A NOVEL TREATMENT OF FRECKLES BY PHOTODYNAMIC THERAPY USING CHITOSAN- METHYLENE BLUE HYDROGEL

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ABSTRACT

Objective: *To evaluate the efficacy of chitosan- methylene blue (MB) hydrogel mediated photodynamic therapy in the treatment of freckles.* ***Patients and methods:*** *Thirty four patients complaining from freckles were recruited in this Prospective, split-face controlled study. Pregnant women, patients with photosensitive dermatosis, other contraindications for phototherapy and use of topical or systemic retinoid treatment in the past 6 months were excluded. Chitosan hydrogel was prepared by dispersing 3g of chitosan in 2% acetic acid solution, and then MB added with continuous stirring until thoroughly mixed to prepare 0.01% MB-chitosan hydrogel. The left side of the face was treated by application of Chitosan- MB hydrogel, 30 min prior to treatments followed by irradiation with diode laser (650 nm wave lengths) twice weekly until complete clearance of the lesions or maximum 12 sessions. Improvement was graded as excellent (≥ 75%), very good (50–74%), Good (25–49%) and poor (< 25%).* ***Results*** *The release of MB from formulation follow first order kinetic model, MB content of the prepared hydrogel was found 99.51 % ± 1.1 of the claimed amount (0.01%w/w). Thirty four patients were enrolled in the study, the age of the patients ranged from 18-46 years and the duration of freckles ranged from 8 months to 17 years. Mean surface area, Density, Darkness and PSI score of freckles was significantly decreased in MB treated side compared with their baseline (P<0.05). Excellent improvement was occurred in 6 (17.6%) cases, very good improvement in 19(55.9%) cases, good improvement in 7 (20.6%) cases and poor improvement in 2 (5.9%) cases. Pain during treatment sessions was the most common complication followed by transient erythematic and post-inflammatory hyper pigmentation.* ***Conclusions*** *Chitosan- MB hydrogel mediated photodynamic therapy is an effective and safe method in the treatment of freckles*

INTRODUCTION

Photodynamic therapy (PDT) has been considered effective and safe technique for treatment of various cancerous and non-cancerous diseases [1,2,3,4].

The process of PDT is simple consisting of photosensitizer (PS) plus light of suitable wave length and cell death is the net result, however; the exact mechanism by which PDT works still unknown [5,6] .

One of the PS that has been used in PDT is methylene blue (MB), several clinical studies indicates the effectiveness of MB in treatment of different tumor tissues, melanoma, Kaposi's Sarcoma, onychomycosis, virus and fungal infections and blood sterilization [7,8,9,10,11].

MB absorbs light strongly in the region 630-680 nm, and has very little absorption elsewhere in the visible spectrum [12].

The higher affinity of MB to pigmented melanoma reflects its selectivity to bind melanin which has been confirmed in several studies [13, 14, 15].

The aim of this study was to evaluate the efficacy of photodynamic therapy using MB in the treatment of freckles using chitosan hydrogel as a delivery system and diode laser as source of light.

PATIENTS AND METHODS

Thirty four patients complaining from freckles were recruited in this Prospective, split-face controlled study. An informed consent was obtained from all patients prior to enrollment. Exclusion criteria were patients with pregnancy, personal history of skin cancer or radiotherapy or other contraindications for phototherapy, topical or systemic retinoid treatment in the past 6 months

MB was purchased from (Sigma-Aldrich, St Louis, MO, USA). Chitosan, Molecular Weight: 100, 000 to 300, 000 was purchased from (across organics, Newjersy, USA), Standard cellophane membranes (molecular cut

range = 10.000) (Sigma Chem.Co., USA). Chitosan hydrogel was prepared by dispersing 3g of chitosan in 2%w/v acetic acid solution with continuous stirring until completely dispersed[16], then MB added with continuous stirring until thoroughly mixed to prepare 0.01%w/w MB-chitosan hydrogel. To predict uniformity distribution of MB in the prepared hydrogel. The drug content was determined by dissolving an accurately weighed quantity of the prepared hydrogel (100 mg) in 10 ml water followed by filtration using 0,45 mm membrane filters then assayed with UV-visible double-beam spectrophotometer.

In-vitro Release of methylene blue from chitosan hydrogel has been studied as followed, One gram of hydrogel under investigation was placed on a circular area ($6cm^2$) of cellophane membrane previously moistened with the receptor phase. The loaded membrane was firmly stretched over one end of a glass tube with fixed cross area the tube was then immersed in a 100 ml beaker containing 50 ml of the release media (phosphate buffer pH 6.8) and placed in thermostatically controlled water bath at 37±1 C^0 at 50 rpm. 5ml of sample was withdrawn at different time intervals up to 2 hr., and then replaced by equal volume of the release medium maintained at the same temperature. The amount of MB released at time intervals was determined spectrophotometrically at λmax 664nm.

To describes the release pattern of the drug. The data of the in vitro release was analyzed according to zero order, first order and Higuchi diffusion model [17,18]

Chitosan- MB hydrogel was applied on the lesions of the left side of the face 30 min prior to treatments, all patients received diode laser with 650 nm wave length with maximum output power 150mW twice weekly until complete clearance of the lesions or maximum 12 sessions. All the patients were asked to use sunscreens with SPF>50 during daytime.

Assessment of treatment outcome on the basis of clinical examination and photography evaluation using camera (Nikon Coolpix S2500, 12MP) were taken at baseline and after 12 sessions, pigmentation severity index score (PSI) was calculated according to the method described by Kimbrough *et al*[19]. Improvement from baseline was rated on a four- point scale, excellent improvement ≥ 75%, very good improvement 50–74%, Good improvement 25–49% and poor < 25%.

Statistical analysis

Data were analyzed and expressed in tables as mean values ±standard deviations (SD). The statistical analysis was performed using SPSS, version 21.0 (SPSS Inc., Chicago, IL, USA). Results were expressed as simple percentage accompanied by qualitative description of comments. The significance of differences between the data of the studied groups and the mean standard deviation values were use t-test. A p-value of 0.05 or less was considered significant.

RESULTS

The release of MB from formulation follow first order kinetic model as confirmed by the values of correlation coefficients (r) (Table 1) and the percentage of MB released from 3% (w/w) chitosan hydrogel was found to be 91.8% after 2 hours (figure 1). Also, MB content of the prepared hydrogel was found 99.51 % ± 1.1 of the claimed amount (0.01%w/w).

Table (1): Kinetic data for MB release from chitosan hydrogel.

Mechanism of release	**Zero**	**First**	**Diffusion**
R	0.93	**0.99**	0.97
K	27.6 (K_0)	1.1 (K_1)	55.9
t(1/2)	1.8	0.62	0.80
Best fitted model	**First order model**		

R: correlation coefficients, K_0: Zero order release constant, K_1: First order release constant

Thirty four patients were enrolled in the study, their ages ranged from 18-46years, 5 patients were males and 29 were females. According to Fitzpatrick classification, 12 cases of skin phototype II, 16 cases of skin phototype III and 6 cases of skin phototype IV. The duration of freckles ranged from 8 months to 17 years, with a mean duration of 9.21 ± 5.02 years.

At the end of treatment, mean surface area, Density, Darkness and PSI score of freckles was significantly decreased in MB treated side compared with their baseline (P<0.05) (Table 2). Excellent improvement (≥75 %) was occurred in 6 cases (17.6%), very good improvement (50-74%) in 19 (55.9%) cases, good improvement (25-49%) in 7 (20.6%) cases and poor improvement (<25 % lightening) in 2 (5.9%) cases (Table 3).

Table 2: Mean ± SD of area, density, darkness and PSI before and after treatment of freckles with chitosan-MB hydrogel mediated photodynamic therapy.

	Treated side		Control side	
	Pre treatment	Post treatment	Pre treatment	Post treatment
Area	2.9 ± 1.13	1.21 ± 1.1*	2.9 ± 1.13	2.6 ± 0.89
Density	3.11 ± 0.76	1.4 ± 0.76*	3.11 ± 0.76	2.8 ±1.01
Darkness	3.02 ± 0.93	1.86 ± 1.02*	3.02 ± 0.93	2.81 ± 0.88
PSI score	8.21 ± 9.55	6.02 ± 10.1	8.21 ± 9.55	7.6 ± 10.2

PSI: The pigmentation area and severity index

* Significant $p<0.05$

Table 3: Degree of improvement in freckles treated with chitosan- MB hydrogel mediated photodynamic therapy.

	No	%
Excellent (≥ 75 %)	6	17.6
Very good (50 >75%)	19	55.9
Good (25 > 50%)	7	20.6
Poor (0-25%)	2	5.9

The reported side effects among this study was pain during treatment in all cases followed by transient erythema in 28 cases (82.4%) and post-inflammatory hyperpigmentation in 4 cases (11.8%).

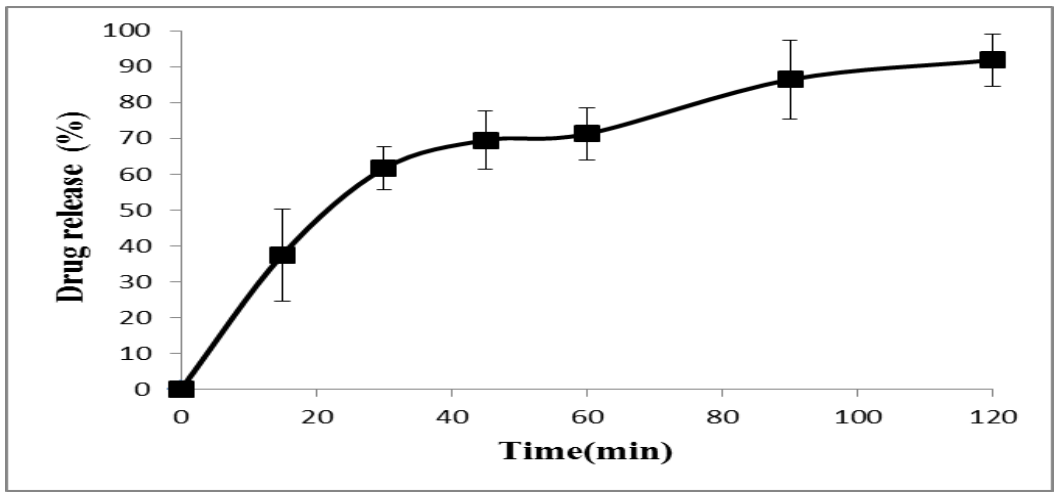

Figure 1: In vitro release of MB from 3% chitosan hydrogel.

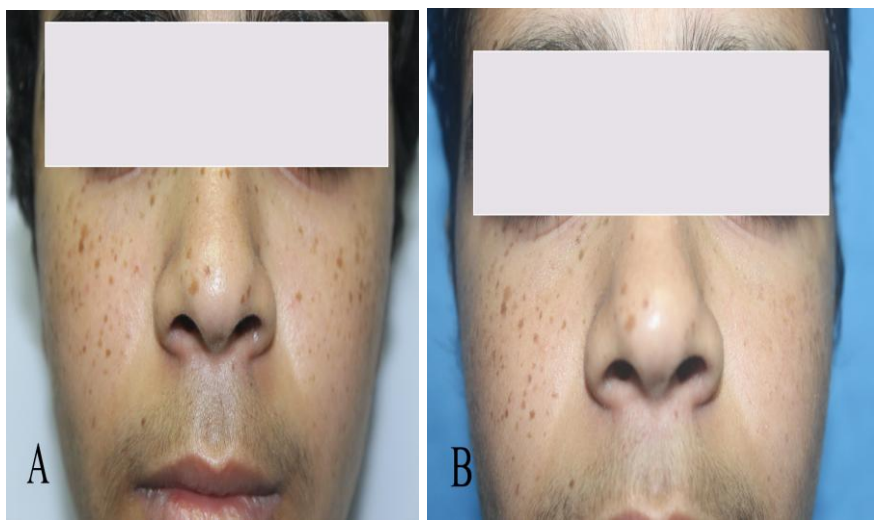

Figure 2:

A 19 years-old male with freckles, the left side of the face treated with Chitosan- MB hydrogel mediated photodynamic therapy (A) before treatment, (B) 4 weeks after 9 treatment sessions. Significant lightening was observed on photodynamic therapy treated side.

DISCUSSION

The higher release of MB from chitosan hydrogels may be attributed to the repulsion between the same charges (positive charge) that both MB and chitosan hold, This result was in accordance with lee *et al*.(2002)[20],who found that, the rapidly release of cationic drug from cationic hydrogel was due to charge repulsion.

Treatment of freckles is not usually necessary as they are asymptomatic and tend to fade during winter months, but cosmetically undesired lesions can be treated by different methods [21].Different modalities of treatment may have reduced the occurrence and severity of freckles, but it is still with a relatively high rate of recurrence[22].

Photodynamic therapy (PDT) is a therapeutic method used with increasing frequency in dermatology[23]. The therapeutic effect is achieved by light activation of a photosensitizing agent, and in the presence of oxygen, reactive oxygen intermediates are formed. These intermediates irreversibly oxidize essential cellular components, causing apoptosis and necrosis[24,25] .

In dermatology, PDT is used to treat non-melanocytic skin cancer treatment and other inflammatory and proliferative non-neoplastic diseases such as psoriasis, Darier disease, sarcoidosis and lipoidic necrobiosis. In recent decades, PDT progressed from experimental therapeutic resources for the first option of treatment of lesions as actinic keratosis and other extensive superficial lesions such as superficial basal cell carcinoma and Bowen disease.

The selective binding of MB to melanin has been previously studied[13,14,26]. MB forms a strong complex with melanin and may provide a means of selective delivery of radionuclides to melanoma cells, useful for noninvasive diagnosis as well as for therapy of disseminated disease [15,27].

Chitosan is the most interesting natural polymer used in pharmaceutical technology which has several advantages being biocompatible, bidegradable and considered as penetration enhancer. It has also bacteriostatic and wound healing property. Also, it is considered as bioadhesive which increases retention at the site of application[28].

In the present study, there is significant improvement in mean area, density, darkness and PSI score of freckles among patients treated with chitosan- methylene blue hydrogel photodynamic therapy in comparison to the control side; improvement (>50%) was reported in 73.5% of patients.

Several studies evaluated the efficacy of PDT in regard to dyschromia. Improvement in mottled hyperpigmentation was significantly better with IPL-PDT than in the IPL-only side[29,30]. In addition, Key *et al*[31]. attained removal of brown spots with pulsed dye laser - PDT, while pulsed dye laser treatment alone was not successful. However, one IPL split-face study failed to show additional improvement after pretreatment with ALA, as a result of the excellent results which had already been achieved through IPL-only treatment[29]. To present, there are no data about the effect of photodynamic therapy in the treatment of freckles before our study.

Pain during treatment session was the most common side effect reported in our study followed by erythema and post-inflammatory hyperpigmentation. The same observation was reported by Langan and Collins, 2006[32], they observed that pain during illumination is the most prominent side-effect of PDT. Pain usually mild to moderate and common with red or blue light PDT, whereas PDT using pulsed light was usually better tolerated[32].

CONCLUSION

Chitosan- MB hydrogel mediated photodynamic therapy is an effective and safe method in the treatment of freckles.

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