

Original Article

Comparison of efficacy of conjugated estrogen cream 0.625% plus clobetasol 0.05% vs. clobetasol 0.05% alone in the treatment of vitiligo patients

A.Asilian, M. Shabaram, G. Faghihi

Department of Dermatology, Medical Science University of Isfahan, School of Medicine, Iran

Abstract *Background:* Vitiligo is a pigmentary skin disorder characterized by hypo- or depigmented patches. Topical steroid creams are used as a common standard method in vitiligo therapy.

Objective The goal of this study was to compare the efficacy of topical clobetasol (as a standard method for treatment of vitiligo) versus combination of clobetasol + topical estrogen in treatment of vitiligo.

Patients and methods In this double-blind clinical trial, 37 patients who had at least 2 vitiligo patches were treated with topical clobetasol on one side of the body for three months while a combination of clobetasol and estrogen was applied on the other side. Three months after termination of therapy, the patients were visited again for improvement of disease. The results were evaluated with statistical analysis of paired t test, McNemar and Wilcoxon tests in SPSS software.

Results Overall 37 patients, 21 women and 16 men with minimum age of 14 and maximum of 45 years old, completed the study. The difference of average area of lesions before and after treatment in clobetasol and estrogen group was significantly more than clobetasol only group ($P=0.001$). There was no significant difference between clobetasol and clobetasol + estrogen treatment for two age groups of 14-30 and 31-45 years. Also the pigmentation score of two treatment groups measured by paired sample test was not significant for both sexes ($P> 0.05$).

Conclusion Addition of estrogen to clobetasol increases its efficiency in treatment of vitiligo without any side effects. Also it seems that estrogen may decrease the topical clobetasol complications such as atrophy, erythema and telangiectasia.

Key words

Vitiligo, melanocyte, treatment, clobetasol, estrogen

Introduction

Vitiligo is an acquired skin disorder characterized by white and depigmented patches enlarging and becoming more numerous with time.¹ It is the most common pigmentary

disorder affecting about 1% of the world's population, irrespective of race and gender.² There is a high prevalence of this disease in Iran with respect to skin type in the country. It may appear in any age, but half the patients develop it before age 20.³ Usually the disease spreads gradually, but sometimes a number of patches develop quite quickly and then remain static for months or years without changing. The depigmentation process may be partial or complete.⁴ The hair color is usually normal, but

Address for correspondence

Dr. M. Shabaram
Dermatology Department,
Al-Zahra Hospital, Isfahan, Iran,
P.O. Box 895,
Email: M.Shabaram@gmail.com

in earlier lesions it may be amelanotic.^{2,5} Vitiligo may be polygenic or autosomal dominant with variable penetration. It has been observed in monozygotic twins also.⁶ There are many theories about etiology of vitiligo. Autoimmune hypothesis proposes that an immune system disorder results in destruction of melanocytes.⁷ It is supported by the frequent observation of several autoimmune disorders in association with vitiligo. A significant association of vitiligo has been demonstrated with thyroid dysfunction and/or thyroid antibodies in particular.^{8,9} However, most vitiligo susceptibility genes detected so far are associated with both vitiligo and other autoimmune disorders¹⁰; moreover, one of these genes was recently identified as NALP1, a regulator of the innate immune response,¹¹ offering strong support for the autoimmune hypothesis. Destruction of melanocytes may be directly mediated by autoreactive T cells. Activated T cells have been demonstrated in perilesional vitiligo skin.¹² Neurogenic hypothesis says that nerve endings in the skin release a chemical that is toxic to the melanocytes or probably restrain the melanogens.^{13,14} Self-destruct hypothesis suggests that melanocytes destroy themselves due to toxin released in the process of melanin synthesis.¹⁴

The patient's age, distribution of lesions and their edge hyperpigmentation are the diagnostic criteria.¹⁵ Considering the serious psychological results of vitiligo which affects the social life of the patients impressively, treatment of the disease has been earnestly required.^{16,17} Up to now, several treatment methods have been suggested for vitiligo.

By increase of pigmentation in some parts of the body during pregnancy, effect of estrogen on skin initially was identified in 1940.^{15,18} Several studies demonstrated the effect of estrogen on

skin collagen and thickness. Varila *et al.* showed that not only topical estrogen increases the collagen amount by measuring hydroxyproline of skin, but also it leads to increase of procollagen type I and procollagen type III.¹⁹ Both systemic and topical glucocorticoid therapy can produce cutaneous atrophy. Earliest change is thinning of epidermis which results from a reduction of mitotic activity in germinal layer. Topical steroids also inhibit the activity of enzymes involved in collagen biosynthesis,^{20,21} and they have been shown to depress synthesis of types I and III collagen *in vivo*.^{22,23} The existing data supports that topical estrogen can prevent the atrophogenic complication of clobetasol.¹⁹ Also several studies performed on the effects of estrogen treatment on tyrosinase activity, reported that this melanogenic enzyme is stimulated by estrogen.^{24,25}

Although there was no sign of systemic complications by topical estrogen application, further studies are required to determine the topical effective dose that produces no systemic complications.

Since topical estrogen does not have complications, the goal of this research was to study its effect on vitiligo patients, so that a cheap treatment with least complications but good response was achieved.

Patients and methods

This double-blind clinical trial was conducted from November, 2007 to October, 2008 and 37 patients, with at least two patches of vitiligo on their bodies, were enrolled. The sampling method was random and gathering of information was performed by precise visit of the patients. Inclusion criteria were as following: vitiligo patients with at least 2 lesions on the body, 14-45 years old, lack of follicular

pigmentation in the lesion, lack of white hair in the lesion and duration of disease less than 6 months. On the other hand the following patients were excluded from the study: those who had dermatomal and segmental vitiligo, those who had received treatment during the last 2 months or had any sensitivity or allergy to estrogen, those who had any dysplastic nevus, had vitiligo around fingers and nails or had follicular pigmentation and finally those who had any other autoimmune disorders such as thyroid and diabetes (which could be diagnosed by specific tests) besides vitiligo.

Patients were explained about the study protocol and informed consent was taken. Then patients were evaluated for exclusion criteria. Enrolled patients were meticulously examined for follicular pigmentation, photographs of lesions were taken and the area of lesions was measured by graph papers.

Clobetasol 0.05% cream and its combination with estrogen cream 0.625% were dispensed in identical tubes and encoded. Two creams for topical use over the lesions of each patch were delivered to the patients who were not aware of the contents. The patients were instructed how the drugs should be applied. The duration of study was three months and patients were followed-up monthly for three months.

At each follow-up, patients were evaluated for efficacy and side effects. Evaluation of lesions was performed according to scoring system, taking photographs and precise examination of the patients. The scoring system of the patients was as following: 0 for lack of perifollicular pigmentation, 1 for perifollicular pigmentation in less than 25% of the lesion area, 2 for perifollicular pigmentation in 25-50% of the lesion area and 3 for presence of perifollicular pigmentation in more than 50% of the lesion

area. The area of lesions in cm^2 was measured by graph paper and complications such as atrophy, erythema and telangiectasia were studied by observation of lesions.

Finally the statistical analysis of the results was carried out by using SPSS software. Pigmentation score in age groups was evaluated by Mann-Whitney test. Study of complications of each treatment method was performed by t-test while McNemar test was used to evaluate the improvement of lesions area.

Results

Totally 37 patients, 16 men and 21 women, completed the study. The mean age of patients was 27 years varying in range of 14-45 years. The mean duration of the disease was 4 months with minimum of 2 and maximum of 6 months. At baseline, the mean area of the lesions in clobetasol and clobetasol-estrogen groups was $13.9 \pm 8.8 \text{cm}^2$ and $15.49 \pm 8.4 \text{cm}^2$, respectively ($P > 0.05$). Similarly, the perifollicular pigmentation score in both groups was zero before treatment.

When the results for lesions area in each treatment method were compared before and after the treatment ($P = 0.001$) i.e. both groups had considerable recovery (**Table 1**).

At the end of treatment, both groups showed considerable improvement in the perifollicular pigmentation score as compared to baseline score ($P < 0.05$, paired t test) i.e. both of the groups had remarkable improvement. Comparing the two groups, mean perifollicular pigmentation scores after using topical clobetasol only and a composition of clobetasol and estrogen were 1.41 ± 0.50 and 2.10 ± 0.75 , respectively ($P < 0.001$, Wilcoxon test). Therefore, it can be deduced that pigmentation

Table 1 Mean and standard deviation of disease area before and after treatment for two treatment methods.

Method	Before treatment	After treatment	Area difference before and after therapy
	Mean (cm ²)	Mean (cm ²)	
Clobetasol	13.92±8.75	10.56±7.05	P= 0.010
Clobetasol +estrogen	15.48±8.40	10.19±6.49	P = 0.013
Area variation difference in two treatment methods	Mean = -1.95	Standard deviation = 2.30	P = 0.001

Table 2 Mean, median and standard deviation of score pigmentation for treatment methods in two age groups.

Method	14-30 years		30-45 years		Exact P
	Mean	Median	Mean	Median	
Clobetasol. score	1.4±1	0.50	1.4±0.5	1	0.97
Clobetasol + estrogen score	2.1±2	0.8	2.0±0.67	2.0	0.66



Figure 1 Lesions of first and second patients before treatment.



Figure 2 Lesions of first and second patients after treatment.

score has been increased in later group.

Figure 1 and **2** show the lesions image before and after treatment.

Considering the patients sex in evaluation of the results, the mean lesions area in clobetasol group

was 4.3±3.85 for men and 4.29±2.75 for women and hence the difference of two treatment groups was not significant for both of the sexes. Also the pigmentation score of two treatment groups compared by paired t test was not significant amount for both sexes (P> 0.05).

There was no significant difference between clobetasol and clobetasol + estrogen for two age groups ($P > 0.05$). The values for age groups of 14-30 and 31-45 years old were 1.74 ± 2.0 and 2.5 ± 2.9 , respectively (**Table 2**).

Considering the safety profile of two groups, for clobetasol + estrogen group there was not any side effects as atrophy, erythema and telangiectasia, but in clobetasol group, 4 cases of erythema and telangiectasia were observed. These complications resolved after a 3 months follow up. Statistical studies of complications in both groups did not show a significant difference.

Three months after the treatment, 18 of 37 visited for follow up and increase of follicular pigmentation and decrease of lesions size was observed for 4 patients (2 men and 2 women).

Discussion

A meta analysis of existing data shows that corticosteroids of class III and UVB therapy are the most effective and safe topical treatment methods for generalized vitiligo.^{26,27} Topical corticosteroids have proved effect in some cases. A study showed an improvement rate of 80% and 55% on face and body lesions respectively after topical clobetasol 0.05% use for 6 months period and less.²⁷

Effect of estrogen on wound healing and skin aging has been regarded by researchers since past years.^{15,18} Effect of estrogen on skin wrinkles and aging-derived dryness, has been characterized by its effect on thickness, collagen and moisture of skin. Not only skin but also its appendages such as hair are also affected by estrogen. Another research compared the effect of estradiol 0.01 % and estradiol 0.3 % and the

results showed a significant decrease of wrinkles without any minor complication.²⁸

Decrease of skin elasticity has been observed after menopause for women and the thickness of elastic fiber has increased after applying estradiol ointments over these women's skin. There is also some evidence that fluctuations of these hormones during the menstrual cycle may affect epidermal pigmentation in some women. In one study 62 of women consistently noticed darkening of the periorcular skin towards the end of their menstrual cycle immediately prior to menstruation.²⁹ The use of dermal ointments containing estrogens has also resulted in intense pigmentation of the genitals, mammary areola and linea alba of the abdomen in both sexes.³⁰

Recently, Sungbin *et al.* reported the presence of estrogen receptors in normal melanocytes of human by using immunohistochemistry and RT-PCR.³¹ Several researches have reported the therapeutic effect of estrogen on tyrosinase activity and stimulation of this melanogenic enzyme which is a regulator key for pigmentation of skin.^{32,33} Estrogen increases tyrosinase activity by cAMP enhancement. The cAMP itself is activated by stimulation of MC1R which is a member of G protein transmembranous family. The cAMP activation increases both of melanocyte proliferation and intracellular synthesis.³⁴

Presence of estrogen receptors in anagen hair follicles (especially in dermal papilla and bulge part of outer root sheath) has been demonstrated by immunohistochemical studies for both sexes. Hair follicular dermal papilla serves as a reservoir for skin melanocytes. Therefore, vitiligo lesions associated with hair whiteness are more resistant to treatment. By this assumption, it seems that estrogen affects dermal papilla and outer root sheath and provides

conditions for migration of melanocytes to the follicular surrounded skin.

Considering the limited *in vitro* studies about the theory of estrogen effects on melanocytes and also presence of few studies about effect of estrogen on skin, we tried to investigate the effect of estrogen on vitiligo treatment in this study. The purpose of this study was to compare the efficiency of topical clobetasol (as a standard method for treatment of vitiligo) with a combination of clobetasol and topical estrogen.

In initial visit of the patients before treatment by two treatment methods, lesions area and pigmentation score were similar in two groups. Nevertheless, when the two groups i.e. clobetasol + estrogen group and clobetasol-only were compared before and after the treatment, the difference was significant, suggesting that the response to treatment in clobetasol + estrogen group has been significantly more than clobetasol-only one.

Considering the patients sex in evaluation of the treatment methods, the difference of two treatment groups was not significant for both of men and women, i.e. using topical estrogen for men or women's skin does not have a significant difference.

There was not any significant difference between two treatment methods for two age groups of 14-30 and 30-45 years old, i.e. the patient's age did not affect the response of melanocytes to topical estrogen.

Topical steroids, especially potent and high potent, produce cutaneous atrophy, erythema and telangiectasia, particularly if used for a long time and in specific places. It has been recommended when clobetasol 0.05% is applied over the body, follow up of patient is advisable

every 1-2 months. Then, if there is not any response during three months, the treatment has to be stopped.²⁷ This is the reason why the treatment period in this research was three months and the patients were visited every month. By studying the complications of each treatment method, for clobetasol + estrogen group there was not any side effects such as atrophy, erythema and telangiectasia, but in clobetasol only group 4 cases of erythema and telangiectasia were observed. These complications were resolved after a 3 months follow up.

Three months after the treatment, 18 of 37 patients referred again for visit and in three months later follow up, increase of follicular pigmentation and decrease of lesions size was observed for 4 patients (2 men and 2 women). It seems that migration of melanocytes and their stimulation has been continued after interruption of treatment.

References

1. Koranue RV , Sachdeva KG. Vitiligo. *Int J Dermatol* 1988; **27**: 676-81.
2. Lerner AB. On the etiology of vitiligo and gray hair. *Am J Med* 1971; **51**: 141-7.
3. Ortonne J-P, Mosher DB, Fitzpatrick TB, eds. In: Vitiligo and other Hypomelanoses of Hair and Skin. New York: Plenum medical; 1983. P. 129-310.
4. Handa H, Dogra S. Epidemiology of childhood vitiligo: *Ped Dermatol* 2003; **20**: 107-10.
5. Oh H S, Smart R C. An estrogen receptor pathway regulates the telogen-anagen hair follicle transition and influences epidermal cell proliferation. *Proc Nat Acad Sci USA* 1996; **93**: 12525-30.
6. Mohr J. Vitiligo in a pair of monozygotic twins. *Acta Genet* 1951; **2**: 252-5.
7. Cui J. Artia Y, Bystryjn J-C. Cytolytic antibodies to melanocytes in vitiligo. *J Invest Dermatol* 1993; **100**: 812-15.
8. Betterle C, Peserico A, Bersani G. Vitiligo and autoimmune polyendocrine deficiencies

- with autoantibodies to melanin – producing cells. *Arch Dermatol* 1979; **115**: 364.
9. Woolfson H, Finn OA, Mackie RM *et al.* Serum anti – tumour antibodies and auto-antibodies in vitiligo. *Br J Dermatol* 1975; **92**: 395-400.
 10. Spritz RA. The genetics of generalized vitiligo and associated autoimmune diseases. *Pigment Cell Res* 2007, **20**: 271-8.
 11. Jin Y, Mailloux CM, Gowan K *et al.* NALP1 in vitiligo-associated multiple autoimmune disease. *New Eng J Med* 2007; **356**: 1216-25.
 12. Kemp FH, Waterman EA, Hawes BE *et al.* The melanocortin receptor 1, a novel target of autoantibody responses in vitiligo. *J Clin Invest* 2002; **109**: 923-30.
 13. Lerner AB. Vitiligo. *J Invest Dermatol* 1959; **32**: 285.
 14. Dell' Anna ML, Picardo M. A review and a new hypothesis for non-immunological pathogenetic mechanisms in vitiligo. *Pigment Cell Res* 2006; **19**: 406-11.
 15. Calvin M. Oestrogens and wound healing. *Maturitas* 2000; **34**: 195-210.
 16. Dolatshahi M, Ghazi P, Feizi V, Hemami M R. Life quality assessment among patients with vitiligo. *Indian J Dermatol Venereol Leprol* 2008; **74**: 700.
 17. Papadopoulos L. Psychological therapies for dermatological problems. In Walker C, Papadopoulos L, eds. *Psychodermatology: The psychological impact of skin disorders*. Cambridge, UK: Cambridge University; 2005. P. 101-115.
 18. Ashcroft GS, Ashworth JJ. Potential role of estrogen in wound healing. *Am J Clin Dermatol* 2003; **4**: 737-43.
 19. Varila E, Rantala I, Orikarinen A *et al.* The effect of topical estradiol on skin collagen of postmenopausal women. *Br J Obstet Gynaecol* 1995; **120**: 985-9.
 20. Sauerbonn AV, Fonseca AM, Bagnoli VR *et al.* The effect of systemic hormone replacement therapy on the skin of the postmenopausal women. *Int J Gynecol Obstet* 2000; **68**: 35-41.
 21. Risteli J. Effect of prednisolone on the activities of intercellular enzymes of collagen biosynthesis in rat skin. *Biochem Pharmacol* 1977; **26**: 1295-8.
 22. Autio P, Oikarinen A, Melkko J *et al.* Systemic glucocorticoids decrease the synthesis of type I and III collagen in human skin in vivo, whereas isotretinoin has little effect. *Br J Dermatol* 1994; **131**: 660-3.
 23. Nuutinen P, Riekkki R, Parikka M *et al.* Modulation of collagen synthesis and mRNA by continuous and intermittent use of topical hydrocortisone in human skin. *Br J Dermatol* 2003; **148**: 39-45.
 24. Verdier-Sevrain S, Bonte F, Gilchrist B. Biology of estrogens in skin. *Exp Dermatol* 2006; **15**: 83-94.
 25. Thornton M.J. I. The biological actions of estrogens on skin. *Exp Dermatol* 2000; **11**: 487-502.
 26. Grimes PE. New insights and new therapies in vitiligo. *JAMA* 2005; **293**: 730-5.
 27. Njoo MD, Spuls PI, Bosy D *et al.* Non surgical repigmentation therapies in vitiligo. Meta-analysis of the literature. *Arch Dermatol* 1998; **134**: 1532-40.
 28. Philips TJ, Dermicary Z, Sahu M. Hormonal effects on skin aging. *Clin Geriatr Med* 2001; **17**: 661-672
 29. Snell R.S, Turner R. Skin pigmentation in relation to the menstrual cycle. *J Invest Dermatol* 1966; **47**: 147-5.
 30. Beas F, Vargas L, Spada RP, Merchak N. Pseudoprecocious puberty in infants caused by a dermal ointment containing estrogen. *J Pediatr* 1969; **75**: 125-30.
 31. Im S, Lee ES, Kim W *et al.* Donor specific response of estrogen and progesterone on cultured human melanocytes. *J Korean Med Sci* 2002; **17**: 58-64.
 32. Ranson M, Posen S, Mason RS. Human melanocytes as a target tissue for hormones. in vitro studies with 1 alpha-25, dihydroxy vitamin D3, alpha-melanocyte stimulating hormone and beta-estradiol. *J Invest Dermatol* 1988; **91**:593-8.
 33. Kippenberger S, Lotisch S, Solano F *et al.* Quantification of tyrosinase, TRP-1 and TRP-2 transcripts in human melanocytes by reverse transcriptase- competitive multiplex PCR regulation by estroid hormones. *J Invest Dermatol* 1998; **110**: 364-7.
 34. Nussey SS, Wwhitehead SA, eds. *Endocrinology: An integrated approach*, 1st edn. Oxford: BIOS Scientific Publishers; 2001.