COMPARISON OF TREATMENT WITH TACROLIMUS 0.03% AND SUPEROXIDE DISMUTASE AND CATALASE IN VITILIGO

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ABSTRACT

Objective: *To compare the efficacy and safety of topical tacrolimus 0.03% with superoxide dismutase and catalase in vitiligo.*

Material and methods: *This hospital based quasi-experimental study was carried out in out patients department of Dermatology Unit-II, Mayo Hospital Lahore in year 2006-2007. Patients were selected by non-probability purposive sampling method after obtaining an informed consent. Both sexes (above 5 years) were included with head and neck vitiligo. They were randomly divided into 2 groups; group 1: superoxide dismutase and catalase; group 2: tacrolimus by using random number table.*

Results: *There were 34(56.7%) females and 26(43.3%) males. Age of the patients ranged from 5 to 60 years with the mean age of 20.52±14.34 years. Overall 31(62%) patients showed repigmentation of varied grades. Repigmentation was shown by 14(46.6%) patients in superoxide dismutase & catalase group and 17(56.6%) patients in tacrolimus group.*

Conclusion: *Topical superoxide dismutase & catalase and tacrolimus are effective treatment modalities in patients with vitiligo. There is no significant difference regarding efficacy and safety between both forms of treatment.*

Key words: *Vitiligo, Depigmentation, Tacrolimus, Superoxide dismutase and catalase.*

INTRODUCTION

Vitiligo is a common, acquired and often familial, depigmenting skin disorder characterized by the loss of functioning epidermal melanocytes because of multifactorial and overlapping pathogenetic mechanisms [1].

It may be associated with hyperthyroidism, hypothyroidism, pernicious anemia, diabetes mellitus and Addison's disease [1]. In Pakistan, the reported incidence varies from 4.4% to 7.5% [2, 3]. In about 30% of cases there is familial clustering of cases. Incidence decreases with increasing age [1].

Vitiligo causes cosmetic disability and in many cases, especially on exposed parts of body, it leads to severe depression and suicidal tendencies [4].

Different modalities of treatment have been used in vitiligo like phototherapy with psoralens, steroids, heliotherapy, laser, vitamin-D analogues and skin grafting [1].

Newer therapies are emerging with minimum side effects and safety. Among them are Tacrolimus and Superoxide dismutase & catalase.

As the pathogenesis of this disease is still obscure, the treatment of vitiligo has generally been unsatisfactory and often disappointing. Topical tacrolimus (FK506) ointment has recently been added to the armamentarium against this pigmentary disorder. Direct interaction between FK506 and keratinocytes creates a favorable milieu for melanocytes growth and migration [5].

According to recent hypothesis, vitiligo results from a failure of mechanisms detoxifying melanocytes [6]. Presence of an imbalance in the oxidant-antioxidant system might play a role in the pathogenesis of vitiligo. Results support the concept that free radical-mediated damage may be the initial pathogenic event in melanocyte degeneration in generalized vitiligo[7].

Deficit in the main detoxifying enzymes,

catalase and thioredoxin reductase was demonstrated in patients presenting with vitiligo[8]. Tacrolimus is a highly lipophilic macrolide lactone and immunosuppressive drug that has been used widely in organ transplantation. It inhibits the action of calcineurin and consequently inhibit T-cell activation and the production of various cytokines. Tacrolimus has stimulatory action on tyrosine activity which leads to melanin biosynthesis[9].

Topical tacrolimus ointment (0.03% or 0.1%) is produced by Streptomyces tsukubaensis .In Japan it was approved for the treatment of atopic dermatitis in 1999, the United States in 2000 and Europe in 2001. Pimecrolimus and tacrolimus are two topical calcineurin inhibitors used in pediatric patients with atopic dermatitis. It is an effective and well tolerated therapy for head and neck vitiligo[10].

Tacrolimus can be used on a domiciliary basis for longer period of time without aggressive monitoring. It is applied to the affected area twice daily and continued for 1 week after signs and symptoms resolve[10].

Side effects include infections, pyrexia, burning, pruritus, erythema and papules in the application area[11].

Significant higher increase in erythrocytes superoxide dismutase activities was observed in active vitiligo[12]. Superoxide dismutase (SOD) is a group of metalloenzymes that protects cells from the toxic effects of superoxide radicals. Recent results support the removal of epidermal hydrogen per oxide in the successful treatment of vitiligo[13].

Superoxide dismutase and catalase is considered to be effective, well tolerated treatment of vitiligo due to its ability to re-establish the physiological equilibrium of free radicals in epidermal cells i.e. melanocytes and keratinocytes[14]. It can be applied to periorbital area with total safety.

Oxidative stress has a definite role in several diseases. Research at molecular level demonstrated a deficiency of antioxidant substances in vitiliginous skin. Reactive oxygen species like super oxide anion and hydroxyl radicals are produced following ultraviolet damage to epidermis. These inhibit tyrosinase enzyme and are cytotoxic to melanocytes. Low level of catalase enzyme, which function as scavenger of reactive oxygen radical is also reduced in vitiliginous skin[15].

Previously these therapies were found successful in patients with type I skin. This study was conducted to see their effect and safety in type IV and V skin (Fitz Patrick Classification).

MATERIAL AND METHODS

This hospital based quasi-experimental study was carried out in Out Patients Department of Dermatology Unit-II, Mayo Hospital Lahore in year 2006-2007. Sixty patients of vitiligo (diagnosed clinically) were selected by non-probability purposive sampling method after obtaining an informed written consent. Both sexes (above 5 years) were included with head and neck vitiligo and patients with stable vitiligo i.e. those cases in which vitiligo did not show any change in size and color for at least last six months. They were randomly divided into 2 groups (super oxide dismutase & catalase and tacrolimus) by using random number table. Patients in group I were treated with twice daily application of topical superoxide dismutase and catalase. The patients in group II were treated with twice daily application of topical tacrolimus 0.03%.

Efficacy of tacrolimus and superoxide dismutase and catalase was measured by clearance of vitiligo i.e. area of repigmentation in six months. Therapy was considered inefficacious if no response was visible in three months. Safety was measured by evaluating the number of side effects e.g. burning, pruritis, dryness, erythema and scaling.

Vitiliginous area was measured in centimeters at start of therapy.

Response of treatment was graded as following

Grade	Area of Repigmentation	Percentage Response
0	0%	Nil
1	1 -25%	Poor
2	26 -50%	Mild
3	51 -75%	Moderate
4	76 -95%	Marked
5	96 -100%	Excellent

A total of sixty patients with head and neck vitiligo coming through OPD of Dermatology unit II Mayo hospital Lahore, after taking prior informed and written consent were assigned to either group by using random number table.

Diagnostic criteria used for vitiligo was well circumscribed milky white cutaneous macules and patches. No history of previous disease at the site of lesion and no signs of inflammation. Inclusion exclusion criteria was followed for enrollment of patients.

All the patients of more than 5 years of age of either sex with stable vitiligo involving head and neck i.e. those cases in which vitiligo did not show any change in size and color for at least in last six months and no evidence of spontaneous repigmentation or hypersensitivity to super oxide dismutase / catalase or tacrolimus were included in the study. The patients with unstable Vitiligo, lip tip vitiligo, systemic or topical treatment over four weeks preceding the study, vitiligo associated with non melanic- hair, any systemic disease or autoimmune disorder were excluded from the study. Pregnant and lactating women were also excluded.

After excluding patients as per exclusion criteria of this study, a total of 60 patients with vitiligo were enrolled and a formal consent was obtained from all of them. Two patients lost to follow up in tacrolimus group.

Pre-devised proforma was completed including bio-data of patients. All the relevant details regarding history, examination, treatment, type of vitiligo, sites of involvement and extent of the disease were recorded.

Detailed history was taken, clinical examination performed to rule out associated diseases and exclude other diseases with same morphology. Relevant investigations were performed where required to rule out any systemic disease or autoimmune disorder. For example blood glucose level to exclude diabetes, thyroid function tests to rule out thyroid dysfunction and serum electrolytes for evidence of Addison's disease.

Patients were photographed before commencement, after three months and at the end of therapy. Topical medicine was applied twice daily. Repigmentation was documented according to grading.

Patients were followed up every month for a maximum period of six months. If no response was visible in three months the therapy was stopped.

Side effects were observed and noted down. If any side effects were recorded the medicine was stopped.

Outcome variables were area of repigmentation to determine the efficacy and erythema, burning, pruritus, sore lips, rashes, scaling, papules, headache and muscle pain to evaluate the side effects.

Confounding Variables were spontaneous Repigmentation, use of Steroids, unusual exposure to sunlight, vitamin therapy including folic acid, vitamin B12 and vitamin C and any systemic or autoimmune disease.

Risk benefit ratio was explained to patients regarding aetiology and pathogenesis of vitiligo and its associated diseases. They were informed about the disadvantages of not being treated. For example sunburn and possibility of focal vitiligo proceeding towards generalized disease and were explained about spontaneous repigmentation, advantages and side effects of medicine.

Data analysis was done by computer using SPSS 11.

RESULTS

Sixty patients suffering from vitiligo were included in the study. Out of these 30(50%) patients were in the superoxide dismutase and catalase group and 30(50%) patients were in the tacrolimus group.

There were 34(56.7%) female and 26(43.3%) male patients with an overall female to male ratio of 1.3:1.

In the tacrolimus group 11(36.66%) patients were <20 years of age, 13(43.33%) patients in the age range of 20-40 years and 6(20%) patients were more than 40 years of age. Majority of patients i.e. 24(80%) in the tacrolimus group and 29(96.63%) in the superoxide dismutase and catalase group were < 40 years of age.

Duration of disease ranged from 1 month to 7 years with the mode of 6 months. In the superoxide dismutase and catalase group duration of disease ranged from 1 month to 6 years with the mode of 1 year. Duration of vitiligo was less than 6 months in 9(30.0%) patients, 6months to less than 2 years in 15(50%) cases and in 6(20%) patients it was more than 2 years.

In the tacrolimus group duration of disease ranged from 2 months to 7 years with the mode of 6 months. Duration of vitiligo was less than 6 months in 15(50%) patients, 6months to less than 2 years in 11(36.66%) cases and more than 2 years in 4(13.33%) patients.

Majority of patients i.e. 24(80%) in the superoxide dismutase and catalase group and 26(86.6%) in the tacrolimus group had vitiligo for <2 years.

Sites of vitiliginous involvement were eyelid 17(28.3%), cheek 10(16.66%), forehead 9(15.0%), neck 7(11.7%), chin 3(5.0%) and moustache area in 2(3.3%) patients. More than one site was involved in 12(20.0%) patients. Sites of involvement in superoxide dismutase & catalase and tacrolimus groups are shown in table 1.

Table 1: Sites involved in both groups (n=60)

Sites Involved	Group 1 (n= 30)		Group II (n = 30)	
	No. of Patients	Percentage	No. of Patients	Percentage
Forehead	3	10.00	6	20.00
Eyelids	9	30.00	8	26.66
Cheeks	4	13.33	6	20.00
Chin	1	3.33	2	6.66
Mouchtache Area	1	3.33	1	3.33
Neck	5	16.66	2	6.66
More than one site	7	23.33	5	16.66

**Two patients in tacrolimus group were lost to follow up.*

Size of the lesions were <5 cm in 19(31.66%) patients, 5-10 cm in 32(53.33%) cases and >10 cm in 9(15.0%) patients.

In 17(28.3%) patients the lesions were symmetrical, while in 43(71.7%) patients the lesions were asymmetrical.

Excellent response was seen in 6(10%) patients, while 10(16.7%) showed marked response, 9(15%) moderate, 4(6.66%) mild and in 2(3.33) patients there was poor repigmentation. In 27(45%) patients no response was seen.

Five patients in tacrolimus group showed an excellent response as compared to those treated with superoxide dismutase and catalase only one patient had similar response.

No response was seen in 11(36.6%) patients treated with tacrolimus and 16(53.33) cases treated with superoxide dismutase and catalase.

Repigmentation as occurred in the tacrolimus and the superoxide dismutase and catalase groups is shown in table 2.

In 56 (93.33.0%) patients, side effects of treatment did not occur. Skin color papules were noted in 1(1.7%) patient. Erythema/ burning was also recorded in 1 patient (1.7%).Both were from tacrolimus group (Table 3).

Two (3.4%) patients were lost to follow up. Side effects observed in the tacrolimus and the superoxide dismutase and catalase groups are shown in table 4.

Table 2: Repigmentation in both groups (n=60)

Repigmentation	Group 1 (n= 30)		Group II (n = 30)	
	No. of Patients	Percentage	No. of Patients	Percentage
Nil	16	53.33	11	36.66
Poor	1	3.33	1	3.33
Mild	1	3.33	3	10.00
Moderate	3	10.00	6	20.00
Marked	8	26.66	2	6.66
Excellent	1	3.33	5	16.66
Total	30		28*	

**Two patients in tacrolimus group were lost to follow up.*

Table 3: Comparison of overall repigmentation in both groups

Repigmentation	No of Patients	Group 1	Percentage	Group 2*	Percentage	P-value
Nil	27	16	53.33	11	36.66	0.95**
Present	31	14	46.66	17	56.66	

**Two patients in tacrolimus group were lost to follow up.* ***Not Significant*

Table 4: Comparison of side effects in both groups

Side Effects	No of Patients	Group 1	Percentage	Group 2*	Percentage	P-value
Nil	56	30	100	26	86.66	0.95**
Present	2	00	00.00	02	6.66	

**Two patients in tacrolimus group were lost to follow up.* ***Not Significant*

DISCUSSION

Despite significant advances made in the past few years, treatment of vitiligo still remains a challenge. Different treatment modalities have been tried for this disease but results are not satisfactory for many patients[16]. Relative failure to achieve desired effects with existing therapies in this cosmetically disfiguring disease has always stimulated research[17, 18]. Several treatment modalities with varying success rates are currently in use. The therapeutic effect varies greatly. For effective treatment of vitiligo, it is as important to arrest the progression of the disease and induce repigmentation[19].

Previously published studies in our region regarding treatment of vitiligo focused on existing therapies like Psoralen Ultra Violet A, Ultra Violet B, corticosteroid, calcipotriol etc. It is the first time that a study comparing superoxide dismutase and catalase with tacrolimus conducted in Pakistan with no such comparison even from abroad to the best of our knowledge.

Defects in cell mediated and humoral immunity[20] as well as oxidative stress have been hypothesized as aetiological factors in vitiligo. To address these factors superoxide dismutase / catalase and tacrolimus were chosen for treatment of vitiligo in type IV and V skin.

Vitiligo may result from failure of mechanisms detoxifying melanocytes, imbalance of oxidant and antioxidant system, free radical damage and deficit in detoxifying enzymes [6-8]. Research at molecular level showed a deficiency of antioxidant substance in vitiliginous skin. Reactive oxygen species such as superoxide anions, hydroxyl radicals etc are produced following ultra violet induced damage to epidermis which are cytotoxic to melanocytes and inhibit tyrosinase enzymes. Low level of catalase enzymes which function as scavenger of reactive oxygen radical is found in vitiliginous skin[21].

In the superoxide dismutase and catalase group, overall 14 (46.66%) patients showed repigmentation. Majority of those who responded (8 out of 14 cases) had marked repigmentation and one patient had total recovery. Repigmentation did not occur in 16 (53.33%) patients.

Topical superoxide dismutase and catalase by balancing oxidant and antioxidant system and fulfilling the deficit in detoxifying enzymes probably caused repigmentation in this group.

Vitiligo is a disease in which pathogenesis is still not completely understood. In non responders imbalance of oxidant-antioxidant system may not be playing major role and there may be other factors involved in pathogenesis.

International studies using superoxide dismutase and catalase showed higher rate of repigmentation but all of them used combination therapies[22, 23].

In a study by Schallreuter KU, a combination of pseudocatalase, calcium chloride and suberythemogenic UVB showed excellent repigmentation in majority of cases. Focal vitiligo showed 90-100% repigmentation in all cases [22].

Another study by Khemis A, using UVB phototherapy with superoxide dismutase and catalase showed 60% repigmentation[23].

In the tacrolimus group, overall 17 (56.66%) patients showed repigmentation. Excellent results were shown in 5 patients while one quarter of patients responded with moderate to marked repigmentation. Repigmentation did not occur in 11(36.66%) patients.

Tacrolimus blocks T cell activation by inhibiting the initial signal transmitted to T cell nucleus after antigen has bound to the T cell receptor. One of the key intermediate molecule in this signal transduction process is a phosphatase calcineurin. This inhibitory activity suppresses gene activity and block production of various cytokines including IL-2, IL-3, IL-4, granulocyte macrophage colony stimulating factor (GM-CSF) and interferon[24]. Our response rate was comparable with most of the international studies[24, 25].

Study carried out by Almeida P et al[25] showed repigmentation of over 50% in their patients treated with tacrolimus. Similarly thirteen patients (68%) treated with tacrolimus by Grimes et al [24] had greater than 75% repigmentation of face and neck lesions.

However a different report was given by Silverberg NB et al [26] in which almost 90% of

patients of head and neck vitiligo responded partially to treatment with tacrolimus. Significant feature was that all the patients were children. No adult was included in study.

International studies have been carried out in type 1 & II skin while our patients mainly have type IV and V skin (Fitz Patrick Classification).

Since our findings are comparable with other studies in both treatment groups except that of Silverberg (carried in children), skin type does not seem to be affected by either modality of treatment.

In superoxide dismutase and catalase group none of the patients demonstrated any side effect which appears to be a major advantage of this treatment group. Only two patients in tacrolimus group had side effects but there was no statistical significance between two treatment groups (p value = 0.95) (Table 3).

Similarly the difference in the success rate between two treatment groups was also statistically insignificant (p value = 0.95) (Table 2).

The study hypothesis that topical tacrolimus ointment is superior to superoxide dismutase and catalase ould not be proved in this study.

Limitations of study included small sample size, non availability of treatment modalities to non affordable patients and usage of drugs in stable vitiligo involving head and neck region. Results can't be generalized as sampling technique was Non probability purposive.

Both drugs were effective in almost half of the patients with minimal side effects seen only in tacrolimus group. Studies need to be carried out on larger sample involving other areas of body as well.

CONCLUSION

Topical tacrolimus and superoxide dismutase and catalase both are effective treatment modalities in vitiligo patients with type IV and V skin with no superiority of one over the other in terms of efficacy or side effects.

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