

Lipid Peroxidation and Serum Total Antioxidant Status in Patients with Recurrent Aphthous Ulceration Treated by Herbal Medicine

Farhad N. Hussein

College of Pharmacy, Hawler Medical University, Erbil, Iraq

Abstract

Background: Recurrent aphthous is a common but poorly understood oral mucosal disorder. Impaired oxidant/antioxidant balance is associated with the tissue damage in recurrent aphthous. **Aim:** To evaluate lipid peroxidation and serum total antioxidant status (TAS) in aphthous patients treated locally by herbal medicine. **Patients and methods:** Patients were diagnosed and treated for recurrent ulceration in Outpatient Clinic, Mosul, Iraq, from September 2011 to February 2012. A total of 42 patients with recurrent aphthous ulceration (31 males and 11 females) and 25 healthy subjects as controls (19 males and 6 females) were included in the study. The patients were treated with aphthous cream that contained clove, thyme, mint, and liquorice, applied six times daily until cure or for two weeks. The patients were advised to use the cream for two weeks even the aphthous is healed. Blood samples were taken (5 mL) from patients before and after two weeks of treatment and other blood samples from the control group and analyzed for serum malodialdehyde (MDA) and total antioxidant status (TAS) by colorimetric methods. **Results:** In aphthous patients, serum MDA was significantly higher ($p \leq 0.001$) than that in controls (1.82 ± 0.19 vs 1.06 ± 0.12 $\mu\text{mol/L}$), while serum TAS was significantly lower ($p \leq 0.001$) than in the control group (1.16 ± 0.11 vs 1.70 ± 0.16 mmol/L). After treatment, serum MDA was decreased significantly ($p \leq 0.001$) compared with the value before treatment (1.82 ± 0.19 vs 1.74 ± 0.17 $\mu\text{mol/L}$); however, serum TAS was increased significantly ($p \leq 0.05$) after treatment compared with the value before treatment (1.16 ± 0.11 vs 1.2 ± 0.08 mmol/L). Both serum MDA and TAS in patients after treatment did not return to the control levels. The aphthous healed in most of the treated patient after 5-7 days, only two patients cured after 10 days of treatment. **Conclusion:** Aphthous ulceration in patients raises lipid peroxidation and reduces antioxidants. Aphthous treated with cream contained clove, thyme, ment and liquorice, healed within a week and the cured patients were associated with reduced lipid peroxidation and raised antioxidants status.

مستويات زناخة الدهون ومضادات الاكسدة الكلي في مصلى مرضى التهاب الفم
القلاعى المتكرر الخاضعين لعلاج عشبى موضعى

فرهاد نعمة الله حسين

الخلاصة

تعتبر قرحة الفم المتكررة من الامراض الشائعة ولكن من الصعوبة معرفة سبب الخلل فى غشاء الفم. ان الخلل فى توازن عامل الاكسدة يكون مصحوبا بتدمير النسيج فى قرحة الفم المتكررة. أهداف البحث: لتقييم زناخة الدهون ومستوى مضادات الاكسدة الكلي فى مرضى قرحة الفم والخاضعين للعلاج الموضعى العشبى. المرضى

وطرائق العمل: تم تشخيص مرضى التهاب الفم المتكررة وعلاجهم في العيادة الخارجية في المستشفى الجمهوري التعليمي في الموصل / العراق للفترة من شهر ايلول 2011 لغاية شهر شباط 2012. تضمنت الدراسة 42 مريضا" بالتهاب الفم المتكرر (31 ذكرا" و 11 اناث) مع 25 شخص سليم كمجاميع سيطرة (19 ذكر ، 6 اناث) . تم اخضاع المرضى للعلاج بـكريم (افتوس) والحاوي على الزيوت الاساسية مثل القرنفل والزعر والنعناع والسوس والذي اعطى للمرضى بست جرح يوميا"المدة اسبوعين. وأوصي المرضى بأستخدام الكريم لمدة اسبوعين اضافيين حتى بعد شفاء القرحة الفموية . اخذت عينات الدم (5 مل) من المرضى قبل وبعد اسبوعين قبل وبعد اسبوعين من العلاج وكذلك من مجاميع السيطرة وتم تحليل العينات لقياس مستويات مالو داي الديهايد ومضادات الاكسدة الكلية بواسطة المطياف الضوئي. **النتائج:** كان مصل مالو داي الديهايد في مرضى قرحة الفم اعلى معنويا" ($P \leq 0.001$) منه في السيطرة (0.19 ± 1.82 مقابل 0.12 ± 1.06 مايكرو مول / لتر) . بينما كان مصل مضادات الاكسدة الكلي اقل معنويا" ($P \leq 0.001$) منه في السيطرة (0.11 ± 1.16 مقابل 0.16 ± 1.7 مل مول). وبعد اعطاء العلاج قل مصل مالونديهايد معنويا" ($p \leq 0.001$) مقارنة منه قبل العلاج (0.19 ± 1.82 مقابل 0.17 ± 1.74 مايكرو مول / لتر). بينما ازداد مصل مضادات الاكسدة الكلي معنويا" ($p \leq 0.05$) مقارنة منه قبل العلاج (0.11 ± 1.16 مقابل 0.08 ± 1.2 مل مول) ولم يرجع كلامص مالنديهايد ومضادات الاكسدة الكلي الى مستوى السيطرة . وشفيت القرحة في معظم المرضى خلال 5-7 ايام عدا مريضين شفيت القرحة بعد 10 ايام. **الاستنتاج:** ترفع قرحة الفم في المرضى من زناخة الدهون وتقلل من مضادات الاكسدة. وشفي علاج قرحة الفم المستعمل موضعيا" بالكريم الذي يحتوي على القرنفل والزعر والنعناع والسوس المرضى خلال اسبوع وشفاء المريض كان مصحوبا" بنقص في زناخة الدهون مع زيادة في مضادات الاكسدة الكلي.

الكلمات المفتاحية: زناخة الدهون، مضادات الاكسدة الكلي، قرحة الفم المتكررة.

Introduction

Recurrent aphthous is a common but poorly understood oral mucosal disorder that affects almost 20% of population worldwide (Clover 99). The point prevalence of recurrent aphthous stomatitis (RAS) was 1.23%, while lifetime prevalence was 37% according to the national survey of oral health in schoolchild in USA (Kleinman, et al, 1994). Although the exact pathophysiology of aphthous remains unclear. Several factors have been identified including predisposing factors for RAS such as genetic backgrounds, immunological and nutritional deficiency, and infectious diseases (Ship 96). Local mechanical trauma, smoking, emotional stress, medication, vitamin and trace element deficiency have also been identified as potential factors (Chavan 2012).

It has been reported in few studies that impaired oxidant/antioxidant balance is associated with the tissue damage in

recurrent aphthous (Saral et al., 2005; Momen-Beitollahi, 2010). However, most of these studies only included individual antioxidants and lipid peroxidation by measuring serum malondialdehyde (MDA), without measuring serum total antioxidant status (TAS) (Arikan, et al. 2009; Gurel, et al. 2007). Measurement of oxidant/antioxidant status by follow up patients with RAS treated locally has not been studied. Recent studies used application of extracts of herbal medicine and essential oil with promising results were obtained by shortening the healing time and severity of pain for the RAS patients (Martin, et al., 2008; Babae, et al. 2010). Aim and objectives: This study was conducted in order to evaluate lipid peroxidation and serum TAS in aphthous patients after local treatment with cream containing herbal medicine.

Patients and methods

Patients were diagnosed and treated for recurrent aphthous ulceration in the Outpatient Clinic, Mosul, Iraq, from September 2011 to February 2012. The study was approved by ethical committee of Ninevah Directorate (Health Medical Research Ethical Committee). Diagnosis and treatment of the patients were under supervision of a dentist. A total of 42 patients with recurrent minor aphthous ulceration (31 males and 11 females) and 25 healthy subjects as a control group (19 males and 6 females). The age range of the patient group was between 18-47 years (mean \pm S.D: 30.1 ± 8.5 years). The age range for the control group was between 20-50 years (mean \pm S.D: 29.5 ± 8.2 years). The patients were treated with aphthous cream that contained cloves, thyme, mint, and licorice, applied locally 6 times per day until cure or for two weeks. The patients were advised to dry

the area by a clean tissue before applying the cream, in order to allow the drugs to remain long enough to give their effects. The patients were also advised to continue the use of the cream for two weeks even the aphthous was healed. Blood samples were taken (5 ml) from patients before treatment and after two weeks of the treatment and other blood samples were taken from the control group. The samples were analyzed by colorimetric methods for serum MDA (Aust and Bage, 78) and TAS (Meller et al., 93). Exclusion criteria for patients and controls were any disease or medication taken during the study period. Smoking or alcohol drinking were also excluded from this study. Data are presented as mean \pm SD and were analyzed by using paired t-test for the follow up study and independent t-test to compare parameters between the control and patient groups.

Results

Table (1):- Serum MDA and TAS in patient with aphthous treated locally with cream containing herbal medicine

	Serum MDA $\mu\text{mol/L}$	Serum TAS mmol/L
Control subjects	1.06 ± 0.12	1.70 ± 0.16
Patients Before treatment	1.82 ± 0.19^a	1.16 ± 0.11^a
After treatment	1.74 ± 0.17^b	1.2 ± 0.08^c

^a $p \leq 0.001$ vs controls, ^b $p \leq 0.001$ vs before treatment,

^c $p \leq 0.05$ vs before treatment

Only 42 patients were included in the study, since 17 patients were missed during the

follow up study. The aphthous healed in most of the treated patient after 5-7 days,

only two patients cured after 10 days of treatment. Only patients with minor recurrent aphthous ulcer were included in the study. The minor ulcer is characterized by round, small painful ulcers with shallow necrotic centre, raised margins and erythromatus halos in the labial, buccal and floor of the mouth mucosa (Jurge et al. 2006). In aphthous patients, serum MDA was significantly higher ($p \leq 0.001$) than in the control group, while serum TAS was significantly lower than in the control group (Table 1). After treatment for two weeks, serum MDA was decreased significantly ($p \leq 0.001$), while serum TAS was increased significantly ($p \leq 0.05$) compared with the patients before treatment. Both serum MDA and TAS did not return to the control levels (Table 1).

Discussion

In the aphthous patients, lipid peroxidation was higher than controls, while serum TAS was lower than controls. The increase of lipid peroxidation in the aphthous patients was consistent with other studies (Cimen, 2003; Saral, 2005; Arikan, 2009). However, Khademi et al. found no change in lipid peroxidation compared with healthy subjects. Stress factors may play a key role in the disease. The imbalance between free radical and antioxidants caused many inflammatory oral soft tissue diseases varying from infection to cancer (Scully, et al. 2003, Beevi, et al. 2004). Serum TAS was lower than controls, in the present study, the result was consistent with other workers (Bilgili, et al., 2013). However, plasma TAS was not affected

by aphthous disease (Momen-Beitollahi, 2010). Individual antioxidants showed controversial values in aphthous patients (Karincaoglu, et al., 2005; Khademi, et al. 2012; Saral, et al., 2005; Saxena, 2010). Serum TAS includes all enzymatic and non enzymatic antioxidants. Lamont et al. found that the monitoring of individual antioxidants in physiological samples is more important than plasma TAS, since the reaction kinetics in vivo are not the same and may interact with each other. Thus TAS of a sample is a quantitative measurement of the state of balance of these various components under specified reaction conditions. Lipid peroxidation after treatment was decreased significantly, while serum TAS increased significantly. However, these values still did not reach the levels of the control values. It is not known whether the disease by itself disturbs the antioxidant defense system or the oxidative stress induces the disease. Further studies are needed to evaluate effect of oxidative stress in aphthous disease. Serum MDA and TAS should be studied for several months in order to evaluate the effect of oxidative stress on serum MDA and TAS. This study only included two visits for the patients within two weeks without placebo group. Therefore, the efficacy of the drug cannot be assessed. However, the aim of the study only included the assessment of oxidant/antioxidant status in aphthous patients before and after treatment. The cream used in the present treatment contained essential oils. The therapeutic activity for these ingredients could be due to their antibacterial activity. Licorice caused a significant

reduction in the diameter of the inflammatory halo and necrotic centre (Moghadmnia, et al. 2009). Licorice has been found to have potential benefit for preventing and treating oro-dental disease (Messier et al. 2012). In conclusion, aphthous ulceration in patients raises lipid peroxidation and

reduces antioxidants. Aphthous treated with cream contained clove, thyme, ment and licorice, were healed within a week and the cured patients were associated with reduced lipid peroxidation and raised antioxidants status

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