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CLINICAL EFFECTS OF LEVAMISOLE AND ANTIOXIDANTS AND THEIR EFFECTS ON SERUM IMMUNOGLOBULINS IGG, IGA AND IGM: A COMPARATIVE CLINICAL STUDY IN MANAGEMENT OF OSMF.

Oral Medicine

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ABSTRACT

BACKGROUND AND OBJECTIVES: Oral submucous fibrosis (OSMF) is considered as an oral precancerous condition that shows characteristic inflammation and progressive fibrosis of the submucosal tissues that leads to marked rigidity as well astrismus. OSMF till date remains a dilemma to the oral pathologists due to its ambiguous pathogenesis and overlapping classification systems.OSMF is linked with alterations in serum immunoglobulins levels.

AIM: This study was conducted to evaluate the clinical effects of levamisole and antioxidants and their effects on serum immunoglobulins IgG, IgA and IgM levels.

MATERIALS AND METHODS: 45 study participants were enrolled for the study. Subjects were assigned randomly into 3 sub-groups. There were 15 patients per group; group I comprised of patients who received levamisole, 50 mg thrice daily for three alternate weeks, group II comprised of patients who received 2 capsules of antoxid daily for six weeks, group III comprised of patients who received levamisole and antioxidant both.

RESULTS: Paired't' test and unpaired't' test were used to assess the study results. The results were clearly indicative of the advantage oftaking levamisole, antoxid and the combination of levamisole and antoxid in improving mouth opening and reducing burning sensation of mouth significantly. Significant decrease in serum IgG, IgA and IgM was observed in group I and combination group III whereas in the group II significant reduction was only seen in serum IgA and IgM levels.

CONCLUSION: Levamisole alone has a higher advantage in clinical management of OSMF when compared to that of antioxidant therapy alone or in combination. Antioxidants proved to have no added benefit in aiding to the reduction in burning sensation or in opening of restricted oral cavity.

KEYWORDS

Oral Submucous Fibrosis,immunoglobulins, Levamisole, Antoxid

INTRODUCTION

Oral submucous fibrosis (OSMF) is considered as a chronic disease of delusive onset characterised byprogressive fibrosis of thesubmucous layer of the palate, cheeks, lips, larynx,pharynx and esophagus. The underlying muscles of mastication might also get involved leading totrismus and disability.[1] The pathogenesis is however not clearly understood and the disease treatment becomes quitechallenging for clinicians as well as oral pathologists.OSMF was previouslybelieved to be a disease of the Indian subcontinent where near about 5 million patients have been reported.[2] A worldwide distribution is however seen due to immigration hence a handful of cases have also been reported in other races. This chronic condition is precancerous in nature since it carries a relatively higher risk for malignant metamo rphosis.[3]

Over the past decades, shift has been shifted to the various immun ologic alterations that have been found associated with OSMF. There have been disputing reports pertaining to the levels of serum Igs in patients with OSMF with one or more of them being statistically high. [2,5-7]

Due to poorly understood pathogenesis and overlapping features till date no analysis has yet been published suggestive of any kind of spontaneity in regression of condition as well as a definitive treatment approach. The following approaches have been tried, namely nutritional supplements, intralesional injections of placental extracts, corticosteroids, hyaluronidase and chymotrypsin, antioxidants and surgical excision of fibrotic bands with placement of grafts.[8-13]

While examining closely all the current literature available, it was observed that the effect of immunomodulators like levamisole,has yet to be explored adequately for effective management of OSMF.

Therefore, this current study was conducted to compare the efficacy of levamisole with antioxidants and to assess the levels of serum immunoglobulins (IgG, IgM, IgA) pre and post treatment.

METHODOLOGY

The present study was randomized and single blinded category of research that was undertaken in the Department of Oral Medicine Diagnosis and Radiology. At first 60 subjects were taken for the study but later only 45 subjects of either sex gave their consent for participating into the study protocol. The age group of recruited subjects was above 15 years of age.

OSMF was diagnosed on the basis of natural course (history) and characteristic clinical signs and symptoms which included reduced or restricted mouth opening, intolerance to hot and spicy food, burning of oral mucosa, characteristic blanching and marked palpable fibrotic bands. Oral mucosa also showed lack of suppleness. The final diagnosis was confirmed after careful and meticulous histopathological examination categorising OSMF

INCLUSION CRITERIA:

- Apparently healthy subjects, well oriented (time, space and as a person)
- Satisfying clinical characteristics of OSMF
- Histopathologically confirmed cases of OSMF
- Patients with burning sensation
- Subjects with restricted mouth opening
- Subjects complying to quit their Khaini, gutkha chewing habits, tobacco in any form etc.
- Patients willing to obey follow-up visits and comply by study protocols
- Patients who had the above mentioned features and were not taking any medication for the same.

EXCLUSION CRITERIA:

- Subjects with already diagnosed condition of OSMF and taking medication for the same
- Subjects having any past or present systemic conditions (e.g. autoimmune disorders diabetes, hypertension, heaptic disorders or renal diseases),
- Subjects withother mucosal lesions,
- Patients suffering from acute or chronic infection, patients with a known allergy or contraindication to the study drugs

Immunomodulator drug levamisole (50 mg tablets, trade name(TN) - Vermisol) and antioxidant capsules (TN: ANTOXID withbeta carotene 10 mg)wereadministered. To assess serum immunoglobulins level, 5 ml of blood was drawn from the ante-cubital fossa. The drawn blood was then allowed to clot and the serum collected was stored at

4°C until it was analysed for radial immunodiffusion. Serum IgA,IgGand IgM were estimated by Serial Radial Immunodiffusion (SRI). This procedure was carried outpost treatment also.

The selected subjects were randomly allocated to 3 subgroups. All participantswere administered for their first therapy dosage 1 week after biopsy was carried out. Participants were recalled for six weeksat weekly intervals, and then at an interval of 1 month for the subsequent two months. At each visit patients were examined and administered the subsequent doses. Group I subjects took 1 tablet of levamisole 50 mg, thrice daily, for three consecutive days in a week for 3 alternative weeks, group II subjects took one capsule of antioxidant twice times daily for 6 weeks, group III patients took 1 tablet of levamisole 50 mg, three times daily, for three consecutive days in a week for three alternate weeks and one capsule of antioxidant two times daily for six weeks.

The interincisal mouth opening was recorded using divider and metallic scale from the mesio-incisal angle of maxillary central incisor to the mesio-incisal angle of mandibular central incisor. All measurements were recorded in millimeters. Visual Analogue Scale (VAS) of 0–100 was used to determine the intensity of burning sensation using a with 10 mm division, where 0 is no burning sensation and 100 is the worst possible burning sensation. The participants were asked to mark the VAS at a point that best depicted the burning sensation at that visit.

Post treatment follow-up involved the evaluation of the patient once in 1 month over a period of the next 2 months. Post treatment serum Igs estimation was carried out one month post completion of treatment. All the relevant data collected was entered in a proforma. Demographic details, physical examination (extra oral and intra-oral) records and laboratory data gathered together were sorted, tabulated and subjected to appropriate statistical analysis.

RESULTS

Descriptive data that included mean, numbers and percentages were calculated for each group and were used for analysis. Paired 't' test was used for intra group comparison, and unpaired 't' test was used for inter group comparison using the SPSS package (10th version). For all the tests, a p-value of 0.05 or less was considered for statistical significance.

At the end of treatment the improvement in mouth opening [Table 1] in patients treated with levamisole (groupwas 7.1%, in group II 6.7% and in group III 8%. At the end of the two-month follow-up period the improvement in mouth opening in group I was 10.7%. In groups II and III the improvement remained the same (6.7 and 8% respectively). No statistically significant difference was found between the three groups.

Table 1: Mouth opening (in cm)

Groups		Baseline	5th Visit	Diff. (BL-5th)	7th Visit	Diff. (BL-7th)
I	Mean ± SD	2.8 ± 0.6	3.0 ± 0.7	0.2 ± 0.1	3.1 ± 0.7	0.3 ± 0.1
	%			7.1%		10.7%
	P-Value			*P* < 0.001		*P* < 0.001
II	Mean ± SD	3.0 ± 1.1	3.2 ± 1.0	0.2 ± 0.2	3.2 ± 1.0	0.2 ± 0.1
	%			6.7%		6.7%
	P-Value			*P* < 0.001		*P* < 0.001
III	Mean ± SD	2.5 ± 1.0	2.7 ± 1.0	0.2 ± 0.4	2.7 ± 0.8	0.2 ± 0.3
	%			8%		8%
	P-Value			*P* < 0.05		*P* < 0.05

Diff b/w groups

I–II	P=1.0	P = 0.22	Diff. between Groups	I–II	*P* = 0.71	*P* = 0.93
I–III	P=1.0	P = 0.22		I–III	*P* < 0.05	*P* < 0.05
II – III	P=1.0	P=1.0		II – III	*P* = 0.15	*P* = 0.13

Table 2: Burning sensation (using VAS) before and after treatment

Groups		Baseline	5th Visit	7th Visit
I	Mean ± SD	51.7 ± 19.9	1.0 ± 2.8	0.7 ± 2.6
	% Reduction		98%	98.6%
	P-Value		*P* < 0.001	*P* < 0.001
II	Mean ± SD	58.0 ± 29.7	10.1 ± 13.8	7.7 ± 12.7
	% Reduction		82.4%	86.7%
	P-Value		*P* < 0.001	*P* < 0.001
III	Mean ± SD	39.3 ± 16.9	2.7 ± 6.8	1.3 ± 3.0
	% Reduction		93.1%	96.7%
	P-Value		*P* < 0.001	*P* < 0.001

Statistically highly significant reduction in burning sensation was seen in all the three groups. Inter group comparisons did not reveal statistically significant differences [Table 2].Significant reduction was seen in the serum IgG, IgA and IgM [Table 3] in the levamisole group (14.8, 28.1, 28% respectively) and the combination group (13.4, 21.9,18.3% respectively) which was statistically significant (p<0.001), while in the antoxid group significant reduction was seen in serum IgA and IgM (17, 26.8% respectively). The reduction in mean serum IgG was significantly better in the levamisole group (group I) and the levamisole and antoxid combination group (group III) than in the antoxid group (group II) [group I v/s group II (*P*< 0.01) and group III v/s group I (*P*<0.01)]. The intergroup comparison for reduction in serum IgA and IgM was not statistically significant.

Table 3: Serum IgG, IgA, IgM before and after treatment

Groups		IgG (mg/dl)			IgA (mg/dl)			IgM (mg/dl)		
		Pre	Post	Diff.	Pre	Post	Diff.	Pre	Post	Diff.
I	Mean ±	1554.0 ±	1323.8 ±	230.2 ±	319.6 ±	229.9 ±	89.7 ±	153.1 ±	110.3 ±	42.8 ±
	SD	156.1	156.1	101.3	93.9	81.9	68.9	61.8	36.6	35.8
	%			14.8%			28.1%			28%
	P-Value			< 0.001			< 0.001			< 0.001
II	Mean ±	1695.8 ±	1468.4 ±	227.4 ±	357.3 ±	279.0 ±	78.3 ±	154.4 ±	126.1 ±	28.3±
	SD	200.3	17.5	79.7	101.0	103.9	27.0	67.5	52.0	17.8
	%			13.4%			21.9%			18.3%
	P-Value			< 0.001			< 0.001			< 0.001
Intergroup										
I–II	*P-Value*			< 0.01, S			= 0.09			= 0.71
I–III	*P-Value*			= 0.93			= 0.54			= 0.70
II – III	*P-Value*			< 0.01			= 0.05			= 0.14

DISCUSSION

The etiological factors for OSMF include local irritants such as Chilli consumption, areca nut chewing, tobacco smoking and chewing. Systemic factors include anemia (iron deficiency), vitamin deficiencies (B-complex and folate) together with the malnourished state (protein deficiency), genetic predisposition to the disease and autoimmunity.

Areca nut use is considered to be most important etiological factor in pathogenesis OSMF. The basic constituent of areca nut is either raw or dried or boiled or baked. Diverse agents including lime, tobacco, catechu, cloves, saffaron, and leaf of piper betel leaves may form a part of formulation. [6]

Various methods have been tried to bring relief from burning sensation, and to decrease fibrous bands, and improve mouth opening. These include intralesional injections of corticosteroids, placental extracts or hyaluronidase either alone or in combination, intralesional injections of IFN γ, micronutrient supplementation, physiotherapy, and surgery. [8-13]

Levamisole is an immunomodulator which alters both cellular and humoral immunity.[9] This property has been used in treatment of several oral mucosal lesions with favourable results. However its effects on OSMF are not known. ANTOXID is an antioxidant formulation that contains beta carotene, zinc, copper, manganese and selenium. It has been found that these micronutrients have antioxidant properties and enhance cellular immunity. The serum levels of beta carotene have found to be significantly reduced in OSMF patients and supplementation with BC was associated with clinical improvement and increase in serum BC levels as well.[1]

It has been found that some of the serum immunoglobulins are elevated in OSMF patients;[3-6] however it is not known whether it can decrease after treatment with levamisole and antioxidants. The present study was thus conducted to treat OSMF patients with levamisole, antoxid or a combination of the two and to estimate the serum level of IgG, IgA, and IgM before and after treatment in all the patients. There are no reported randomized clinical trials of levamisole in the treatment of OSMF. In our study the patients were administered 50 mg levamisole thrice a day for three consecutive days for three alternate weeks. The regimen for antoxid group was two capsules per day, each day for six consecutive weeks.

Restriction of mouth opening is a major disability associated with OSMF. To date there is no treatment protocol that can restore the mouth opening to normal, but an improvement of a few millimeters has been seen. In our study a highly significant improvement was seen in all the three groups and the improvement was better in the levamisole and antoxid combination group i.e., 8% (group III), when compared to 7.1% in the levamisole (group I) and 6.7% in the antoxid (group II) groups. However at the end of follow-up it was seen that the mouth opening continued to improve in patients who had taken levamisole alone (group I) i.e. 10.7%, but the differences among the groups were not statistically significant. The distribution of samples was not uniform across the three groups. The more severe the restriction in mouth opening, the longer it takes to regain some improvement, therefore the significant result is probably masked by an improper sample distribution.

Burning sensation when eating spicy food or even normal food is common among OSMF patients due to which they switch over to bland diet, which is generally not nutritionally adequate. Although the exact mechanism causing burning sensation is not clear, intolerance to spices could be due to the atrophic and permeable epithelium. In our study, patients treated with levamisole (group I) had a reduction in burning sensation by 98% at the end of treatment and 98.6% at the end of the follow up period. Patients treated with antoxid (group II) had 82.4% reduction at the end of treatment and 86.7% reduction after the follow-up period. Patients who were treated with the combination of levamisole and antoxid (group III) had a 93.1% reduction at the end of treatment and 96.7% reduction at the end of the follow-up period. Maher *et al*. have also reported similar findings.[8] In their study multiple micronutrient supplements produced relief from burning sensation in 85% of the patients. Treatment of OSMF with intralesional injections of hyaluronidase and/or corticosteroids (dexamethasone or triamcinolone acetonide) has been reported to reduce burning sensation by 75-100%. Treatment with intralesional placental extracts[13] and IFN γ[12] have resulted in reduction in burning sensation by 40-51% and 60% respectively. The patients in our study showed reduction in burning sensation comparable to that seen in patients treated with intralesional injections of hyaluronidase and/or corticosteroids and better than that in patients treated with intralesional placental extracts and IFNγ. Since our treatment was non-invasive and not associated with side effects as in intralesional injections, we can expect better patient compliance and consequently better results. Though the reduction in burning sensation was sustained in all the three groups, levamisole alone seems to be more effective when compared to antoxid or combination of levamisole and antoxid. There were no statistically significant differences between the groups. The relief from burning sensation in patients treated with levamisole is probably due to the anti-inflammatory effects of levamisole and its ability to modulate inflammatory cytokines.

The reduction in mean serum IgG and IgA was better in the levamisole group (group I) and the levamisole and antoxid combination group (group III) than in the antoxid group (group II) but the reduction was significant only for IgG. The reduction in mean serum IgM was better in the levamisole group (group I) and the antoxid group (group II) than in the levamisole and antoxid combination group (group III) but the difference was not statistically significant. OSMF is associated with altered immunoglobulin profile. Various studies have reported an increase in one or more of the five immunoglobulins in OSMF patients.[3-6] There have been no reports in which serum immunoglobulins IgG, IgA, IgM were evaluated before and after treatment of OSMF with levamisole or antioxidants.

Levamisole has been found to modulate both cellular and humoral immunity in patients with recurrent aphthous ulcers and chronic hepatitis.[6] These reports are similar to the findings in our study. The reductions in the levels of IgG, IgA and IgM as seen in our study suggest that the humoral response in OSMF is modified, probably due to slowing down of the chronic inflammatory process. It has been shown that beta carotene not only has antioxidant properties but also improves cellular immunity by increasing the number of circulating lymphocytes and number of helper T cells, enhancing the proliferation and induction of cytotoxic T cells, and enabling natural killer cells to be more effective on supplementation in those who are beta carotene deficient. The reduction in serum IgG, IgA and IgM due to antioxidants in our study is probably secondary to reduction in inflammation. None of the patients in our study reported any side effects due to study drugs.

CONCLUSION

Levamisole alone has a higher advantage in clinical management of OSMF when compared to that of antioxidant therapy alone or in combination. Antioxidants proved to have no added benefit in aiding to the reduction in burning sensation or in opening of restricted oral cavity.

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