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A comparative study between the use of the combination of trichloroacetic acid peeling with hydroquinone and hydroquinone alone in patients with melasma

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ABSTRACT

Background: Melasma is a common acquired pigmentary disorder that is aesthetically displeasing. Kligman's and Modified Kligman's formula using topical steroids, hydroquinone and retinoids, and various other depigmenting agents is being widely used all over for melasma with varying results. Chemical peeling is newly added to the therapeutic armamentarium and is showing encouraging results worldwide in patients with melasma. However, comparative studies are lacking in abundance in our part of the world. **Aims and Objectives:** To determine if serial trichloroacetic acid peels provide additional benefits when combined with time-tested topical therapy with hydroquinone 4% in patients with melasma. **Materials and Methods:** Fifty melasma patients were divided into two groups of 25 each. One group received serial trichloroacetic acid peel combined with topical hydroquinone 4%. The other group only received topical hydroquinone 4% cream. The results were evaluated by a clinical investigator both subjectively and with photographs taken at baseline, 12 weeks, and 21 weeks. For clinical evaluation, the melasma area and severity index (MASI) was used. **Results:** A significant decrease in MASI score from baseline to 21 weeks was observed in both groups ($P<0.001$). The group receiving the trichloroacetic acid peel 20% showed a trend toward more rapid and greater improvement, with statistically significant results ($P<0.001$). Only a few side effects were observed in the peel group. **Conclusion:** This study demonstrates that serial trichloroacetic acid peels provide an additional effect to a topical regimen of 4% hydroquinone cream for treating melasma in Fitzpatrick skin types III and above if used judiciously and under supervision. It demonstrates that superficial chemical peels can be used as an adjunct with better efficacy to treat patients with melasma.

Key words: Melasma; Chemical peeling; Hydroquinone

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INTRODUCTION

Melasma is a common acquired pigmentary disturbance characterized by hyperchromic macules with varying color tones ranging from light brown to black. It is common in areas with maximum sun exposure: Forehead, dorsum of the nose, upper lip, infra and supraorbital margins, zygomatic process, and angle of the jaw. Three distinct facial patterns: Malar, centro-facial and mandibular have been conventionally defined for melasma. Similarly, three histological patterns of melasma have been identified based on the location of pigment accumulation, epidermal, dermal, and mixed. It is more prevalent among females (95%), with Fitzpatrick skin types IV-VI and among Asian descendants and Hispanics. Family history is present in 30% of cases. Various factors have been implicated in the causation of melasma. Photo-stimulation of melanocytes is the main etiological factor. Other factors include increased

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estrogen in pregnancy, use of hormonal contraceptives, dysfunction of thyroid, ovarian, and adrenal glands, random use of cosmetics and various medications including quinacrine, phenylhydantoin, etc.[1]

Treatment is mainly focused in sun protection to combat the melanocytic stimulation by ultraviolet radiation. There is often a worsening of melasma during summer and its improvement during the winter. The use of sun protection alone is sufficient to promote improvement in melasma. Hydroquinone is the most used depigmenting agent that blocks melanogenesis. It's a tyrosinase inhibitor and inhibits the transformation of tyrosine into dopa and dopaquinone and, consequently into melanin. It is the most widely used chemical agent with very low rate of side effects such as irritative contact dermatitis, allergy, post-inflammatory hyperpigmentation and discoloration of nails and eyebrows.[2]

Chemical peelings are widely used these days in the treatment of aging and skin dyschromia. Superficial peelings are recommended in the treatment of melasma. More aggressive treatments such as medium and deep peeling, the use of lasers, and IPL have not been shown to be effective and rather pose a greater risk for post-inflammatory pigmentation, hypochromia, and formation of scars and keloids.[3]

Peels are a well-known modality of treatment for melasma, having shown promising results in many clinical trials. However, in darker races, the choice of a peeling agent becomes relatively limited and there is an additional need of priming agents and maintenance peels. Although a number of new agents have come up, there is little evidence supporting their use in day-to-day practice. The basic mechanism of the action of chemical peels in melasma is the removal of unwanted melanin by causing a controlled chemical burn to the skin. They have proved to be useful agents melasma both as a sole treatment as well as an adjunct to other topical therapies.[4]

There are conflicting results in the literature about the benefits of superficial and medium peels in melasma. Of the peels available, TCA is one of the economic peels among all and in a lower concentration, 20% may be as effective as GA peels for the treatment of epidermal melasma. It's more effective while used among lighter skin types. However, caution should be exercised while using these peels in darker skin types and dermal melasma due to the higher frequency of adverse effects like post-inflammatory hyperpigmentation and recurrence of the condition.[5]

Melasma is a common notorious dermatosis often resistant to treatment and commonly encountered with serious psychosocial repercussions. It's very common to come across patients exhausted with the length of treatment when mere depigmenting agents, single or in combination, are being used. With emerging recent practices of adding chemical peels to the treatment of melasma, TCA 20% is one of the easily available peels and comes out to be economical for patients as well, for early and better response.[6]

Taking especial caution while applying this peel on higher Fitzpatrick skin types and with the selection of patients with epidermal melasma in this study, the efficacy of TCA 20% in melasma can be assessed, validating the findings of only a limited number of studies done in melasma in our part of the country. If the efficacy comes out to be substantial, a big financial burden of having to use depigmenting agents for a long run can be in check. Psychosocial aspects can also be attended to that way by preventing the patients from cumbersome and lengthy treatment. Furthermore, the introduction of peels in treatment modality in melasma in our part of the world helps in preventing the common side effects such as exogenous ochronosis, poikiloderma of civatte and also prevents the misuse of OTC hydroquinone and non-hydroquinone depigmenting agents as well as the misuse of steroids which is so prevalent in our region.[7]

Aims and objectives

To determine if serial trichloroacetic acid 20% peel provides additional improvement when combined with time-tested hydroquinone 4% cream topical therapy.

MATERIALS AND METHODS

Fifty melasma patients presenting to the Department of Dermatology at Pokhara Academy of Health Sciences were included in this study. An open pilot study was conducted that lasted for 21 weeks. All the risks involved were explained in details, and the study was carried out among those who signed the informed consent. Our exclusion criteria were those under 16 years of age, those suffering from severe psychiatric illness, those refusing to participate after being explained, those with deep, dermal melasma, with keloidal tendency, who underwent peeling in the last 3 months, took oral retinoids within last 3 month, hydrantoin and quinacrine and those with unrealistic expectations.

A detailed history regarding age, sex, duration of melasma, any precipitating factors, any associated chronic illnesses, medications, and previous treatment received were taken, and proforma for each were thoroughly filled up. A clinical examination and Wood's lamp examination were carried out in each patient and those with epidermal melasma were only taken for the study.

Twenty-five patients were allocated in each group, one group receiving daily 4% hydroquinone cream and the second group receiving six serial 3-weekly chemical peels in addition to daily topical hydroquinone 4% cream. All the patients were advised to use a liberal amount of sunscreen throughout. A written informed consent was obtained from each patient before the study. A test peel was applied in the post-auricular region and left for 15–20 min to determine hypersensitivity to any ingredients of the peeling agent. The patients in the peel group were primed with 0.025% tretinoin peel for 2 weeks before the first peel and with hydroquinone 4% to check for sensitivity with the same. One group received only 4% hydroquinone daily throughout the treatment.

The peel group used hydroquinone 4% daily and received six serial trichloroacetic acid 20% peels at 3 weeks interval. For this group, hydroquinone 4% was discontinued two days prior to receiving peel and was restarted 3 days after the peel. Every patient were advised to wash their face with soap and water before the peel. After patting the face dry, cleansing with gauzes soaked with methyl alcohol or acetone was done to clean off the cutaneous greases. 20% TCA peel was then applied in mild strokes with the help of a brush with fanned-out bristles. A contact time was till the frosting appeared, which was usually 1–2 min. The face was then rinsed with cool water. Patients were advised to use only sunscreen, mild soap, and petrolatum jelly for three days after the peel. Hydroquinone 4% was again started after three days in the peel group.

Evaluation of melasma severity was done at baseline, at week 12 and week 21 based on melasma area and severity index (MASI) determined by Kinbrough-Green et al.[8] According to the MASI score, face is divided into four areas-forehead, right malar region, left malar region and chin corresponding 30%, 30%, 30%, and 10% areas respectively. Melasma in each of these areas was graded on three variables: Percentage of total area involved, on a scale of 0 (no involvement) to 6 (90–100% involvement); darkness, on a scale of 0 (absent) to 4 (severe); and homogeneity, on a scale of 0 (absent) to 4 (maximum). MASI was then calculated with the following formula:

MASI=0.3 (DF+HF) AF+0.3 (DMR+HMR) AMR+0.3 (DML+HML) AML+0.1 (DC+HC) AC,

Where D is darkness, H is homogeneity, A is area, F is the forehead, MR is the right malar, ML is left malar, C is chin, and the values 0.3, 0.3, 0.3, and 0.1 are respective percentages of the total facial area.

A high MASI score correlates with severe hyperpigmentation. Student's t-test and paired t-test were used as the statistical tools for comparisons of findings and results tabulated. At 12 weeks and at 21 weeks, patients were asked to give their subjective assessment of their clinical response to the peels as excellent, good, fair, or poor. Photographs were taken in a standardized position at baseline, week 12, and at week 21.

RESULTS

Patient's data

Fifty melasma patients were enrolled in our study. Patient's ages ranged from 26 to 47 years

(Mean 35.6 years). Forty-five females and five males were enrolled in the study (male: Female ratio 1:9). The duration of melasma ranged from 9 to 30 months (mean 1.63 years). Patient's demographic data and characteristics are presented in Table 1.

Their final evaluation was at 21 weeks (3 weeks after receiving the sixth chemical peel). Both groups experienced significant overall lightening of the melasma according to MASI score, subjective evaluation and by photography.

Evaluation and analysis of MASI score

The mean MASI score for the peel group decreased from 20.140 (SD 1.70) at baseline to 8.93 (SD 2.29) at 12 weeks and to 3.53 (SD 1.69) at 21 weeks as shown in Table 2. This shows a percentage change of 55.67% at week 12 and 82.48% at week 21, respectively, as shown in Figure 1, which is highly

Table 1: Demography and characteristics of melasma patients

General data	Chemical peel group (TCA 20%)	Topical regimen (hydroquinone 4% cream)
Age (years)[a]	35.6 (26–47)	34.56 (27–44)
Sex (male: Female)	3:22	2:23
Duration (years)[a]	1.75 (0.84–2.5)	1.47 (0.84–2.34)
Precipitating factors		
Pregnancy	3	2
Oral contraceptives	1	3
Sunlight	14	12
Idiopathic	7	8
Pattern		
Centro-facial[b]	5 (20)	6 (24)
Malar[b]	20 (80)	19 (76)

[a]values are expressed as mean (range). [b]values are expressed as percentages

Table 2: MASI score in both groups

MASI	Chemical peel group	Hydroquinone cream 4%
Baseline	20.14 (SD1.70)	19.40 (SD2.09)
12 weeks	8.94 (SD 2.29)[c,d]	16.82 (SD 2.50)[c,d]
21 weeks	3.53 (SD 1.69)[c,d]	9.52 (SD 2.43)[c,d]

[c]Stastistically significant improvent within the group (paired t-test), P<0.001. [d]statistically significant difference between the peel and topical regimen group by student's t-test, P<0.001. MASI: Melasma area and severity index

significant (P<0.0001). In the control group,the mean MASI score at the baseline was 19.40 (SD 2.09), which decreased to 16.82 (SD2.50) at week 12 and to 9.52 (SD 2.43) at week 21 representing 13.30% and 50.92% change in pigment intensity, respectively which was highly significant (P<0.001), as shown in Figure 1. The difference between the two groups was also found to be significant both at week 12 and 21 (P<0.0001), with the better and rapid response in the form of lightening of melasma being experienced in the group receiving the chemical peels. The photographs were also in agreement with these results as shown in Figures 3 and 4.

Analysis of patient's subjective assessment

At the end of the study, a subjective improvement was noticed by most of the patients. In the peel group, 70% of the patients graded their improvement as excellent, 20% as good, and 10% as fair. In the control group, 40% of the patients graded improvement in their melasma as excellent, 30% as good, and 30% as fair, as shown in Figure 2.

Assessment of side-effects

Erythema was observed in 10 (40%) of the peel patients lasting for 3–4 days that resolved on its own. Three (12%) patients in the peel group reported mild discomfort with burning sensation, which was treated with betamethasone 0.05% cream applied twice a day. Three (12%) of the patients in the topical regimen group (hydroquinone 4% cream), reported mild acneiform eruptions, which was treated with benzoyl peroxide 0.5% applied locally at night. None of the side effects were severe enough to be necessitate the discontinuation of treatment.

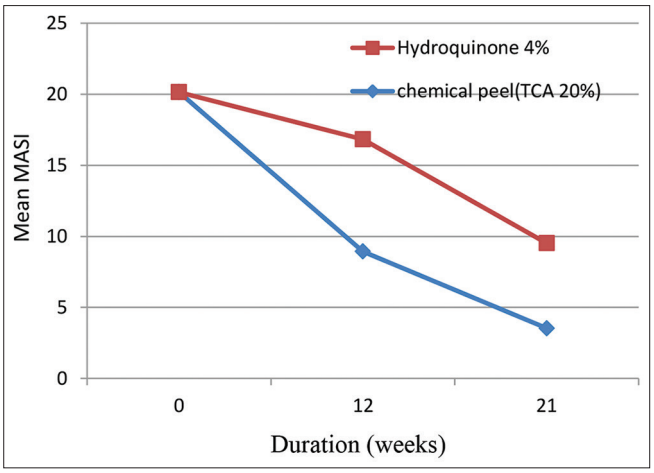

Figure 1: Decrease in mean MASI score in both the groups

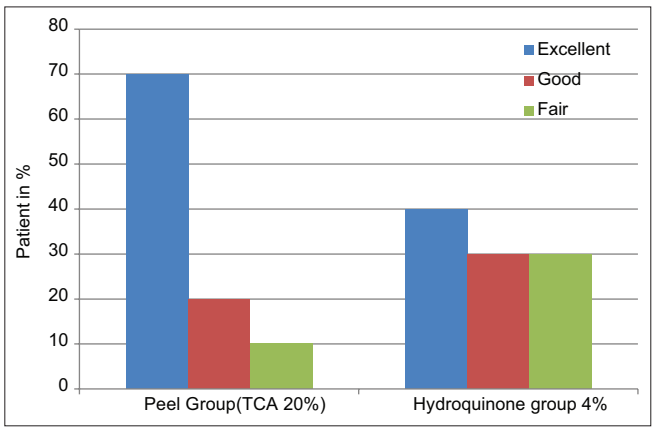

Figure 2: Subjective assessment in each group

DISCUSSION

Melasma is a symmetric progressive hyperpigmentation the facial skin that occurs in all races but has a predilection for darker skin phenotypes. Depigmenting agents, laser, and chemical peeling have been used alone and in combination for the treatment of melasma.[9] Of various topical hypopigmented agents, hydroquinone and kojic acid are well-establishedmonotherapeutic agents for treating melasma. 4% hydroquinone cream is an agent with the rapid rate of clinical improvement. Hydroquinone is believed to have additional actions such as degradation of melanosomes, destruction of melanocytes, and inhibition of DNA and RNA synthesis, contributing better to skin lightening. Clinicians often use chemical peels as an adjunct to medical therapy because they produce complementary

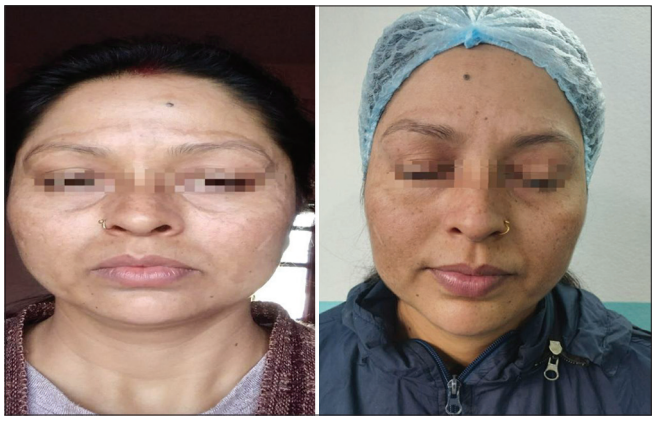

Figure 3: Baseline and 21 week photograph of patient in hydroquinone group

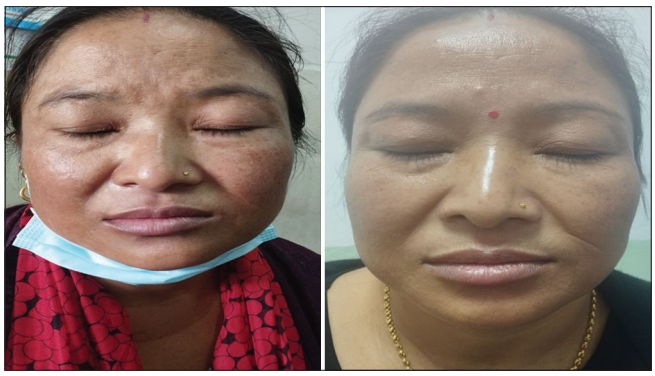

Figure 4: Baseline and 21 week photograph of combination group (TCA 20% plus hydroquinone)

rapid therapeutic effects and improvement in skin appearance and texture.[10]

Superficial chemical peels have been considered in the treatment of melasma that's refractory to topical depigmenting agents. In melasma, the enhanced risk of developing post-inflammatory hyperpigmentation restricts the use of medium depth to deeper chemical peels in most cases.[11]

The gold standard for the chemical peeling agent is trichloroacetic acid, it is very well studied and has versatile use due to its ability to create superficial, medium, and deep peels. It is stable, inexpensive, and causes no toxicity. It is easy to perform, as the peel depth is assessed with the intensity of skin frosting and there is no need to neutralize a TCA peel.[12]

Since there is high risk of post-inflammatory hyperpigmentation with medium and deep-depth TCA peel, we use superficial TCA 20% as there is risk of intense ultra-violet exposure in a tropical place like ours.

The preponderance of younger female patients in our study conforms to previous studies and shows that melasma is more cosmetically disturbing for them and they form the major group demanding treatment. The mean age of patients in the peel group and the topical therapy group was 35.6±7.28 years and 34.56±5.76 years, respectively. The cases included in this study varied in age from 26 to 44 years. About 93% of patients belonged to the age group of 20–40 years. Male: Female ratio in our study was 1:10 showing male preponderance. The age and sex preponderance in our study is in concordance with studies by Kalla et al.,[2] and Javaheri et al.[13] The mean duration of melasma in our study was 9–30 months (mean 1.63 years) which was consistent with finding by Javaheri et al.[13]

The precipitating factors for melasma in our study was found to be sunlight 52% followed by idiopathic 30%, pregnancy 10% and oral contraceptives 8% similar to the study done by Chun et al.,[5] and Dogra et al.[14]

In our study the pattern of melasma observed was malar 78% and centro-facial in 22% which is consistent with the study done by Grover and Reddu[15] and Dogra et al.[14]

Though there have been many comparative studies between varieties of peels and also among peel and various topical regimens, not much studies are there comparing the efficacy of gold standard peel TCA as an adjunct with time-tested hydroquinone 4% cream even when both individually also have been proven to be beneficial in patients with melasma.

Our study demonstrated that serial tricloroacetic acid 20% peels can enhance the efficacy of a topical regimen while treating melasma in patients with Fitzpatrick skin type III and above. The findings were significant in both the chemical peel and topical hydroquinone 4% group as shown in Table 2, but the trend was definitely more rapid and greater in the TCA 20% group as shown in Figure 1. Both combination and control groups showed a statistically significant decrease in mean MASI score within the group and also among the groups as compared to the baseline, with rapid and better lightening noticed with the combination group. The reduction in mean MASI score and difference in mean percentage decrease in MASI score was significantly higher in the combination group compared to the control group both at 12 weeks and at the end of the therapy, as shown in Table 2 and Figure 1.

This reflects the higher efficacy of the combination therapy with superficial peel like TCA 20% with topical regimen as compared with the efficacy of either of them alone. It corroborates with the findings of Soliman et al.,[16] and Moy et al.,[17] as both had also shown a statistically significant decrease in the mean MASI and percentage decrease in MASI in the combination group as compared to the control group at the completion of the study. Furthermore, the subjective assessment in our study also showed more patients, 70% reported excellent responses in the combination group compared to the topical group alone 40% reinforcing the findings as shown in Figure 2.

Adverse effects were not severe enough needing discontinuation of treatment in any group. Mild side effects included erythema in 40% of peel group that resolves on its own and 12% in the peel group reported mild burning sensation and discomfort which was treated with topical betamethasone cream 0.05% LA BD and 12% in the hydroquinone group reported acneiform eruptions which was treated with benzoyl peroxide. Post-inflammatory hyperpigmentation, as noticed with TCA peel in many studies like in one done by Dogra et al., was not seen in any patients in our study, probably because of adequate priming before peel, superficial peeling only up to the point of frosting and due to advise on adequate sun protection with religious use of sunscreen.

By and large, there is still the scarcity of comparative studies of hydroquinone 4% cream in combination with TCA superficial peels; as the esthetic realm is moving toward newer and expensive peels and newer topical options, we thought of bringing back in attention the presence of equally efficacious time tested gold standard peel which is affordable as well in adjunct with time-tested topical modality of choice for melasma hydroquinone 4% producing good results as shown in our study.

Limitations of the study

A limited sample size so, it may have a limitations in generalisation of results. Hence, more studies with large

number of patients are required to confirm these findings and to analyse the outcome of the study.

CONCLUSION

Three weekly TCA 20% peel along with hydroquinone 4% cream in melasma patients has added benefits and rapid response in lightening of melasma compared to use of hydroquinone 4% cream alone. Side effects like post inflammatory hyperpigmentation in Fitzpatriks skin type III and above can be taken care of with preprocedural priming with topical retinoids and postprocedural care with topical hydrocortisone 1% in case of inflammation. The beneficial results can be maintained with regular use of sunscreen and topical depigmenting agents. TCA has an added advantage of facial rejuvenation.

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REFERENCES

1. Pasricha JS, Khaitan BK and Dash S. Pigmentary disorders in India. Dermatol Clin. 2007;25(3):343-352, viii.
   https://doi.org/10.1016/j.det.2007.05.004
2. Kalla G, Garg A and Kachhawa D. Chemical peeling--glycolic acid versus trichloroacetic acid in melasma. Indian J Dermatol Venereol Leprol. 2001;67(2):82-84.
   https://doi.org/10.4103/0378-6323.66602
3. Kumari R and Thappa DM. Comparative study of trichloroacetic acid versus glycolic acid chemical peels in the treatment of melasma. Indian J Dermatol Venereol Leprol. 2010;76(4):447.
   https://doi.org/10.4103/0378-6323.66602
4. Monheit GD. The Jessner's-trichloroacetic acid peel. An enhanced medium-depth chemical peel. Dermatol Clin. 1995;13(2):277-283.
   https://doi.org/10.1016/s0733-8635(18)30081-0
5. Chun EY, Lee JB and Lee KH. Focal trichloroacetic acid peel method for benign pigmented lesions in dark-skinned patients. Dermatol Surg. 2004;30(4 Pt 1):512-516.
   https://doi.org/10.1111/j.1524-4725.2004.30166.x
6. Nanda S, Grover C and Reddy BS. Efficacy of hydroquinone (2%) versus tretinoin (0.025%) as adjunct topical agents for chemical peeling in patients of melasma. Dermatol Surg. 2004;30(3):385-388; discussion 389.
   https://doi.org/10.1111/j.1524-4725.2004.30106.x
7. Obagi ZE, Obagi S, Alaiti S and Stevens MB. TCA-based blue peel: A standardized procedure with depth control. Dermatol Surg. 1999;25(10):773-780.
   https://doi.org/10.1046/j.1524-4725.1999.98178.x
8. Kimbrough-Green CK, Griffiths CE, Finkel LJ, Hamilton TA, Bulengo-Ransby SM, Ellis CN, et al. Topical retinoic acid (tretinoin) for melasma in black patients. A vehicle-controlled clinical trial. Arch Dermatol. 1994;130(6):727-733.
   https://doi.org/10.1001/archderm.1994.01690060057005
9. Ortonne JP and Passeron T. Melanin pigmentary disorders: Treatment update. Dermatol Clin. 2005;23(2):209-226.
   https://doi.org/10.1016/j.det.2005.01.001
10. Atzori L, Brundu MA, Orru A and Biggo P. Glycolic acid peeling in the treatment of acne. J Eur Acad Dermatol Venereol. 1999;12(2):119-122.
    https://doi.org/10.1111/j.1468-3083.1999.tb01000.x
11. Manalato RM and Alster T. Erbium: Yag laser resurfacing for refractory melasma. Dermatol Surg. 1999;25(2):121-123.
    https://doi.org/10.1046/j.1524-4725.1999.08103.x
12. Lawrence N and Leonhardt JM. Trichloroacetic acid (TCA) peels. In: Rubin MG, Dover JS and Alam M. Chemical Peels. 9th ed. Netherlands: Elsevier Inc.; 2006. p. 778.
13. Javaheri SM, Handa S, Kaur I and Kumar B. Safety and efficacy of glycolic acid facial peel in Indian women with melasma. Int J Dermatol. 2001;40(5):354-357.
    https://doi.org/10.1046/j.1365-4362.2001.01149.x
14. Dogra A, Gupta S and Gupta S. Comparative efficacy of 20% trichloroacetic acid and 50% glycolic acid peels in treatment of recalcitrant melasma. Pak J Dermatol. 2006;16(2):81-87.
15. Grover C and Reddu BS. The therapeutic value of glycolic acid peels in dermatology. Indian J Dermatol Venereol Leprol. 2003;69(2):148-150.
16. Soliman MM, Ramadan SA, Bassiouny DA and Abdelmalek M. Combined trichloroacetic acid peel and topical ascorbic acid versus trichloroacetic acid peel alone in the treatment of melasma: A comparative study. J Cosmet Dermatol. 2007;6(2):89-94.
    https://doi.org/10.1111/j.1473-2165.2007.00302.x
17. Moy LS, Murad H and Moy RL. Glycolic acid peels for the treatment of wrinkles and photoaging. J Dermatol Surg Oncol. 1993;19(3):243-246.
    https://doi.org/10.1111/j.1524-4725.1993.tb00343.x

Authors' Contributions:
SA- Concept and design of the study, prepared first draft of manuscript, statistical analysis and interpreted the results; **SK-** Concept and co-ordination, Reviewed the literature, manuscript; **AN-** Concept and co-ordination, reviewed the literature, manuscript; **KRG-** Statistical analysis and result interpretation

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