Relative assessment of serum vitamin B_{12} and its metabolites in recurrent aphthous stomatitis. A short communication

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KEYWORDS

Recurrent aphthous stomatitis, Vitamin B_{12}, Homocysteine, MMA, Subclinical vitamin B_{12} deficiency.

ABSTRACT

Aim: *Recurrent aphthous stomatitis (RAS), is characterized by recurrent, ulcerative oral lesions which are often painful, single or multiple, round or ovoid ulcers, present during childhood or adolescence and recur for variable periods of time. Although, exact pathogenesis of RAS is unclear, suppression of cell-mediated immunity and changes in the cells of the tongue and buccal mucosa, which include atrophy and thinning of the oral epithelium and enhanced penetration of exogenous antigens. The aim of the present study was to evaluate the serum levels of vitamin B12 and its metabolites, homocysteine (Hcy) and methylmalonic acid (MMA) in RAS and healthy controls and to establish its role in the etiology and pathogenesis of RAS.*
Materials and methods: *A group of 15 subjects of either gender diagnosed with RAS on the basis of clinical history and features and 15 healthy controls was selected. The data obtained were statistically analyzed using Student's t-test, Chi-square test and Fisher exact probability test.*
Results: *The values of serum vitamin B12 were normal, however those of Hcy were higher in the study group as compared with controls. The values of serum MMA were lower in the study group as compared with the controls. The levels in some cases of the study group were in the range indicating a sub-clinical vitamin B12 deficiency.*
Conclusion: *A normal serum concentration of vitamin B12 does not rule out tissue deficiency of vitamin B12. If clinical symptoms suggest deficiency, measurement of MMA and Hcy may be considered, even if serum vitamin B12 concentrations are normal.*

Introduction

Recurrent aphthous stomatitis (RAS), is a oral mucosal disorder, characterized by recurring ulcers with no other signs of disease, affecting one subject in five of the population at some time in their lives (1). The condition affects 5%-25% of the general population and can start in childhood, adolescence or young adults in the age group below 30 years. The diagnosis is based on patient history and physical examination (2). The cause of RAS is unknown, however several factors including local and systemic conditions, genetics, immunological factors, stress, bacterial and viral infections (human herpes virus 6), smoking habit, nutritional deficiencies (vitamin B1, B2, B6, B12, iron, folate), diet, hormonal changes, gastrointestinal disorders may play a role (3,4).

Vitamin B12 (cobalamin) is unique among all the vitamins in that it contains not only a complex organic molecule, but also an essential trace element: cobalt. It plays an important role in DNA synthesis and has important immuno-modulatory and neurotrophic effects. Deficiency of vitamin B12 can lead to a wide spectrum of disorders that can often be reversed by early diagnosis and prompt treatment. It is widely recognized as a possible predisposing factor for RAS. There are associated abnormalities in cell structure

and keratinization pattern of the oral epithelium, which are similar to those observed in the blood and bone marrow in cases of insufficient DNA synthesis. It has also been postulated that the presence of a deficiency allows the expression of an underlying tendency to ulceration (5).

Homocysteine (Hcy) is a sulfur-containing amino acid produced during the metabolism of the essential amino acid methionine in all cells through the normal methylation process. Hcy is the strong stimulus for T-cell production, which induces cellular activity and differentiation along with cytokine-induced cell death and apoptosis (6). Hcy has various effects on function of immune system, blood circulation and tissues. High levels of Hcy are reported in case of vitamin B12 or folate deficiency. This high serum Hcy level may result in increased frequency of thrombosis in the feeding arterioles that supply the oral epithelial cells. This in turn leads to a breakdown of the oral epithelium and finally results in oral ulceration. Taken these together, hematinic deficiencies and elevated Hcy levels can decrease oral epithelial barrier and thus increase the frequency of RAS occurrence (7).

Methylmalonic acid (MMA) is an intermediate of amino acid metabolism which results from the conversion of propionyl-coenzyme A to succinyl-coenzyme A in a vitamin B12-dependent reaction, and is regarded as an early, sensitive biomarker of vitamin B12 deficiency. MMA is a valuable marker to identify and optimize the effect of vitamin B12 supplementation and for monitoring the success of the B12 treatment. MMA concentration decreases approximately 1–2 weeks after initiation of vitamin B12 supplementation, depending on the dosage and the initial MMA concentration.

It has been suggested that Hcy and MMA tests are more effective in the diagnosis of suspected cobalamin deficiency states (8). To increase specificity and sensitivity in the diagnosis of vitamin B12 deficiency, the concept of measuring serum levels of Hcy and MMA has aroused great interest (9). Hcy concentration provides information on whether the intracellular methionine cycle is functioning (which depends on vitamin B12), whereas MMA specifically documents the effectiveness of vitamin B12 dependent reactions (10). The high level of Hcy and MMA indicates sub-clinical vitamin B12 deficiency, although there may be no clinical symptoms.

However, to the best of our knowledge the measurements of Hcy and MMA in recurrent aphthous stomatitis has not been studied extensively. Therefore, the identification and measurement of functional vitamin B12 deficiency remains controversial. Therefore, the aim of this study is to assess vitamin B12 and its metabolites Hcy and MMA in patients with RAS and healthy controls and their possible role in the etiology and pathogenesis of RAS.

Patients and methods

A total of 30 patients in the age range of 20-58 years of both genders attending the Out Patient Department of a private dental college in Bangalore, with active episodes of RAS as well as healthy controls were included in the study after obtaining their informed consent. Among these, two groups were selected with 15 subjects affected with recurrent aphthous ulcer patients and 15 healthy controls.

The procedure was explained to the subjects involved in the study.

The patients were selected based on the following criteria.

Inclusion criteria

The study included the patients with minimum 2 years duration of RAS. The control group consisted of healthy individuals with clinically normal oral mucosa who reported to the dental hospital for routine diagnostic and therapeutic purposes and who were not under any medications or vitamin therapy.

Exclusion criteria

Patients with conditions such as Behcet syndrome, Celiac, Crohn's disease, traumatic ulcers and any other systemic disease and those patients who were under iron supplementation and/or multi-vitamin therapy. Patients with history of smoking and alcohol were also excluded from the study.

To all the participants the need for undergoing a thorough clinical examination and blood investigations were explained at the start of the study. Only those patients who gave their informed consent were included in the study.

Clinical procedure

After clinical examination, and establishing diagnosis of RAS and signing a written consent form, approximately 5 ml of blood were sampled by venipuncture using a sterile disposable syringe from 15 patients with RAS and 15 healthy controls and then drawn into centrifuge tubes. Blood was subjected to centrifugation at 3000 rpm for 3 min to obtain serum, which was stored in Eppendorf tubes at -800 C.

For the study of vitamin B12, Elecsys 2010 (Roche Diagnostics, Mannheim, USA) analyser was used. For the study of the Hcy and MMA Agilent 7820A Gas Chromatography analyser was used.

Results

In the 15 study subjects, 8 (53.3%) were in the age group of 18-25 years, 2 (13.3%) subjects were in the age group of 26-30 years and 5 (33.3%) were in the age group of 31-58 years with a mean age of 28.33

years.

In the 15 controls, 8 (53.3%) were in the age group of 18-25 years, 5 (33.3%) were in the age group of 26-30 years and 2 (13.3%) were in the age group of 31-58 years with a mean age of 23.89 years.

In the present study out of 30 samples 19 were males and 11 females. In the study group 8 subjects (53.3%) were males and 7 (46.6%) were females; in the control group 11 (73.3%) were males and 4 (26.6%) were females.

The mean serum vitamin B12 level in the study group was 233.9333 with a standard deviation of 108.04726. Out of 15 controls, mean value of serum vitamin B12 level was 170.8000 with a standard deviation of 33.62652. Student's t-test is used to test the mean difference of vitamin B12 between study group and control group. There is a significant difference in mean vitamin B12 between study group and control group.

In the present study, the mean value of serum homocysteine level in the study group was 13.7453 with a standard deviation of 1.93094. In controls, mean value of serum homocysteine level was 11.3533 with a standard deviation of 2.10928.

The mean value of serum methyl malonic acid level in the study group was 0.3927, with a standard deviation of 0.42635, in controls it was 0.8213 with a standard deviation of 0.47211 (Table 1).

Discussion

Recurrent aphthous stomatitis (RAS) is one of the most common oral mucosal lesions, seen by the general dental practitioner and oral physicians. Typically RAS lesions are characterized by painful, recurrent, single or multiple ulcers of the oral mucosa which are usually round or oval in shape. The margin usually appears edematous, inflamed and red, giving a crater like appearance to the lesion. The central area is often coated with a characteristic grey-white fibrino-purulent pseudomembrane.11

Discomfort in patients with RAS is relatively high. The patients' life quality is affected by these painful mucosal lesions. The presence of ulcerations increase the flow of saliva and interfere with eating, drinking and speaking. Since the etiology of RAS is unknown, many patients are receiving medications only to relieve pain (12). Factors related to the etiology of the disease include the oxidant-antioxidant balance of the organism directly or indirectly involved and factors that trigger free radical formation (13).

RAS patients with anemia and lower hemoglobin levels have reduced capacity of the blood to carry oxygen to the oral mucosa, that may eventually result in atrophy. Oral epithelial cells have a high turnover rate, therefore deficiencies of iron, vitamin B12 and folic acid may result in oral epithelial atrophy. Atrophic oral epithelium in hematinic deficient patients may explain why some patients with deficiencies of hematinics are prone to have RAS (14).

In humans, only two enzymatic reactions are known to be dependent on vitamin B12. In the first reaction, methylmalonic acid (MMA) is converted to succinyl-CoA using vitamin B12 as a cofactor. Vitamin B12 deficiency, therefore, can lead to increased levels of serum MMA. In the second reaction, homocysteine (Hcy) is converted to methionine by using vitamin B12 and folic acid as cofactors. In this reaction, a deficiency of vitamin B12 or folic acid may lead to increased Hcy levels (15). Furthermore, measurements of metabolites such as MMA and Hcy levels have been shown to be more sensitive in the diagnosis of vitamin B12 deficiency than measurement of serum B12 levels alone.

This functional B12 imbalance is characterized by increased Hcy and MMA concentrations in the serum without any clinical evidence of vitamin B12

deficiency. The exact prevalence of clinically significant B12 deficiency is not known, the range of symptoms is wide and the new markers enable the detection of vitamin deficiency notably more often (10).

Use of MMA and Hcy levels in the diagnosis of vitamin B12 deficiency has led to some surprising findings. In approximately 50 percent of patients with RAS, there is sub-clinical vitamin B12 deficiency without any clinical manifestations. In such cases, one

Study parameter	Groups	Mean ± SD	P- value between study and control group
Vitamin B12	Patient Control	233.9333 ± 108.04726	(p<0.05).
		170.8000 ± 33.62652.	
Homocysteine	Patient Control	13.7453 ± 1.93094	(p<0.01).
		11.3533 ± 2.10928	
Methylmalonic acid	Patient Control	0.3927 ± 0.42635	(p<0.01).
		0.8213 ± 0.47211.	

Table 1 Comparison of mean serum vitamin B12, homocysteine and methylmalonic acid levels in study and control group

can suspect increase in MMA and Hcy levels above normal limits. If in these patients there is normalization of MMA and Hcy levels with vitamin B12 replacement therapy, then these levels can be used as diagnostic criteria for vitamin B12 deficiency (15).

It is possible that even when the serum cobalamin level is high, treatment with vitamin B12 can correct defects caused by deficiency of other biologically active substances that regulate the system and try to keep it in balance such as thiamine, riboflavin, iron, folic acid and zinc. It has been suggested that increasing or supplementing dietary intake of vitamin B12 and folate may be of value in preventing RAS episodes (5, 16).

The measurements of vitamin B12 metabolites such as MMA and Hcy have been shown to be more sensitive in the diagnosis of vitamin B12 deficiency than measurement of serum B12 levels alone, which is one of the etiological factor in RAS (17, 18, 19). It has been found that as cobalamin deficiency develops, a diagnostic elevation of the serum MMA is typically an early event, often preceding the decrease in serum cobalamin or increase of the total Hcy. There is evidence that these serum metabolites of B12 are sensitive indicators of the presence of tissue deficiency of cobalamin. The serum level of cobalamin, therefore cannot be considered to be 100% sensitive in the detection of cobalamin deficiency. It is also apparent that serum metabolite levels obtained before and after specific treatment with vitamin B12 may be useful and more accurate in identifying patients with atypical findings with greater certainty (5). Therefore to increase specificity and sensitivity in the diagnosis of vitamin B12 deficiency, the concept of measuring Hcy and MMA has aroused great interest.

There are no generally accepted guidelines for the definition, diagnosis, treatment, and follow-up of cobalamin deficiency. It is likely that, even if the serum level of cobalamin is within normal limits, treatment with vitamin B12 could correct defects caused by other biologically active substances. The probability of sub-clinical vitamin B12 deficiency decreases with increasing levels of vitamin B12. This is called as “Master Key” effect. This potential beneficial effect can be tested by treating diseases such as RAS with vitamin B12, even if its serum level is normal (10).

Many studies were conducted which highlights the role of treating RAS (15, 20). The current evidence is suggestive that for patients with RAS, daily intake of vitamin B12 supplementation may be beneficial and may be useful as a simple, inexpensive treatment in both acute and long term care.20

This study, has thus brought forth conflicting results to many studies conducted so far. However, it includes a smal number of subjects in an urban population to detect the presence of vitamin B12 deficiency in RAS patients. There is need for similar studies to be carried out on a larger scale in the rural and the underprivileged and other populations. Additionally, the test for vitamin B12 has several pitfalls. Most laboratories set normal limits at 200 to 900 pg/mL, but sensitivity and specificity vary greatly, depending on the method used. First, the studies which are carried out for presence of vitamin B12 deficiency in any subset of population should use a validated tool to quantify dietary intake of vitamin B12. These observations, then need to be corroborated with serum vitamin B12, Hcy and MMA levels.

Therefore, it is recommended that studies should be conducted on groups with larger sample size to evaluate the role of vitamin B12, Hcy and MMA in patients with RAS. Also consuming sufficient amount or supplementing the diet with vitamin B12, irrespective of its serum level, is beneficial in the management of RAS.

Conclusion

HThe results of our study have indicated the importance of sub-clinical vitamin B12 deficiency or functional vitamin B12 imbalance, which can probably play a role in several body functions and also in RAS. Further studies have to be carried out with respect to Hcy and MMA in RAS subjects as no studies have been found in the literature.

Disclaimers

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