International Journal of Advanced Research in Medicine

E-ISSN: 2706-9575
P-ISSN: 2706-9567
IJARM 2019; 1(1): 88-91
Received: 22-01-2019
Accepted: 27-02-2019

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Role of methylene blue in treatment of oral lichen planus

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Abstract
Introduction: Methylene blue, widely used as a histologic dye, has potential photochemical and photo biological properties favoring its use as a photosensitizer in photodynamic therapy (PDT) of varied diseases. This study was designed to assess the efficacy of MB mediated PDT in treatment of Oral Lichen Planus.
Materials and Methods: A sample selection of 37 patients was done based upon a predefined set of inclusion and exclusion determinants from the Outpatient Department Radiotherapy, IMS and SUM Hospital. This study encompassed anon-randomized, non-comparative prospective split mouth study protocol. Pre-treatment assessment of clinical presentation and symptoms was done using REU and VAS score respectively. The subjects were treated with a single session of MB-PDT and followed up after 2 weeks, 1 month and 3 months from baseline. The post treatment REU and VAS recorded at each visit for the test and control site separately.
Findings: For the final sample of 35 recruits, repeated measure ANOVA was used for analysis of REU and VAS score among various visits in test group. With respect to REU scores, statistically significant results were obtained for baseline with 3 months follow up, for 2 weeks with 3 months follow up and for 4 weeks with 3 months follow up. The VAS score was statistically significant amongst all comparisons.
Conclusion: MB is photo chemically and photo biologically a dynamic molecule which can be effectively harnessed to target various premalignant and malignant pathologies to prevent its progression.

Keywords: Methylene blue, photodynamic therapy, REU score

Introduction
Photodynamic therapy (PDT) is a promising modality for the management of various tumors and nonmalignant diseases, based on the combination of a photosensitizer that is selectively localized in thetarget tissue and illumination of the lesion with visible light, resulting in photodamage and subsequentcell death [1, 2]. Early preparations of photosensitizers for PDT, based on a complex mixture of porphyrins called haematoporphyrin derivatives were first generation photosensitizers [3]. Ongoing research in photo chemotherapy has uncovered new photosensitizers that belong to the different classes of compounds including porphyrins, chlorins, phthalocyanines, texafrins and phenothiaziniums [4]. Methylene blue is a widely known histological dye. Chemico-structurally MB is a phenothiazinium compound. Its favorable photochemistry and photo physics confers upon this molecule a great potential for application in PDT. This molecule has a well characterized and effective photochemistry that triggers both photosensitization mechanisms type I and type II [5]. Its photodisruptive effect damages biomolecules and efficiently induces death in several target cells, tissues and organisms. Therefore, upon photo activation, it can be used to treat an array of cancerous and non-cancerous diseases.

Material and Methods
a) Research design and study sample
The subsequent research design relied upon a non-randomized, non-comparative prospective split mouth study protocol. The study recruited participants from amongst the patients attending the Outpatient Department of Department Radiotherapy, IMS and SUM Hospital. Sample selection was done based upon the following outlined inclusion and exclusion criteria.

b) Inclusion criteria

1. Clinical diagnosis of any one of the typical variants of oral lichen planus (OLP) of buccal mucosa present bilaterally, with histopathological confirmation
2. Patients previously diagnosed and under conventional treatment for oral lichen planus who where nonresponsive or calcitrant
3. Dissatisfied or reluctant to continue the conventional treatments (Patients included as per criteria 2 and 3 were clinically and histopathologically reassessed prior to their inclusion in the study.)

c) Exclusion criteria

1. Histopathologic non-confirmation of oral lichen planus
2. Dysplastic features histopathologically
3. Lichenoid reactions with clinical improvement after removal of suspected etiology
4. Systemic compromise or debility and pharmacologic treatments that could predispose to and/or alter the course of OLP and effect of treatments instituted in the study hence
5. OLP with dermatologic manifestations
6. Documented allergy to phototherapy and photosensitizers
7. Pregnancy, lactation
8. Patients with deleterious oral habits (smoking and smokeless tobacco, alcohol) and parafunction.

An informed consent was obtained from all subjects after detailed explanation of study protocol, advantages and disadvantages with a clause allowing voluntary discontinuation of participation on behalf of the subject at any point during the study.

d) Methodology

A sample of 37 patients was recruited into this study based upon the outlined norms. Pretreatment evaluation included 3 stages: assessment of clinical characteristics of the lesions, symptom evaluation, and histopathological confirmation. During recruitment of patients, it was ensured that lesions were present on bilateral buccal mucosa with similar clinical manifestations. Pretreatment assessment (Stage-1) of the intraoral lesions on buccal mucosa was done in corroboration with the REU scale[6]. Lesion severity in each site was scored according to the following: presence of reticular/hyperkeratotic/white papular (R) lesions (0-none, 1-presence), presence of erosive/erythematous (E) lesions and/or ulcerative (U) lesions (0-none, 1-lesions smaller than 1cm^2, lesions from 1 to 3 cm^2, 3-lesions larger than 3 cm^2). The total weighted score was a summation of reticulation score, erythematous score (weighted 1.5), and ulcerative score (weighted2.0).To measure dimensions using REU scale, transparent flexible grid with marking squares of 1x1 mm^2 of minimum dimension was used. The clinical examination was performed by two examiners (Oral Medicine specialist and Oral Pathologist) separately to rule out any bias concerning the scoring. Also a recording of symptom (burning and pain) was done using VAS score (pretreatment stage-2). These were baseline pretreatment data. Following this, a punch biopsy was taken from the most representative site of buccal mucosa (pre-treatment stage-3). Only unilateral biopsy was done with the side of biopsy being treated as control and the contralateral lesional buccal mucosa was treated as test site (PDT intervention), to avoid any untoward effects of wound healing on appearance hence subsequent scoring of lesions. The biopsy specimen was harvested as aseptically as possible under local anesthesia (2% lignocaine with 1:200000 adrenalin) using a 4mm diameter and depth disposable punch instrument. The specimen was transported in screw capped vials with 10% buffered formalin and sent for histopathological confirmation. A period of 2 to 4 weeks was allowed to lapse with supervision for adequate healing of biopsy site.

Before application of the photosensitizer, an atopic patch testing was done. None of the subjects tested positive. The lesions on test and control site were cleaned with cotton wool soaked in a soap free cleansing lotion. The subject was instructed to swish with freshly prepared 5% MB solution (Sigma, Life Sciences, purity grade≥97%, molecular weight-319.85) for 5 minutes (fig-1,2). The patient refrained from consumption of food and water during the test period. Twenty minutes after application of the dye, the test site was given a radiant exposure of 120 J/cm2 with a wavelength range of 625-675nm of red light, at irradiances of 100-130 mW/cm2 using a light emitting diode source (Epic10 Biolase, operating voltage-100 V to 240 V, wavelength-940+/-10nm, maximum power output-10W, continuous power mode, fig-3, 4) for a period of 120 seconds. The control site was spared of the above procedure. However, the aiming beam of the laser equipment was flashed at control site. The distilled water application with the aiming beam was chosen as placebo to MB-PDT. The subjects were assigned a follow up visits after 2 weeks, 1 month and 3 months from baseline with post treatment REU and VAS recorded at each visit for the test and control site separately. During the study period, 2 patients were lost to follow up at 2 weeks and were excluded from the study giving a net sample strength of 35 patients.

Fig 1: dispensing of MB powder and distilled water to prepare 5% solution

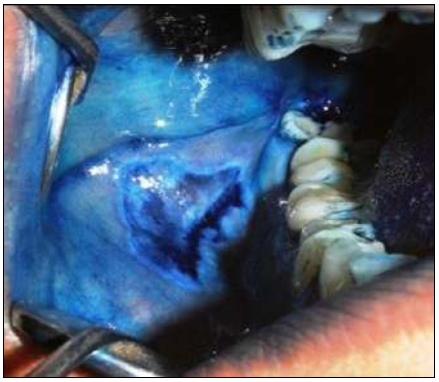

Fig 2: Lesional site after swishing mouth with prepared solution

Fig 3: Epic10 Biolase, operating voltage-100 V to 240 V, wavelength-940+/-10nm, maximum power output-10W

Fig 4: Irradiation at 120 J/cm^2 with a wavelength range of 625-675nm of red light

Findings

The intra and intergroup (test and control sites) comparisons of both objective and subjective parameters (REU and VAS) at baseline and subsequent follow up visits have been documented in table 1. Out of 35 patients recruited in the final sample, 14 were males and 21 were females giving a percentage distribution of 60% and 40% in favor of female predilection. Statistically significant change in both REU and VAS score was observed at the test site in contrast to the side of nonintervention.

Table 1: Intra and inter group comparisions of clinical parameters (mean ± SD).

	Test site					Control site				
	Baseline (Mean+ SD)	**2 weeks (Mean+ SD)**	**4 weeks (Mean+ SD)**	**3 month (Mean+ SD)**	***p***	**Baseline (Mean+ SD)**	**2 weeks (Mean+ SD)**	**4 weeks (Mean+ SD)**	**3 month (Mean+ SD)**	***p***
Age (years)	41.45±10.46				-	41.45±10.46				-
Gender (M/F)	14/21				-	14/21				-
REU	2.67±0.48	2.67±0.48	2.54±0.25	1.72±0.76	0.000*	2.50±0.00	2.50±0.00	2.50±0.00	2.50±0.00	-
VAS	4.60±8.11	4.20±0.71	3.14±0.80	1.88±0.796	0.000*	4.33±0.86	3.97±0.73	2.66±0.92	2.38±1.10	1.00

Table 2: Analysis with Repeated measure ANOVA of REU score among various visits (in weeks and months) with Bon ferroni correction in PDT test group.

Visit		**Mean difference**	***P***
Baseline	2 Weeks	0.000	-
	4 Weeks	0.129	0.499
	3 Months	0.943	0.000*
2 Weeks	4 Weeks	0.129	0.499
	3 Months	0.943	0.000*
4 Weeks	3 Months	0.814	0.000*

Table 3: Analysis with Repeated measure ANOVA of VAS score among various visits (in weeks and months) with Bon ferroni correction in PDT test group.

Visit		**Mean difference**	***P***
Baseline	2 Weeks	-0.40	0.002*
	4 Weeks	1.45	0.000*
	3 Months	2.714	0.000*
2 Weeks	4 Weeks	1.057	0.000*
	3 Months	2.314	0.000*
4 Weeks	3 Months	1.257	0.000*

Table 4: Mann Whitney U test, $p<0.05$ significant. Inter group comparison of REU and VAS score at various visits between study groups.

Study interval	**Variable**	**Case Site (Median ± SD) N=35**	**Control site (Median ±SD) N=35**	**Mann Whitney U**	**P**
Baseline	REU	2.50±0.48	2.50±0.00	542.500	0.041
	VAS	5.00±0.81	3.00±0.49	157.500	0.000*
2ND Week	REU	2.50±0.35	2.50±0.00	542.500	0.041
	VAS	4.00±0.71	3.00±0.50	270.000	0.000*
4TH Week	REU	2.50±0.25	2.50±0.00	595.000	0.317
	VAS	3.00±0.80	4.00±0.32	217.000	0.000*
3 Months	REU	1.00±0.76	2.5±0.00	297.500	0.000*
	VAS	2.00±0.79	4.00±0.65	0.000	0.000*

In table-2 and table-3, repeated measure ANOVA was used for analysis of REU and VAS score among various visits in test group. With respect to REU scores, statistically significant results were obtained for baseline with 3 months follow up, for 2 weeks with 3 months follow up and for 4 weeks with 3 months follow up. The VAS score was statistically significant amongst all comparisons.

Table-4 elucidates inter group comparison of REU and VAS scores at various visits between study groups. Statistically significant results were obtained for VAS at all follow up visits and at 3 months for REU.

Conclusion

Nearly a century ago, the antibacterial characteristics ofMB which is a phenothiazine dye were described and attributed to its photodynamic properties. MB itself hasbeen used in medical practice for more than 100 years and is recognized as having very low tissue toxicity. Clinical usesof MB include the treatment of ifosfamide encephalopathy, methemoglobinemsia, urolithiasis, and cyanide poisoning [7, 8]. Photodynamic therapy (PDT) has been considered as an alternative/complimentary therapeutic modality for the management of premalignant lesions. The results from this

study highlight the potential role of methylene blue mediated PDT in alleviating subjective symptoms whilst also causing regression in clinical lesion. MB is photo chemically and photo biologically a dynamic molecule. Its photo action occurs in direct contact with membranes, polyelectrolytes and in the presence of reducing agents. Photosensitization reactions induced by MB excitation are known to cause damage to several biomolecules [9, 10]. These mechanisms can be effectively harnessed to target various premalignant and malignant pathologies to prevent its progression.

Conflicts of Interest
None to declare.

Source of Funding
Self.

Ethical clearance
Duly obtained from Instituitional Ethical Committee.

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How to Cite This Article
Sanjay Mishra MD, Ifterkhar S. Role of methylene blue in treatment of oral lichen planus. International Journal of Advanced Research in Medicine 2019; 1(1): 88-91.