Egyptian Dermatology Online Journal
Volume 7 Number 2

Immunological skin tests and hematological indices in Nigerian users of skin lightening creams

Arinola O.G, Afolabi K.A, *Olopade C.O

Department of Chemical Pathology and Immunology, College of Medicine, University of Ibadan, Nigeria,
*Section of Pulmonary and Critical Care, University of Chicago, Chicago, Illinois

Egyptian Dermatology Online Journal 7 (2):3

Correspondence: Arinola O.G (PhD).
E-mail: drarinolaog64@yahoo.com

Submitted: October 26, 2010
Accepted: July 10, 2011

Abstract

Background/Objective: Skin lightening is a common practice in various black communities worldwide. This affects the epidermal layer of the skin that plays important role in innate immune function of the skin. This study was carried out to assess immunologic skin tests and hematological indices in the users of skin lightening creams.

Subjects and Methods: Sixty participants took part in this study. Thirty of them were those who had used skin lightening creams for at least two years while the remaining thirty who had never bleached served as controls. The laboratory investigations performed included full blood count, packed cell volume (PCV), total and differential white blood cell count), Mantoux skin test (In vivo measurement of Type IV hypersensitivity) and skin prick test (In vivo detection of Type 1 hypersensitivity).

Results: The mean values of PCV and neutrophils were significantly lower while the mean value of lymphocytes was higher in the users of skin lightening creams when compared with the controls. There was significantly increased diameter of skin reaction to Mantoux test in the users of skin lightening creams when compared with the controls. Skin prick test also showed significantly increased reaction diameter with dog epithelia antigen in the users of skin lightening creams when compared with controls. Significantly higher proportions of the users of skin lightening creams were positive to GS2 cockroach antigen, standardized mite antigen and mouse epithelia antigen when compared with the controls.

Conclusion: The present study shows that skin lightening creams cause disruption in the normal immunologic functions (Types I and IV hypersensitivity states) of the skin and certain hematological parameters. There is need for public awareness programs to enlighten the populace about the danger involved in the practice of skin lightening.

Introduction

The surface of the skin is a primary site for the deposition and introduction of microorganisms. The outermost skin layer (the epidermis) provides the first line of defense against pathogens [1]. Therefore, the deeper layers of the skin (dermis) remains free of infection suggesting that skin has the ability to protect against invading microbes.

Lightening of skin is practiced by black people at all ages [2], and in both sexes with higher prevalence in young, unmarried and educated women [3]. Skin lightening involves the use of a wide range of products, applied to specific or widespread area of the skin to lighten the normal black skin for fame, cosmetic purpose and also due to misconceptions about the presumed superiority and desirability of fair skin [4]. Most skin lightening creams contain mercury, lemon, citric acid and even cement water. Various local skin lightening creams have no identified active agent [9].

Studies have shown that 52.7%, 25%, 39%, and 49% of women use lightening agents in Dakar (Senegal), Bamako (Mali), Quagadougou and Bobo-Dioulasso (Burkina Faso) respectively [5]. A similar study also revealed an increased in prevalence of skin lightening cream users in Lagos, Nigeria [5].

Skin lightening disrupts primary innate immune function of the epidermal skin bleaching skin leading to susceptibility of the users to localized or systemic infections since lightening creams used for long duration, on a large body surface area and under hot humid conditions enhanced percutaneous absorption [6]. In addition, higher susceptibility to infections in these people may lead to an increased of phagocytes in response to infections which generated free radicals, increased utilization of antioxidants; thus lowering the antioxidant potential [7] which may lead to a state of oxidative stress and increased in the rates of skin cancer [8]. Other side effects of skin lightening creams are damage of elastic fibers of the skin, skin wrinkling, ochronosis, acrodynia, contact allergy, stretch marks and ache [9]. This study is designed to investigate the possible effects of skin lightening cream on skin immune status by determining the skin responses to environmental antigens. Hematogical indices of the users were also assessed.

Subjects and Methods

A total number of sixty (60) subjects participated in this study. Thirty (30) of them were skin bleachers who had used lightening creams for average of 4.9 years. Another thirty (30) apparently healthy people who had never use skin lighten creams served as controls. The subjects were recruited from various locations within the city of Ibadan, Nigeria. All the participants gave willing consent after the nature and objectives of the study had been explained to them. Those with history of asthma, tuberculosis infection or had BCG vaccine (confirmed by the mark at the upper arm) as well on medication (e.g. immunosuppressive or antibiotic drugs) were excluded from the study. The study had the approval of the University of Ibadan/University College Hospital Joint Ethical Review Committee, Ibadan, Nigeria.

a. Determination of percentage PCV and counting of white blood cells:

Two (2) ml of blood specimen was collected from each participant into an EDTA tube for full blood count. PCV was determined by standard method. Well mixed whole blood was taken into the capillary tube through capillary attraction. One end was sealed and the sealed capillary tube

positioned correctly in the hematocrit centrifuge. The centrifuge was switched on at 10,000 rpm for 5 minutes. The percent ratio of the packed red blood cells to the whole blood is the PCV read on the hematocrit reader.

White blood cells were counted as described in standard texts. Diluting fluid (0.38ml) was dispensed into a tube and 20µl of blood sample was added. Improved Neubauer ruled counting chamber (hemocytometer) was filled by holding capillary tube, containing the blood sample, at an angle 450 and slightly touched the tip against the edge of the coverglass. The chamber was left undisturbed for 2 minutes after which it was placed on microscope stage and the white blood cells were counted in a specified area using 10 x objective.

b. Skin Prick Test:

The test was performed on the forearm. The forearm was sterilized with methylated spirit, and a drop of commercially - produced allergenic extracts (Standardized cat hair, Dog epithelia mixed breeds, GS9 Southern grass mix, GS2 Cockroach mix, Mango blossom Magnifera indica, Mixture of standardized mite, Mouse epithelia and GS mold #3 (GREER brand, USA) were placed onto marked areas of the skin using a dropper. Histamine was used as a positive control and saline solution as the negative control. With a sterile special lancet, a small prick through the drops was made. This was to allow a small amount of allergens to enter the skin. The diameter of induration was measured after 20 minutes of skin prick using ruler. The diameter of induration greater than 3mm is regarded as positive.

c. Mantoux test:

The test involved the injection 0.1ml of 5IU of purified protein derivative (PPD) into the skin intradermally using insulin syringe after been sterilized with alcohol. The subjects were instructed not to scratch the site of injection and the area was examined at 48-72 hours after the injection. The wheal and flare reaction was measured by measuring the diameter of induration with meter ruler.

Results

Sixty participants took part in this study. Thirty of these were those using skin lightening creams while the remaining thirty that were not using skin lightening cream served as controls. All participants in this study were females. The age mean of subjects was 26.9±2.7 years while that of controls was 25.9±3.8years. There was no statistically significant difference between the ages of the test and control subjects.

In Table 1, the results of full blood count comprising packed cell volume (PCV), total and differential while blood cells count were presented. The mean percentage value of PCV in test subjects was 36 ±3 % while it was 38± 2% in controls. There was statistically significant decrease in the mean level of test subjects when compared with controls. The mean value of total white blood cells count in test subjects was 5,200±1100/mm^3 while it was 5,400±900/mm^3 in controls. There was no statistically significant difference in the mean value of test subjects compared with controls. The mean value of neutrophils in test subjects was 2,900±69/mm^3 while it was 3,200±43/mm^3 in controls. There was statistically significant decrease in the mean value of test subjects when compared with controls. The mean value of lymphocytes in test subjects was 2,200±64/mm^3 while it was 2,000±46/mm^3 in controls. There was statistically significant increase in the mean value of subjects when compared with controls. The mean values of monocytes,

eosinophils, basophils in the test subjects were 125±11/mm^3, 42±9/mm^3, and 4±3/mm^3 while corresponding values in the controls were 119±7/mm^3, 34±5/mm^3, and 4±2/mm^3. There were no statistically significant differences.

Table 2 presents comparison (Mean ± S.D) of diameter of skin reaction during Mantoux and skin prick test. The mean diameter of Mantoux test in skin lightening cream users was 4.2±2.8mm while it was 1.4±2.3mm in controls. There was statistically significant difference in the mean value of bleachers when compared with controls. The mean diameter of skin prick test using allergic extracts of standardized cat hair, dog epithelia mixed breeds, GS9 southern grass mix, GS2 cockroach mix, mango blossom Magnifera indica, mixture of standardized mite, mouse epithelia and GS mold mix #3 were 2.8±0.9mm, 3.3±0.5mm, 3.0±0.9mm, 3.3±0.7mm, 2.6±0.8mm, 2.9±9mm, 3.0±0.9mm, 2.1±0.3mm respectively while the corresponding values in controls were 2.0±0.6mm 2.6±0.5mm, 3.3±0.5mm, 3.0±0.8mm, 2.5±0.8mm, 2.3±.0.5mm, 2.3mm±0.2mm. There was statistically significant increase in skin prick diameter in test subjects using dog epithelia mixed breeds antigen when compared with controls while other allergic extracts showed no statistically significant differences in test subjects compared with controls.

Table 3 presents the comparison (Chi-square) prevalence of positive skin prick reactions in subjects and controls. The prevalence of positive skin prick reactions in the subjects using allergic extracts of standardized cat hair, dog epithelia mixed breeds, GS9 southern grass mix, GS2 cockroach mix, mango blossom Magnifera indica, mixture of standardized mite, mouse epithelia and GS Mold mix #3 were 6.7%, 20%, 16.7%, 56.7%, 13.3%, 26.7%, 23.3%, 3.3% respectively while the corresponding prevalence in controls were 0%, 16.7%, 13.3% 23.3% 10.0%, 6.7% 3.3%, 0%. There were significant higher prevalence of positive skin reactions to GS2 cockroach mix and mixture of standardized mite in the subjects when compared with controls.

Parameters	Tests (n=30)	Controls (n=30)	t-	p
PCV (%)	36±3	38±2	2.732	0.008*
WBC (/mm^3)	5,200±1,100	5,400±900	0.916	0.346
Neut. (/$mm^{3)}$	2,900±69	3,200±43	3.27	0.002*
Lymph (/mm^3)	2,200±64	2,000±46	3.388	0.001*
Mono. (/mm^3)	125±11	119±7	0.727	0.47
Eosin. (/mm^3)	42±9	34±5	0.901	0.371
Baso. (/mm^3)	4±3	4±2	1.439	0.155

*=significantly different from the controls.

Table1. Mean and standard deviation of Full Blood Count in test subjects compared with controls.

Parameters (mm)	Test (n=30)	Controls (n=30)	t-	p
Mantoux	4.2±2.8	1.4±2.3	4.266	0.000*
Standardized cat hair	2.8±0.9	2.0±0.6	0.701	0.534
Dog epithelia mixed breeds	3.3±0.5	2.6±0.5	2.53	0.026*
GS9 Southern grass mix	3.0±0.9	2.6±0.5	1.075	0.302
GS2 Cockroach mix	3.3±0.7	3.3± 0.5	0.167	0.869
Mango blossom-Magnifera indica	2.6± 0.8	3.0±0.8	0.808	0.435
Mixture of standardized mite	2.9±0.9	2.5±0.8	0.854	0.404
Mouse epithelia	3.0±0.9	2.3±0.5	1.566	0.141
GS mold mix #3	2.1±0.3	2.3±0.2	0.289	0.779

*=significantly different from the controls.

Table 2: The diameter (Mean ± S.D) of skin reaction to various antigens in test subjects and controls.

Parameters	Positive	Negative	Chi-square	P
Standardized cat hair:				
Test subjects	2	28		
Controls	0	30	2.069	0.15
Dog epithelia:				
Test subjects	6	24		
Controls	5	25	0.111	0.739
GS9 Southern Grass:				
Test subjects	5	25		
Controls	4	26	0.131	0.718
GS2 Cockroach Mix:				
Test subjects	17	13		
Controls	7	23	6.944	0.008*
Mango blossom:				
Test subjects	4	26		
Controls	3	27	0.162	0.688
Mixture of mites:				
Subjects	8	22		
Control	2	28	4.32	0.038*
Mouse Epithelia:				
Test subjects	7	23		
Controls	1	29	5.192	0.023*
GS mold Mix #3:				
Test subjects	1	29		
Controls	0	30	1.017	0.313

*=significantly different from the controls.

Table 3: The comparison of prevalence of positive skin prick reaction in test subjects and controls.

Discussion

The epidermis layer of the skin plays important role in the body immunological defense against pathogens. To knowledge of the investigator of the present study, no report has related the act of skin lightening with disruption of innate immune function of the skin.

Whenever the skin immune defense mechanism is impaired, the skin is expected to become prone to various infections. The presence of infectious agents will mobilize the polymorphonuclear cells (PMN) to the site of infection, thereby causing reduced circulating neutrophils. In this study,

the mean level of circulating neutrophils was found to be significantly lower while the mean level of circulating lymphocytes was found to be significantly higher in test subjects compared with the controls. Thus, suggesting possibility of localized infection of the skin. It was previously reported that in a localized bacterial infection, there is mobilization of the blood neutrophils to the sites of the infection causing reduced level of circulating in the system circulating [11]. Moreover, users of skin lightening creams have been reported to be prone to various skin infections of bacterial or fungi origins [10]. There was no such report or finding in the users of lightening creams in this environment.

The mean level of packed cell volume in test subjects was significantly lower in the test when compared with the controls. This might be due to RBC destruction by skin lightening creams that entered blood circulation through permeabilised skin. According to Halder and Nootheti [12], free radicals are generated by components of skin lightening creams which causes red blood cell membrane damage through lipid peroxidation or direct RBC attack.

Positive Mantoux skin test reaction suggests the presence of activated phagocytes due to infection by *Mycobacterium tuberculosis* or environmental Mycobacteria. The diameter of Mantoux skin reaction to purified protein antigen was significantly higher in test subjects compared with controls. The Mantoux skin reaction diameter of 10mm and above is accepted as exposure to *M. tuberculosis,* therefore part of diagnosis of pulmonary tuberculosis. The result of larger diameter of Mantoux test in subjects using skin lightening creams could not be readily explained but it may be hypothesized that may be cross-reaction antigens in the skin of lightening cream users that mimicked *Mycobacterium tuberculosis*.

The skin prick test is a diagnostic tool in the assessment of immediate hypersensitivity. The results from skin prick tests can be used to guide in the management of patient with asthma and hay fever, e.g. desensitization to a certain allergen, removal of a family pet, and avoidance of certain foods. The mechanism of reaction involves preferential production of IgE which has very high affinity for its receptor on mast cells and basophils. These mast cells may also be triggered by chemicals but such reaction mediated by chemical agents without IgE-allergen interactions are not classical Type 1 hypersensitive reactions. In the present study, the users of skin lightening creams were found to positively react to dog epithelia mixed breeds, GS2 Cockroach mix, and mixture of standardized mite and mouse epithelia when compared with controls. This suggested possible sensitization of immune cells in those using skin bleaching creams probably due to chemical assault on the skin. The results of skin prick test might also be due to the fact that certain components of skin lightening cream mimicked immune responses to dog epithelia, cockroach, mite and mouse epithelia allergens. Biebl and Warshaw [13] previously reported the occurrence of contact dermatitis cosmetic users.

In conclusion, the skin lightening creams disrupts the normal immunologic functions of the skin. Thus, the use of Mantoux skin test to screen for tuberculosis infection or Type IV hypersensitivity state and the use of skin prick test to screen for Type 1 allergic reaction to environmental allergen may not be useful to the users of skin lightening creams.

References

1. Gorbach SL. Lactic acid bacteria and human health. Ann Med. 1990; 22:37-41.

2. Bongiorno MR, Arico M. Exogenous ochronosis and striae atrophicae following the use of bleaching creams. Int J Dermatol. 2005; 44:112-115.

3. Traore A, Kadeba J-C, Niamba P, Barro F, Ouedraogo L. Use of cutaneous de-pigmenting products by women in two towns in Burkina Faso: epidemiologic data, motivations, products and side effects. Int J Dermatol. 2005; 44 Suppl 1:30-32.

4. Ajose FOA. Consequences of skin bleaching in Nigerian men and women. Int J Dermatol. 2005; 44 Suppl 1:41-43.

5. Mahe A, Blanc L, Halna JM, et al. [An epidemiologic survey on the cosmetic use of bleaching agents by the women of Bamako (Mali)]. Ann Dermatol Venereol. 1993; 120:870-873.

6. Olumide YM, Akinkugbe AO, Altraide D, et al. Complications of chronic use of skin lightening cosmetics. Int J Dermatol. 2008; 47:344-353.

7. Babior BM. The respiratory burst of phagocytes. J Clin Invest. 1984; 73:599-601.

8. Taylor SC. Skin of color: biology, structure, function, and implications for dermatologic disease. J Am Acad Dermatol. 2002; 46:S41-S62.

9. Engler DE. Mercury "bleaching" creams. J Am Acad Dermatol. 2005; 52:1113-1114.

10. Mahe A, Ly F, Aymard G, Dangou JM. Skin diseases associated with the cosmetic use of bleaching products in women from Dakar, Senegal. Br J Dermatol. 2003; 148:493-500.

11. Lubbe J. Secondary infections in patients with atopic dermatitis. Am J Clin Dermatol. 2003; 4:641-654.

12. Halder RM, Nootheti PK. Ethnic skin disorders overview. J Am Acad Dermatol. 2003; 48:S143-S148.

13. Biebl KA, Warshaw EM. Allergic contact dermatitis to cosmetics. Dermatol Clin. 2006; 24:215-32, vii.