



Potential effect of decreased levels of folic acid and vitamin B12 on herpes simplex virus keratitis reactivation

Mogući uticaj sniženih nivoa folne kiseline i vitamina B12 na reaktivaciju herpes simpleks virusnog keratitisa

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Abstract

Background/Aim. Most cases of herpetic keratitis present a recurrent disease as a result of herpes simplex virus type 1 reactivation from latency in the nearest sensory ganglia. Therefore, understanding the mechanisms of latency and reactivation of the latent virus is an important link in comprehending the onset of the recurrent eye disease itself. Epigenetic regulation of virus reactivation, as a result of the presence of transcriptionally active Latency-Associated Transcript (LAT) region in the latent viral genome, has already been demonstrated in several studies. The activity of the LAT region is directed to the chromatin arrangement. Epigenetic modulation of DNA methylation is associated with folate and vitamin B12 intake or their serum concentrations. To our knowledge, there is no report on the potential role of vitamin B12 and folic acid in herpes simplex virus keratitis reactivation. The aim of this study was to analyze the potential role of folic acid and vitamin

B12 in the control of ocular herpes simplex keratitis reactivation. **Methods.** The study included 50 patients older than 18 years of age with recurrent herpes simplex virus eye disease. Levels of vitamin B12 and folic acid were measured in the acute phase of the disease. All patients were followed up for at least one year and episodes of recurrent herpetic eye diseases were recorded. **Results.** The recurrence rate of herpetic keratitis was statistically significantly lower in patients with a higher blood level of vitamin B12. In addition, the recurrence rate of herpetic keratitis was lower in patients with a higher blood level of folic acid. However, statistical significance was lower in comparison with that for vitamin B12. **Conclusion.** The decreased levels of vitamin B12 and folic acid might have a vital role in herpes simplex keratitis reactivation.

Key words: herpesvirus 1, human; folic acid; keratitis; recurrence; risk factors; vitamin b 12.

Apstrakt

Uvod/Cilj. Herpetični keratitis u većini slučajeva je rekurentna bolest, a nastaje kao rezultat reaktivacije herpes simpleks virusa tipa 1 iz latentnosti u najbližoj senzornoj gangliji. Zbog toga je razumevanje mehanizma latentnosti i reaktivacije latentnog virusa važna karika u razumevanju nastanka recidivne bolesti oka. Sve više studija potvrđuje epigenetsku regulaciju reaktivacije virusa kao posledicu prisustva transkripciono aktivnog transkripta vezanog za latenciju (LAT) regiona u latentnom virusnom genomu. Aktivnost LAT regiona usmerena je na hromatinsko uređenje. Epigenetska modulacija metilacije DNA je povezana sa unosom folata i vitamina B12 ili njihovim koncentracijama u serumu. Prema našim saznanjima, ne postoji izveštaj o

potencijalnoj ulozi vitamina B12 i folne kiseline u reaktivaciji HSV keratitisa. Cilj rada bio je analiza moguće uloge folne kiseline i vitamina B12 u kontroli reaktivacije okularnog herpetičnog keratitisa. **Metode.** Studijom je bilo obuhvaćeno 50 bolesnika starijih od 18 godina sa različitim formama herpetičnog keratitisa, kao posledicom reaktivacije herpes simpleks virusa tipa 1. Nivoi vitamina B12 i folne kiseline mereni su u akutnoj fazi bolesti. Svi bolesnici su praćeni najmanje godinu dana, a beležen je broj recidiva virusne infekcije. **Rezultati.** Stopa recidiva herpetičnog keratitisa bila je statistički značajno niža kod bolesnika sa višim nivoom vitamina B12 u krvi. Pored toga, stopa recidiva herpetičnog keratitisa je bila niža i kod bolesnika sa višim nivoom folne kiseline u krvi, ali sa nižom statističkom značajnošću u poređenju sa onom za vitamin B12.

Zaključak. Sniženi nivoi vitamina B12 i folne kiseline mogu imati važnu ulogu u reaktivaciji herpetičnog keratitisa.

Ključne reči: herpesvirus 1, humani; folna kiselina; keratitis; recidiv; faktori rizika; vitamin b12.

Introduction

The results of recent studies have shown that 50%–90% of adult humans have serum antibodies to herpes simplex virus (HSV) type 1 (HSV-1)^{1,2}.

The annual incidence of all types of new ocular HSV infections has recently been estimated at 11.8 to 31.5 per 100,000 persons a year^{3,4}. The epithelial dendritic lesion is the most frequent type of recurrent keratitis, with prevalence as high as 56.3%, followed by stromal keratitis, 29.5%⁴. The clinical manifestations of primary HSV ocular infection are rare⁵. Reactivation of the latent virus in the ophthalmic branch of trigeminal ganglion can result in its shedding with subsequent infection of the overlying corneal epithelium^{6,7}. Herpetic keratitis occurs in various forms, and this largely depends on the depth of virus penetration into a corneal tissue. The direct effect of the virus and potent immune response to the viral proteins trigger corneal inflammation and neovascularisation leading to corneal thinning and scarring⁸.

Most cases of herpetic keratitis represent a recurrent disease that occurs as a result of HSV-1 reactivation from latency. Due to its recurrent nature, after cataract, herpes virus keratitis is the second leading cause of corneal blindness in the developed world. Therefore, understanding the mechanism and causes of HSV-1 reactivation from the latent state has long been the holy grail of herpes virologists. In animal models and later humans, the latency of the virus may have an epigenetic regulation, primarily because the latent viral genome has a transcriptionally active Latency-Associated Transcript (LAT) region. The activity of the LAT region is directed to chromatin arrangement without the encoding of known proteins⁹. Although viral mutants lacking LATs are still able to establish and maintain reactivation from latency, recent findings indicate that the LAT-region increases the reactivation efficiency and, in some way, controls the latency of the virus itself^{10,11}.

The aim of this study was to analyze the potential role of folic acid and B12 vitamin in the control of ocular HSV-1 reactivation. To our knowledge, there is no report on the potential role of vitamin B12 and folic acid in keratitis reactivation.

Methods

This study was conducted in compliance with the institutional review board regulations, the informed consent regulation, and it adhered to the tenets of the Declaration of Helsinki. It included 50 patients older than 18 years of age, regardless of gender, with recurrent ocular HSV-1 disease. Recurrent herpetic keratitis was confirmed by slit lamp

examination based on clinical findings. The recurrences were classified as epithelial keratitis, stromal keratitis, endothelitis, iridocyclitis, or as combinations of these conditions. Exclusion criteria were as follows: existing history of associated ophthalmic comorbidities, previous ocular surgery, some form of anemia, or systemic and neurological diseases.

All patients received at least a one-year follow-up between January 2017 and January 2018 at the Clinic for Eye Diseases, Clinical Center of Serbia in Belgrade. In all patients, levels of vitamin B12 and folic acid were measured during the acute phase of the recurrent ocular HSV disease.

All patients were fasting for 8 hours before having their blood samples taken. In addition, none of the included patients took any form of vitamin B complex supplementation for at least 12 months prior to the blood sample harvesting. Two milliliters of venous blood was collected in a standard biochemical tube. Vitamin B12 level was measured on the "Roche Cobas 6000" analyzer, (ECLIA Method) and folate level on the "Roche Cobas E411" analyzer, (ECLIA Method). Blood samples were analyzed in the same laboratory, certified by the Total Quality Management (TQM) quality system. Reference values for serum levels of vitamin B12 and folic acid were 6.9–44.4 ng/mL and 4.6–18.7 ng/mL, respectively. Statistical analysis was performed using SPSS Statistics 17.

Results

The analysis of our results showed that in all patients, blood levels of vitamin B12 and folic acid were in the lower reference range. According to the scatter diagrams (Figures 1 and 2), there was a drop in the number of relapses (as dependent variable) depending on both the blood level of vitamin B12 and folic acid (independent variable).

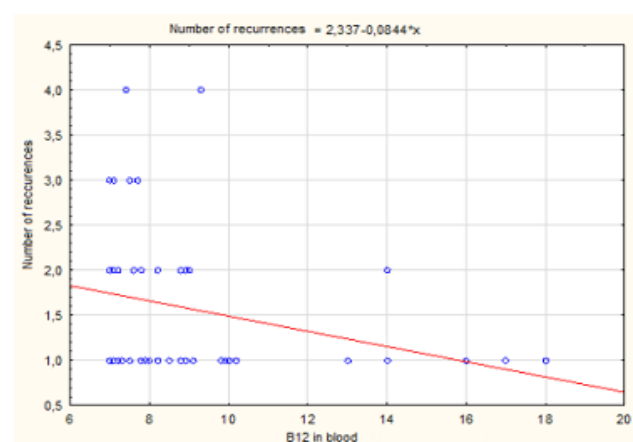


Fig. 1 – Scatter plot for vitamin B12 levels vs. number of herpetic keratitis recurrences.

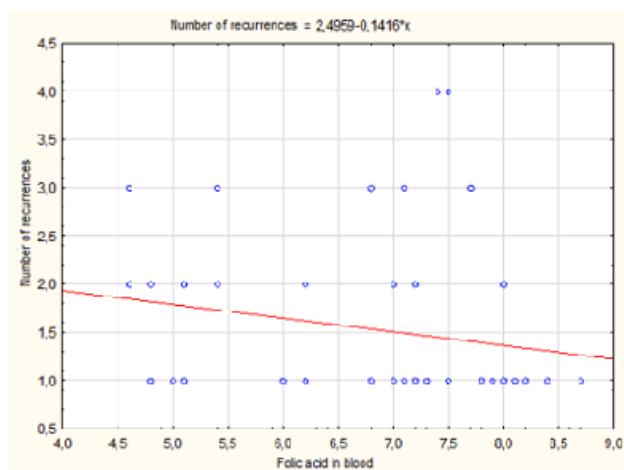


Fig. 2 – Scatter plot for folic acid levels vs. number of herpetic keratitis recurrences.

Analysis of Variance (ANOVA) and F-test ($F = 5.031$) (Table 1) showed that there were highly statistically significant difference between dependent (number of recurrences) and independent variables (B12 level in the blood); thus, the model had a statistical significance.

Pearson's correlation coefficient (Table 2) showed a statistically significant correlation between the level of vitamin B12 in the acute phase of the disease recurrence and the number of HSV keratitis recurrences. A higher level of vitamin B12 was associated with a reduced rate of disease recurrences. A higher folate level also had an impact on the

decrease of the number of HSV keratitis recurrences; however, it was not as significant as vitamin B12 impact.

Discussion

Both vitamin B12 and folic acid are involved in the methylation process of DNA molecules. Methylation of DNA molecules is associated with folate intake and serum folate concentrations in the body¹². A better insight into the epigenetic nature of the virus itself might help control HSV reactivation by using additional supplements in patients at higher risk of the disease recurrence. Interestingly, a case study from 1956 did not consider the epigenetic nature of virus reactivation. However, additional vitamin B12 supplementation significantly improved the clinical course of herpetic keratitis. Those patients experienced a milder clinical picture of recurrent herpetic eye disease^{13, 14}.

Reactivation of the virus from a latent phase of the disease into active HSV-1 keratitis may depend on the minimal deficiency of vitamin B12 or folic acids.

In our study, all patients had lower reference values of these vitamins in the acute phase of the disease. Therefore, this may be a potential trigger for virus reactivation and more severe clinical manifestations of herpetic keratitis.

Several studies have also found similar results with other viruses. Interestingly, Piyathilake et al.¹⁵ evaluated the influence of plasma folate and vitamin B12 concentrations on cervical cancer risk. Folate and vitamin B12 may play a critical role in lowering the human papillomavirus (HPV) 16

Table 1

Differences between number of herpetic keratitis recurrences and vitamin B12 level in blood

Model		Sum of squares	df	Mean square	F	Sig.
1	Regression	3.265	1	3.265	5.031	0.030
	Residual	31.155	48	0.649		
	Total	34.420	49			

Dependent variable: number of recurrences; Predictors: (constant), vitamin B12 level in blood

Table 2

Correlation between the levels of vitamin B12 and folic acid and the number of herpetic keratitis recurrences

Variable	B12 in blood	Folic acid in blood	Number of recurrences
B12 in blood	1		
correlation coefficient		0.587	-0.308
sig. (2-tailed)		0.000	0.030
Folic acid in blood		1	
correlation coefficient			-0.200
sig. (2-tailed)			0.164
Number of recurrences			1
correlation coefficient			
sig. (2-tailed)			

sig. – significance.

methylation-associated risk of developing higher grades of cervical intraepithelial neoplasia. Likewise, Lopes et al. ¹⁶ revealed that vitamin B12 intake was inversely associated with nononcogenic HPV persistence. Recently, it has been observed that recurrent aphthous stomatitis, including herpetic etiology, was also related to iron and vitamin B12 deficiency ¹⁷.

HSV establishes a latent infection in sensory neurons. The fact that the latent viral genome has a transcriptionally active LAT region that encodes the protein and transcriptionally inactive lytic gene regions suggests epigenetic regulation. The LAT region itself records various forms of histone arrangement ^{9, 18, 19}.

Immunological control of virus reactivation should also be considered. The environmental and physiologic factors that induce HSV-1 reactivation from latency include exposure to UV light, stress, and immune suppression, suggesting a possible role for T cells in preventing viral reactivation ^{21–23}. Studies in rabbits and mice also demonstrated that T cells infiltrate sensory neurons of the eye region around 8–10 days after corneal infection and remain there ^{24, 25}.

The virus does not produce proteins in the latency period, and in that way, it 'hides' from the immune system. Therefore, what maintained the attraction of CD8+ T cells for latently infected neurons was unclear. At this juncture, a definition of the terms latency and reactivation is important.

The virus is able to hide from the host immune system during latency since the immune system can only respond to viral protein synthesis.

As recently postulated, asymptomatic virus latency may also be related to the epigenetic nature of the virus. In our study, higher blood levels of both folic acid and vitamin B12 were associated with a reduced rate of recurrent herpetic keratitis. Future clinical and molecular epigenetic studies are necessary to clarify this further.

Conclusion

Our study showed that all patients in the acute phase of the disease had lower reference values of vitamin B12 and folic acid. Moreover, the recurrence rate of herpetic keratitis was lower in patients with higher blood levels of vitamin B12 and folic acid during the follow-up period.

Here, it is assumed that the reactivation of the HSV virus may be related to the minimal deficiency of vitamin B12 and folic acid during the latent phase of the disease.

Therefore, additional supplementation with vitamin B12 and folic acid may be helpful in preventing the reactivation of herpetic keratitis, potentially due to the epigenetic nature of virus reactivation. Further molecular epigenetic research and clinical studies may contribute to understanding and applying epigenetic therapy in herpetic eye disease.

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