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A CLINICAL AND HISTOLOGICAL EVALUATION FOR THERAPEUTIC EFFICACY AND SAFETY OF TRANEXAMIC ACID LOCAL INFILTRATION WITH DERMAPEN IN COMBINATION WITH TOPICAL TRANEXAMIC ACID EMUGEL OR KLIGMAN'S FORMULA CREAM IN PATIENTS WITH MELASMA: A COMPARATIVE STUDY

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ABSTRACT

Background: Tranexamic acid (TA), an anti-fibrinolytic lysine derivative, seems to be a promising agent for melasma treatment. But, little is known about its optimal administration route, dose, frequency, use duration, or its role in skin whitening of hypermelanotic patches. **Aims and Objectives:** To evaluate clinical and histological efficacy and safety of TA microinjection plus its topical use in treatment of melasma, and compare this lone use versus TA microinjection plus of Kligman's formula. **Materials and Methods:** Egyptian melasma patients (n=40) were given TA(100 mg/mL per week) using dermapen for 12 weeks. Moreover, some patients used topical TA3% emulgel (group I, n=20), while others used Kligman's formula (group II, n=20). Outcome measures were clinical assessment, Melasma Area and Severity Index (MASI) improvement percent, patient satisfaction and histopathological study including immunohistochemical staining. **Results:** At the end of 12th week, both groups showed a significant decrease in MSAI, that was more pronounced in group I (p=0.04), and significant improvement in pigmentation, solar elastosis, mast cell count and blood vessel density. No significant adverse effects were observed. **Conclusion:** Tranexamic acid (TA) is an effective and safe therapeutic agent for melasma treatment. Topical use of TA in between dermapen sessions has an improving consequence superior to that of Kligman's formula.

KEYWORDS: Tranexamic acid, Melasma, Dermapen, Kligman's formula.

INTRODUCTION

Melasma is a common acquired hypermelanotic disorder that presented by asymptomatic light-to-dark brown-colored irregular macules and patches on sun-exposed area, mainly the face.[1] Typically, it affects women of reproductive age, especially those having Fitzpatrick skin type IV-VI, though the condition can also occur in men.[2] Its prevalence widely varies from 1.5-33.3%.[3] Genetic factors, chronic ultraviolet exposure, estrogen and thyroid disease, pregnancy and drugs like phenytoin are considered risk factors for melasma development, however, the precise cause of melasma still remains unknown, and its pathogenesis has not yet been fully elucidated.[4] Various dermal changes as well as increased epidermal pigmentation are characteristic features of melasma.[5] In addition to its relapsing tendency, these dermal changes may represent hurdles in melasma treatment, and mostly show partial response to treatment with hydroquinone and triple combination creams, the gold standard in melasma treatment.[6]

Recently, role of tranexanic acid (TA) was proposed as a line in melasma management, after reporting of its skin whitening effects, incidentally, during its use in the treatment of aneurysmal subarachnoid hemorrhage. [6] TA is, an anti-fibrinolytic lysine analog, a plasmin-inhibitor classically used in bleeding control such as menorrhagia and hemophilia.[7]

TA skin whitening effects may be through its anti-fibrinolytic action, hold the position of an adjuvant in pigmentary disorders like melasma and ultraviolet induced pigmentation.[8] TA inhibits ultraviolet-induced plasmin activity in epidermal keratinocytes resulting in decrease of phospholipase A2 precursors, and limited free arachidonic acid with subsequently decreased prostaglandins and leukotrienes production and release of fibroblast growth factor, a powerful melanocyte growth factor. In addition it decreases melanocyte tyrosinase activity.[9] However, the exact mechanism of TA skin whitening effect, ideal TA administration route,

frequency, and duration of its use are not established yet and need to be fully elucidated.

The aim of this current study was to assess clinical and histological efficacy and safety of TA in treatment of melasma (dermapen delivered TA plus TA emulgel topically), in addition to compare this lone use versus dermapen delivered TA in combination with Kligman's formula.

MATERIALS AND METHODS

Ethics and study settings

This cohort study was carried out on Egyptian melasma patients (n=40) from Dermatology Outpatient Clinic, Faculty of Medicine Menoufia University Hospital during the period from September 2015 to August 2016. All procedures tracked in this study were in accordance with the ethical standards of the Committee of Human Rights in Research of Menoufia and with the Helsinki Declaration of 1975, as revised in 2000 (http://www.wma.net/e/policy/17-c_e.html). A written informed consent was signed by every participant before study initiation.

Inclusion criteria include melasma patients aged > 18 years old who did not receive any topical or systemic treatment for melasma within the last one month. Exclusion criteria included presence of any bleeding disorder, severe hepatic, renal or endocrinal disorders, history of malignancy, keloid tendency or allergic reaction to any component of drugs.

Additionally, pregnant or breast-feeding females and those taking contraceptive pills, systemic retinoids or photosensitizing drugs or having any dermatological diseases other than melasma were also excluded.

Patients' classification and medication

All participants in this study were subjected to complete history taking, skin photo typing,[10] and physical examination. A complete cutaneous examination was performed to evaluate melasma and its pattern. Wood's light was used to classify the type of melasma, in which color of epidermal melasma was enhanced and mixed type showed color enhancement only in some areas, however, the color of dermal melasma demonstrated no difference. Assessment of severity of melasma was performed using Melasma Area and Severity Index (MASI) score.[11]

Every patient in this study received dermapen skin microneedling weekly session with TA (100mg/ml), for 12 weeks. TA was available as 5 mL ampoule containing 500 mg (Kapron; Amoun Pharmaceuticals Co., El-Obour city, Egypt). For topically used medication in-between sessions, subjects were randomly divided into two groups: group I (n=20); used topical emulgel (3% TA) twice daily, and group II (n=20); used Kligman's formula (5% hydroquinone, 0.1% tretinoin and 0.1% dexamethasone) at night.

TA emulgel Preparation

A-The phases of the gel formulation is prepared by dispersing 1gm Carbopol 940 to q.s. purified water with continuous stirring at a moderate rate using a mechanical shaker, then the pH was adjusted to 6-6.5 by the three ethanol amine (TEA).

B-The phases of the emulsion formulation is prepared by, 1-Emulsion Oil phase; that prepared by dissolving 1gm span 20 in 7.5gm light liquid paraffin. 2-Emulsion aqueous phase; prepared by,-0.5gm tween20 was dissolved in purified water, and - 0.003gm methyl and 0.001gm propyl parabens were dissolved in 5gm propylene glycol, plus TA 10 % aqueous solution from kapron ampoules.

Both the oily and aqueous phases are separately heated to 70-80 C, and then the oily phase was added to the aqueous phase with continuous stirring until the assigned cooled to room temperature. The obtained emulsion was mixed with the gel in a ratio of 1: 1 with stirring to give TA emulgel.

Microneedling session

Patient's skin was cleansed with 70% ethyl alcohol then topical anesthetic cream (Pridocaine cream; GLOBAL NAPI Pharmaceuticals, October City, Giza, Egypt) was applied for 30 minutes. The dermapen was prepared, and then TA was intra-dermally injected with circular then horizontal and vertical motions, for four times each session. Skin was washed with sterile saline then cold packs were applied for five minutes. Patients were instructed to avoid skin rubbing and sun exposure for 12 hours. Additionally, sunscreen cream with sun protection factor of ≥30 may be used every 3 hours during day time if needed.

For comparative analysis, digital photographs were taken in the first visit (pretreatment), and one week after the last session, after washing and degreasing the face to prevent reflection, using a Sony Cyber-shot (model # DSC-W350, Sony America Corporation, NY). These photos were examined by an independent, experienced dermatologist.

Evaluation of therapeutic efficacy and Safety

One week after the last session, t efficacy of each therapeutic regimen in this study was clinically evaluated by MASI score compared to pre-treatment and calculated as MASI score improvement percent [(pretreatment MASI score – post-treatment MASI score) / Pre-treatment MASI score values) x 100].[12] To estimate patient satisfaction by treatment outcome; degree of improvement according to patient opinion was also evaluated at the end of the study using a scoring system in which, the patient's self-assessment of melasma improvement was ranked along four scales: excellent, >75% lightening; good, 51-75% lightening, fair, 26-50% lightening and poor, 0-25% lightening.[8] For safety assessment of TA, complete blood count and

international normalized ratio (INR) were evaluated in the first session and one week after the last session.

Skin Biopsy

Under local anesthesia 2 mm punch biopsies were taken from skin lesions of studied patients before treatment and one week after the last session. All specimens were fixed in 10% neutral-buffered formalin for routine tissue processing and preparation of formalin fixed paraffin embedded blocks. For Every patient, we have prepared 4 slides for; 1.Hematoxylin and eosin (H&E) staining for morphological assessment of epidermal region for pigmented basal cell layer, pendulous melanocytes and basement membrane status as well as dermal area for melanin incontinence and inflammatory infiltrates changes. In addition, type of melasma was confirmed. 2. Van Gieson's stain (VG stain)*:* to evaluate the degree of solar elastosis. 3/4. Immunohistochemical (IHC) study for CD31 and c kit (CD117) single staining using rabbit polyclonal antibodies anti-CD31/ PECAM-1 (Catalog number # PA5-29166,) and anti-c Kit (Catalog number # PA5-16770) both from Life Technologies Europe BV, Bleiswijk, Netherlands). IHC stained slides were examined using a light CH2 microscope (Olympus Ltd, Tokyo, Japan) with wide angle (field size of 0.274 mm^2 and field diameter of 0.59 mm^2). Micro-vessels density (MVD) was assessed by counting CD31+ve (cytoplasmic) cells in ten fields at 200× magnification (hot spot),[13] and mast cell density (MCD) from c kit +ve cells (nuclear or nucleocytoplasmic) using a ×400x magnification.[14]

Statistical Analysis

Data was examined for its distribution to apply an appropriate statistical test for comparison between patients groups such as χ^2 test, student t-test or Mann Whitney U-test. Results were presented as mean +/- SD and range or number and percentage according to data type. $P < 0.05$ was considered significant.

RESULTS

Personal and clinical data of the studied groups

Demographic and clinical criteria of the investigated patients were demonstrated in table 1. This study patients groups were age and gender matched as shown in Table 1. Our cohort patients groups were comparable regarding melasma patients' clinical parameters including skin phototypes, melasma typing and pattern. There were no significant differences in **table 1**.

MASI scores of melasma patient groups

Before treatment, MASI score in group I (8.62 ± 9.57) was comparable to group II (12.57 ± 9.41) (p= 0.22). One week after the last session, both group 1and II showed significant clinical improvement **(Photo 1, 2)** in the form of decreased MASI score mean values than their corresponding pretreatment values (**3.56 ± 7.04** post Rx vs 8.62 ± 9.5 pre Rx in group I and **4.72 ± 5.45** post Rx vs 12.57 ± 9.4 pre-Rx) respectively (p < 0.001 for both). Moreover, patients in group I showed a significant decrease in post Rx MASI score compared to group II (3.56 ± 7.04 vs 4.72 ± 5.45 (p= 0.04) (**Table 2**).

Percent of MASI score improvement

The percentage of MASI score improvement was high in group I than group II (64.05 ± 34.54% vs 55.80 ± 31.56%), however this difference could not reach level of significance (p=0.42) (**Table 3**).

Patient satisfaction in studied melasma groups

At the end of this study, levels of patient satisfaction were (45%, 30%, 20% & 5% vs 35%, 35%, 10% & 20%) for excellent, good, fair and poor satisfaction in group I and group II respectively, with no significant difference between both groups (p= 0.43) (**Table 3**).

Results of histopathological study in melasma patients

In pretreatment H/E stained sections (**Figure**. 1A), pigmented basal cell layer and pendulous melanocytes showed significant decrease after treatment in both groups (**Figure**. 2A; 3A) (P= 0.000, 0.008, 0.02 and 0.09 respectively). Similarly, Pretreatment melanin incontinence and chronic inflammatory infiltrates showed significant decrease after treatment (**Figure**. 2A; 3A) in group I and group II (P=0.006 and 0.000) (P=0.02 and 0.001) respectively (**Table** 4).

Moreover, solar elastosis (Figure. 1B) showed significant improvement in both group I and II (**Figure**. 2B; 3B) (P= 0.00 and 0.003) respectively.

Patients in both group I and group II (**Figure**.1C; 1D) revealed significant low post treatment micro-vessel density (MVD, CD31+ve) (**Figure**. 2C; 3C) (P=0.0003 and 0.009) in group I and II respectively as well as mast cell density (MCD, c kit +ve) (**Figure**. 2D; 3D) (P<0.001and 0.0004) in group I and II respectively. Furthermore, a significantly lower MVD in group II than group I was noted (P=0.02) (**Table 4**).

Relationship between percentage of MASI score improvement and studied parameters

In both studied groups, there were no significant differences among melasma types or skin phototypes regarding MASI score improvement percentage (p> 0.05 for both). Also, no significant associations between percentages of MASI score improvement and all other examined patients parameters (personal, clinical or histopathological markers) in both melasma groups (p> 0.05 for all). However, a significant association was observed between patient satisfaction and percent of MASI score improvement in group I as well as in group II (P= 0.001, P=0.028) respectively (data not shown).

Assessment of side effects

Few patients have reported erythema, local bruising and burning feeling which they occur immediately after sessions which were self-limited and get resolved within 1-2 days (**Table** 5). Skin dryness which was tolerable by patients in both groups treated with topical TA emugel or

Kligman's formula cream. Only one patient exited this study, and replaced by another one, following hyperpigmentation developed post inflammatory.

Table 1: Demographic and clinical characteristics of studied patients'groups.

Parameters	**Group I (n=20)**	**Group II (n=20)**	**Test of significance**	**P value**
Age (years)				
Mean ± SD	37.95±5.17	38.95 ± 3.11	t-test=0.47	0.46
Range	29-47	35-47		
Gender	**No (%)**	**No (%)**	χ^2	
Females	20 (100)	19 (95)	1.03	0.31
Males	0(0)	1 (5)		
Marital status				
Married	19 (95)	17 (85)	2.11	0.35
Divorced	0 (0)	2 (10)		
Widow	1 (5)	1 (5)		
Occupation				
Outdoor	9 (45)	10 (50)	0.10	0.75
Indoor	11 (55)	10 (50)		
Family history of melasma				
Present	7 (35)	6 (30)	0.11	0.74
Absent	13 (65)	14 (70)		
Aggravating factors				
OCPs	8 (40)	6 (30)	0.51	0.78
Pregnancy	4 (20)	4 (20)		
Sun exposure	8 (40)	10 (50)		
Duration of illness (years)				
Mean ± SD	5.15±2.54	4.00±2.73	1.59	0.11
Range	1-10	1-10		
Skin phototyping	# (%)	# (%)		
II	7 (35)	6 (30)	8.15	0.06
III	6 (30)	7 (35)		
IV	6 (30)	6 (30)		
V	1(5)	1(5)		
Melasma pattern				
Centrofacial	7 (35)	8 (40)	1.07	0.59
Malar	13 (65)	12 (60)		

OCPs: Oral contraception pills; t-test = student t–test; χ^2 = Chi–square test; U test= Mann.

Whitney test

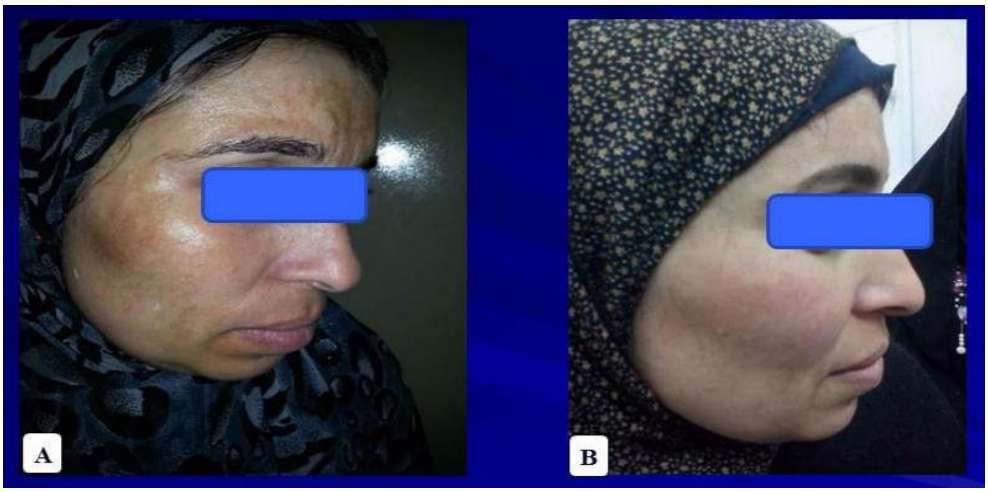

Photo 1: Group I patient; a: Before treatment: Very severe degree of melisma. (Melasma Area and Severity Index score = 30.6), b: After treatment: Mild degree of melasma with a significant decrease in Melasma Area and Severity Index score (MASI=1.2).

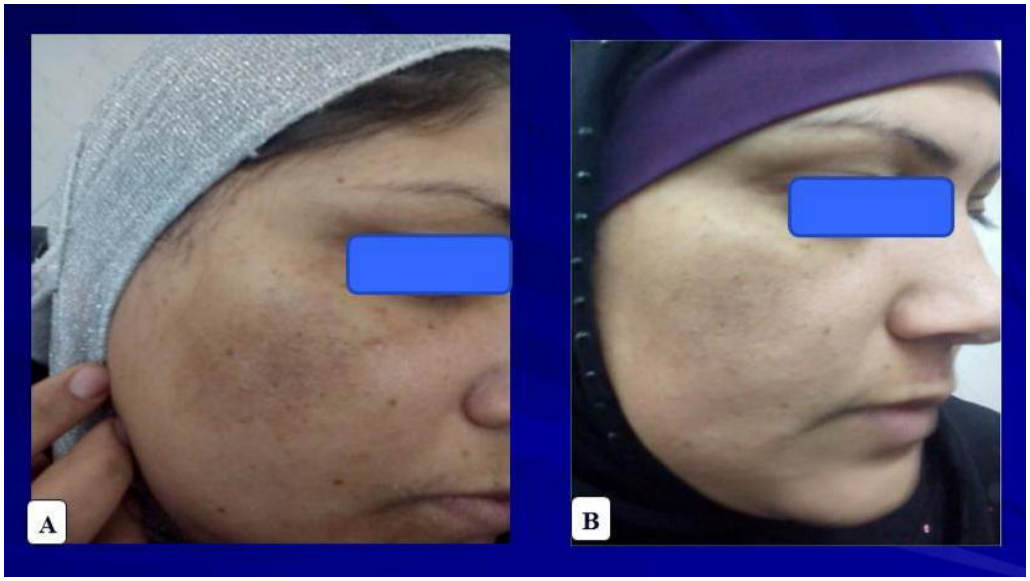

Photo 2: Group II Patient; a: Before treatment: Moderate degree of melisma (Melasma Area and Severity Index score =7.2), b: After treatment: Mild degree of melasma with a significant decrease of Melasma Area and Severity Index score (MASI=0.9).

Table 2: Melasma Area and Severity Index (MASI) score in patients groups before and after treatment.

MASI score	Group I (n=20)	Group II (n=20)	Test of significance	P value
Pre-Treatment				
Mean ± SD	8.62±9.57	12.57±9.41	U	
Range	1.2 – 39.5	2.1-30.6	1.23	0.22
Post-Treatment				
Mean ± SD	3.56±7.04	4.72±5.45	U	
Range	0 – 31	0 – 19.2	2.05	**0.04**
Test	3.89	3.51		
P value	<0.001	<0.001		

-U = Mann Whitney U test.

Table 3: Therapeutic efficacy by clinical evaluation through degree of improvement (percentage of MASI score decline) and patient satisfaction among studied groups.

	Group I (n=20)	*Group II (n=20)*	*test*	*P value*
Improvement of MASI (%)			***U***	
Mean ± SD	64.05±34.54	55.80±31.56	0.80	0.42
Range	0-100	0-100		
Patient satisfaction	N (%)	N (%)	X^2	
Excellent	9(45)	7 (35)		
Good	6 (30)	7 (35)	2.79	0.43
Fair	4 (20)	2 (10)		
Poor	1 (5)	4 (20)		

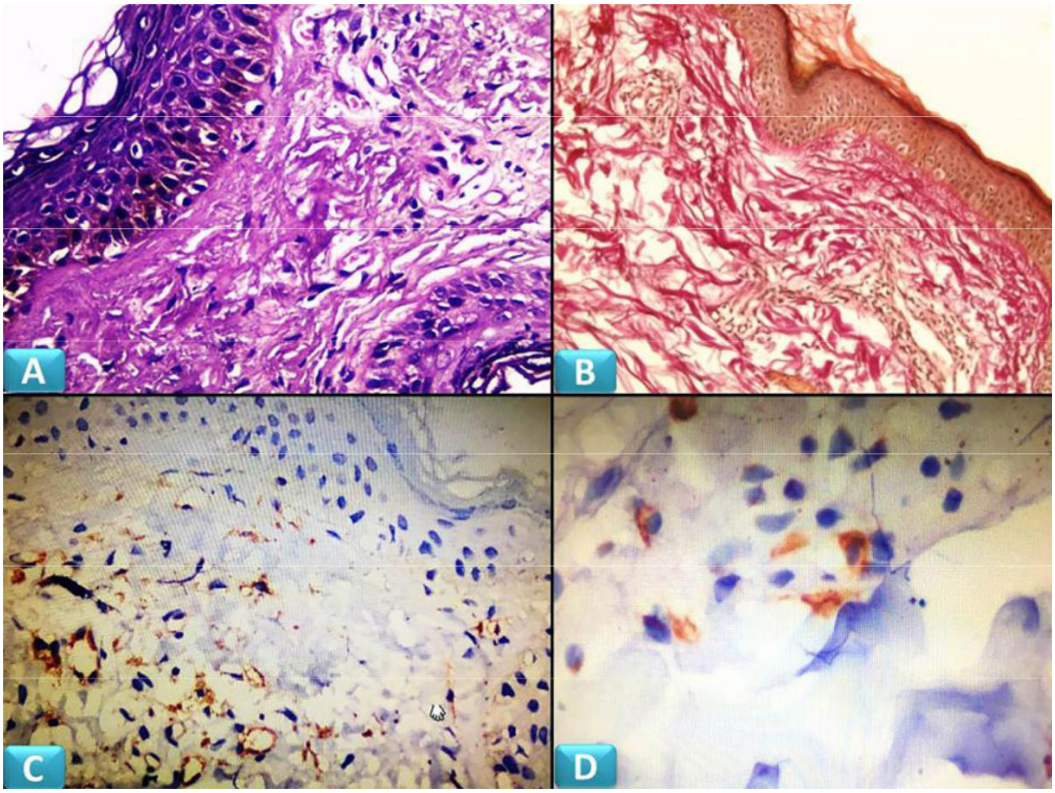

Figure 1: Pretreatment melasma lesions; A) Hematoxylin and eosin stained section showed epidermis exhibiting intensely stained pigmented basal cell layer with many pendulous melanocytes. Dermis revealed moderate degree of inflammatory infiltrate, B) Van Gieson's stained section reported severe solar elastosis, C) CD 31 stained section showed moderate degree of micro-vessels density and D) c-kit stained section demonstrated severe degree of mast cell density (A, X 200, B, X 100, C, 200 and D, 400).

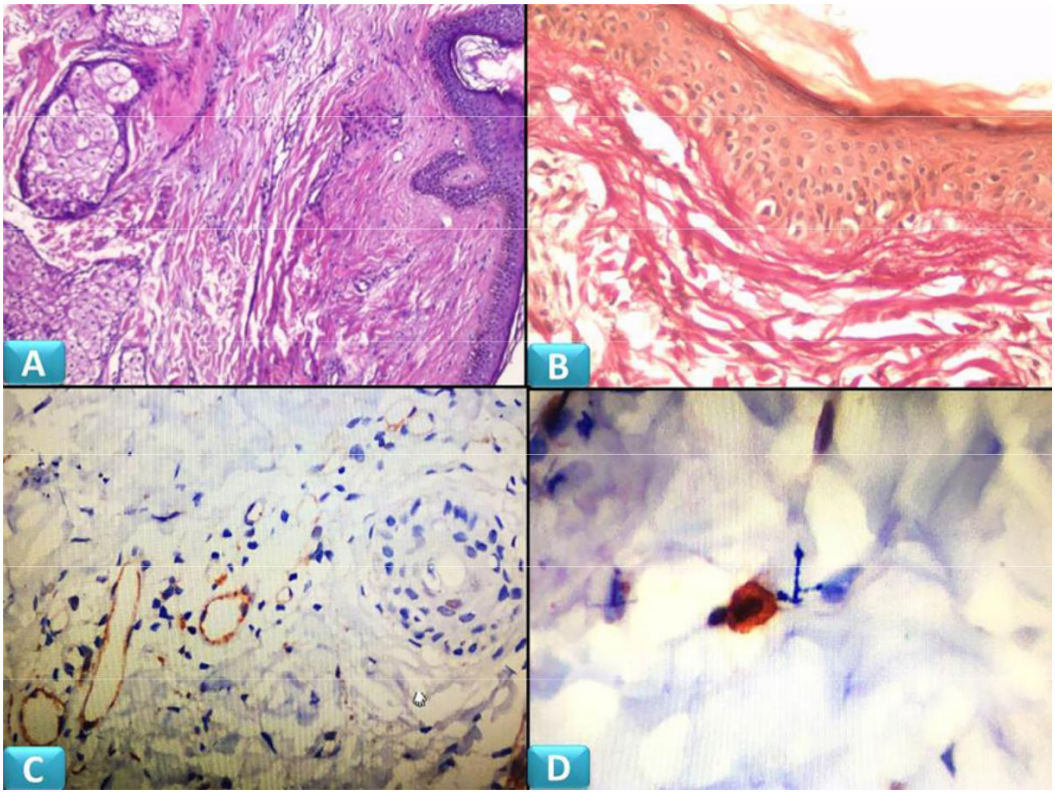

Figure 2: Post treatment biopsy (group I); A) Hematoxylin and eosin stained section showed epidermis exhibiting mildly stained pigmented basal cell layer with few pendulous melanocytes. Dermis demonstrated mild degree of inflammatory infiltrate, B).

Van Gieson's stained section showed mild degree of solar elastosis, C) CD 31 stained section revealed low degree of micro-vessels density and D) c-kit stained section showed mild degree of mast cell density (A, X 40, B, X 100, C, 400 and D, 400).

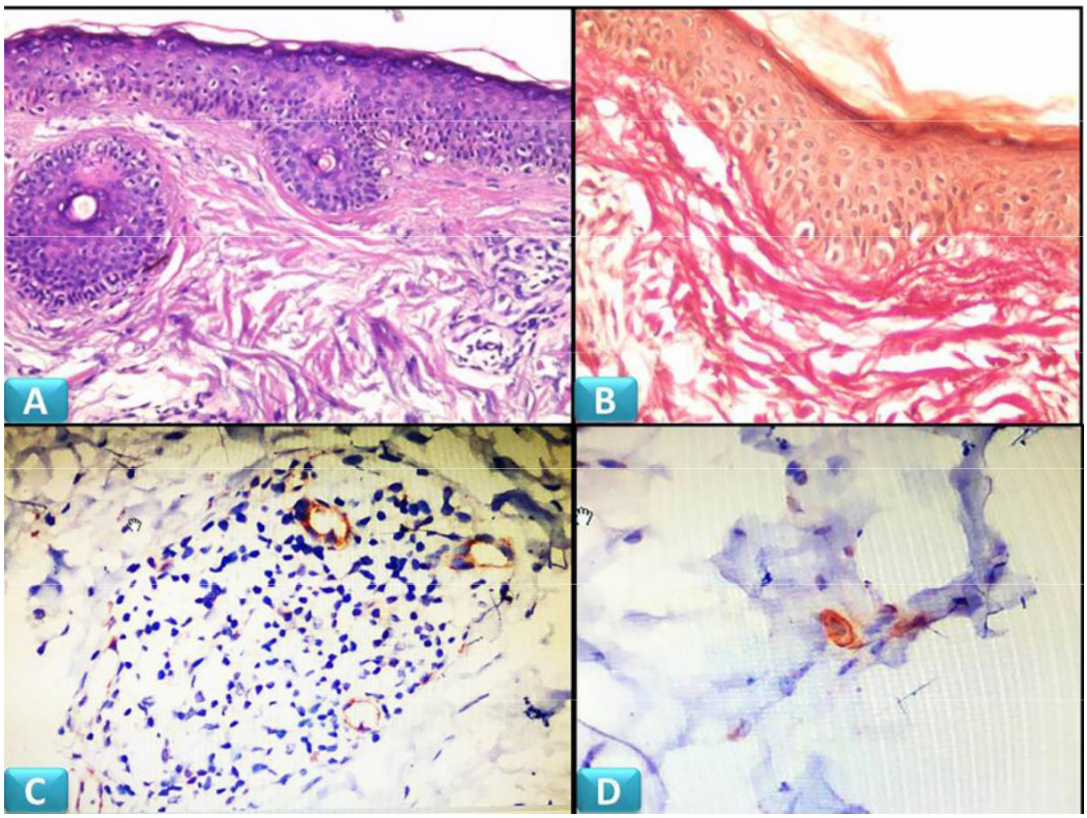

Figure 3: Post treatment biopsy (group II); A) Hematoxylin and eosin stained section showed epidermis exhibiting faintly stained pigmented basal cell layer without pendulous melanocytes. Dermis showed mild degree of inflammatory infiltrate, B) Van Gieson's stained section revealed mild degree of solar elastosis, C) CD 31 stained section reported low degree of micro-vessels density and D) C kit stained section showed mild degree of mast cell density (A, X 100, B, X 100, C, 400 and D, 400).

Table 4: Pathological findings pre and post treatment among both studied groups.

Histopathological changes	***Group I* (n=20)**		***Group II* (n=20)**		***χ2***	***P***
	Pre Rx	***Post Rx***	***Pre Rx***	***Post Rx***		
Epidermal changes						
Pigmented basal cell layer						
Absent	0 (0.0)	9 (45)	0 (0.0)	6 (30)	0.40	0.527[1]
Mild	9 (45)	9 (45)	11 (55)	11 (55)	1.80	0.62[2]
Moderate	11(55)	2 (10)	9 (45)	2 (10)	22.0	**0.000**[3]
Sever	0 (0.0)	0 (0.0)	0 (0.0)	1 (5)	11.6	0.02[4]
Pendulus melanocytes					0.40	0.53[1]
Absent	9 (45)	17(85)	11 (55)	16 (80)	0.17	0.68[2]
Present	11 (55)	3 (15)	9 (45)	4 (20)	7.03	**0.008**[3]
					2.85	0.09[4]
Dermal						
Degree of melanin incontinence						
Absent	4 (20)	15 (75)	7 (35)	16 (80)	3.31	0.19[1]
Mild	8 (40)	3 (15)	10(50)	3 (15)	0.37	0.83[2]
Moderate	8 (40)	2 (10)	3 (15)	1 (5)	12.4	**0.006**[3]
					8.29	**0.02**[4]

Melasma type						
Epidermal	4 (20)		7 (35)		1.13	0.29[1]
Mixed	16(80)		13 (65)			
Degree of inflammatory infiltrates						
Absent	0 (0.0)	10 (50)	0 (0.0)	10 (50)	3.96	***0.047****[1]
Mild	10 (50)	10 (50)	16 (80)	7 (35)	3.53	***0.17***[2]
Moderate	10 (50)	0 (0.0)	4 (20)	3 (15)	20.0	***0.000***[3]
					13.67	***0.001***[4]
Degree of fibrosis					0.11	0.74[1]
Mild	12(60)	13 (65)	13 (65)	17 (85)	2.50	0.11[2]
Moderate	8 (40)	7 (35)	7 (35)	3 (15)	0.11	0.74[3]
					2.13	0.14[4]
Degree of Solar elastosis by VG stain					3.60	0.17[1]
Mild	0 (0.0)	16 (80)	3 (15)	12 (60)	1.90	0.47[2]
Moderate	11 (55)	4 (20)	11 (55)	8 (40)	28.3	**0.000**[3]
Severe	9 (45)	0 (0.0)	6 (30)	0 (0.0)	11.87	**0.003**[4]
MVD					0.24	0.81[1]
Mean ±SD	10.05±1.36	5.0±*0.79*	10.15±*1.31*	4.35±*0.93*	2.37	**0.02**[2]
Range	8- 12	4 – 6	8 – 12		18.9	**0.000**[3]
					20.9	**0.000**[3]
MCD						
Mean ±SD					0.74	0.46[1]
Range	4.0±*0.86*	0.95±*0.96*	4.20±*0.83*	1.05±*0.76*	0.42	0.67[2]
	3 – 6	0 – 2	3 – 6	0 – 2	15.4	**0.000**[3]
					11.9	**0.000**[4]

1 = comparing between group I and group II pretreatment; 2 = comparing between group I and group II post treatment; 3 = comparing between pretreatment and post treatment in group I; 4 = comparing between pretreatment and post treatment in group II.

Table 5: Safety of therapeutic regimens was measured by incidence of side effects among the studied groups.

	Group I (n=20)	***Group II (n=20)***
Erythema,	2 (10%)	3 (15)
Local bruising	---	1 (5%)
Burning sensation	2 (10)	2 (10%)

DISCUSSION

Tranexamic acid (TA) is as a promising treatment for melasma, was suggested by many investigators. Response to TA was evaluated in many studies using various modalities for drug delivery including intradermal microinjections,[15] dermarollar microneedling method,[12] topical preparations,[8] and oral route.[5] Till now, no one evaluated using dermapen for intradermal microinjection of TA. In addition, no trial to evaluate the use of TA combined with stranded treatment such as kligman's formula was found. Although all early studies have evaluated TA clinically, only one report showed an interest in histological changes.[5]

Herein, we aimed to evaluate a new regimen for melasma treatment using dermapen for intra-dermal micro-injections of TA solution, in combination with its 3% topical use in-between sessions (group I) or with Kligman's formula to identify any additive effects to TA. Our evaluation depends on clinical parameters as well as biomarkers using molecular and histological changes achieved after treatment.

In this current study, MASI score results showed evident improvement, with significant marked decrease in its post treatment than the pretreatment mean values in both enrolled patient groups. Nevertheless, against our expectation, patients in group I revealed significant reduction in post treatment MASI score than those in group II. This means that topical TA has an additive effect with TA mesotherapy superior to Kligman's formula. This surprising unexpected result seems to be against the scientific description as patients in group II received two different drugs with different mechanism of

actions. This finding that needs further and more extended work to be confirmed and interpreted.

However, Padhi and Pradhan on their open labeled randomized comparative trial concluded that addition of oral TA to fluocinolone-based triple combination cream results in a faster and sustained improvement in melasma treatment.[16] This discrepancy could be attributed to different routes of TA administration in each study.

Excellent and good patient satisfaction was noted in majority of our patients in both groups with no significant differences between them concerning this item. Likewise, Budamakuntla et al. reported a significant decrease in MASI score and a significant improvement of photographic evaluation and patients self-assessment, after three microneedling sessions of TA (using dermaroller once/ month) than before treatment on their study on 30 Indian patients with melasma.[12] Also, Steiner et al. demonstrated the same results after intradermal injection of TA (4mg/ml) weekly for 12 weeks, on their clinical trial on 9 women having melasma.[15] Besides, only topical use of TA preparations showed significant improvement after 12 weeks of twice daily application, comparable to microinjection in both trials.[12,15]

In line with previous studies,[7,8,17,18,19] our pretreatment findings showed pigmented basal cell layer, pendulous melanocytes and thickened disrupted basement membrane, with melanin incontinence and chronic inflammatory infiltrates (H & E), additionally, solar elastosis (Van– Geison's stain),vascular changes (CD31), and infiltration of excess mast cell (c-kit) were observed. Based on these changes, particularly the vascular one, melasma is considered not only a pigmentary disorder but also a unique phenotype of photo damage throughout aging process.[4] The assessed histopathologic parameters in this study showed significant noticeable improvement after treatment. In accordance with these findings, Na et al had reported significant decrease in epidermal pigmentation using Fontana-Masson staining, blood vessel density (CD31), and number of mast cells (expending anti tryptase antibody).[5]

In melasma, mast cells play an important role in solar elastosis development, induce vascular proliferation by many angiogenic factors such as VEGF, FGF2 and TGF ß.[20] Moreover, released mast cell tryptase might participate in disrupted basement membrane observed in melasma.[21] In addition, histamine motivates the proliferation besides migration of melanocytes.[22] Being antiplasmin agent, TA has anti-angiogenic action. As plasmin participates in angiogenesis through conversion of bound VEGF into its free form,[23] and induces release of bFGF.[24]Along with decreased blood vessel density, our result showed a significant decrease in mast cell number after treatment that may affect and reflect the improved vascular and dermatopathic changes. From these significant improvements especially in the histopathologic parameters, with particular the dermal component, we may suggest that, hypomelanogenic effect, antiangiogenic action, and mast cell suppressing deed could be the mechanisms by which TA might participate in treatment of melasma.

In line with Steiner et al.[15], results of CBC and INR in our study were within normal ranges and showed no changes at the end of the study, even we used very high doses of TA than that of Steiner et al. study (100mg/ml versus 4mg/ml). Moreover, no one in our study got any systemic side effect. Furthermore, only minor dematologic adverse effects were noted such as erythema, burning sensation and bruising, approximating to the results reported by Lee et al. after TA microinjection.[25]Also, Budamakuntla et al. reported no serious side effects after TA microinjection or dermaroller microneedling sessions apart from erythema, mild discomfort and burning sensation.[12] As well topical use of TA revealed good response without significant side effects.[8]

In the present study, as previously noticed,[12] no significant associations were observed between the percent in MASI score improvement and skin phototypes. Moreover, we observed non-significant difference in treatment result among type of melasma, denoting that melasma patient having different melasma types and skin colors can gain benefits from using this amazing drug. However, Ebrahimi and Naeini concluded that TA has a quite good rapid result, especially for epidermal type.[8] This difference could be attributed to the used method in each study as they used topical solution of TA, but in our study we used dermapen to deliver TA which distributes TA evenly and deeper into the skin.

However, the limitations of this work were small sample size, and lack of the overall patient satisfaction and inadequate follow up period by which any possible late side effects or recurrence could be detected.

Based on our findings, we can conclude that, TA is a hopeful therapeutic agent for melasma treatment, with a prober safety and efficacy. TA delivered dermapen may be an innovative therapeutic option that may address this challenging problem. Topical use of TA in between dermapen sessions has an additive effect superior to that of Kligman'sformula. Additionally, TA might contribute to melasma improvement through its hypomelanogenic and antiangiogenic effects, as well as its suppressing act on mast cells.

Finally, we recommend further large scale studies to validate our findings and to determine the optimal dosage, application frequency and long-term evaluation. Adequate follow up period to identify any recurrence or late side effects is required. Additionally, trials to evaluate using this method with any of other treatment

modalities are needed to optimize for any additive effect for better melasma treatment.

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