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MOGUĆA ULOGA FOLNE KISELINE I VITAMINA B12 U REAKTIVACIJI HERPES SIMPLEKS VIRUSNOG KERATITISA

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POTENTIAL ROLE OF FOLIC ACID AND VITAMIN B12 IN HERPES SIMPLEX VIRUS KERATITIS REACTIVATION

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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 the nearest sensory ganglia. Therefore, understanding the mechanisms of latency and reactivation of latent virus is an important link in understanding the onset of recurrent eye disease itself. Epigenetic regulation of virus reactivation as a result of the presence of transcriptionally active LAT region (Latency-Associated Transcript - LAT) 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 folat and vitamin B12 intake or their serum concentrations. 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.** Recurrence rate of herpetic keratitis is lower in patients with a higher blood level of vitamin B12. There is statistical significance between these two variables. In addition, recurrence rate of herpetic keratitis is lower in patients with higher blood level of folic acid. However, statistical significance is lower in comparison with vitamin B12. **Conclusion.** Vitamin B12 and folic acid might have important role in herpes simplex keratitis reactivation. To our knowledge there is no report on potential role of vitamin B12 and folic acid in HSV keratitis reactivation.

**Key words:** Herpes simplex virus reactivation, HSV-1, keratitis, vitamin B12, folic acid, epigenetics.

Apstrakt

**Uvod/Cilj.** U većina slučajeva herpetični keratitis predstavlja rekurentnu bolest, kao rezultat reaktivacije herpes simpleks virusa tipa 1 iz latentnosti u najbližoj senzornoj gangliji. Prema tome razumevanje mehanizma latentnosti i reaktivacije latentnog virusa predstavlja važnu kariku u razumevanju nastanka recidivne bolesti oka. Sve je više studija
The results of recent studies have shown that 50-90% of adult humans have serum antibodies to herpes simplex virus type 1 (HSV-1). The annual incidence of all types of new ocular HSV infections has recently been estimated at 11.8 to 31.5 per 100,000 person a year. Epithelial dendritic lesion is the most frequent type of recurrent keratitis, with prevalence as much as 56.3%, followed by stromal keratitis, 29.5%. The clinical manifestations of primary HSV ocular infection are rare. Reactivation of latent virus in the ophthalmic branch of trigeminal ganglia can result in its shedding with subsequent infection of the overlying corneal epithelium. Herpetic keratitis occurs in various forms, and this largely depends on the depth of virus penetration into a
corneal tissue. Direct effect of virus and potent immune response to the viral proteins trigger corneal inflammation and neovascularisation leading to corneal thinning and scarring.

Most cases of herpetic keratitis represent recurrent disease that occurs as a result of HSV-1 reactivation from latency. Due to its recurrent nature, herpes virus keratitis is the second leading cause of corneal blindness, after cataract, in 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, latency of the virus may have epigenetic regulation, primarily because latent viral genome has a transcriptionally active LAT region. The activity of the LAT-region is directed to chromatin arrangement without encoding of known proteins. 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.

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 potential role of vitamin B12 and folic acid in HSV keratitis reactivation.

Methods

This study was conducted in compliance with the institutional review board regulations, informed consent regulation and 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 on the basis of clinical findings. Recurrences were classified as epithelial keratitis, stromal keratitis, endothelitis, iridocyclitis or as combinations of these conditions. Exclusion criteria were as follows: history of associated ophthalmic comorbidities, previous ocular surgery, some form of anemia or systemic and neurological diseases.

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

All patients were fasting 8 hours before taking blood samples. In addition, none of the included patients took any form of vitamin B complex supplementation for at least 12 months prior to 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. Correlation analysis was performed using SPSS Statistics 17.

Results

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

Analysis of Variance (ANOVA) table and F-test (F = 5.031) Table 1, show that there is highly statistically significant differences between dependent (number of recurrences) and independent variables (B12 level in the blood); thus, the model has a statistical significance.

![Graph showing correlation between B12 in blood and number of recurrences]
**Fig. 1** Scatter plot for vitamin B12 levels vs. number of recurrences

![Scatter plot for vitamin B12 levels vs. number of recurrences](image)

**Fig. 2** Scatter plot for folic acid levels vs. number of recurrence

**Table 1** Differences between number of recurrences and vitamin B12 level in the blood

<table>
<thead>
<tr>
<th>Model</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>3,265</td>
<td>1</td>
<td>3,265</td>
<td>5,031</td>
<td>.030</td>
</tr>
<tr>
<td>Residual</td>
<td>31,155</td>
<td>48</td>
<td>.649</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>34,420</td>
<td>49</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* a. Dependent Variable: Number of recurrences
* b. Predictors: (Constant), B12 in blood

Pearson correlation coefficient **Table 2** shows that there is 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. The higher level of vitamin B12 was associated with a reduced rate of disease recurrences. The higher folate level has also an impact on decrease the number of HSV keratitis recurrence; however, not as significantly as vitamin B12.
Table 2 Correlation between the level of vitamin B12 in and the number of HSV recurrences

<table>
<thead>
<tr>
<th></th>
<th>B12 in blood Pearson Correlation</th>
<th>Folic acid in blood Pearson Correlation</th>
<th>Number of recurrences Pearson Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>Sig. (2-tailed)</td>
<td>Sig. (2-tailed)</td>
</tr>
<tr>
<td>B12 in blood</td>
<td>1.587**</td>
<td>-0.308</td>
<td></td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.000</td>
<td>0.030</td>
<td></td>
</tr>
<tr>
<td>Folic acid in blood</td>
<td>1 -0.200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.164</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Discussion

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

Reactivation of the virus from a latent phase of 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 disease, thus it 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. Interesting, Piyathilake CJ et al. 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 HPV 16 methylation-associated risk of developing higher grades of cervical intraepithelial neoplasia. Likewise, Lopes et al. revealed that vitamin B12 intake was inversely associated with non oncogenic HPV persistence. Recently, it has been observed that recurrent aphthous stomatitis, including herpetic etiology was also related with iron and vitamin B12 deficiency.

Herpes simplex virus 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.

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

The virus does not produce proteins in the latency period and in that way it “hides” from the immune system. Therefore, it was unclear what maintained the attraction of CD8+ T cells for latently infected neurons. 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 be related to the epigenetic nature of the virus, as well. In our study, lower blood reference values of both, folic acid and vitamin B12 were associated with reduced rate of recurrent herpetic keratitis. Future clinical and molecular epigenetic studies are necessary to further clarify this.

**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, recurrence rate of herpetic keratitis was lower in patients with higher blood level of vitamin B12 and folic acid during follow-up period.

Here, we assume that reactivation of the HSV virus may be related with the minimal deficiency of vitamin B12 and folic acid during latent phase of disease.

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

**References**


15. Piyathilake CJ, Macaluso M, Chambers MM, Badiga S, Siddiqui NR, Bell WC, Edberg JC, Partridge EE, Alvarez RD, Johanning GL. Folate and vitamin B12 may play a critical role in lowering the HPV 16 methylation-associated risk of


