other correlations were identified.

Prognostic Significance of EBV DNA Copy Number

Next, we analyzed the influence of the number of EBV DNA copies in saliva samples on the development of clinical complaints in patients. The linear regression method was used to calculate the coefficient of determination R2 (R Square) using the F test and calculate the standardized beta coefficient (ß) with a 95% confidence interval. (Table 5).

Table 5: The Influence of the Number of EBV DNA Copies on the Development and Progression of Clinical Complaints in Patients in the General Group

Complaints	Coefficient of determination (R^2)	Fisher's test (F)	p	Standardized coefficient ß
Subfebrile temperature	0,297	31,736	0,0001	0,545
Lymphadenitis	0,214	20,403	0,0001	0,462
Throat pain	0,298	31,907	0,0001	0,546
Postnasal drip	0,228	22,182	0,0001	0,478
Weakness	0,322	35,668	0,0001	0,568
Chills	0,365	43,036	0,0001	0,604
Sleep disturbance	0,219	20,997	0,0001	0,468
Irritability and tearfulness	0,184	16,886	0,0001	0,429

The relationship between the patient's age and the number of EBV DNA copies in saliva samples was also identified: R2 = 0.306 F = 34.893 p = 0.0001 β = 0.554 (Figure 4).

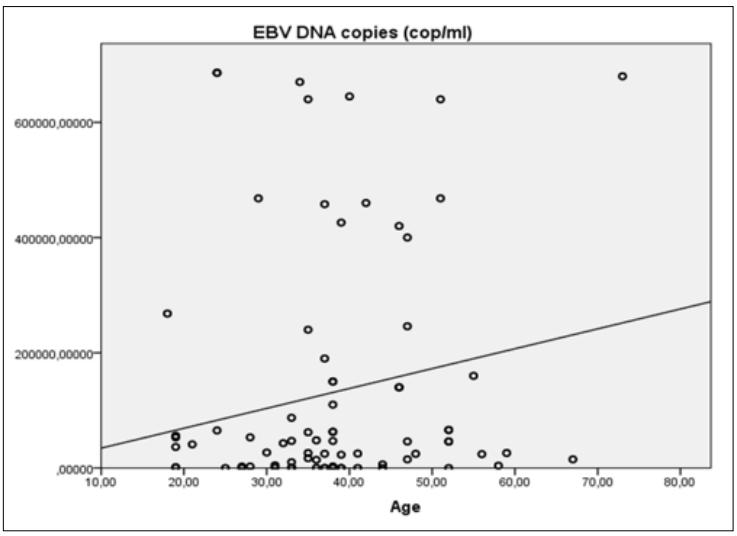

Figure 4: Relationship between the Number of EBV DNA Copies in Saliva Samples and the Patient's Age in the General Group of Patients with CVEB Infection

Discussion

Most people become infected with EBV during childhood or adolescence. This leads to the development of persistent, mostly latent EBV infection. Numerous studies have addressed the question of why some patients develop infectious mononucleosis and others do not. It has been suggested that it is not the EBV infection itself that plays a role (viremia or expansion of B cells), but the severity of the immune system response [19-22]. The state of chronic inflammation aggravates the patient's painful and depressive-like behavior, which develops in response to acute peripheral inflammation. The primary immune response to infection leads to the development of stereotypical clinical manifestations, such as fever, weakness, fatigue, sleep disturbances, arthralgia, irritability and cognitive impairment. The brain cytokine system is a conductor of an ensemble of neural circuits and neurotransmitters that organize physiological and pathological behavior [23]. Krzyszton C.P., et al. in their work showed that pro-inflammatory cytokines IL-1β, IL-6 and TNF-α can cause the development of fever, fatigue, sleep disturbances, depressive and cognitive impairment [24]. This immune response has been shown to be induced by the production and action of proinflammatory cytokines and other acute phase proteins. The next stage is the development of changes in the central nervous system through activation of resident microglial cells, secondary production of mediators and changes in neural transmissions. Our work shows that the level of induced TNF-α correlates with the development of headaches. There is evidence in the literature indicating an increase in the level of proinflammatory cytokines in the blood of children with headaches, which allowed the authors to express the opinion that neurogenic inflammation is involved in the development of headaches [25]. Tension headache patients showed higher levels of TNF-α (SMD 0.64, 95% CI: 0.33, 0.96, p =0.0001) compared to healthy controls. These findings support the findings of others that migraine patients typically have elevated levels of proinflammatory cytokines, indicating the presence of neuroinflammation [26-28]. A systematic review and meta-analysis of studies measuring peripheral blood cytokine levels in primary headache disorders was recently conducted and published. The study was conducted in patients with migraine, tension-type headache, cluster headache, primary headache, and new daily persistent headache. A total of 2,904 participants took part in the studies. Cytokine levels in headache patients have been shown to be elevated when compared with healthy donors: IL-1β (SMD = 0.75; P < 0.001), IL-6 (SMD = 1.18; P < 0.001), and TNF -α (SMD = 0.69; P = 0.003) These results also support the role of inflammation in the development of headaches. [29]. A high level of TNF-α production in the blood was detected in patients with episodic and chronic headache (P < 0.001) [30]. Togha M. and colleagues hypothesized that as levels of inflammatory factors increase, headaches tend to become more chronic [31]. There is evidence in the literature that the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α are involved in both the onset and persistence of pain through their direct effects on nociceptive sensory neurons and central sensitization [32]. The correlation between TNF-α levels and headache was demonstrated only by Pearson correlation R=0.223 p=0.040. Using the linear regression method, a very weak connection model was obtained: R2 = 0.050 F = 4.364 p = 0.040 β = 0.402, which we did not take into account. This is probably due to the peculiarities of blood sampling for TNF-α testing, which was carried out regardless of the presence or absence of headache at the time of sampling. Also important is the fact that a high level of TNF-α production was detected in only 11 patients out of 90. And in 30 patients the level was within normal values. Perhaps this can be explained by the course of the disease for more than 3 years.

It is hypothesized that infection may contribute to the development of changes in the patient's behavior due to inflammatory markers that are released due to the infectious immunological problem. In 2023, data were published studying the assessment of depression and the production of pro-inflammatory cytokines TNFα, IL6 in patients recovering from a bacterial infection. The authors of the work revealed a correlation between the infectious pathogen and the production of IL6 (r = 0.499, p <0.001) and TNFα (r = 0.235, p = 0.007) and suggested that pro-inflammatory cytokines can mediate the painful behavior of patients (depression, headaches, sleep disturbances) during acute inflammation [33].

U. Vollmer-Conna et al. conducted a correlation analysis between the spontaneous production of pro-inflammatory cytokines IL-1beta and IL-6 and clinical complaints (fever, malaise, fatigue, pain, decreased concentration and poor mood) in patients with acute EBV infection. The authors of the work identified a correlation between the level of IL-1beta and IL-6 production

and clinical complaints and suggested that cytokines are sensitive markers of the disease state in patients with acute infection [34]. Our work demonstrated a correlation between the level of induced TNF-α and the severity of postnasal drip in patients in the general group r = -0.231 p = 0.033. Our data confirm previously published results of studies of the content of cells producing proinflammatory cytokines and the level of cytokines in patients with sinusitis. According to the literature, the influence of TNF-α level on the development of refractory chronic rhinosinusitis has been shown [35]. Data have been published studying the presence of the non-histone nucleoprotein HMG B1 (High Mobility Group Box 1 chromosomal protein) when it is released into the extracellular space as a late mediator of inflammation by activated macrophages/monocytes. HMG B1 functions as an inflammatory cytokine and plays a role in damage-associated molecular patterning. The authors identified a correlation indicating that TNF-α-positive cells may be involved in the regulation of HMGB1 (R Sq log = 0.31) and showed increased cellular expression of TNF-α in patients with sinusitis ($P < 0.05$). [36,37]. Using the example of patients with Sjögren's disease, it was shown that HMGB1, together with TNF-α and IL-1β, can form a pro-inflammatory loop that contributes to chronic inflammation of the nasal mucosa and paranasal sinuses [38]. A positive correlation was also shown between increased levels of circulating TNF-α and an increased risk of developing allergic rhinitis (OR = 1.01478, 95% CI = 1.00225–1.02746, p = 0.02067), that is, the presence of a cause-and-effect relationship [39]. These results are consistent with our findings.

Previous work demonstrated by polymerase chain reaction amplification that EBV inhibits the production of TNF-α mRNA [15]. That is, EBV acquired the ability to suppress TNF-α production, thus avoiding the inhibitory effect of this cytokine and became a potent inhibitor of TNF-α production [40]. However, earlier work has shown that TNF-α can directly inhibit the replication of DNA viruses in vitro, playing a role in both virus-induced macrophage activation and the control of herpesvirus replication, independent of T and B cells [41]. TNF-α-dependent activation of macrophages during primary infection may play a role in establishing viral latency. A study of the effect of recombinant rTNF-α on the activation and differentiation of human B cells showed inhibition of EBV activation. Experimental work demonstrated a dose-dependent effect of rTNF-α on the proliferation and differentiation (Ig secretion) of EBV-infected B cells. Inhibition of B cell activation occurred in the presence of a significant number (25%) of plastic adherent macrophages in the B cell population. Macrophages, in response to rTNF-alpha, produced factors that alone or synergistically with rTNF-alpha inhibited B cell activation. The authors concluded that rTNF-α can modulate certain normal immune responses in vitro [41]. Another study demonstrated that serum TNF-α mRNA expression was significantly higher in patients than in healthy donors. The authors of the work showed that cytokine mRNA expression was detected in EBV-infected cells [42]. TNF-α has an antiviral effect inducing apoptosis in virus-infected cells and stimulating the inflammatory response at the site of infection [43, 44].

In our study, we analyzed the relationship between the number of EBV DNA copies and the production of induced TNF-α in the general group of patients using exponential regression and showed the existence of this regression model (R2 = 0.307). EBV infection of patients lasted for at least 3 years, and the longer the duration of infection, the lower the level of induced TNF-α. That is, at the early stage of infection, production is higher; as chronic EBV infection progresses, the level of induced TNF-α decreased to reference values. This is confirmed by the obtained regression model, indicating the significance of the effect of disease duration on the level of production of induced TNF-α in the general group of patients using linear and exponential regression (R2 = 0.386 and R2 = 0.735, respectively). Previously published studies have shown that in patients with acute infectious mononucleosis, the level of EBV replication in saliva samples does not correlate with the severity of symptoms [19, 45]. However, our linear regression study demonstrated the influence of DNA copy number in saliva samples on patients' clinical complaints. It was not possible to detect EBV DNA in blood samples due to the chronic course of infection, when EBV replication occurs in the oropharynx and the virus is released into saliva for a long time [46]. This can probably be explained not only by the duration of EBV infection, but also by the fact that clinical complaints persist for many years if the patient does not receive antiviral therapy.

Conclusion

Thus, a study of patients with chronic Epstein-Barr viral infection showed that patients with long-term illness have an increased level of production of induced TNF-α and a small number of copies of EBV DNA in saliva samples. The results of linear and exponential regression showed that the duration of the disease can influence changes in the level of production of induced TNF-α in the general group of patients. The level of TNF-α contributes to the development and progression of headaches and postnasal drip in patients in the general group. In the future, it is planned to study the production of interleukin-1 and interleukin-6 in patients with CVEB infection.

Data Sharing Policy: The Statistical Code, Dataset Used in Support of the Findings of This Study are Included within the Article.

Funding: The study had no sponsorship

Conflict of Interest: The authors declare no conflict of interest

Authors' Contribution: All the authors have made substantial contribution to this study and approved final script version.

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