Clinical research on the efficacy of a gel based on hyaluronic acid and essential oils in the treatment of oral aphthosis

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Oral aphthosis, in its different clinical manifestations, is one of the most common diseases affecting the mucous membranes of the oral cavity. The etiology of the lesion has not yet been definitively clarified, although the involvement of the immune-regulatory district of the human body is known. Three clinical forms of this mucositis are distinguished: canker sores simplex, recurrent aphthous stomatitis, and aphthous herpetiform stomatitis. The lesions can be single or multiple. Depending on their size, they are then divided into major or minor forms. Each lesion is circular in shape, surrounded by an edematous border and with a surface covered with a yellowish-gray patina. The symptoms complained of by patients are multiple and acute: pain, functional limitation, fever and satellite adenopathy. Many general and local therapies have been proposed for this pathology with the aim of achieving rapid healing and effective relief from the pain symptom. The results of these therapeutic protocols, however, still seem to be limited and insufficient. The aim of this clinical research was to evaluate the clinical effects of a new gel, specific for this pathology, which can be used for local application in a group of 40 adult subjects, all suffering from various forms of aphthous stomatitis. The active ingredients present in the gel are represented by: cetylpyridinium chloride, essential oils with anti-inflammatory and re-epithelializing action (Tea-tree oil and Manuka oil), low molecular weight hyaluronic acid (oligomers), PVP-hydrogen peroxide complex at 0.1%, allantoin, bisabolol, vitamin E. All these substances have been made very adhesive thanks to the use of specific natural rubbers and resins in the gel. The clinical results obtained can be summarized as follows: 1) Patients report substantial pain relief on the first day.

2) Most patients experienced healing of the aphthous lesions within the first 6 days of treatment, reporting a shortening of the healing time compared to previous experiences. 3) The results of pain relief and healing are identical in every clinical form of aphthous stomatitis. 4) Patients perceive the gel as very adhesive, well tolerated, of acceptable flavor and easy to use. No side effects have been reported.

Keywords: *Aphthous stomatitis, Hyaluronic acid, Essential oils, Adhesive gel, Healing time, Pain relief.*

INTRODUCTION

The dentist and the dental hygienist are constantly in the need to make diagnoses, even if they differ from the between various lesions affecting the soft tissues of the oral cavity. Some of these lesions affect the periodontium, with typical signs and symptoms, normally well known and addressed by these professionals. In other circumstances, however, the mucous lesions present involve diagnostic and therapeutic questions that are not easy to resolve. Patients suffering from these acute pain condition and oral functional limitation.

Consequently, they ask for immediatediagnostic and therapeutic intervention. Sometimes, even the most experienced specialist is notable to fully understand the problem, considering the multiplicity of pathologies and the overlapping of symptoms. The diagnosis may therefore be incomplete or summary and the therapeutic intervention, even if only palliative, incongruous or useless.

Some stomatitis or mucositis are, of course, more typical and better known for their unmistakable clinical picture. However, even in the presence of a certain diagnosis, the therapeutic possibilities of rapid resolution of the lesion and timely relief of the patient's symptoms are noteasily obtainable.

This situation is typically found in oral aphthosis, the most common of stomatitis, affecting 10 to 30%

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of the general population and being, therefore, frequently diagnosed in dental patients[23].

Terminologically, the term "aphthosis" refers to the various pathologies in which canker sores are present[29]. Canker sores[6] are ulcers, usually of reduced size, affecting the oral mucosa. A common feature of all canker sores[9] is their rounded shape with clear margins with an edematous halo and a yellow/pearly background. They induce a clear pain symptomatology and very often have the possibility of recurring periodically in the same patient ("Recurrent Aphthous Stomatitis" - S.A.R.).

The etiology of the disease has notyet been definitively identified, although aphthosis is classified among the immunological disorders linked to T lymphocytes, with multiple predisposing factors[26]. These include psycho-physical stress, trauma[17], pharmaceutical and food intolerances, the luteal phase of the menstrual cycle and also a certain familiarity. Canthous lesions are also associated with a number of diseases such as iron deficiency anemia and vitamin deficiencies, Crhon disease and ulcerative colitis, celiac disease[4,30], Beçhet's disease, HIV infection and other immune-suppressive/deficiency situations[28]. Aphthous ulcers are classified into three clinical variants: minor aphthous ulcers, major aphthous ulcers (Sutton's ulcers) and herpetiform ulcers.

From a therapeutic point of view, despite a varied range of protocols proposed in the literature, the clinical results that can be obtained are not completely satisfactory in all types of aphthosis.

In most cases, these are symptomatic therapies, notentirely effective or free from side effects, which do noteliminate or prevent the disease and its periodic recurrence.

This clinical inadequacy is also evident in paediatric cases, for which the recommendations of the Ministry of Labour, Health and Social Policies of the Italian State[18] would requireadequate monitoring of mucositis, especially ulcerative mucositis and apossible referral of patients to appropriate specialists, including the paediatric dentist.

Many active therapeutic principles havebeen proposed but, as well pointed out by Baccaglini and Coll.[3] In a recent critical review,the studies that support them are not alwayschecked and the risk of errors or biases is frequent. These active ingredients include chlorhexidine 0.2% in mouthwash, topical applications of benzidamine, zinc chloride and polycresulene, levamisole[32]. A discreet therapeutic action is attributed to both tetracycline and lidocaine 1%[7]. Many ulcers, however, and especially those that recur, arerefractory to these devices. Among the possible causes of failure of the proposed protocols, it must certainly be included the fact that, regardless of the substance used, it is generally retained in situ only for ashort time. Bio-adhesion to the mucosa affected by canker sores is, in fact, the indispensable pre-condition for clinical efficacy, as shown by some research on both animals[16] and humans[5].

In cases of more intense symptoms, steroid drugs are also used, some with specific preparations for dental use such as adhesive tablets, gums orgels[15].

In severe cases, steroid therapy is also prescribed systemically or with intra-lesional injections. A further approach to systemic therapy of relapsing aphthosis is thalidomide[24], although there is apossible risk of side effects.

Finally, in recent years, aphyto-therapeutic approach has been spreading, especially aimed at the smallest forms of aphthosis. Much literature, even recent, has described the results, more or less satisfactory, obtained in the control of pain and in the healing of aphthous ulcers with the use of various plant extracts including Alchemilla vulgaris[31], Myrtus communis[1], Berberine gelatin[34], Licorice[19], Quercetin[11] and, above all, Aloe vera[2].

PURPOSES

With this clinical research we wanted to preliminarily evaluate the effectiveness of aninnovative gel in the treatment of aphthous stomatitis. In particular, we wanted to evaluate whether the use of the gel quickly reduces the pain referred to the aphthous lesion and whether the healing time of the same undergoes a parallel modification. Finally, the aim was to obtain information on the effect of the gel in the different clinical forms of canker sores taken into consideration, on the possible presence of side effects related to the use of the product and on other aspects of the applications (taste, consistency, adhesiveness, tolerability, practicality).

MATERIALS AND METHODS

Our research can be defined as a "phase II controlled clinical trial towards baseline", i.e. aimed at preliminarily measuring the impact of anintervention. The value of the method and also its limitation, lies in the fact that in it the recruited subjects constitute both the "test group" and the "control group", thus providing both the "base-line" data and those collected after the conclusion of the treatment, for appropriate comparisons. These subjects constituted a group of 40 cases, 29 women and 11 men, aged between 18 and 73 years. All the subjects had already suffered from aphthous lesions: 12 of them were suffering from S.A.R. (Recurrent Aphthous Stomatitis), 3 presented with canker sores in the symptomatic picture of systemic diseases. Considering the ongoing lesions, 33 subjects had "minor" type canker sores, in 7 cases the aphthosis was with "major" lesions. In 12 cases the canker sores were multiple, in the remaining single. Inclusion in the research sample presupposed the appearance of oral canker sores immediately before the application of the gel on the first day. Exclusion criteria were the absence of aphthous lesions in previous times and the use of other local or systemic drugs that could interfere with the proposed treatment.

After ananamnestic interview with the subjects and after the direct clinical evaluation of the oral clinical picture, the clinical research in question was described, collecting the appropriate informed consent. All subjects then received the tubes of gel and wereinstructed on how to apply them (directly from the dispenser or via a fingertip or cotton swab) to cover the entire area of the lesion. Applications ranged from 3 to 5 times a day, possibly after meals, until the clinical picture was remitted. All subjects were provided with aquestionnaire to be filled in and returned after recovery. This questionnaire was divided into 3 parts: in fact, it contains data referring to previous aphthous lesions, those of current canker sores and those commenting on the outcome of the treatment and the characteristics of the gel used. To collect data on pain intensity and remission, a 10 cm long visual analogue scale (V.A.S.) was used, which did not, however, have a graphic display of units or numbers.

The gel proposed for the treatment of canker sores in these subjects consists of an association of different active substances, already known in the medical or dental literature to be effective in the treatment of mucositis, at dosages such as to avoid or limit any side effects. The origin of the gel consists in the association of the active ingredients, which in the gel aremade particularly adhesive to the mucosa by specific natural rubbers and resins. The active ingredients present arerepresented by hyaluronic acid and its oligomers (different molecular weights), allantoin, bisabolol, vitamin E, cetylpyridinium chloride, PVP-oxygenated water complex, specific essential oils (Melaleuca and Manuka) of the myrtaceae group, much richer in terpenes than other similar plant extracts.

RESULTS

The numerous data, derived from the questionnaire administered to the subjects participating in the research, werestatistically processed in order to be able to make comparisons between the previous lesions and those treated with the proposed gel. Below are the results obtained highlighted in special graphs.

a. Pain reduction. To evaluate this parameter,the pain threshold values of current canker sores were compared with those of previous lesions. The t-test carried outdoes not detect substantial differences for these values (which are both about 7 cm in the V.A.S.). If, on the other hand, the difference after gel treatment is evaluated, the t-test shows a statistically significant mean difference ($P < 0.001$): in this case, in fact, the average V.A.S. of the treated patients is about 1 cm on the scale. The treated subjects therefore report in almost all cases that they have obtained relief from the pain symptom that afflicted them during the course of the disease. In particular (Fig. 1) 43% of subjects report having experiencedimmediate relief after the first application of the gel, while afurther 18% of cases had a lot of relief after the first day of treatment. 37% of the subjects experienced enough relief from the cycle of applications carried out, while only 2% of cases reported that they did not alleviate their pain symptoms.
b. Injury healing time. By comparing the average healing time of other previous episodes of oral aphthosis, which involved a time frame of about 8 days, according to patients, we were able to detect a significant change (Fig. 2). In fact, 45% of subjects report having seen a healing time of 3 days for the ongoing lesion treated with gel applications. To this nple who showed a

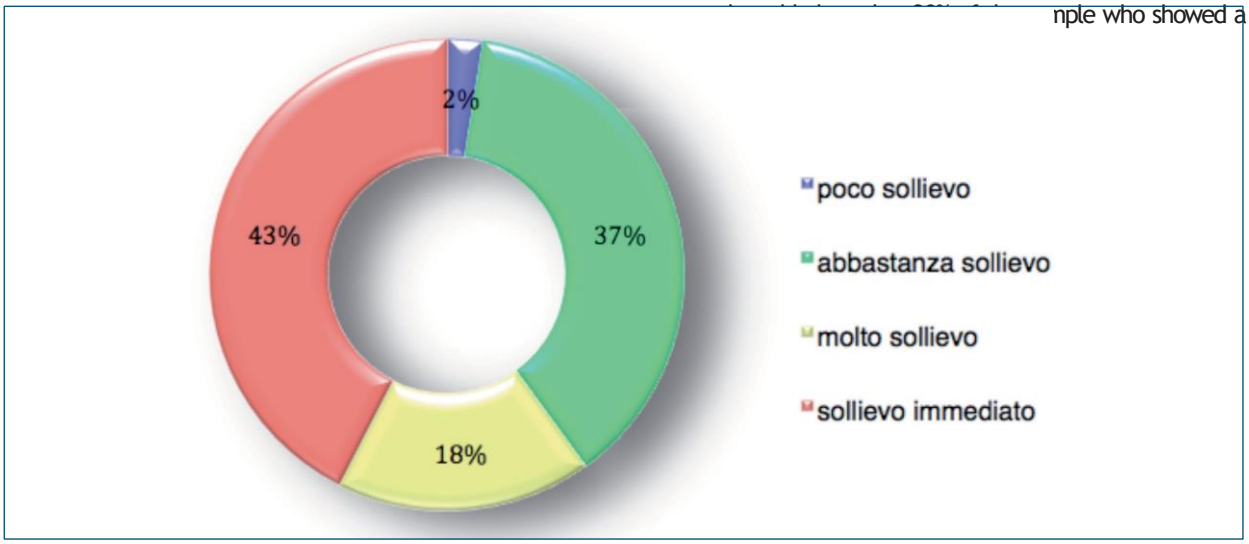

Fig. 1 The high level of pain relief caused by canker sore obtained on the first day with the gel tested in the treated patients.

within 6 days of the start of treatment. 25% of subjects, on the contrary, report a healing time of between 7 and 12 days of treatment.

c. Differences in clinical efficacy between the different types of canker sores observed. Figures 3 to 6 indicate that there are no statistically significant differences in clinical outcome (pain relief and healing time) in the different types of canker sores considered (canker sores major versus canker sores - comparison between canker sores simplex/R.A.S./canker sores related to systemic diseases). The gel therefore appears to be effective in all clinical forms observed.

d. Product Features. In the evaluation of the subjects included in the research, the tested gel was judged positively in all the parameters covered by the specific question. In particular, the taste is acceptable in 72.5% of cases, as well as the tolerability (70% of cases) and ease of use (62.5% of cases). The most interesting clinical data is represented by the perception of adhesion to the mucosa, which is a salient feature of the product formulation. Figure 7 shows how 78% of subjects appreciate the tenacious adhesiveness of the product.

e. Reduction of lesion-related symptoms and possible side effects. Figure 8 shows that some lesion-related symptoms (size, edematous border, fever and satellite adenopathy) suggest significant improvements during treatment. The pigmented patina present in the heart of the aphthous lesion is less modified by topi- gel treatment, at least until the canker sores disappear. Finally, in no case have any collate-related effects been reported worthy of mention.

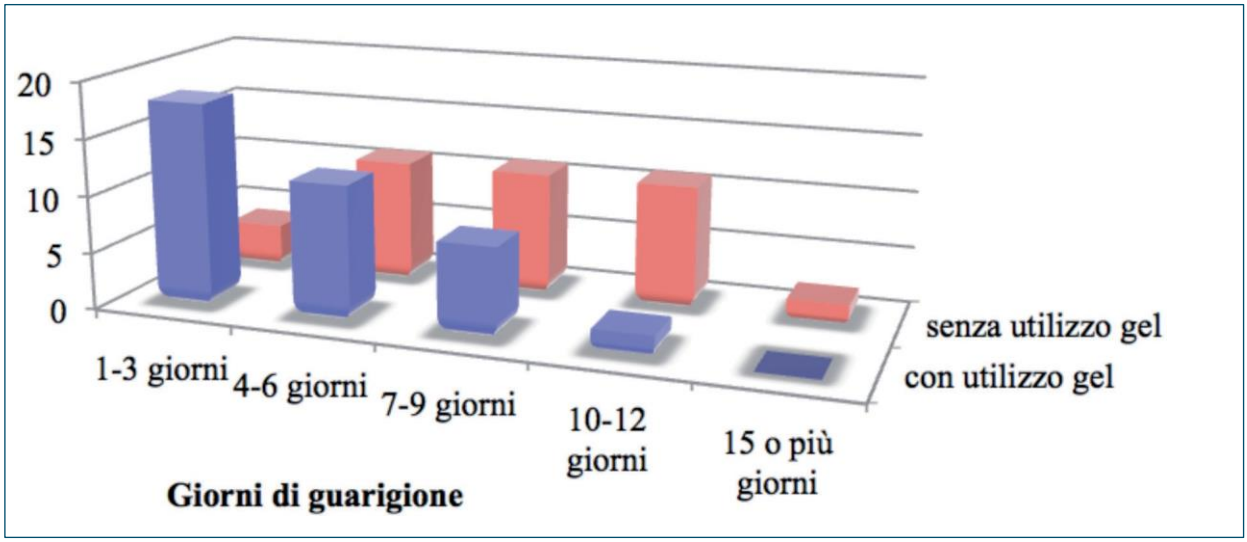

Fig. 2 75% of patients treated with the tested gel declare that they have achieved healing of the sultry lesion within the first 6 days of application.

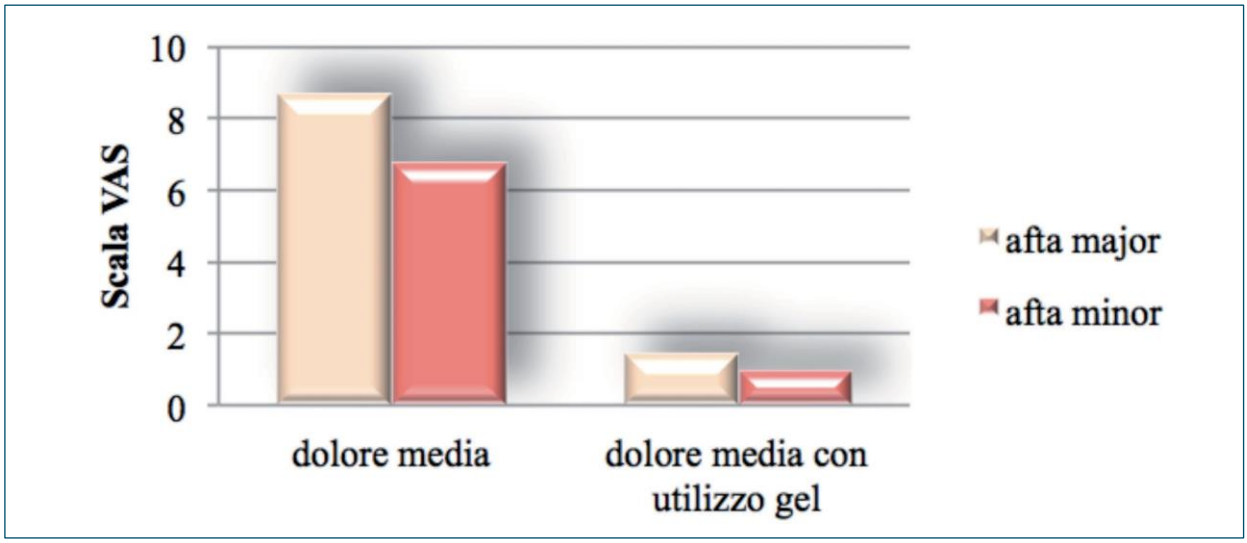

Fig. 3 Significant pain relief of the aphthous lesion was rapidly obtained in both major and minor canker sores.

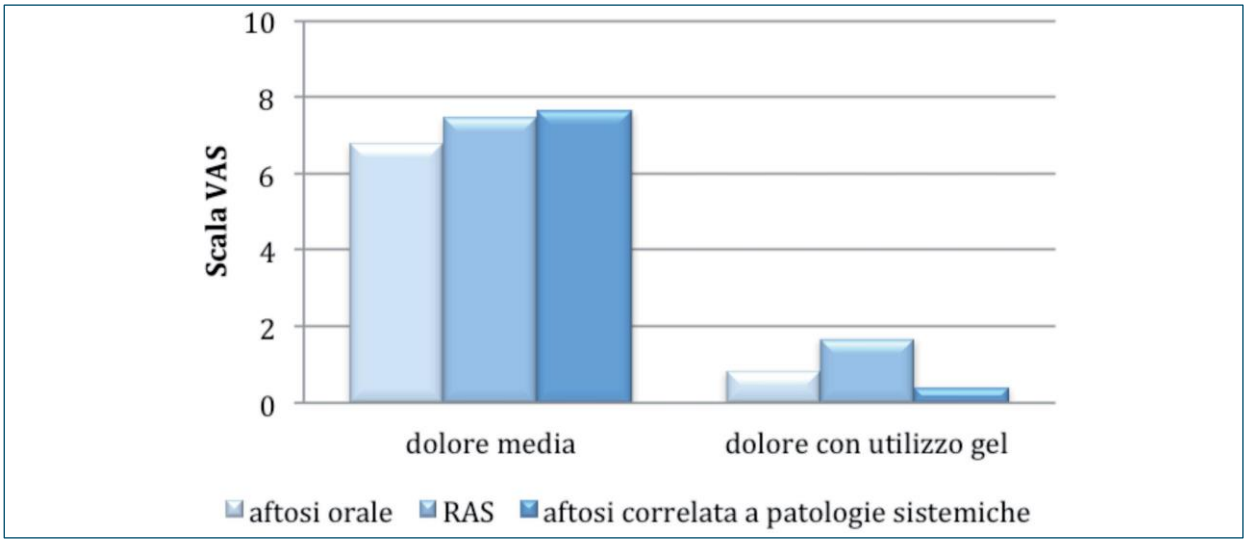

Fig. 4 The tested gel induces uniform pain relief in each subtype of canker sores (simplex, recurrent aphthous stomatitis, canker sores related to systemic diseases).

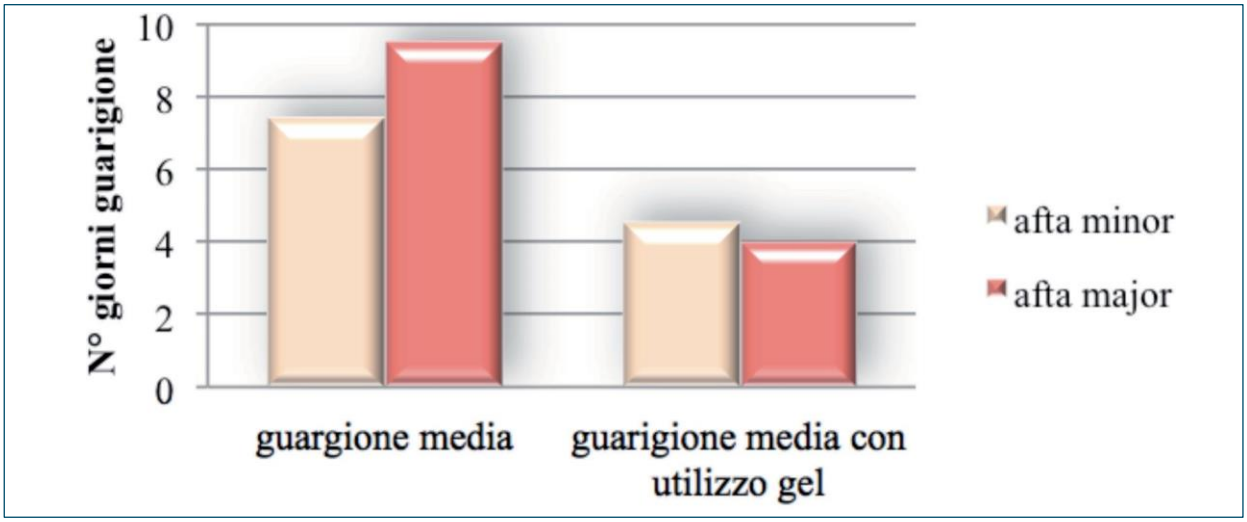

Fig. 5 The average rapid healing time is obtained in both major and minor canker sores.

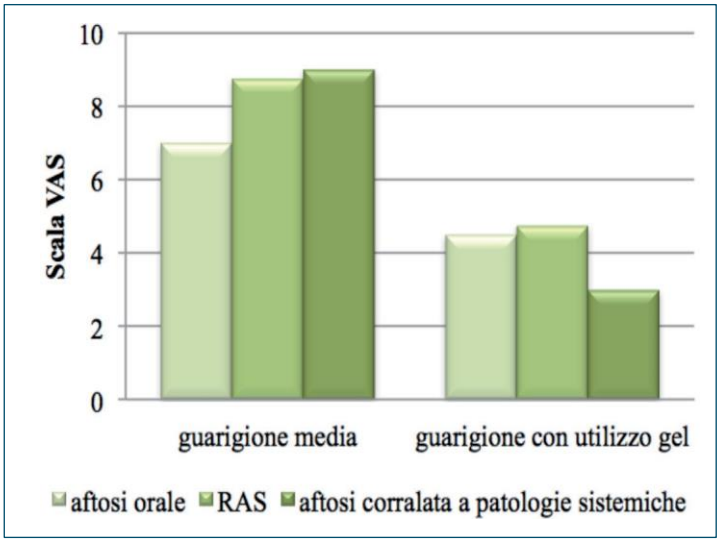

Fig. 6 There are no differences in the healing time of canker sores, regardless of their clinical subtype.

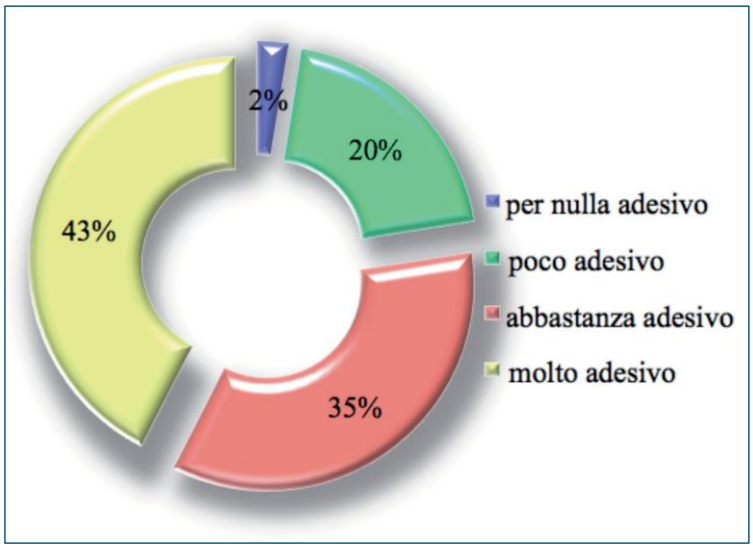

Fig. 7 The adhesiveness of the tested gel is clearly perceived by the treated patients.

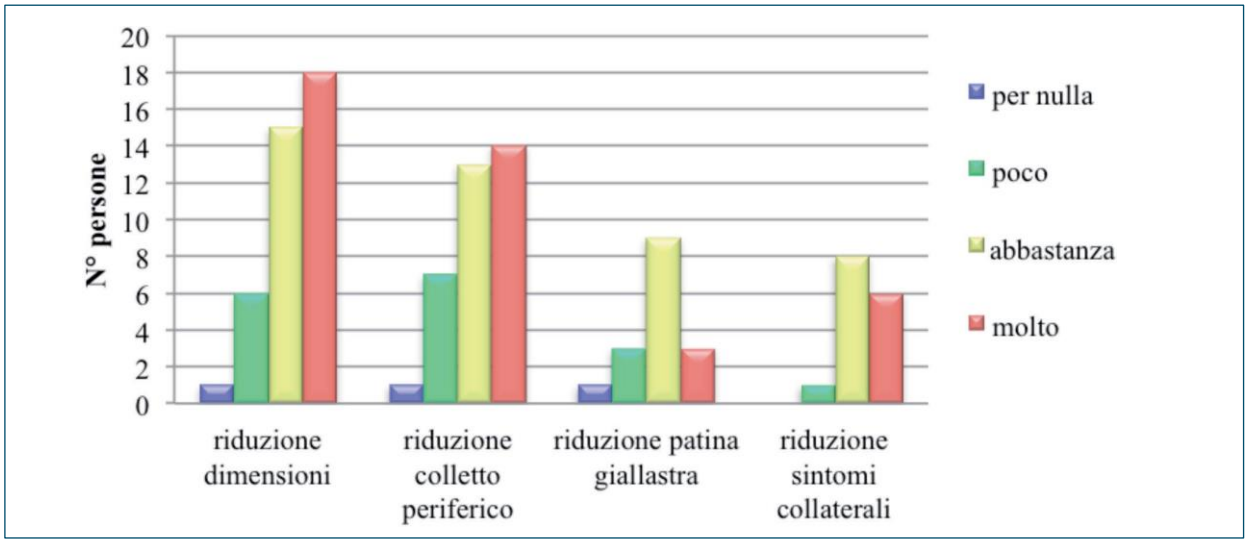

Fig. 8 The symptoms related to canker sores (lesion size, peripheral collar, fever, satellite adenopathy) are intensely reduced by the use of the tested gel.

DISCUSSION

To discuss the encouraging results we have obtained, it should be noted that the use of each active ingredient for topical application on the oral mucous membranes must be subject to careful evaluations that, on the basis of adequate parameters and validated scientific literature, can demonstrate its effectiveness and safety. With this in mind, the American Dental Federation has set up aspecial scientific committee whose purpose has been to divide the topic active ingredients into groups that integrate clinical efficacy with safety for the patient. The Committee's conclusions include in "category 1" (the one in which the product is considered "effective in the absence of side symptoms") both cetylpyridinium chloride and several essential oils. These same active ingredients areeither present in the gel we tested and, in addition, substantively

Used in anoriginal medium of natural rubbers and resins with high adhesion in order to obtain a prolonged persistence on the lesion. Still on the subject of active ingredients for topical use, the most prestigious Italian dental reference institutions (Board of Teachers in Dentistry, A.N.D.I., A.I.I.D., A.N.I.D.) have expressed theirreasoned opinion, drawing up the "Italian Guidelines on the use of mouthwashes". In this comprehensive document, developed using the technique of systematic review and meta-analysis, cetylpyridinium chloride and essential oils are shown to be able to effectively improve all inflammatory indicators. The interesting clinical results we have obtained are therefore based on the use of these same substances. Let us consider the knowledge available today for cetylpyridinium chloride in the dental field to be broad and definitive: this derivative of quaternary ammonium has, in fact, long been used for a long time.

locally in the therapy of some diseases of the oral cavity. As far as essential oils are concerned, however, much research is underway[8,10,33], in an attempt to demonstrate, for this wide range of organic substances of plant origin, their presumed anti-inflammatory, or anti-bacterial, efficacy at the level of oral soft tissues. Most of this clinical research has made use of essential oils in the preparation of mouthwash, which, by its very nature, cannot imply the prolonged persistence "*in situ*" such as that offered by agel, as in the protocol of our work, which involves the use of Melaleuca oil (tea-tree oil) and Manuka.

Tea-tree essential oil (Melaleuca) is rich in alcohols and monoterpenes that allow it a strong bactericidal and antiseptic action that has also been appreciable in Intra-oral soft tissue area[21]. The gel we take in It also contains asecond important essential oil: Manuka oil. This extract of myrtacea australian and New Zealand is rich in monoterpenes, sequiterpenes and triketones that enhance its anti-bacterial, anti-inflammatory and healing abilities on ulcers, such as to consider it 20/30 times more active than Melaleuca[14,22].

In arecent clinical study[12], Lauten et al. studied the effects of these same essential oils used in intra-oral liquid dilution and found that they tasted unpleasant and were notmore effective than other substances. The authors point to the need for further studies on the subject.

Our research responds to this need: we believe that the use in gel preparation and the appropriate processing of the oil by the manufacturer explains the excellent results obtained on the aphthous ulcers considered.

In addition, the gel we tested makes use of the association of hyaluronic acid and hydrogen peroxide, now considered a Interesting and innovative therapeutic possibility in the topical treatment of both mucous and cutaneous inflammation[25]. Hydrogen peroxide has an anti-oxidant and disinfectant action, while hyaluronic acid[20] promotes the restoration of tissue structure, with a soothing effect: the association of the two principles seems to enhance the effects. It should be remembered that, even for these substances, the clinical efficacy is still linked to their adhesion to the injured tissue, which is partly natural, for hyaluronic acid[13] and partly obtained with the original elaboration of the formulation of the gel we have taken into consideration. In a recent evaluation[21], Pasini et al. described this increased adhesion capacity to mucosal tissue, which can be appreciated to the touch due to the presence of alipophilic substrate, but made even more evident thanks to the inclusion of nourishing and gelling resins (mixed Na/Ca salt of the methyl-vinyl-ether copolymer, polyvinylpyrrolidone and Na carboxymethylcellulose).

Still on the subject of hyaluronic acid and its precursors (oligomers), present in the gel we have evaluated, for their healing action, it should be noted that the different molecular weight of them in the mixture has played a fundamental role. In fact, it is considered acquired[27] that hyaluronic acid with low molecular weight (less than 9 K Daltons) can adequately and quickly penetrate the injured tissue. This has the characteristic of binding water and promoting cell migration. Hyaluronic acid precursors (oligomers) with even lower molecular weights can alsobe used by fibroblasts for the bio-synthesis of native hyaluronic acid.

This mix of two different molecular weights is appropriately present in the formulation of the tested gel.

CONCLUSIONS

From what has been said so far, the therapeutic efficacy of the gel we evaluated in the treatment of recurrent aphthosis and in the repair of the affected mucosa is evident.

The shortening of the healing time, the immediate analgesic effect and its good tolerability make it an effective and useful device in the clinical approach of this active and, in many ways, disabling pathology.

The positivity of the clinical results that we have described and discussed here seem to us to refer, in summary, to:

1. perfect adhesion to the oral mucous membranes obtained with the original mix of resins and natural rubbers;
2. the complete antibacterial action of cetylpyridinium-chloride;
3. the anti-inflammatory and re-epithelializing action of high-dose essential oils specially selected and used (manuka and tea tree) for their superior effectiveness compared to other substances of this type;
4. the association between hyaluronic acid, its oligomers and the PVP-hydrogen peroxide complex that enhances the regranulation effect of the tissue, while performing a sanitizing action;
5. the additional presence of active ingredients with a soothing, antioxidant and re-epithelializing action (allantoin, bisabolol, vitamin E).

The clinician therefore has aneffective, easy-to-apply and safe product available to resolve the persistent algic symptomatology of aphthosis and allow the patient to recover his oral function.

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