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Altered Copper Level and Renal Dysfunction in Nigerian Women Using Skin-Whitening Agents

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Abstract The purpose of the study was to determine the concentration of trace elements in serum samples of women who are chronic users of skin-whitening agents as well as the hepatic and renal effects of these agents on these women. The study was conducted among 23 skin-whitening users while 25 women served as the controls. The serum concentrations of Zn, Mn, and Mg were not significantly changed in these women compared with controls ($p>0.05$), but Cu was significantly increased in skin-whitening agent users compared with controls ($p<0.05$). Serum urea and creatinine were significantly increased compared with the controls ($p<0.05$). Moreover, ALT, AST, albumin, total protein, and bilirubin were not significantly changed ($p>0.05$). The significant increase in the levels of renal indices shows that these agents might be nephrotoxic after prolonged usage while increase in copper level with accompanying renal dysfunction may be an indication that copper mediates in oxidative-induced renal dysfunction. However, further study is needed to identify the cause and source of high serum copper as many of the herbal extracts may be rich sources of copper. Moreover, a large population study may be necessary to examine the exact correlation between copper and renal indices.

Keywords Copper · Renal dysfunction · Skin-bleaching agents · Herbal extract

Introduction

Hydroquinone, like other cutaneous depigmenting agents, is used widely by dermatologists to treat pigmentary disorders. In sub-Saharan Africa, the use of skin-whitening or bleaching agents is a highly prevalent cultural practice of applying these agents to the whole or some parts of the body; this is especially common among dark-skinned women [1]. In Mali,

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bleaching is particularly common in unmarried and literate women as well as female students [2]. Whereas Sylla et al. [3] have identified that females of all ages and of different social status use these agents in Dakar (Senegal). Nnoruka and Okoye [4] also confirmed the presence of active ingredients for skin whitening in cosmetics and toiletries of patients who attended the dermatology clinic of the University of Nigeria Teaching Hospital, Enugu (Nigeria).

The use of skin lightening (bleaching) cosmetics and toiletries is not restricted to the sub-Saharan Africa. A link between skin-bleaching agents and exogenous ochronosis has been reported in some other parts of the world [5, 6]. Apart from exogenous ochronosis, these agents are also associated with a wide range of side effects, e.g., mercury poisoning, dyschromia, acne, and hypertrichosis [7–10]. In some cases, systemic complications such as hypertension, hypercorticism, and mercurial nephropathy have also been identified [1]. Their cytotoxic effects have also been established; specifically, they exhibited both chromatoclassic and mitoclassic effects, and these increased with increase in duration of exposure [11].

Skin-bleaching agents act by altering melanin formation through a decrease in the activity of the enzyme tyrosinase [12, 13]. The possibility of cancer is the likely long-term effect, especially as renal adenomas and leukemia were induced in experimental animals, an indication that these agents may be both nephrotoxic and carcinogenic. Moreover, exposure to Hg during critical developmental periods has been implicated to play a role in the development of autism spectrum disorders [14]. This is especially dangerous because many of these users are still within the reproductive period [15]. The purpose of this study is to determine the effect of these agents on serum levels of Zn, Cu, Mn, and Mg and the contribution of a possible alteration in their levels to the widely reported association between skin-whitening agent usage and whitening cream-induced nephropathy.

Methodology

Participants for this study were women selected randomly from some saloons in the city of Ibadan. Informed consent was obtained from each participant. Each participant filled a detailed standardized questionnaire through which information on age, duration of exposure, type of whitening or bleaching cream used, frequency or consistency of exposure, and reason for usage were obtained from each participant. Twenty-three females who had used bleaching cream for a prolonged period of at least 5 years were assigned to the group of skin-whitening agent users. They were aged 29–55 years and the duration of exposure ranged from 5 to 30 years.

Exclusion criteria included the following conditions: pregnancy, lactation, malnutrition, malabsorption, hypertension as well as cases of renal and hepatic disorders. Twenty-six age-matched females were also recruited in the same saloons to serve as the control group. These had not consciously used any of the bleaching agents. Some of the skin-bleaching creams used included Tura, Top gel, Ikb, Dorot, and Mililo as well as locally prepared herbal extracts. The type of active ingredients in the bleaching cream was determined by reading the content from the attached leaflets, but the active ingredient of the local herbal extracts could not be determined. More than 90% of the subjects volunteered the information that both local extract and the bleaching agents were frequently mixed together before application on the skin. In most cases, the application was generously carried out on all parts of the body, with an average of two application times per day per subject.

Non-fasting blood sample was obtained from each subject through venipuncture between the period of 1100 hours and 1500 hours, and the collected blood was centrifuged for 10 min at 3,000 rpm to obtain serum; this was immediately stored at −20°C until utilized for biochemical analysis. Biochemical analyses were carried out to estimate the levels of the following: urea, creatinine, total protein, albumin, globulin, alanine and aspartate aminotransferases, and bilirubin. Serum levels of zinc, manganese, magnesium, and copper were estimated using atomic absorption spectrometry. Hitachi 902 Automated machine (Roche Diagnostic, Germany) was used for blood biochemistry while Buck Scientific 205 Atomic Absorption (Buck Scientific, East Norwalk, Connecticut, USA) was used for the determination of serum elements. The level of significance between skin-whitening agent users and the control group was determined using Student's *t* test with the level of significance being $p<0.05$.

Results

The results of this study are shown in Tables 1 and 2 below. Chronic usage of whitening agents on the skin for cosmetic purposes resulted in significant increase in the levels of both urea and creatinine ($p<0.05$). Table 1 shows the mean±SEM of urea and creatinine, and these were 42.05±2.17 mg/dl and 127.65±2.60 μmol/L for the skin-whitening agent group and 13.60±0.85 mg/dl and 76.09±1.70 μmol/L for the controls, respectively. Estimation of other biochemical parameters of both chronic users and control group revealed a lack of significant difference for total protein, albumin, globulin, and bilirubin ($p>0.05$). In Table 1 also, the mean±SEM of total protein, albumin, globulin, and bilirubin are 7.31±0.19 g/dl, 4.14±0.10 g/dl, 3.18±0.16 g/dl, and 4.84±1.09 μmol/L for the bleaching agent group and 7.11±0.19 g/dl, 3.96±0.11 g/dl, 3.42±0.26 g/dl, and 4.15±0.95 μmol/L for the control group, respectively.

Results of Table 2 show that zinc, manganese, and magnesium were equally not significantly different ($p>0.05$) in skin-whitening users with the mean±SEM being 105.95±5.6 μg/dl, 3.57±0.28 μg/dl, and 1.74±0.03 mg/dl while that of the controls were 101.55±5.1 μg/dl, 3.64±0.21 μg/dl, and 1.86±0.04 mg/dl. Copper, on the other hand, was significantly increased ($p<0.05$) in skin bleaching group (106.05±5.0 μg/dl) compared with controls (86.41±19.45 μg/dl). The mean±SEM of the liver enzymes alanine aminotranferease (ALT) and aspartate aminotransferase (AST) were 25.91±1.90 and

Table 1 Effects of skin-bleaching agents on some biochemical parameters

Biochemical parameters	Skin-bleaching agent users (mean±SEM)	Controls (mean±SEM)
Urea (mg/dl)	42.05±2.17[a]	13.60±0.85
Creatinine (μmol/L)	127.65±2.60[a]	76.09±1.70
Total protein (g/dl)	7.31±0.19	7.11±0.19
Albumin (g/dl)	4.14±0.10	3.96±0.11
Globulin (g/dl)	3.18±0.16	3.42±0.26
ALT (IU/L)	25.91±1.90	25.75±1.81
AST (IU/L)	24.67±2.01	22.67±1.47
Total bilirubin (μmol/L)	4.84±1.09	4.15±0.95

[a] Significant difference at 0.05

Table 2 Age, duration of exposure, and serum element levels in women using skin-bleaching agents and controls

	Skin-bleaching agent users (mean±SEM)	Controls (mean±SEM)
Copper (μg/dl)	106.05±5.0[a]	86.41±19.45
Zinc (μg/dl)	105.95±5.6 (μg/dl)	101.55±5.1
Manganese(μg/dl)	3.57±0.28 (μg/dl)	3.64±0.21
Magnesium (mg/dl)	1.74±0.03 (mg/dl)	1.86±0.04
Age (years)	40.05±1.63 (years)	40.80±1.72
Duration of exposure (years)	17.73±1.58 (years)	

[a] Significant difference at 0.05

24.67±2.01 IU/L for the skin bleaching group and controls being 25.75±1.81 and 22.67±1.47 IU/L and therefore not significantly different ($p>0.05$).

None of the subjects in the skin bleaching agent group was using the product for any clinical condition but for cosmetic purposes only. Seventy-five percent of subjects in skin-bleaching agent group indicated a constant use of both traditional herbal extracts and industrial skin bleaching agents.

Discussion

Topical application of skin-whitening or skin-bleaching agents on the skin for cosmetic purposes is widespread in sub-Saharan Africa, and this practice has been reported to induce a number of diseases such as diabetes, exogenous ochronosis, impaired wound healing, and wound dehiscence, the fish odor syndrome, and steroid addiction syndrome [16–18]. Its nephrotoxic effects have also been documented [19]. The result of this study of a significant increase in the level of serum urea and creatinine in skin-whitening agent group compared with controls ($p<0.05$) is in agreement with the results of past studies [1, 16]. The use of glucocorticoid has been reported to negatively affect the nephrons, blood vessels, and the heart, consequently leading to hypertension [20]. The nonsignificant changes ($p>0.05$) observed for indices of hepatic damage and function, ALT, AST, bilirubin, albumin, and total protein, show that these agents may not be hepatotoxic even with prolonged period of exposure, which in this case was 17.73±1.58 years.

Herbs capable of inhibiting the activity of tyrosinase, and therefore, melanin formation are commonly used all over the world; examples include *Pharbitis nil*, *Sophora japonica*, *Spatholobus suberectus*, and *Morus alba* tyrosinase (China); *Sideroxylon inerme* L. (South Africa) and Thai mango *Mangifera indica* L. (Thailand) [21–23] and many of these herbal extracts have not been tested for their nephrotoxic effects, especially as studies have revealed that many traditional medicinal herbs induce one type of tissue damage or another [24]. A significant increase ($p<0.05$) in the level of serum copper in whitening agent group compared with the controls was observed in this study, but an association between copper and anti-tyrosinase activity of Thai mango had earlier been identified by Nithianakool et al. [21] through molecular docking studies. They revealed that the binding orientations of the phenolic principles of *Mangifera indica* L. were in the tyrosinase/hydrophobic binding pocket surrounding the binuclear copper active site, which means that the phenolic principles of *Mangifera indica* L. probably acted by chelating copper atoms required for the catalytic activity of tyrosinase, an indication that a possible mode of their anti-tyrosinase

activity may involve this process. Their study though did not identify the mechanism by which the chelated copper from the active site was eliminated from the body.

In addition, many herbs have been reported to contain varying levels of elements; many of these herbal extracts may be rich sources of copper. High cadmium content in food has been reported to be linked with liver cancer. High cadmium content was more positively correlated with hepatocellular carcinoma than either aflatoxin exposure or hepatitis B virus infection [25]. The mechanism by which a possible high copper content in herbs may reflect in the systemic circulation may be through percutaneous absorption; Olumide et al. [16] have revealed that skin-bleaching agents are easily and rapidly absorbed through the subcutaneous route. In this case, this is possible due to the generous and frequent application of these agents by these subjects and a favorable hot humid weather condition found in many parts of Nigeria which enhance percutaneous absorption. The enzymes which regulate copper homeostasis or metabolism might have been induced or suppressed by anti-tyrosinase agents in these products or herbal extracts or some inactive content. Edible alcohol–ethanol has been reported to induce the microsomal enzyme P450 2E1 responsible for the metabolism and therefore the serum level of some agents with therapeutic usefulness [26].

Intake of high levels of Cu induced oxidative stress and kidney dysfunction in diabetic condition [27]. The plasma copper levels were found to be significantly raised in acute renal failure (ARF) patients in comparison with controls such that significantly higher levels of plasma copper were more common in ARF nonsurvivors in comparison with survivors, an indication that it could be one of the indices in predicting mortality in AFR patients [28], and the production of free radicals generated from both active to inactive content of the products and herbal extract might have cause renal injury and played a role in the pathogenesis of skin-bleaching agent-induced nephropathy [28].

That an elevated copper serum level may play a role in many of the complications of prolonged use of skin bleaching is evident by the fact that most of them are dermal in nature (e.g., dyschromia, exogenous ochronosis, acne, and hypertrichosis, prominent striae, tinea corporis, pyoderma, erysipelas, scabies, and contact dermatitis), and a number of skin diseases have also been reported to be associated with high plasma copper. In psoriasis, serum Zn and Cu mean levels were higher as compared to controls, and in varicose ulcers, mean levels were significantly increased compared to control group [29]. High copper level and the associated oxidative stress may be a cause of the long-term effect (carcinogenesis) of these agents. Studies have also attributed DNA damage and inhibition of apoptosis of mutated cells to prolonged exposure to bleaching agents. Damaging systemic effects are also possible since in most instances, these agents are applied to the whole body over a long period of time.

Although impaired wound healing and wound dehiscence and predisposition to infections are some other complications of prolonged use and zinc has been reported to be involved in the wound healing process, a nonsignificant change ($p<0.05$) was observed for zinc with the mean±SEM of the bleaching agent group being 105.95±5.6 μg/dl) while that of the control was 101.55±5.1 μg/dl. This suggests that alteration in zinc level is not responsible for delayed wound healing usually associated with chronic exposure to skin-bleaching agents, rather, some other mechanisms not yet identified might be involved.

Conclusion

This study has raised the possibility of an elevation in serum copper level as a result of exposure to skin-bleaching agents, but the source/cause of this elevation could not be identified due in part to usage of a mixture of herbal extract and industrially processed

agents. Further study may be required to identify which of the two is the cause of the elevation, this is important because a number of people require skin-bleaching agents for therapeutic means, for the treatment of such disorders as hyperpigmentation. In the meantime, it may be penitent to encourage users of this mixture to monitor their essential trace element levels because persistently high levels of trace elements such as copper can lead to oxidative stress with its consequent effects. Moreover, there may be a need to test some of the herbs being used for skin-bleaching purposes for their trace element content and determine the toxic effects of these herbs on experimental animals.

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