Original Article

Assessment of acute phase proteins and oxidative stress status of Nigerians using bleaching agents

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ABSTRACT

Objective: The disruption of primary innate immune function of the epidermal layer of the skin accounts for the susceptibility of individuals using bleaching agents to localized or systemic infections. This subverted innate immunity in these people may lead to other pathological conditions. The resultant effects of skin bleaching and phagocytes activation in response to infections have not been studied in Nigerians using bleaching agents. The present study therefore assessed the levels of C-reactive protein (CRP), albumin, total antioxidant potential (TAP), total plasma peroxides (TPP), oxidative stress index (OSI) and malondialdehyde (MDA) in the users bleaching agents.

Methodology: Thirty (30) people who had used bleaching agents for average of 4.9+1.2 years participated in this study. They were recruited from various schools and markets within the city of Ibadan, Oyo State, Nigeria. Thirty apparently healthy staffs of University College Hospital Ibadan, Ibaadan, Nigeria, who had never used bleaching agents served as controls. All the subjects used for this study had no metabolic abnormality and tested negative to both HIV and hepatitis B infections.

Result: The mean value of TAP ($p < 0.01$) was significantly lower in individuals using bleaching agents when compared with the controls. The mean levels of CRP ($p<0.01$), TPP ($p <0.01$), OSI ($p<0.01$) and MDA ($p <0.05$) were significantly higher in the users of bleaching agents when compared with the controls. But there was no significant difference in the mean value of albumin ($p>0.20$) when compared with the controls.

Conclusion: Oxidative stress and chronic inflammation are possible consequences of skin bleaching. The users of skin bleaching agents may need antioxidant therapies to avert the risks of oxidative stress.

KEY WORDS: Skin bleaching, Oxidative stress, Acute phase proteins.

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INTRODUCITON

Bleaching agents are common cosmetics used to lighten complexion or improve the appearance of the skin.[1,2] The bleaching agents, in the process of lightening the skin disrupt the primary innate immune function of the epidermal layer of the skin and make the users prone to various infections such as bacterial, fungal, parasitic and viral infection.[3] Infection of the epidermal layer of the skin leads to the activation of the phagocytes, production of cytokines, and mobilization of circulating neutrophils to the site of infection.[4-6] The activation of the

phagocytes then leads to the production of free radicals.[7,8]

Release of inflammatory cytokines initiates the production of spectrum of acute phase proteins (such as C-reactive protein, haptoglobin, transferrin etc) by the liver cells. C-reactive protein (CRP) is considered as one of the initiators of opsonization, phagocytosis and lysis of invading organism such as bacterial and viruses. The C-reactive protein activates macrophages, possesses anti-proteolytic activity and presumably block the migration of cells into the lumen of blood vessels thus helping to prevent the establishment of a generalized systemic inflammation.[9]

CRP is complexed in the presence of cations such as Ca^{2+}, activates the classical complement pathway and binds to membrane phosphorylcholine complement activation. Plasma level of CRP usually rises dramatically after myocardial infarction, stress, trauma, infection, inflammation, and surgery or neoplasm proliferation. The serum concentrations of CRP that increase several hundred folds in response to bacterial infection therefore make it an attractive diagnostic test for neonatal sepsis.[8]

The constituents of some bleaching agents activate the phagocytes or cause allergic reaction. The consequence of phagocyte activation is the oxidative burst characterized by high plasma levels of reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI). The ROS and RNI are by-products of cellular metabolism that have the potential to damage the various intracellular organelles and components (nucleic acid, lipid, proteins) on which normal cells depend.[7] The pathological effects of free radical attack include lipid peroxidation, gene mutation, enzyme and protein denaturation and cell membrane damage.[7]

Mahe et al[10] stated that prolong use of bleaching agents causes the loss of protective effect of melanin pigment which leads to an increased risk of skin cancer. Some bleaching agents containing steroids increase the risk of infection by fungi and scabies.[11,12] Other consequences of prolong exposure to bleaching agents are high blood pressure, diabetes, neurological and kidney problems.[13]

Although the disruption of the epidermal layer of the skin and the susceptibility of the users of bleaching agents to various infectious agents have been established, there is no previous study showing the influence of bleaching agents on the levels of markers of oxidative stress and CRP. This study was therefore designed to bridge this gap in knowledge by assessing the levels of CRP, albumin, TPP, OSI, TAP and MDA in Nigerians using bleaching agents.

METHODOLOGY

Thirty (30) Nigerians who had used bleaching agents for average of 4.9+1.2 years volunteered to participate in this study. Another thirty apparently healthy Nigerians who had never used bleaching agents served as controls. The subjects were recruited from various schools and markets within the City of Ibadan, Oyo State. Nigeria. Five (5) ml of blood was collected from each participant into heparin container, separated within one hour and stored at -20°C for a week before analyses.

Estimation of MDA: Level of lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) using the method of Varshney and Kale.[14] The principle is based on the fact that malondiahydehyde (MDA) produced from the peroxidiation of membrane fatty acid, reacts with the chromogenic reagent; 2-thiobarbituric acid (TBA) under acidic conditions to yield a pink–coloured complex measured spectrophotometrically at 532nm. 1, 1, 3, 3-tetramethoxylpropane was used as standard.

Estimation of TAP: TAP was determined using the ferric reducing / antioxidant power (FRAP) assay.[15,16] 1.5 ml of working pre-wormed 37°C FRAP reagent (300mM acetate buffer - pH 3.6, 10mM 2,4,6-tripyridyl-s-triazine in 40mM HCl and 20mM $FeCl_3$ at ratio 10:1:1) was vortex mixed with 50µl of test sample and standards. Absorbance was read at 593 nm against a reagent blank. The result was reported as µmol Trolox equiv. / L.

Estimation of total plasma peroxide (TPP):

Principle: Ferrous-butylated hydroxytoluene-xylenol orange complex reacts with plasma hydrogen peroxide to form a colour complex measured spectrophotometrically at 560mm. H_2O_2 was used as standard.[16] 1.8ml of reagent 6 (F0X2) was mixed with 200µ1 of plasma. This was incubated at room temperature for 30 minutes. 100µMol H_2O_2 was used as standard. The mixture was centrifuged and the supernatant separated for reading at 560nm.

Determination of oxidative stress index (OSI): OSI, an indicator of the degree of oxidative stress is the percent ratio of the total plasma peroxide (µmol H_2O_2 / L) to the total antioxidant activity (µmol/L)[16]

Estimation of albumin: The albumin concentration was determined by using a commercially prepared reagent (brilliant cresol green solution) purchased from Dialab Production and Vertrieb vonchemichtechnischen, Wien- Panikengasse. Albumin is a marker of secretory function of the liver and was used to assess the secretory activities of the liver in PTB patients before and after treatment.

Table-I: Levels of CRP and albumin in users of bleaching agents and controls.

	Users of bleaching agents	*Controls*	*t-value*	*p-values*
CRP (mg/dL)	15.7±6.2	6.2±1.4	8.3	<0.01*
Albumin (g/dL)	4.0±1.4	4.2±0.9	0.9	>0.2
N	30	30		

* = significantly different from the controls.

Estimation of CRP: CRP was quantified by single radial immunodiffusion method.[17] A volume of an optimally diluted anti-CRP antiserum was mixed with noble agar and poured on glass plate. Wells of equal diameters were cut in the antibody-agar mixture. The wells were filled with test or standard sera. After incubation, the diameters of precipitin rings were measured using a Hyland viewer with a micrometer eyepiece.

RESULT

Table-I show that the mean level of CRP (15.7+6.2 mg/dL) increased significantly in those who used bleaching agents when compared with the controls (6.2+1.4 mg/dL). But there was no significant change in the mean level of albumin (4.0+1.4g/dL) when compared with the controls (4.2+0.9g/dL).

Table-II shows the mean level of TAP (1258+373µmol Trolox equiv/l) was significantly decreased in those who used bleaching agents when compared with the controls (1825+456µmol Trolox equiv/l). There were significantly higher levels of MDA (8.7+3.9 nmol/ml), TPP (18+3.20µMol H_2O_2/ L) and OSI (1.44+0.9%) in those who used bleaching agents when compared with the controls (5.9+2.2 nmol/ml, 10.4+2.8µMol H_2O_2/ L and 0.64+0.6% respectively).

DISCUSSION

Significantly higher level of CRP was observed in the users of bleaching agents recruited for this study, when compared with the controls. This finding could be due to the response of the hepatocytes to the skin infections commonly encountered in the users of bleaching agents. Ikawa et al[18] linked the activities of the phagocytes in an infected tissue to the increased production of cytokines and complement factors which stimulate all hepatocytes to produce the entire spectrum of acute phase proteins.[18] The acute phase proteins regulate immune responses, function as mediators and inhibitors of inflammation, act as transport proteins for products generated during the inflammatory process (the haem-binding protein hemopexin, and haptoglobin), function as opsonins, activate complement, bind cellular remnants like nuclear fractions[9,18], scavenge free haemoglobin and radicals, modulate the host's immune response, prevent apoptosis and / or play an active role in tissue repair and tissue remodeling. Therefore, it can be inferred from this study that increased level of CRP is a protective immunologic measure, employed by the hepatocytes to reduce the risks associated with the use of bleaching agents.

Albumin is a negative acute phase protein and a chain breaking antioxidant that intercepts damaging free radical species. It is a sacrificial molecule that protects other molecules from free radical attack, and the plasma concentration decreases when performing this antioxidant role.[19] The mean level of albumin in the users of bleaching agents recruited for this study did not show any significant change when compared with the controls. Since there is no previous report on the level of albumin in the users of bleaching agents, it could be inferred from this study that the activities of the liver in the users of bleaching agents increase in response to the cytokines and complement factors generated through the skin infection. Whether the albumin level will be exhausted

Table-II: Levels of TAP, TPP, OSI and MDA in users of bleaching agents and controls.

	N	*TAP(µMol Trolox equiv./L)*	*MDA (nMol/ml)*	*TPP(µMol H_2O_2/L)*	*OSI(%)*
Users of Bleaching agents	30	1258±373	8.7±3.9	18±3.20	1.44±0.9
Control	30	1825±456	5.9±2.2	10.4±2.8	0.64±0.6
t, p value		7.4, <0.01*	3.4,<0.05*	8.2,<0.01*	5.8,<0.01*

*= significantly different from the controls.

in prolonged use of bleaching agents is to be investigated by interested workers in future.

In this study, significantly higher level of TPP was observed in the users of bleaching agents when compared with the controls. This higher level of TPP in this study could be due to the continuous activation of the phagocytes as a result of the disrupted epidermal layer of the skin that predisposes the users of bleaching agents to fungal, bacteria and parasitic infections. Increased TPP in the users of bleaching agents could also result from the metabolism of these bleaching agents. This study is the first to establish higher level of TPP in the users of bleaching agents. But evidences from previous workers show that the disrupted epidermal layer of the skin in these subjects predispose them to several infectious agents, such as bacteria, fungi and scabies infections[10,20] which ultimately lead to activation of phagocytes and production of free radicals.[7,8]

Significantly lower level of TAP was observed in the users of bleaching agents. The lower level of TAP in these users of bleaching agents could be due to the exhaustion of the antioxidants, in the process of neutralization of excess free radicals generated from the activated phagocytes. Under normal physiological conditions, the free radical load is controlled by both enzymatic and non-enzymatic antioxidants.[7,15] But in a chronic infection, the antioxidant system is overridden and the balance between the free radicals and antioxidant system shifts in favour of the free radicals.[7] Our result agrees with the previous reports that increased rate of antioxidant utilization in chronic infections result in antioxidant depletion.[7] This imbalance in the levels of TPP and TAP in the users of bleaching agents may account for the significantly higher level of OSI observed in this study. Significantly higher level of MDA observed in the users of bleaching agents is an indication of higher lipid peroxidation and a marker of oxidative stress.[21] This increased level of lipid peroxidation in the users of bleaching agents could be associated with high free radical load (TPP) observed in them, since lipid peroxidation is the consequence of free radical attack on the poly unsaturated fatty acids.[14,16]

In conclusion, oxidative stress, lipid peroxidation and inflammatory responses are features of skin bleaching. Users of bleaching agents may therefore need antioxidant therapy to avert the risk of oxidative stress in them.

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