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## Exposure Assessment and Antioxidant Vitamin Levels among Cosmetologists and Heavy Cosmetic Users in Benin City, South-South Nigeria

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### Abstract

Beauty enhancement is well craved after among people of the Black race. Most toxic contents of cosmetics and makeups are intentionally undisclosed by manufacturers. Occupationally exposed persons and heavy cosmetics users may be exposed to cosmetics-borne chemicals, including toxic metals. Readily available antioxidants; vitamins A, C and E may hold some promise of mitigation and protection. This study therefore aimed to determine the blood levels of toxic metals and antioxidant vitamins among cosmetologists and heavy cosmetic users in Benin City, South-South, Nigeria. The cosmetologists (n=50; mean age, 25.4 years) comprised of female nail and hair care workers who have had occupational exposure for a minimum of three years, and the heavy cosmetic users (HCU) comprised of apparently healthy females (n=25; mean age, 23.7 years) who had frequently used a variety of cosmetics for a minimum of three years; while apparently healthy, age-matched, and cosmetics-naive female participants (CNP) (n=25, mean age 20.0 years) served as controls. Exposure assessment was carried out using a structured and validated questionnaire. From blood samples obtained, toxic metals: Lead, Arsenic, Mercury, Chromium and Cadmium were determined using the inductively coupled plasma mass spectrometry while vitamins A, C and E levels in serum were determined spectrophotometrically. Results showed that the

frequency of exposure among the cosmetologists was  $\geq 6$  hours/day;  $\geq 6$  days/week while the route of exposure for the cosmetologists included the hands. In addition, similar routes were observed among HCU which included dermal contact, nasal cavity, eyes, oral cavity and the environment. The level of awareness of exposure risk was very low among the cosmetologists (6%). Additionally, of the total number of cosmetologists, 4% were frequent users of personal protective devices, 32% were occasional users, while 64% were non-users. Cadmium levels ( $\mu\text{g}/\text{dL}$ ) in cosmetologists ( $1.885 \pm 0.031$ ) were significantly higher than values obtained from HCU ( $0.538 \pm 0.010$ ) and CNP ( $0.721 \pm 0.020$ ). Lead level ( $\mu\text{g}/\text{dL}$ ) was also significantly higher among cosmetologists (occupationally exposed participants) ( $22.667 \pm 0.428$ ) than in HCU ( $22.389 \pm 0.305$ ) and CNP ( $8.801 \pm 0.326$ ). Arsenic level ( $\mu\text{g}/\text{L}$ ) was also significantly higher in cosmetologists (occupationally exposed participants) ( $11.277 \pm 0.208$ ) than in HCU ( $4.928 \pm 0.152$ ) and CNP ( $9.150 \pm 1.679$ ). Mercury levels ( $\mu\text{g}/\text{L}$ ) were also significantly increased in cosmetologists ( $1.853 \pm 0.032$ ) than in HCU ( $0.529 \pm 0.010$ ) and in CNP ( $0.708 \pm 0.019$ ). Also, Chromium levels ( $\mu\text{g}/\text{L}$ ) were significantly increased in cosmetologists ( $27.064 \pm 0.499$ ) than in HCU ( $10.521 \pm 0.365$ ) and CNP ( $14.777 \pm 0.467$ ). Additionally, vitamins A and C levels ( $\mu\text{mol}/\text{L}$ ) in HCU ( $2.31 \pm 0.08$ ;  $120.04 \pm 1.13$ ) were significantly higher compared with cosmetologists ( $2.01 \pm 0.04$ ;



88.15  $\pm$ 3.69) and CNP (1.34  $\pm$ 0.72; 93.27  $\pm$ 1.67) respectively. Vitamin E ( $\mu$ mol/L) in CNP (12.78  $\pm$ 0.24) was significantly higher compared with both cosmetologists (9.69  $\pm$ 0.27) and HCU (7.92  $\pm$ 0.19). Occupational exposure and frequent cosmetics usage may contribute to increase in the body burden of toxic metals and optimal levels of antioxidant vitamins may be useful in mitigating toxic effects of cosmetics-borne chemicals.

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**Keywords:** Exposure Assessment, Antioxidant Vitamins, Cosmetologists, Toxic metals, Occupational exposure.

## Introduction

Globally, concerns and interests have been raised about the contents and manner of use of various cosmetics and beauty care products (Orisakwe and Otaraku, 2013). They include a range of products such as toothpaste, shampoo, conditioners, mascara, aftershave lotion, styling gel, creams, lotions, powders, perfumes, lipsticks, fingernail and toenail polish, eye and facial make-ups, permanent waves, hair colours, hair sprays and deodorants (Hepp *et al.*, 2009). According to Claeysens (2009), 'makeup' is classified as subset of cosmetics that has to do with beautifying of the face. Schneider *et al.* (2001) defined skincare products or cosmetics as mixtures of synthetic or natural chemical compounds used to improve the appearance or smell of the body. The use of cosmetics dates back to around 10000BC (Price, 2015). Cosmetic preparations should be safe for health, however recently, there has been increasing concern about their safety (Orisakwe and Otaraku, 2013; Nnorom *et al.*, 2005). More than 9,000 chemicals have been reported to be found in cosmetic products (Halliday-Bell *et al.*, 2009).

Among these chemicals are Parabens, which are used in makeup, moisturizers, shampoos etc. Parabens may interfere with hormonal function, associated with breast cancer. Parabens are the most widely used preservative in cosmetics. Parabens easily penetrate the skin and are suspected to

interfere with hormone function (endocrine disruption). Parabens can mimic estrogen, the primary female sex hormone. In one study, Parabens were detected in human breast cancer tissues, raising questions about a possible association between Parabens in cosmetics and cancer. They may also interfere with male reproductive functions. In addition, studies indicated that methylparaben applied on the skin reacts with UVB leading to increased skin aging and DNA damage (Health Canada, 2010; FDA, 2009 and Filon *et al.*, 2009).

Dibutyl Phthalate (DBP) is another chemical found in nail products and some hair sprays. It has been reported to be toxic to reproduction and may interfere with hormone function and may be harmful to fish and other wildlife. Dibutyl phthalate or DBP is used mainly in nail products as a solvent for dyes and as a plasticizer that prevents nail polishes from becoming brittle. Phthalates are found in some nail polishes and hair sprays, and are commonly hidden on ingredient labels under the term "fragrance". DBP is absorbed through the skin. It can enhance the capacity of other chemicals to cause genetic mutations, although it has not been shown to be a mutagen itself. In laboratory experiments, it has been shown to cause developmental defects, changes in the testes and prostate and reduced sperm counts. The European Union classifies DBP as a suspected endocrine disruptor on the basis of evidence that it interferes with hormone function, and as reproductive toxic on the basis that it may cause harm to the unborn child and impair fertility (Health Canada, 2010; FDA, 2009 and Filon *et al.*, 2009).

Formaldehyde-Releasing Preservatives are widely used in hair products, moisturizers, etc. Formaldehyde is a listed carcinogen (ASTDR, 1998). These formaldehyde-releasing agents are used as preservatives in a wide range of cosmetics. Other industrial applications of formaldehyde include production of resins used in wood products, vinyl flooring and other plastics, permanent-press fabric, and toilet bowl cleaners. While formaldehyde occurs naturally in the environment at low levels, worldwide industrial production tops 21 million tons per year (Nigam, 2009; EPA, 1986).

**Table 1: Toxic metal components found in makeup products (Filon *et al*, 2009; FDA, 2009; Health Canada, 2010).**

PRODUCT TYPE		PRODUCT/PRODUCT ITEM	Mercury	Arsenic	Beryllium	Cadmium	Nickel	Lead	Selenium	Thallium
Foundation (5 items total)	<b>MOST</b>	<i>Clinique Stay True Makeup (Stay Ivory)</i>		●	●	●	●	●		●
	<b>LEAST</b>	<i>Marcelle Satin Mousse Makeup (Natural Beige)</i>			●	●	●			●
Concealer (4 items total)	<b>MOST</b>	<i>Laura Mercier Secret Camouflage (Light)</i>		●	●	●	●	●		
	<b>LEAST</b>	<i>Laura Mercier Secret Camouflage (Dark)</i>			●	●	●	●		●
Powders (4 items total)	<b>MOST</b>	<i>Sephora Sculpting Powder Trio (Brown and Pink)</i>			●		●	●		●
	<b>LEAST</b>	<i>Mary Kay Mineral Powder Foundation (Bronze 2), Sephora Sculpting Powder Trio (Beige)</i>			●		●	●		
Blush/Bronzer (5 items total)	<b>MOST</b>	<i>MAC Sheerton Shimmer Blush (Springsheen), Physician's Formula Summer Eclipse Bronzing &amp; Shimmery Face Powder (Bronze and Gold)</i>			●		●	●		●
	<b>LEAST</b>	<i>Quo Faux Glow Bronzing Powder (Sun Drenched), Sephora Sun Disk (01 Copper)</i>			●		●	●		
Mascara (7 items total)	<b>MOST</b>	<i>L'Oreal Bare Naturale (Black/Brown) Avon Astonishing Lengths (Black A01)</i>		●	●	●	●	●	●	●
	<b>LEAST</b>	<i>Maybelline Colossal Volum' Express Drenched)</i>					●	●		
Eye liner (2 items total)	<b>ALL</b>	<i>Fashion Flare Eye Liner Pencil (Midnight Black); Cover Girl Perfect Point Plus (Black Onyx)</i>			●	●	●	●		
Eye shadow (14 items total)	<b>MOST</b>	<i>Too Faced Eye Shadow Duo (I know what boys want - Grey), Almay Intense i-color Trio (02-Trio for Blues - Dark Grey), Almay Intense i-color Trio (02-Trio for Blues - Brown), The Body Shop Shimmer Cubes (Palette 16 - Midnight Black)</i>			●	●	●	●		●
	<b>LEAST</b>	<i>Annabelle Mineral Pigment Dust (Solar)</i>			●		●			
Lip tints/ glosses/sticks (8 items total)	<b>MOST</b>	<i>Benefit Benetint Pocket Pal (Red Tint)</i>		●	●	●	●	●	●	●
	<b>LEAST</b>	<i>Urban Decay XXX Shine Cooling Lipgloss (Guys Love Betsey)</i>			●		●	●		

**Materials and Methods**

**Study Area**

This study was carried out in the metropolitan city of Benin which is the capital of Edo State located in South-South Nigeria. Benin City is situated at 6.34<sup>0</sup>North latitude, 5.64<sup>0</sup>East longitude and 80

meters elevation above the sea level. It is a city approximately 25 miles North of the Benin River and lies 200 miles along the main highways from Lagos, to the Niger Bridge at Asaba and the eastern states. The city is also linked by roads to Sapele, Siluko, Okene and Ubiaja and is served by air and the Niger River delta ports of Koko and Sapele.



### Study Design

This study is designed as a cross-sectional study with purposive approach in the recruitment of participants into three study groups. The groups comprised of:

- (i) Nail and hair care workers otherwise known as cosmetologists/beauticians, who were occupationally exposed to cosmetic-borne chemicals for a minimum of three years duration
- (ii) Heavy cosmetic users, who were frequent users of nail and hair care products, make-up cosmetics etc. for a duration not less than three years.
- (iii) Non-users of nail and hair care products, makeup cosmetics, etc., and who were also occupationally unexposed (cosmetics naïve) for a minimum of three years duration.

### Study participants

A total of hundred (100) female participants comprising of fifty (50) occupationally exposed workers (cosmetologists); twenty-five (25) HCU and twenty-five (25) CNP were recruited from the University of Benin, Ugbowo campus and the neighboring Ekosodin community. All the participants were apparently healthy and age-matched.

### Inclusion Criteria

Inclusion criteria included age ( $\geq 18$  years), non-alcohol and tobacco use, non-menstruating (women), with  $\geq$  three (3) years of exposure to cosmetics-borne chemicals

### Exclusion Criteria

The following individuals who did not meet the inclusion criteria were excluded from participation

in the study; non-adults (<18 years), cosmetic naïve, participants who had been exposed to cosmetics-borne chemicals/metals from cosmetics usage or occupational sources for < 3 years at the time of sample collection.

### Ethical Consideration and Informed Consent

Ethical approval for this study was sought and obtained with reference number H.A 577/Vol/166 from Ministry of Health, Edo State, Benin City. Verbal informed consent was obtained from each participant to indicate voluntary participation in the study.

### Administration of Questionnaires

A pretested structured questionnaire containing both open and closed ended questions were administered to all subjects to obtain socio-demographic information and information about exposure burden.

### Anthropometric and Basic Health Indices

Standing height and weight was measured using standard methods. Body Mass Index (BMI) was calculated from height and weight using the formula:  $BMI = \text{Weight (kg)} / \text{Height}^2 (\text{m}^2)$  as previously described by Romero-Corral *et al.*, (2008)

### Sample Collection

Approximately 7.0 millilitres of venous blood were collected from each participant using standard phlebotomy technique. Table 1 shows the summarized analytical methods.



**Table 1: Summarized Analytical Methods**

Parameters	Sample	Methods
<b>Toxic Metals:</b> Cadmium, Lead, Mercury, Arsenic, Chromium,	Whole Blood	ICP-MS (Fong <i>et al.</i> , 2007)
<b>Antioxidant Vitamins:</b> Vitamin A Vitamin C Vitamin E	Serum	Spectrophotometry Rutkowski <i>et al.</i> , 2006 Grzegorcyk, 2007 Rutkowski <i>et al.</i> , 2005

ICP-MS: Inductively Coupled Plasma -Mass Spectrometry

**Statistical Analysis**

Statistical analyses including descriptive statistics was carried out using the Statistical Package for Social Sciences (SPSS) version 16.0. All values were expressed as Mean ± Standard Error of Mean. The single factor ANOVA/ Post Hoc Analysis was used to determine significant differences in test parameters between the test and controls groups using a two-tailed test. A  $p \leq 0.05$  was accepted as significant.

**Results**

This study investigated cosmetologists (n=50; mean age, 25.4 years) and heavy cosmetic users (HCU) (n=25; mean age, 23.7 years) as subjects while apparently healthy, age-matched cosmetics-naive female participants (CNP) (n=25, mean age 20.0 years) were monitored as controls. The basic health and demographic indices of cosmetologists (occupationally exposed), HCU and CNP, are indicated in table 2. Table 3 shows the pattern of occupational exposure and risk awareness levels of cosmetologists, HCU and CNP. Table 4 shows heavy metal levels in cosmetologist, HCU and CNP and table 5 shows levels of antioxidant vitamins in cosmetologists, HCU and CNP.

The pattern of occupational exposure and risk awareness levels of cosmetologists, HCU and CNP indicated that 38% of the cosmetologists were hair stylists, 8% were nail care workers, while 54% were both nail and hair care workers. The duration of exposure for the cosmetologists was from 3 years and above; the exposed users (HCU) were exposed to these cosmetics by their lifestyle, while there was no occupational/lifestyle exposure in the cosmetics-naive participants (CNP). The frequency of exposure for the cosmetologists was  $\geq 6$  days per week. The

route of exposure for the cosmetologists includes the hands, in addition to similar routes experienced by the heavy users, including: dermal contact, nasal cavity, eyes, oral cavity and the environment. The non-users' participants, had minimal exposure, which was mainly environmental. The level of awareness of exposure risk was very low among the cosmetologists group (6%), while there was no response from the other groups on their exposure risk awareness level. A total of 4% of the cosmetologists' participants were frequent users of personal protective devices, 32% were occasional users, while 64% were non-users. The personal protective devices used include: gloves only (55.5%), gloves and aprons (33.3%), aprons only (5.6%) and nose masks (5.6%).

Table 4 shows heavy metal levels in cosmetologist, HCU and CNP. Cadmium levels ( $\mu\text{g/dL}$ ) in cosmetologists ( $1.885 \pm 0.031$ ) were significantly higher than values obtained from HCU ( $0.538 \pm 0.010$ ) and CNP ( $0.721 \pm 0.020$ ). Lead level ( $\mu\text{g/dL}$ ) was also significantly higher among cosmetologists (occupationally exposed participants) ( $22.667 \pm 0.428$ ) than in HCU ( $22.389 \pm 0.305$ ) and CNP ( $8.801 \pm 0.326$ ) ( $p=0.00$ ). Arsenic level ( $\mu\text{g/L}$ ) was also significantly higher in cosmetologists (occupationally exposed participants) ( $11.277 \pm 0.208$ ) than in HCU ( $4.928 \pm 0.152$ ) and CNP ( $9.150 \pm 1.679$ ) ( $p=0.00$ ). Mercury levels ( $\mu\text{g/L}$ ) were also significantly increased in cosmetologists ( $1.853 \pm 0.032$ ) than in HCU ( $0.529 \pm 0.010$ ) and in CNP ( $0.708 \pm 0.019$ ) ( $p=0.00$ ) ( $p=0.00$ ). Also, Chromium levels ( $\mu\text{g/L}$ ) were significantly increased in cosmetologists ( $27.064 \pm 0.499$ ) than in HCU ( $10.521 \pm 0.365$ ) and CNP ( $14.777 \pm 0.467$ ) ( $p=0.00$ ). Additionally, vitamins A and C levels ( $\mu\text{mol/L}$ ) in HCU ( $2.31 \pm 0.08$ ;  $120.04 \pm 1.13$ ) were



significantly higher compared with cosmetologists ( $2.01 \pm 0.04$ ;  $88.15 \pm 3.69$ ) and CNP ( $1.34 \pm 0.72$ ;  $93.27 \pm 1.67$ ) respectively ( $p=0.00$ ). Vitamin E ( $\mu\text{mol/L}$ ) in CNP ( $12.78 \pm 0.24$ ) was significantly higher compared with both cosmetologists ( $9.69 \pm 0.27$ ) and HCU ( $7.92 \pm 0.19$ ) ( $p=0.00$ ).

**Table 2 Basic health and demographic indices of cosmetologists, heavy cosmetics users and cosmetics naïve participants**

Observations	Cosmetologists (n=50) (Occupationally Exposed)	Heavy cosmetic Users (n=25)	Cosmetics Participants (n=25) (Unexposed)	Naïve	p-value/level of significance
Gender of participants	Females=100% Males=0%	Females=100% Males=0%	Females=100% Males=0%		Not applicable
Age	$25.40 \pm 0.71$	$23.68 \pm 0.42$	$20.00 \pm 0.42$		Highly Significant ( $P=0.000$ )
Nationality	Nigerian (100%)	Nigerian (100%)	Nigerian (100%)		Not applicable
Use of Tobacco	Frequent (n=0; 0.0%) Occasional (n=0; 0.0%) Passive-users (n=50; 100%)	Frequent (n=0; 0.0%) Occasional (n=0; 0.0%) Passive-users (n=25; 100%).	Frequent (n=0; 0.0%) Occasional (n=0; 0.0%) Passive-users (n=25; 100%).		Not applicable
Use of alcohol	Frequent (n=0; 0.0%) Occasional (n=19 ; 38%) Non-users (n=31 ; 62%)	Frequent (n=0; 0.0%) Occasional (n=9; 36%) Non-users (n=16; 64%)	Frequent (n=0; 0.0%) Occasional (n=0; 0.0%) Non-users (n=25; 100%)		Not applicable
Medical History (Current Medical Condition).	Malaria (n=0; 0%) Typhoid (n=0; 0%) Headache (n=0; 0%) Cough (n=0; 0%) None (n=100%)	Malaria (n=0; 0%) Typhoid (n=0; 0%) Headache (n=0; 0%) Cough (n=0; 0%) None (n=100%)	Malaria (n=0; 0%) Typhoid (n=0; 0%) Headache (n=0; 0%) Cough (n=0; 0%) None (n=100%)		Not applicable
Conditions noted by beauticians to be associated with saloon care products.	Skin burns, nasal and eye irritation, catarrh.	Not applicable	Not applicable		Not applicable
Mean BP(mmHg)	Systolic= $114.70 \pm 2.79$ Diastolic= $76.80^a \pm 1.46$	Systolic= $108.68 \pm 1.71$ Diastolic= $71.24^b \pm 1.56$	Systolic= $111.04 \pm 2.14$ Diastolic= $72.12^b \pm 1.99$		Not Significant ( $P=0.264$ ) Significant ( $P=0.032$ )
Mean BMI	$22.59^a \pm 0.76$	$20.92^b \pm 0.62$	$20.30^b \pm 0.46$		Highly Significant ( $P=0.008$ )
Use of multivitamins	Frequent (n=7; 14%) Occasional (n=10 ; 20%) Non users (n=33 ; 66%)	Non-users (n=25; 100%)	Non-users (n=25; 100%)		Not applicable
Multivitamins used	Multivite Tab (n=1; 5.88%) Omega H <sub>3</sub> (n=2; 11.76%) Trehos (n=1; 5.88%) Others (n=13; 76.48%)	Nil	Nil		Not applicable

\*SEM: Standard Error of Mean.

**Table 3 Pattern of Occupational Exposure and Risk Awareness Level amongst participants**

Observations	Cosmetologists (n=50)	Heavy cosmetic Users (n=25)	Cosmetics Naïve Participants (n=25)
Occupational Description	Hair Stylist=38% Nail care=8% Both=54%	Not applicable	Not applicable
Duration of occupational exposure	≥5.0years	Lifestyle Exposure	No occupational/ Lifestyle exposure
Frequency of exposure	≥6 days per week	Not applicable	Not Applicable
Route of exposure	Hands, Dermal contact, nasal cavity, eyes, oral cavity, Environmental.	Dermal contact, nasal cavity, eyes, oral cavity, Environmental.	Minimal exposure (Environnemental)
Level of awareness of exposure risk	Yes=Basic awareness (n=3; 5%) No=No basic awareness (n=47; 95%)	No Response	No response
Personal protective devices	Frequent (n=2; 4%) Occasion (n=16; 32%) Non-users (n=32; 64%)	Not applicable	Not applicable
Personal protective devices used	Gloves only (n=10; 55.6%) Gloves and apron (n=6; 33.3%) Apron only (n=1; 5.6%) Nose mask (n=1; 5.6%)	Not applicable	Not applicable

**Table 4 Toxic metal levels in cosmetologists, heavy cosmetic users and cosmetics naïve participants**

Variables	Mean ± SEM			p – value	Level of Significance
	Cosmetologists (n=50)	Heavy cosmetic users(n=25)	Cosmetics naïve (n=25)		
Cd (µg/dL)	1.89 <sup>A</sup> ± 0.03	0.54 <sup>B</sup> ± 0.01	0.72 <sup>B</sup> ± 0.02	0.000	Highly Significant
Pb (µg/dL)	22.67 <sup>A</sup> ± 0.43	22.39 <sup>A</sup> ± 0.31	8.80 <sup>B</sup> ± 0.33	0.000	Highly Significant
As (µg/L)	11.28 <sup>A</sup> ± 0.21	4.93 <sup>C</sup> ± 0.15	9.15 <sup>B</sup> ± 1.68	0.000	Highly Significant
Hg (µg/L)	1.85 <sup>A</sup> ± 0.03	0.53 <sup>C</sup> ± 0.01	0.71 <sup>B</sup> ± 0.20	0.000	Highly Significant
Cr (µg/L)	27.07 <sup>A</sup> ± 0.50	10.52 <sup>C</sup> ± 0.37	14.78 <sup>B</sup> ± 0.47	0.000	Highly Significant

Note: Similar superscripts indicate mean that are not statistically significant from each other

Note: Values are in mean ± Standard Error of Mean



**Table 5 Antioxidant vitamins levels in cosmetologists, heavy cosmetic users and cosmetics naïve participants**

Vitamins	Cosmetologists (n=50)	Heavy cosmetic users (n=25)	Cosmetics naïve participants (n=25)	F-value	p-value
<b>Vitamin A</b> (µmol/L)	2.01 <sup>B</sup> ±0.04	2.31 <sup>A</sup> ±0.08	1.34 <sup>B</sup> ±0.72	54.17	0.000
<b>Vitamin C</b> (µmol/L)	88.15 <sup>B</sup> ±3.69	120.04 <sup>A</sup> ±1.13	93.27 <sup>B</sup> ±1.67	44.91	0.000
<b>Vitamin E</b> (µmol/L)	9.69 <sup>C</sup> ±0.27	7.92 <sup>B</sup> ±0.19	12.78 <sup>A</sup> ±0.24	110.55	0.000

### Discussion

In this current study, we investigated the exposure and antioxidant vitamin levels among cosmetologists and heavy cosmetic users in Benin City, Edo State South-South Nigeria.

In this study, some of the cosmetologists indicated that some conditions known to be associated with saloon-care products to include: skin burns, nasal and eye irritation and catarrh. The mean systolic blood pressures for the three groups of participants were not significantly different while the mean diastolic pressure of the cosmetologists in comparison with the other groups (HCU and CNP) was significantly higher ( $p=0.032$ ). The mean body mass index (BMI) in HCU was significantly lower than values obtained in cosmetologists ( $p=0.008$ ). With regards to use of multivitamins, among the cosmetologists, there were 14% frequent users of multivitamins, 20% occasional users and 66% non-users of multivitamins. However, the other groups (the exposed users and the unexposed non-users) were all non-users of multivitamins. The multivitamins used by the exposed workers or cosmetologists included supplements with these trade names: Multivite (tablets), Omega H<sub>3</sub> (capsules), Treho (tablets), etc.

The risk awareness indicators and exposure pattern analysis in this study implied that the cosmetologists and HCU lacked basic awareness of health hazards associated with cosmetic exposure. The frequency of exposure among the cosmetologists was  $\geq 6$  hours/day;  $\geq 6$  days/ week while the route of exposure for the cosmetologists included the hands, in addition to similar routes observed among HCU which included dermal contact, nasal cavity, eyes, oral cavity and the environment. The level of awareness of exposure risk was very low among the cosmetologists (6%). Additionally, of the total number of cosmetologists, 4% were frequent users

of personal protective devices, 32% were occasional users, while 64% were non-users. This lack of basic awareness may be responsible for the workers' poor attitude to the use of personal protective equipment. Metals are known to cause oxidative stress, which usually results from excess reactive species (RS) more than the antioxidant system can clear. In other words, imbalances in redox status may develop cellular oxidative stress. At this state, excess reactive species begin to attack biomolecules and cause molecular damage in the cells (Noori, 2012). While many metals exert their pathologic effect through the generation of RS, some metals cause pathological effects by directly inhibiting important biomolecular processes (Noori, 2012).

Significant increase in heavy metals observed in the cosmetologist group (CG) may be attributed to their chronic and persistent exposure ( $\geq 6$  days/week) to chemicals in the various products they use. A total of 38% of the subjects in this study were cosmetologists were hair stylists while 54% were both hair stylists and nail care workers. Among these, only 4% frequently used protective device.

In this study, we observed a statistically significant increase in Cd level in the CG. This observation is an indication of possible accumulation from cosmetics. Cadmium is the seventh most toxic heavy metal as per ATSDR ranking (Jaishankar *et al.*, 2014). Cd binds to cystein-rich protein such as metallothionein. In the liver, the cystein-metallothionein complex causes hepatotoxicity and then it circulates to the kidney and gets accumulated in the renal tissue causing nephrotoxicity. Cadmium has the capability to bind with cystein, glutamate, histidine and aspartate ligands and can lead to the deficiency of iron (Castagnetto *et al.*, 2002). Cadmium and zinc have the same oxidation states and hence cadmium can replace zinc present in metallothionein, polymerase enzymes and in P53



thereby inhibiting metallothionein from acting as a free radical scavenger within the cell, and can lead to infertility and cancer by its action on polymerase enzymes and P53 respectively (Jaishankar *et al.*, 2014).

Highly statistically significant accumulation of lead was observed in both the CG and the HCU. In adults, approximately 80-95 percent of retained lead is stored in the bone. Lead in bone appears to increase significantly with age. Mean tibia lead concentrations for adolescents have been measured at 3 µg/g, concentrations for adults 30-50 years at 17 µg/g, and at 30 µg/g for adults over 75 years (Wittmers *et al.*, 1988). Lead is a divalent cation; its strong binding capacity for sulfhydryl proteins creates interference with enzymes and structural proteins. The most well known of these distortions involves interference with the haem synthetic pathway, specifically the enzyme delta-aminolevulinic acid dehydratase (delta-ALAD; ALAD). When whole blood lead levels (PbBs) exceed 20 µg/dL the activity of ALAD is inhibited by 50 percent. The CG and HCU are therefore at risk of anaemia which may arise as a result of inhibition of haem synthesis, they may also experience oxidative stress due to the inhibition of catalase activity (Jaishankar *et al.*, 2014). Among the signs of Pb toxicity listed by Patrick (2006) in a previous report include blood lead over 10µg/dl, hypertension, decreased nerve conduction velocity e.t.c.

We observed that the mean blood pressure was significantly higher in the CG as compared to the HCU and CNP. Our finding is consistent with a previous report (Schwartz, 1995) which indicated that an increase in mean BMI was observed in the conducted a meta-analysis examining the relationship between blood-lead concentration and systolic blood pressure in adult males. This analysis showed a significant association, with a reduction in blood-lead concentration from 10mg/dl to 5mg/dl being correlated with a decrease in blood pressure of 1.25mmHg.

Increase observed in mean arsenic level among the CG was also highly statistically significant. The inorganic forms of arsenic such as arsenite and arsenate are found to be more dangerous to human health. They are highly carcinogenic and can cause cancer of lungs, liver, bladder and skin. Lower levels of arsenic exposure can cause nausea and vomiting, reduced production of erythrocytes and leukocytes, abnormal heart beat, pricking sensation in hands and

legs, and damage to blood vessels. Long-term exposure can lead to the formation of skin lesions, internal cancers, neurological problems, pulmonary disease, peripheral vascular disease, hypertension and cardiovascular disease and diabetes mellitus (Jaishankar *et al.*, 2014; Smith *et al.*, 2000). The CG is therefore at high risk of various types of carcinogenesis.

Hg is also significantly increased in the CG. Mercury is a well-known hazardous metal and its toxicity is a common cause of acute heavy metal poisoning with cases of 3,596 in 1997 by the American Association of Poison Control Centres. Methylmercury is a neurotoxic compound which is responsible for microtubule destruction, mitochondrial damage, lipid peroxidation and accumulation of neurotoxic molecules such as serotonin, aspartate, and glutamate (Patrick, 2002). The brain remains the target organ for mercury, yet it can impair any organ and lead to malfunctioning of nerves, kidneys and muscles. It can cause disruption to the membrane potential and interrupt with intracellular calcium homeostasis. Mercury binds to freely available thiols as the stability constants are high (Patrick, 2002) the CG and HCU are therefore at risk of serious illnesses if chronic and persistence exposure to metals in cosmetics continues.

The most commonly occurring forms of Cr are trivalent- Cr<sup>+3</sup> and hexavalent-Cr<sup>+6</sup> with both states being toxic to animals, humans and plants (Mohanty and Kumar, 2013). In our study, mean Cr level was statistically increased in CG. Cr(VI) is a strong oxidizing agent and can be reduced to give ephemeral species of pentavalent and tetravalent chromium that are different from that of Cr(III). The reactions between Cr(VI) and biological reductants like thiols and ascorbate result in the production of reactive oxygen species such as superoxide ion, hydrogen peroxide, and hydroxyl radical, ultimately leading to oxidative stress in the cell causing damage to DNA and proteins (Stohs and Bagchi, 1995). Because of its mutagenic properties, Cr(VI) is categorized as a group 1 human carcinogen by the International Agency for the Research on Cancer (Dayan and Paine, 2001). The CG can be said to be at the risk of carcinogenesis from multi-factorial factors.

In this study, vitamin A and C were found to have highest concentrations in the HCU as compared to the Cosmetologists and the CPN. Vitamin E was observed to be significantly depleted in heavy



cosmetics users. Similar observation was also made among the Cosmetologists. Vitamin E falls into the class of conventional antioxidants which generally consist of phenols or aromatic amines. It has been shown that  $\alpha$ -tocopherol has the highest bioavailability and the body preferentially absorbs and metabolise this form (Herrera *et al.*, 2001). In a previous report by Litov *et al.* (1981) in which lipid peroxidation was initiated by 50 mg methyl ethyl ketone peroxide (MEKP)/kg body wt in rats. *In vivo* lipid peroxidation was monitored throughout a 3-hr period by measuring pentane expired in the breath. Rats fed 30 IU *dl*- $\alpha$ -tocopherol acetate/kg diet showed significantly ( $p < 0.001$ ) decreased pentane production at 20 min by 88% compared to rats fed a vitamin E- and selenium-deficient diet. Rats given five ip injections of 180 IU *dl*- $\alpha$ -tocopherol acetate/kg body wt further reduced expired pentane by 83% ( $p < 0.001$ ) beyond the protection afforded by dietary vitamin E. This shows the importance of vitamin E in the protection of lipid against oxidation. In the same study, it was also observed that neither additive nor synergistic protection was found when other antioxidants were given in combination with vitamin E. Vitamin E was the primary protective antioxidant. Pentane expired by individual rats from the various treatment groups also strongly correlated ( $r = 0.92$ ,  $p < 0.001$ ) with the plasma vitamin E status. Plasma vitamin E above the level of 0.5 mg total tocopherol/g total lipid prevented substantial increases in pentane production and blood haemolysis brought on by MEKP. In our study, unaffected levels of vitamin A and C may be due to the protective effect of vitamin E.

The depletion of vitamin E in the HCU may be an indication of chronic and persistence exposure to harmful chemicals in cosmetic products. Persistence exposure and further depletion of vitamin E and other defensive biomolecules may lead to lipid peroxidation and associated pathological conditions. A product of lipid peroxidation (4-HNE), has been implicated in senescence, 4-HNE can induce premature senescence by a direct suppression of telomerase activity affecting the expression of hTERT. In endothelial cells (EC) isolated and cultured from arterial segments of patients with severe coronary artery disease, chronic treatment with an antioxidant (that significantly decreased the levels of lipid peroxidation, that is, 4-HNE expression) N-acetyl-cystein, NAC, significantly delayed cellular senescence via decrease of DNA damage marker ( $\gamma$ H2AX), decrease of nuclear p53, and an increase in hTERT activity (Voghel *et al.*,

2008). 4-HNE can promote organelle and protein damage leading to induction of autophagy, senescence, or cell cycle arrest at 4-HNE medium level and cells can subsist; and finally, 4-HNE induces apoptosis or necrosis programmed cell death at 4-HNE high or very high level, respectively, and cells die (Antonio *et al.*, 2014). In this way HCU may look older than their age, and this may further increase their dependence on cosmetics since they are always wanting to look acceptably young. Scientific basis of defense or protection is emerging; chemoprevention, nutrigenomics and nutrient-toxicant interaction (Anetor, 2016; Adejuwon *et al.*, 2014 and Anetor, 2014). Vitamins A, C, E and inorganic micronutrients may hold some mitigating promise.

### Conclusion

The craving for beauty enhancement may come with its attendant risks. Occupational exposure and frequent cosmetics usage may contribute to increase in the body burden of toxic metals. Optimal levels of antioxidant vitamins may be useful in mitigating toxic effects of cosmetics-borne chemicals.

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