Stratum corneum integrity is better in vitiligenous skin of patients with Fitzpatrick skin type IV/V

N P A P Nelumdeniya[1], N Schuerer[2], G M P Sirimanna[3]

Sri Lanka Journal of Dermatology, 2012-2014, **16**, 3-7

Introduction

Skin pigmentation is primarily determined by the quantity of melanin[1]. Both the skin physiology and epidermal permeability barrier function vary with skin pigmentation types. For instance, the lighter and darker skins behave in different ways upon sun exposure[2]. Hydration of the skin and skin ageing[4] are different according to ethnicity[3]. In addition darker skin is less susceptible to *Candida albicans* infection[5]. Moreover, there is a significant difference in the transcutaneous penetration of nicotinates, and skin electric resistance[7] between highly and lightly pigmented skin[6].

The basic difference between Fitzpatrick skin types is due to quantity of melanin present and structural difference of melanocytes and melanosomes[8]. Main function of melanin is to protect the skin from ultra violet (UV) damage[9,10,11]. But melanin also plays an important role in regulating cutaneous immunity[12,13,14,15] and skin surface pH[16,17,18]. This is attributable to accelerated barrier recovery in dark skin[19].

However, regarding the difference in epidermal permeability barrier function in darker versus lighter skin, results are inconclusive. Some studies have shown that both basal trans epidermal water loss (TEWL) and percent increase in TEWL following tape stripping is higher in darker skin[20,21,22]. On the contrary, Singh *et al* reported that the basal TEWL was lower in darker skin[23]. In contrast, Reed et al reported that the basal TEWL in darker skin did not differ from that in lighter skin[24]. He further described that the stratum corneum (SC) integrity was significantly lower in lighter skin than in darker skin[24]. These data indicate that cutaneous functions vary with the degree of pigmentation.

Vitiligo is an organ-specific autoimmune disease. It is characterized by the development of well-defined milky white macules, devoid of identifiable melanocytes[25]. Differences in cutaneous biophysical properties between normal and vitiligenous skin has been described. For example, Keratinocytes from involved vitiligo skin have a shorter life span[26] and showed decreased levels of stem cell factor and increased apoptosis[27,28]. In addition stratum corneum (SC) is thicker in vitiligo-involved sites than that in uninvolved sites[29]. Furthermore J. Liu *et al.* described that barrier recovery following stratum corneum perturbation is delayed in vitiligo involved site than uninvolved sites[30].

However, the changes in skin biophysical properties including epidermal permeability barrier function in vitiligo in Fitzpatrick skin type IV/V remain largely unknown.

In current study, stratum corneum hydration, the skin surface pH, integrity and epidermal permeability barrier function in vitiligenous areas of Fitzpatrick skin type IV/V was evaluated.

Materials and methods

A total of 18 human volunteers with stable vitiligo vulgaris with skin types IV or V (Fitzpatrick classification) were enrolled to this study (15 new patients and 3 UV treated patients). Vitiligo was diagnosed clinically by the consultant dermatologist of skin clinic-B at NHSL, Colombo. A clinical examination was done to ensure exclusion of inflammation in both involved and uninvolved sites. All subjects were asked to refrain from using skin moisturizing agents or other topical agents 24 hours prior to and during study. All patients were advised not to wash measuring sites with soaps or surfactants for at least two hours prior to the study.

[1]*Senior Registrar in Dermatology, National Hospital of Sri Lanka, Colombo,* [2]*Professor of Dermatology, Department of Dermatology, University of Osnabrück, Germany,* [3]*Consultant Dermatologist, National Hospital of Sri Lanka, Colombo, Sri Lanka.*

Study was conducted in a controlled air conditioned environment. All subjects rested at 24-26°C, for 30 min before measurements were taken.

Measurements were taken on 6 test areas, each 3×3 cm in size. 3 areas (A,B,C) are from pigmented skin and 3 areas are from depigmented skin - (A,B,C).

Test site A was used to assess basal skin physiology (skin hydration and skin surface pH).

Test site B was used for stratum corneum integrity and barrier recovery.

Test site C was used for barrier cohesion test.

The basal TEWL, SC hydration, skin surface PH, were measured directly on both vitiligo-involved and contralateral uninvolved sites using respective probes (Figure-1).

1. Corneometer CM 825 (Courage and Khazaka, Cologne, Germany) - used to measure the basal skin hydration.
2. Tewameter T 300(Courage and Khazaka, Cologne, Germany) - used to measure the transepidermal water loss (TEWL).
3. Skin pH meter PH 905 (Courage and Khazaka, Cologne, Germany) - used to measure the skin surface pH.

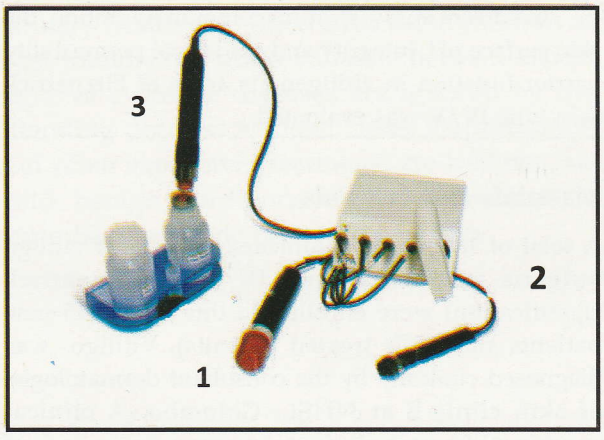

Figure-1

In the next step, for skin stripping 3M blender tape strips were applied to the test areas. These were homogeneously pressed on to the skin by moving a 5kg steel roller to and fro 5 times.

Barrier integrity was taken as number of strippings required to increase the baseline TEWL by three fold.

Barrier recovery was expressed as percentage. This was evaluated 24 hours after perturbation of the epidermal barrier, by measurement of transepidermal water loss and was calculated using following formula.

$$\text{Barrier recovery BR\%} = \frac{(\text{TEWL immediately after stripping} - \text{TEWL after 24hrs})}{(\text{TEWL immediately after stripping} - \text{baseline TEWL})} \times 100$$

Cohesion

To assess stratum corneum cohesion, sequential ten D-squame tapes were applied on the test site C and homogenously pressed on the skin by 5 kg steel roller to and fro 5 times. Each tape was removed and stored in different centrifuge tubes and labeled 1-10. Tubes from different patients were labeled and stored in different envelops. Amount of protein removed per tape on both involved and uninvolved area was estimated using a Bio-rad protein assay in the laboratory.

Statistics

The analytical software SPSS 17.0 was used for statistical analyses. Data will be expressed as mean and standard deviations. The unpaired Student t test was used to draw comparison and significance.

Results

SC hydration (Hydration; 39.2 +/-16.0 vs. 43.1+/-11.9; t = 0.84; p=0.410) of vitiligo-involved sites was not significantly different from that in contralateral uninvolved sites (Figure 3).

This study also demonstrated that the basal TEWL (basal TEWL: 7.04+/-3.09 vs. 6.99+/-1.84; t = 0.07; p=0.948) (Figure-2) and basal skin surface pH (pH:5.320+/-0.443 vs.5.323+/-0.396; t = 0.02; p=0.984) in vitiligo-involved sites (Figure 4) did not differ significantly from that in uninvolved sites.

Barrier recovery was also not significantly delayed in vitiligo-involved sites in comparison with contralateral uninvolved sites (Barrier recovery 0.504+/-0.171 vs. 0.462+/-0.233; t = 0.62; p=0.536) (Figure 5).

As shown in Figure 6, SC integrity between vitiligo-involved and contralateral uninvolved sites is significantly different. (Number of strips 55.7+/-39.2 vs. 30.2+/-12.0, t = 2.63, p=0.01)

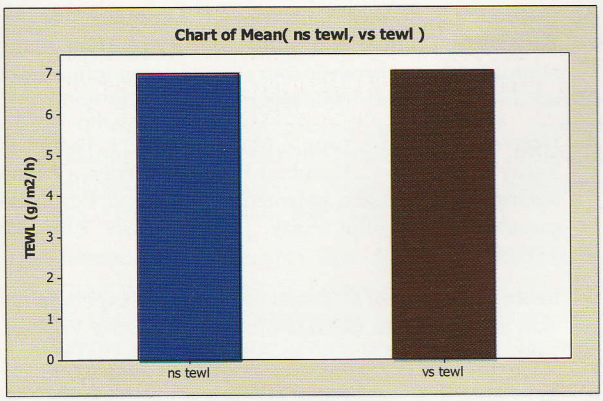

Figure 2.

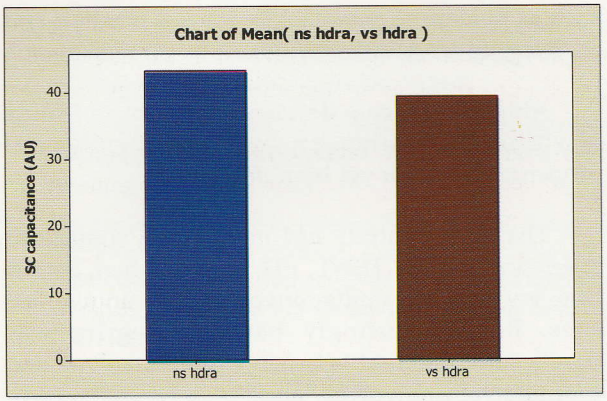

Figure 3.

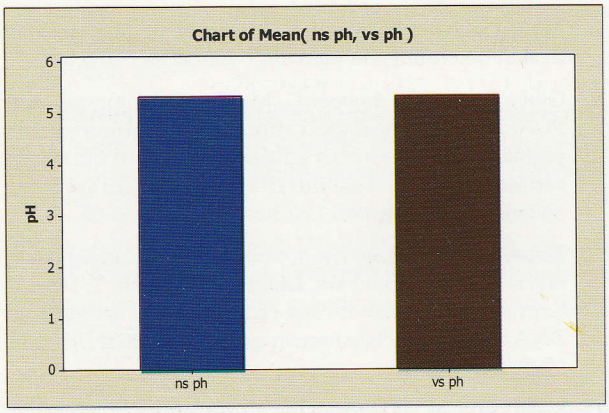

Figure 4.

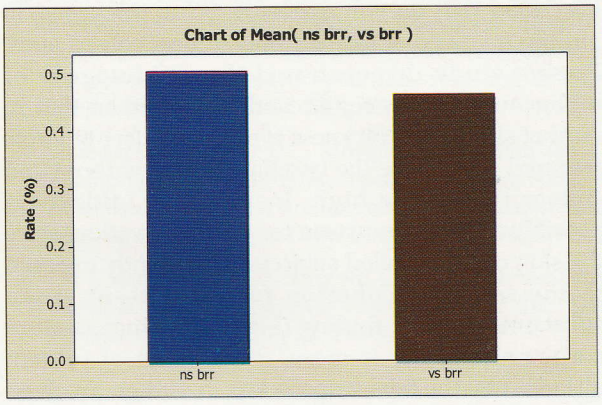

Figure 5.

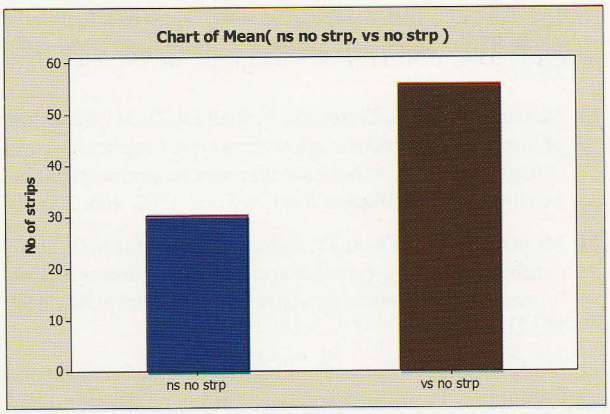

Figure 6.

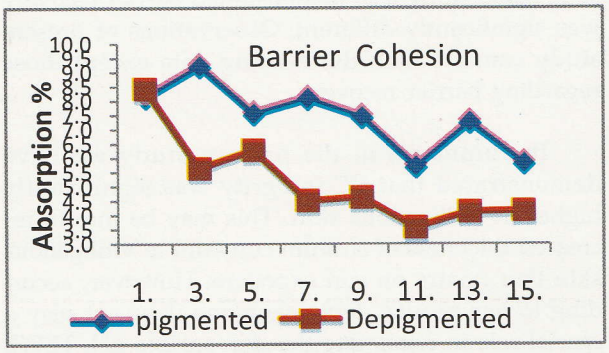

Figure 7.

Discussion

Prime function of melanocytes is to produce melanin which generates a natural protection against UV damage.

Melanocytes also play an important role in regulating cutaneous immunity, expressing sodium/hydrogen (Na +/H +) exchangers which are key regulators of maintaining skin pH[16], and regulating of epidermal proliferation[27,28,29].

In addition cutaneous biophysical properties

such as epidermal permeability barrier function and SC integrity are greatly influenced by pigmentation[19].

Although melanin deficiency is the main feature of vitiligo, alterations in cutaneous biophysical properties have not yet been defined.

The present study did not show a significant difference in basal TEWL, pH, SC hydration, barrier recovery between vitiligo-involved and uninvolved sites. But interestingly barrier integrity was significantly different between vitiligo-involved and contralateral uninvolved sites.

Keratinocytes of vitiligenous skin have a shorter life span[26], decreased levels of stem cell factor and increased apoptosis[27, 28]. However, Gniadecka *et al*[31] described that on sun exposure SC is thickened in vitiligo-involved sites than that in uninvolved sites. Present study demonstrated that SC integrity of vitiligenous skin is significantly higher than that in normal skin. If SC thickness is more, number of strips required to increase the baseline TEWL by three fold (integrity) will be high. Increased SC thickness possibly is the main reason for this observation, since the skin of the enrolled subjects is frequently exposed to the sun. The cohesion test of present study substantiate above finding (Figure 7). Since stratum corneum integrity is more, the amount of protein absorbed by the tape strips is less in vitiligo involved skin when compared with the normal skin.

Lue *et al*[30] demonstrated that there is no significant differences in TEWL, skin pH, SC integrity in vitiligo-involved and contra lateral uninvolved skin. Same study also demonstrated barrier recovery was significantly different. Observations of present study consists with the existing data except those regarding barrier recovery.

In summary, in the present study we have demonstrated that SC integrity was significantly higher in vitiligenous skin. This may be due to increased thickness of stratum corneum in vitiligenous skin that occurs on sun exposure. However, according to our results, depigmentation does not play a crucial role in regulating the skin surface pH, TEWL and barrier recovery in vitiligenous skin in Fitzpatrick skin type IV/V.

References

1. Rawlings AV. Ethnic skin types: are there differences in skin structure and function? *Int J Cosmet Sci.* 2006; **28**: 79-93.
2. Grimes P, Edison BL, Green BA, Wildnauer RH. Evaluation of inherent differences between African-American and white skin surface properties using subjective and objective measures. *Cutis.* 2004; **73**: 392-6.
3. Diridollou S, de Rigal J, Querleux B, Leroy F, Holloway Barbosa V. Comparative study of the hydration of the stratum corneum between four ethnic groups: influence of age. *Int J Dermatol.* 2007; **46**(suppl 1) : 11-14.
4. Nouveau-Richard S, Yang Z, Mac-Mary S, Li L, Bastien P, Tardy I, Bouillon C, Humbert P, de Lacharrière O. Skin ageing: a comparison between Chinese and European populations - a pilot study. *J Dermatol Sci.* 2005; **40**: 187-193.
5. Rebora A, Guarrera M. Racial differences in experimental skin infection with *Candida albicans*. *Acta Derm Venereol.* 1988; **68:** 165-8.
6. Berardesca E, Maibach HI. Racial differences in pharmacodynamic response to nicotinates in vivo in human skin: black and white. *Acta Derm Venereol.* 1990; **70**: 63-66.
7. Johnson LC, Corah NL. Racial differences in skin resistance. *Science* 1963; **139**: 766-7.
8. Matts PJ, Dykes PJ, Marks R. The distribution of melanin in skin determined in vivo. *BJD.* 2007; **156**: 620-8.
9. Gibbs S, Murli S, de Boer G, Mulder A, Mommaas AM, Ponec M. Melanosome capping of keratinocytes in pigmented reconstructed epidermis: effect of ultraviolet radiation and 3-isobutyl-1-methyl-xanthine on melanogenesis. *Pigment Cell Res.* 2000; **13**: 458-66.
10. Kobayashi N, Nakagawa A, Muramatsu T, Yamashina Y, Shirai T, Hashimoto MW, Ishigaki Y, Ohnishi T, Mori T. Supranuclear melanin caps reduce ultraviolet-induced DNA photoproducts in human epidermis. *J Invest Dermatol.* 1998; **110**: 806-10.
11. Kobayashi N, Muramatsu T, Yamashina Y, Shirai T, Ohnishi T, Mori T. Melanin reduces ultraviolet-induced DNA damage formation and killing rate in cultured human melanoma cells. *J Invest Dermatol.* 1993; **101**: 685-9.
12. Mohagheghpour N, Waleh N, Garger SJ, Dousman L, Grill LK, Tusé D. Synthetic melanin suppresses production of proinflammatory cytokines. *Cell Immunol.* 2000; **199**: 25-36.
13. Montefiori DC, Modliszewski A, Shaff DI, Zhou J. Inhibition of human immunodeficiency virus type 1 replication and cytopathicity by synthetic soluble catecholamine melanins in vitro. *Biochem Biophys Res Commun.* 1990; **168**: 200-5.
14. Montefiori DC, Zhou JY. Selective antiviral activity of synthetic soluble L-tyrosine and L-dopa melanins against human immunodeficiency virus in vitro. *Antiviral Res.* 1991; **15**: 11-25.
15. Sidibe S, Saal F, Rhodes-Feuillette A, Lagaye S, Pelicano L, Canivet M, Peries J, Dianoux L. Effects of serotonin and melanin on in vitro HIV-1 infection. *J Biol Regul Homeost Agents.* 1996; **10**: 19-24.
16. Behne MJ, Meyer JW, Hanson KM, Barry NP, Murata S, Crumrine D, Clegg RW, Gratton E, Holleran WM, Elias PM, Mauro TM. NHE1 regulates the stratum corneum permeability barrier homeostasis: microenvironment acidification assessed with fluorescence lifetime imaging. *J Biol Chem.* 2002; **277**: 47399-406.
17. Smith DR, Spaulding DT, Glenn HM, Fuller BB. The relationship between Na^+/H^+ exchanger expression and

tyrosinase activity in human melanocytes. *Exp Cell Res.* 2004; **298**: 521-34.

18. Ancans J, Tobin DJ, Hoogduijn MJ, Smit NP, Wakamatsu K, Thody AJ. Melanosomal pH controls rate of melanogenesis, eumelanin/phaeomelanin ratio and melanosome maturation in melanocytes and melanoma cells. *Exp Cell Res.* 2001; **268**: 26-35.
19. Gunathilake R, Schurer NY, Shoo BA, Celli A, Hachem JP, Crumrine D, Sirimanna G, Feingold KR, Mauro TM, Elias PM. pH-regulated mechanisms account for pigment-type differences in epidermal barrier function. *J Invest Dermatol.* 2009; **129**: 1719-29.
20. Wilson D, Berardesca E, Maibach HI. In vitro transepidermal water loss: differences between black and white human skin. *Br J Dermatol.* 1988; **119**: 647-52.
21. Kompaore F, Marty JP, Dupont C. In vivo evaluation of the stratum corneum barrier function in Blacks, Caucasians and Asians with two noninvasive methods. *Skin Pharmacol.* 1993; **6**: 200-7.
22. Berardesca E, Pirot F, Singh M, Maibach H. Differences in stratum corneum pH gradient when comparing white Caucasian and black African-American skin. *Br J Dermatol.* 1998; **139**: 855-7.
23. Singh J, Gross M, Sage B, Davis HT, Maibach HI. Effect of saline iontophoresis on skin barrier function and cutaneous irritation in four ethnic groups. *Food Chem Toxicol.* 2000; 38: 717-26.
24. Reed JT, Ghadially R, Elias PM. Skin type, but neither race nor gender, influence epidermal permeability barrier function. *Arch Dermatol.* 1995; **131**: 1134-8.
25. Wolff K, Freedberg IM, Fitzpatrick TB (eds). Fitzpatrick's dermatology in general medicine: 7th ed. Vol 1: New York: McGraw-Hill.
26. Bondanza S, Maurelli R, Paterna P, Migliore E, Giacomo FD, Primavera G, Paionni E, Dellambra E, Guerra L. Keratinocyte cultures from involved skin in vitiligo patients show an impaired in vitro behaviour. *Pigment Cell Res.* 2007; **20**: 288-300.
27. Lee AY, Kim NH, Choi WI, Youm YH. Less keratinocyte-derived factors related to more keratinocyte apoptosis in depigmented than normally pigmented suction-blistered epidermis may cause passive melanocyte death in vitiligo. *J Invest Dermatol.* 2005; **124**: 976-83.
28. Lee AY, Youm YH, Kim NH, Yang H, Choi WI. Keratinocytes in the depigmented epidermis of vitiligo are more vulnerable to trauma (suction) than keratinocytes in the normally pigmented epidermis, resulting in their apoptosis. *Br J Dermatol.* 2004; **151**: 995-1003.
29. Gniadecka M, Wulf HC, Mortensen NN, Poulsen T. Photoprotection in vitiligo and normal skin: a quantitative assessment of the role of stratum corneum, viable epidermis and pigmentation. *Acta Derm Venereol.* 1996; **76**: 429-32.
30. Liu J, Man WY, Lv CZ, Song SP. Epidermal permeability barrier recovery is delayed in vitiligo-involved sites. *Skin Pharmacol Physiol.* 2010; **23**: 193-200.