

# THE EFFECTIVENESS OF SUBMUCOSAL INJECTION BIOACTIVE VITAMIN B<sub>12</sub> IN COMPARISON TO TRIAMINOLONE IN PAIN AND FREQUENCY OF APHTHOUS ULCER

Golestannejad Z,<sup>1</sup> Ghalayani P,<sup>2</sup> Mojtahedi N,<sup>3</sup> Foroughi A<sup>4</sup>

1. Associate Professor, Torabinejad Dental Research, Department of Oral Medicine, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran

2. Assistant Professor, Torabinejad Dental Research, Department of Oral Medicine, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran

3. Dental Student, Torabinejad Dental Research, Department of Oral Medicine, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran

4. Dental Student, Torabinejad Dental Research, Department of Oral Medicine, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran

## ABSTRACT

**Aim:** Previous study was observed the effects of systemic administration B<sub>12</sub> in frequency of aphthous. According to the therapeutic effects of B<sub>12</sub> include Master-Key, anti-inflammatory and analgesic, topical administration has the potential to decrease pain and the frequency of aphthous. Cyanocobalamin is needed to metabolize in the liver and there is no possibility to become active in submucosal injection so the purpose of this study was evaluate the effect of bioactive Vitamin B<sub>12</sub> (methylcobalamin).

**Materials & Method:** This clinical trial single-blind was performed on 40 patients who randomly divided into two groups of 20 persons. The intervention group received submucosal injection B<sub>12</sub> (methylcobalamin) whereas the control group took a Triamcinolone. The pain and recovery time was calculated based on numeric scale and achieved to zero pain. Statistical analyses were performed using the SPSS version 22 statistical software. The statistical analyses were performed using an analysis of variance, T-test and survival analysis.  $\alpha=0/05$  was considered to indicate statistical significance.

**Results:** The results of this study indicate pain differences between the two groups was not significant on the first day ( $P=0/716$ ). The third day to sixth in the intervention group was significantly lower ( $P<0/05$ ). Again after seventh day was significant differences in pain between the two groups ( $P>0/05$ ) and the recovery time is significantly lower in the intervention group ( $P<0/001$ ).

**Conclusion:** This study indicates that submucosal injection B<sub>12</sub> (methylcobalamin) is an effective treatment for aphthous stomatitis whereas Triamcinolone.

**Key words:** Aphthous Stomatitis, Methylcobalamin, Vitamin B<sub>12</sub>.

## Introduction

Recurrent aphthous stomatitis is the most common disease of the oral mucosa,<sup>1,2</sup> which is determined by recurrent and painful single or multiple sores limited to the oral cavity.<sup>3</sup> Its prevalence is reported ranging from 5% to 66% in different populations, and its average prevalence is 20%. Its prevalence is higher in women.<sup>4,5</sup> Different etiologic factors have been reported for aphthous that its collection can be placed in three general categories: Immune regulation disorder caused by factors such as stress and anxiety, loss or destruction of oral mucosal barrier caused by trauma, nutritional deficiencies such as iron deficiency and vitamin B<sub>12</sub> deficiency and increasing contact with antigens such as streptococcus, herpes simplex virus and food antigens.<sup>1,2,5</sup>

Due to severe pain in aphthous wastes, many treatment methods have already been proposed for its treatment. But considering that the precise etiology of aphthous stomatitis is still unknown, currently symptomatic treatment is raised as standard treatment to reduce pain, inflammation and lesion period, with the use of corticosteroids.<sup>2</sup> Attention to new methods of treatment is essential due to the lack of efficient treatment and without side effect for aphthous.<sup>6</sup>

Lack of vitamin B<sub>12</sub> is raised as one of the etiologic agents of aphthous. Studies have shown in previous years, systemic prescription of vitamin B<sub>12</sub> in patients with deficiency of this vitamin causes to cut or reduce aphthous attacks.<sup>7,8</sup>

In recent years, again studies have been conducted on prescription of vitamin B<sub>12</sub>. Studies have shown that even in people with normal serum level of vitamin B<sub>12</sub>, systemic prescription of the vitamin in a high percentage of patients reduces the frequency of aphthous attacks.<sup>9,10,11</sup>

Aphthous recovery mechanism following systemic treatment with vitamin B<sub>12</sub> that even is seen in those without deficiency of this vitamin probably is due to the effect of its Master key. Treatment with vitamin B<sub>12</sub> can correct defects caused by the other biologic active material of body, Volkov *et al*<sup>9</sup> called this phenomenon the effect of Master key of vitamin B<sub>12</sub>.

Another treatment mechanism raised are the effect of antioxidant - anti-inflammatory<sup>12,13</sup> as well as anti-nosito of vitamin B<sub>12</sub> against chronic and acute pains.<sup>13</sup> Burgess and Haley<sup>14</sup> in 2008 used the active of vitamin B<sub>12</sub> (Methylcobalamin) as buccal Patch for systemic prescription. They considered the reason for using this drug, the need to metabolize the common form of vitamin B<sub>12</sub> (cyanocobalamin) in the body, and considered the possible reason for the lack of accountability of some patients to the vitamin B<sub>12</sub> prescription in previous studies, the prescription of inactive form of this vitamin.

In the studies that have already been conducted on the therapy impact of vitamin B<sub>12</sub> on aphthous, first treatments have been as systemic prescription of the drug in various forms (capsules - sublingual tablets, buccal Patch) and systemic absorption has been considered in all cases.<sup>7,8,9,14</sup>

Second, in most studies, inactive form of vitamin B<sub>12</sub> is used and only in the Burgess and Haley<sup>14</sup> in 2008, the active form B<sub>12</sub> (Methylcobalamin) is used. Also in these studies, the aim has been to investigate the effect of vitamin B<sub>12</sub> on reducing recurrent aphthous and its effect on pain relief and reducing the recovery period of aphthous has not been investigated.<sup>7,8,9,14</sup> Considering that vitamin B<sub>12</sub> has therapeutic effects include: Master key, antioxidant, anti-inflammatory and anti-nosisto (analgesic against chronic and acute pains)<sup>9,12,13</sup> and now also aphthous treatment is symptomatic treatment with anti-inflammatory and analgesic drugs, the aim of the present study was to evaluate the topical effect of active form of vitamin B<sub>12</sub> (Methylcobalamin) on pain and recovery period of aphthous stomatitis.

The null hypothesis of this study was that the active form of vitamin B<sub>12</sub> (Methylcobalamin) not affects the pain and recovery period of aphthous.

### Materials and Method

This study was conducted as a single- blind clinical trial.

40 samples were selected among patients with aphthous lesions referring to the section of Oral Medicine, dental school of Isfahan in 2015 by simple non-probability sampling method and then randomly divided into two groups of 20 persons.

Inclusion criteria of study consisted of the aphthous lesions with minor clinical view, since the advent of the lesion not to be passed more than 48 hours, not using any topical medicine on the aphthous lesion, the lack of any treatment action to reduce pain or treat aphthous lesion, not using any systemic drug in 3 recent months and not having systemic diseases including Behcet's syndrome and Reiter's syndrome.

Exclusion criteria from study consisted of aphthous lesions with clinical view of major aphthous or herpetiformis, since the advent of the lesion to be passed 48 hours, taking any topical medicine on the recent aphthous lesion, any treatment action to relieve pain or treat the recent aphthous, taking any systemic drug in 3 recent months and having systemic diseases including Behcet's syndrome and Reiter's syndrome.

In the first group, after applying the topical anesthetic using benzocaine gel 20% (Master-Dent, Dentonics Inc., USA), submucosal injection of the active form of vitamin B<sub>12</sub> of Methylcobalamin (Mecobalamin, Eisai Co., Japan) using insulin syringe (Becton, Dickins Co., USA) to the amount of 0.1 cc / cm<sup>2</sup> took place. In the second group, after applying topical anesthesia using benzocaine gel 20%, submucosal injection of triamcinolone (Cortiran, Iran Hormone, Iran) using insulin syringe to the amount of 0.1 cc / cm<sup>2</sup> took place. It was given to patients in both groups a form to determine the pain with numerical scale (Numeric Scale) to soecify the aphthous pain based on the criteria in the day of beginning treatment (first day) and after daily treatment until being zero of the criteria. Recovery period

due to the number of days required to achieve pain to zero (Numeric Scale = 0) was calculated.

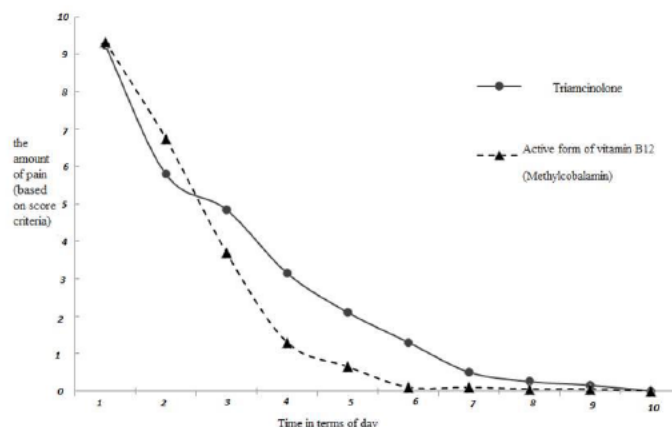
The results were analyzed statistically using SPSS version 22. To study the process of pain during the treatment in each group, individually statistical analysis of variance was used for frequent data and analyses of T test was performed to compare pain between the two groups, as well as compare the recovery time between the two groups was performed with statistical analysis of survival and the significant level was considered  $\alpha = 0.05$ .

### Results

To compare the effect of two drugs on aphthous recovery, average of the amount of pain (based on score criteria) and recovery time (Number of days that lasted until the pain reaches to zero) were determined and analyzed in the two groups of case and control. To study the process of pain during the treatment in each group, individually statistical analysis of variance was used for frequent data and analyses of T test was performed to compare pain between the two groups, as well as compare the recovery time between the two groups was performed with statistical analysis of survival and the significant level was considered  $\alpha=0.05$ .

In the following part, the results and analyses related are described.

In Figure 1, the amount of pain at the day of starting treatment and the days after it (Up to the day of ending pain) in both case and control groups is shown.



Graph1: the amount of pain in treatment days in two groups of case (B<sub>12</sub>) and control (triamcinolone)

Figure 1: The amount of pain in treatment days in two group of cases (B<sub>12</sub>) and control (triamcinolone).

To study the process of pain during treatment in both groups of case and control, statistical analysis of variance was performed for frequent data and in completing it, Pair T test was conducted, the results showed the pain has significantly decreased until sixth day in the case group (treated with active form of vitamin B<sub>12</sub>) and until seventh day in the control group (treated with triamcinolone). ( $0.05 > P$  Value)

For comparison of pain between the two groups, the average and standard deviation of pain was determined at the beginning of treatment (first day) and the days after it (Table 1) and then the pain between the two groups was compared by statistical analysis of T test of.

The results showed difference in pain between the two groups of case and control was not significant in the first day. (P Value=0.716), in the second day, in the control group (treated with triamcinolone) significantly was lower than the case group (treated with Methylcobalamin). (P Value=0.001), but on the third day, fourth, fifth and sixth, pain in the case group was significantly lower than the control group. (Respectively P Value=0.018, P Value=0.000, P Value=0.007, P Value=0.007), from the sixth day later ie the seventh, eighth and ninth days, the amount of pain between the two groups of case and control as the first day was significant without statistical difference. (Respectively P Value=0.206, P Value=0.281, P Value=0.411)

Days of Treatment	Average + SD of Amount of Pain	
	Case Group	Control Group
1 <sup>st</sup> Day	9.35 ± 0.745	9.25 ± 0.966
2 <sup>nd</sup> Day	6.75 ± 0.019	5.80 ± 1.196
3 <sup>rd</sup> Day	3.70 ± 1.592	4.85 ± 1.348
4 <sup>th</sup> Day	1.30 ± 1.592	<b>2.10 ± 1.832</b>
5 <sup>th</sup> Day	0.65 ± 1.384	<b>1.30 ± 1.838</b>
6 <sup>th</sup> Day	0.10 ± 0.447	0.50 ± 1.317
7 <sup>th</sup> Day	0.05 ± 0.223	0.25 ± 0.786
8 <sup>th</sup> Day	0.05 ± 0.223	0.25 ± 0.786
9 <sup>th</sup> Day	0.05 ± 0.223	0.15 ± 0.489

Table 1: Average and standard deviation of pain during treatment days in both groups of case (treated with activated Vitamin B<sub>12</sub>) and control (treated with triamcinolone)

In Table 2, the average of time of aphthous recovery (the number of days that lasted until the pain reaches to zero) in the two groups of case (treated Methylcobalamin) and control (treated with triamcinolone) is shown.

Recovery Time ( Based on Day)	
Control Group	Case Group
6.3 ± 1.4	4.85 ± 1.2

Table 2: Average and standard deviation of aphthous recovery time in the two groups of case and control

T test analysis was used for comparison of aphthous recovery time between two groups of case and control, the results showed that the recovery time in case group (treated with Methylcobalamin) significantly has been lower than the control group (treated with triamcinolone). (P Value<0.001)

## Discussion

The null hypothesis of this study was rejected that the active form of vitamin B<sub>12</sub> (Methylcobalamin) not affects the amount of pain and the aphthous recovery period.

The results of this study showed that submucosal injection of active form of vitamin B<sub>12</sub> (Methylcobalamin) compared with submucosal injection of triamcinolone significantly reduced pain (P Value<0.05) and aphthous recovery time (P Value<0.001).

Since 1954, the Brachman<sup>15</sup> for the first time raised the relationship between RAS and vitamin B<sub>12</sub> deficiency; several studies have shown the effect of prescription this vitamin on reducing the recurrence of aphthous attacks.<sup>9,14,16</sup>

But so far only in the study of Volkov *et al.*<sup>9</sup> in 2009, the effect of prescription of this vitamin on pain relief and aphthous recovery period is examined that the results of it indicate reducing pain and aphthous recovery period. Two differences of present study with study of Volkov is the application of active form of vitamin B<sub>12</sub> (Methylcobalamin) rather than its inactive form (cyanocobalamin) and other topical prescription (as submucosal injection of mentioned drug rather than its systemic prescription). Considering that cyanocobalamin to carry out its activities such as analgesic effect (anti-nosisto) needs to metabolize and convert to the active form (Methylcobalamin) in the liver and there isn't the possibility of metabolize in the liver following topical prescription, in this study Methylcobalamin was prescribed topically.

In the present study because of the use of submucosal form instead of systematic form, there is the possibility of access of drug to the lesion and the onset of the therapeutic effects including anti-inflammatory and analgesic effects predictably. On the other hand, because in the present study, vitamin B<sub>12</sub> is topically used, the possibility of metabolize is not existed in the liver, so the use of the active form of drug (Methylcobalamin) rather than inactive form (cyanocobalamin) has removed the need to metabolize the drug in the liver.

Prescription of the active form B<sub>12</sub> (Methylcobalamin) rather than its inactive form (cyanocobalamin) in patients with RAS is conducted only in the study of Burgess and Haley<sup>14</sup> in 2008. Two differences of present study with study of Burgess is the topical prescription (submucosal) of the drug in the present study rather than systemic prescription in the study of Burgess and the other is to prescribe this drug simultaneously with the incidence of aphthous attacks and as a single dose to reduce symptoms and recovery time in the present study instead of the prescription 6 months and with the aim of reducing recurrence of aphthous attacks in Burgess study.

Since in the present study, the aim of prescribing vitamin B<sub>12</sub> was to achieve the effects of analgesic and anti-inflammatory and ultimately reducing the aphthous lesions, its prescription was only designed during the incident of lesions and as mentioned in the previous section, submucosal injection was with the aim of possibility of access drug to position of its performance ie the submucosal of aphthous lesions.

The limitations of this study were impossibility of investigating histopathologic of aphthous lesions treated with vitamin B<sub>12</sub> (case group) and triamcinolone (control group) and compare the process of pathological improvement of these two with each other.

It is suggested, considering that in this study for the first time, the active form of vitamin B<sub>12</sub> (Methylcobalamin) is topically used for the treatment of aphthous and the results indicated the reducing of pain and recovery time of lesions, other clinical studies with more samples and comparing the prescription of two types of systemic with topical pharmaceutical form (active vitamin B<sub>12</sub>) to be conducted.

### Conclusion

The results of this study showed that submucosal injection of active form of vitamin B<sub>12</sub> (Methylcobalamin) compared to triamcinolone injection significantly improved pain and reduced recovery time of aphthous stomatitis.

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### References

- Mojtahedi N, Golestannejad Z, Shahi HY, Bahrani M. Comparison of antibacterial activity from essential oils of fennel, mint and peppermint against streptococcus mutants. *J Fundamental Applied Sci* 2016;8(2s):3298-314.
- Mojtahedi N, Ghalayani P, Golestannejad Z, Davari A, Shahi HY. Comparative evaluation of two forms of drugs: patch & lotion of clobetasol, on recovery indicators of aphthous stomatitis. *Annals of Dental Specialty* 2017;5(2):27-32.
- Rattan J, Schneider M, Arber N, Gorsky M, Dayan D. Sucralfate suspension as treatment of recurrent aphthous stomatitis. *J Intern Med* 1994;236(3):341-3.
- Sonis S, Fazio R, Fany L. Principle and practice of oral medicine. 2nd ed. Philadelphia: W.B. Saunders Co; 1995, 376-9.
- Weusten B, van de Wiel A. Aphthous ulcers and vitamin B<sub>12</sub> deficiency. *Neth J Med* 1998;53(4):172-5.
- Hoseinpour H, Peel SA, Rakhshandeh H, Forouzanfar A, Taheri M, Rajabi O *et al*. Evaluation of Rosa damascena mouthwash in the treatment of recurrent aphthous stomatitis: a randomized, double-blinded, placebo-controlled clinical trial. *Quintessence Int* 2011;42(6):483-91.
- Scully C, Gorsky M, Lozada-Nur F. The diagnosis and management of recurrent aphthous stomatitis: A consensus approach. *J Am Dent Assoc* 2003;134(2):200-7.
- Porter S, Flint S, Scully C, Keith O. Recurrent aphthous stomatitis: the efficacy of replacement therapy in patients with underlying hematinic deficiencies. *Ann Dent* 1992;51(2):14-6.
- Volkov I, Press Y, Rudoy I. Vitamin B<sub>12</sub> could be a "master key" in the regulation of multiple pathological processes. *J Nippon Med Sch* 2006;73(2):65-9.
- Gulcan E, Toker S, Hatipoğlu H, Gulcan A, Toker A. Cyanocobalamin may be beneficial in the treatment of recurrent aphthous ulcers even when vitamin B<sub>12</sub> levels are normal. *Am J Med Sci* 2008;336(5):379-82.
- Volkov I, Rudoy I, Freud T, Sardal G, Naimer S, Peleg R *et al*. Effectiveness of vitamin B<sub>12</sub> in treating recurrent aphthous stomatitis: a randomized, double-blind, placebo-controlled trial. *J Am Board Fam Med* 2009;22(1):9-16.
- Manzanares W, Hardy G. Vitamin B<sub>12</sub>: The forgotten micronutrient for critical care. *Curr Opin Clin Nutr Metab Care* 2010;13(6):662-668.
- Hosseinzadeh H, Moallem SA, Moshiri M, Sarnavazi MS, Etemad L. Anti-nociceptive and anti-inflammatory effects of cyanocobalamin (vitamin B<sub>12</sub>) against acute and chronic pain and inflammation in mice. *Arzneimittelforschung* 2012;62(7):324-329.
- Burgess JA, Haley JT. Effect of bioactive B<sub>12</sub> in adhering discs on aphthous ulcers. *Inside Dentistry* 2008;4(9):60-64.
- Brachmann F. Treatment of chronically recurrent aphthae with vitamin B<sub>12</sub>. *Zahnarztl Welt* 1954;9(3):3/58-9.
- Zhong D, Mao X., Li A. Observation on effect of compound vitamin B<sub>12</sub> solution to treat patients with oral ulcers induced by nebulization inhalation of surface corticosteroid. *Chinese Gen Nur* 2010;8(8):1988-1989

### Corresponding Author

#### Dr. Navid Mojtahedi

Dental Student,  
Torabinejad Dental Research,  
School of Dentistry,  
Isfahan University of Medical Sciences,  
Isfahan, IRAN  
Email Id: - navid.mojtahedi.nm@gmail.com