Evaluation of the Effect of Probiotic Lozenges in the Treatment of Recurrent Aphthous Stomatitis: a Randomized, Controlled Clinical Trial

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Introduction

Recurrent aphthous stomatitis (RAS) is the most common painful oral mucosal disease. It affects nonkeratinized mucosa in men and women of all ages, races and geographic regions. The prevalence ranges from 0.7–20% in the general population [1]. In children, the prevalence differs according to age, ranging from 1.2–36.2% [2]. Minor RAS is the most common form, which accounts for approximately 70% to 87% of the population with RAS [3,4] and usually has 1 to 5 ulcers at one episode, with a size of less than 1 cm in diameter [5]. For RAS patients, the ulcer pain associated with each episode may severely interfere with eating, speaking, and swallowing.

Although RAS represents a very common oral lesion, its etiology remains unclear. Some studies have showed that several local and systemic factors, such as local trauma, genetic predisposition, immunodeficiency, zinc deficiency and hormonal changes may play a role in the pathogenesis of RAS. Suspected microbiota may also associate with RAS [6-8]. Since the etiology is unknown, no curative therapy has been described to date. All current systemic or topical treatment methods are to relieve symptoms and accelerate healing. Most systemic medications, although effective, have side effects that limit their general use. Therefore, topical agents remain the first choice for the treatment of RAS, due to their effectiveness and safety.

The application of host-modulating bacteria for therapeutic purposes is one of the strongest emerging fields. Probiotics are live microorganisms, which, when administered in an adequate amount, confer a health benefit on the host [9, 10]. Majority of probiotic bacteria belong to the genera, *Lactobacilli*, *Bifidobacterium*, *Propionibacterium* and *Streptococcus*. Lactobacilli play an important role in the maintenance of health by stimulating the innate immunity as well as by contributing to the balance of the microflora [11].

Probiotics have been documented to exert several actions on a wide variety of immune cells shifting it towards the anti-inflammatory pathway [12]. Probiotics modulate mucosal immune mechanism by reducing production of pro-inflammatory cytokines through actions on NFkB pathways, increasing production of anti-inflammatory cytokines such as IL-10 and host defense peptides such as b-defensin, enhancing IgA defenses, influencing dendritic cell maturation and stimulating the activity of Treg lymphocytes [13-15]. Additionally, probiotic action could be augmented by using prebiotics such as inulin. This combination is known as symbiotic [15].

According to our understanding to the mechanism of probiotics in modulating the local and systemic inflammatory immune process and previous studies [16], it is feasible to investigate the effects of this therapy on RAS. Hence; it is one of the conditions that immune mechanism is

implicated. To the best of our knowledge, no study has been conducted in pediatric population. The aim of this study is to explore the effectiveness of lozenges containing *Lactobacillus acidophilus* (*L. acidophilus*) and *Bifidobacterium lactis*, plus inulin in the treatment of minor RAS in adult and pediatric RAS patients.

Patients and Methods

Study design

The study is a randomized, controlled, clinical trial. Study population was recruited among patients referred to the department of Oral Medicine and Periodontology and department of Pediatric Dentistry, Faculty of Dentistry, October 6 University (Egypt) and had a provisional diagnosis of minor RAS. The study protocol and consent form were approved by the Institutional Ethics Committee (IEC)/ 23-2016). After explanation of all aspects of the study, characteristics of the product and the available alternative treatments, a signed consent form was obtained from all patients and parents.

Randomization and treatment groups

We performed power analysis to calculate minimum number of patients for the study. To achieve a significance level (type 1 error) as 0.05 and power (type 2 error) as 0.8, we decided to include 30 patients for each subgroup estimating a dropout rate of 20%. Hence, sixty adult (group A) and 60 children patients (group B) with diagnosis of minor RAS were included in the study. Each group was divided into two subgroups as follows:

Group AI: ChocBalls (*L. acidophilus containing* lozenges, PharmaCare Europe Ltd; West Sussex, RH10 9NQ, UK)

Group AII: (control) Oracure oral gel (15 gm, Amun pharmaceutical company, Egypt)

Group BI: ChocBalls (*L. acidophilus containing* lozenges, PharmaCare Europe Ltd; West Sussex, RH10 9NQ, UK)

Group BII: (control) Oracure oral gel (15 gm, Amun pharmaceutical company, Egypt)

The inclusion criteria for group A is as follows: 1. Males and females aged 18–45 years old, 2. Patients presenting with RAS with the following characteristics: a. Minor aphthous ulcers less than 48 hours' duration prior to enrolment, b. Size no greater than 10 mm in diameter, c. A history that ulcers normally more than 5 days to resolve without treatment. The inclusion criteria for group B is the same except age, children with RAS aged between 3 and 12 years were recruited for the study. The exclusion criteria are as follows: A known history of hypersensitivities, immunologic or systemic diseases, pregnancy, smoking, treatment with systemic steroid or other immunomodulatory agents within 1 month before the study , use of nonsteroidal anti-inflammatory drugs or oral antihistamines within 1 month prior to the study, treatment of the ulcer with any preparation or medication within 72 hours prior to the study, treatment with systemic antibiotics within 2 weeks prior to the study and a history of

adverse reactions to lactose or fermented milk products. Children with a positive family history of RAS were excluded. The nutritional status of the children was evaluated.

After taking a detailed history and clinical examination, all patients eligible for study participation was assigned to the test subgroups (*L. acidophilus containing* lozenges, PharmaCare Europe Ltd; West Sussex, RH10 9NQ, UK) or control subgroups by using a computer-generated random number list (30 patients for each subgroup). Adult lozenge was composed of the following: *L. acidophilus* 1.5 billion cfu, *Bifidobacterium lactis* 1.5 billion cfu and inulin 0.13 g per lozenge. For children, lozenge was composed of the following: *L. acidophilus* 0.5 billion cfu, *Bifidobacterium lactis* 0.5 billion cfu and inulin 0.13 g per lozenge.

Patients were instructed to melt the *L. acidophilus containing* lozenges in the mouth slowly twice daily for 5 days (day 1 to day 5). The baseline parameters were taken and recorded on the day of the first visit. Effectiveness evaluations were made on the morning of day 3 visit and day 5 visit.

Clinical outcome variables:

The index ulcer's size was measured by using a methodology described by Liu et al [17] on treatment days 0, 3 and 5. The investigators measured the maximum and minimum diameters when the ulcer has an oval shape, using a calibrated dental probe with millimeter markings. The two measurements were then be multiplied to represent the cross-sectional areas of the ulcer.

To evaluate pain, a visual analog scale (VAS) consisting of a 10-cm horizontal line between poles connoting no pain (origin) to unbearable pain was used. Subjects was told to mark the line with a vertical line at the point that best represented the present pain level of the ulcer.

The effectiveness indices (EI) of the ulcer size and pain improvement were calculated with the following formula: (V3 and V5 referring to the values measured at Day 3 visit and Day 5 visit, while V1 referring to the baseline value measured before the study entry): $EI = [(V1 - V3 \text{ or } V5) \div V1] \times 100\%$. The effectiveness indices were evaluated on a 4-rank scale: (1) Heal: $EI \geq 95\%$; (2) Marked improvement: $EI<95\%$, but $\geq 70\%$; (3) Moderate improvement: $EI<70\%$, but $\geq 30\%$; (4) No improvement: $EI <30\%$. Participants were asked to estimate the average duration of episodes during the past 6 months and the potential to reduce the outbreak frequency of RAS within the next 6 months was investigated. All clinical outcome variables are recorded by an investigator masked about the treatment modality that has been used.

Statistical Analysis

The data was subjected to statistical analysis using:

a) Wilcoxon signed rank test for intragroup comparison

b) Mann-Whitney U- test for inter-group comparison

The level of significance was set at the probability value $p \leq 0.05$.

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