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Disturbed Levels of Non-Enzymatic Antioxidants and Malondialdehyde among Makeup Users

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Abstract

Makeup products contain heavy metals in variable amounts. Oxidative stress, damage, and aging could happen due to exposure to heavy metals. This study aimed to evaluate the concentrations of non-enzymatic antioxidants, and lipid peroxidation marker (malondialdehyde, MDA) in makeup and non-makeup users. 69 female students were sorted into two groups of 31 of non-makeup users (control group) and 38 of makeup users (case group). In this respect, the case group was also divided into 3 subgroups according to a period of 1.09±0.78 years (as mean±SD). 10 mL venous blood was drawn to determine the serum level of vitamin C, uric acid, glutathione (GSH) and MDA. The gained results were statistically analysed using SPSS. This in turn indicated a significant reduction in vitamin C, uric acid and GSH levels in makeup users compared to the control group. However, MDA concentrations were significantly higher in makeup users. A significant correlation between lipid peroxidation, dropping in non-enzymatic antioxidants and makeup products application was found.

Keywords: *Makeup products; Oxidative stress; Antioxidants; Vitamin C; Lipid peroxidation*

1. Introduction

Every day, many of external aggressors attack the skin. For instance, the UV light exposure, air, microbes and other environmental pollutants are the most important examples. The increased inflammatory response by the skin to these attackers generates reactive oxygen species (ROS). ROS have an unpaired electron in their atomic orbital at the outer shell and therefore they are highly reactive [1]–[3]. Oxidative stress (OS) is a metabolic imbalance state, which occurs in cells that favour pro-oxidants like reactive oxygen species (e.g. superoxide, hydrogen peroxide), rather than non-enzymatic antioxidants (e.g. ascorbic acid, glutathione) and enzymatic antioxidant systems (e.g. catalase, superoxide dismutases). Organic biomolecules like lipids, proteins, and nucleic acids are damaged by oxidation resulting from ROS accumulation [3]. The OS ends with oxidative destruction to the major biomolecules and at all levels. According to the degree of imbalance, the cell may die or it might survive in an altered state. Such stress can lead to cause a wide variety of degenerative states, including chronic inflammatory disease, mutagenesis, atherosclerosis and ischemia/reperfusion injury in the heart and brain [4]–[6]. Makeup products (cosmetics) are widely used for flourishing the face's appearance such as lipstick, eye shadow, mascara, and foundation [7]. However, makeup products may have possible harmful effects [8]. Undoubtedly, makeup products comprise natural fats, oils, and fragrances that are subjected to auto-oxidation (exposure to air) and causes off-odours and other instabilities. Several studies can be found in the open literature have indicated a considerable relationship between heavy metals such as lead (Pb), iron (Fe), nickel (Ni), cobalt (Co), cadmium (Cd), chromium (Cr), aluminum (Al), zinc (Zn), and copper (Cu) and the makeup items [2], [9]–[12].

Nomenclature

Al	Aluminum	Hg	Mercury	SPSS	Statistical Package for Social Sciences
Cd	Cadmium	MDA	Malondialdehyde	Tl	Thallium
Co	Cobalt	Ni	Nickle	UA	Uric acid
Cr	Chromium	OS	Oxidative stress	UV	Ultraviolet
Cu	Copper	Pb	Lead	Vit. C	Vitamin C
Fe	Iron	QF	Questionnaire form	Zn	Zinc
GR	Glutathione reductase	ROS	Reactive Oxygen Species		
GSH	Glutathione	SD	Standard deviation		

This in turn affirmed the high possibility of an adverse health problem of toxicity and OS due to the use of some makeup products. Therefore, there was wide research to investigate the influence of heavy metal-induced toxicity and carcinogenicity [13]–[15]. Moreover, the role of cosmetics in ROS generation in biological systems and their significance were also reported [16]. More importantly, there was a consensus of that metal-mediated formation of free radicals can cause various modifications to DNA. This is specifically interpreted to the molecular level enhanced lipid peroxidation, changes in calcium and sulfhydryl homeostasis [1], [2], [11], [17]. Apart from enzymatic antioxidants, non-enzymatic antioxidants like C and E vitamins with GSH, are excellent for cell protection against oxidative threats [18]. Skin's antioxidants like superoxide dismutase (SOD), catalase, glutathione, vit. C and E, and other defence mechanisms of skin serve in neutralising ROS. However, all these mechanisms can be engulfed by OS action [3]. Malondialdehyde (MDA) is one of the most commonly measured markers of OS, namely of lipid peroxidation. Higher concentrations of MDA are measured in biological samples as compared to health in many diseases [19]. Therefore, elevated OS is generally considered as a pathological condition. Minimizing the concentration of OS's biomarkers can be achieved by (a) changing lifestyle, (b) increasing the nutritional intake of antioxidants or (c) means of pharmaceuticals is overall believed to be beneficial to health [20], [21]. Basically, MDA is a colorless organic compound in a liquid state, with the nominal formula $CH_2(CHO)_2$ and is a highly reactive compound that is denoted as the enol [22]. It occurs naturally and is a marker for oxidative damage (stress), since the MDA is the outcome of the peroxidation of polyunsaturated fatty acids [23]. Non-enzymatic antioxidants like glutathione (GSH), vitamin C (vit. C) and uric acid (UA) were extensively reported to have positive modulatory effects against OS deleterious potential by neutralizing the ROS [24]. Other several studies have shown the link between reductions of UA and vit. C and lipid peroxidation [24]–[26]. Vitamin C (L-ascorbic acid or ascorbic acid), is an enzymatic cofactor commonly found in mammals and consumed in the collagen synthesis. Also, it can be found in various sold foods as a dietary supplement [27], [28]. Vit. C is an important nutrient used in the enzymatic production of specific neurotransmitters, and commonly used to heal the living tissue [29]. Moreover, it is essential for the immune system function and as a treating and preventive agent against scurvy. It also serves as an antioxidant, i.e. potent reducing agent able to scavenge several ROS rapidly [2], [30]. Vit. C acts in scavenges hydroxyl and superoxide radical anion (types of ROS) [31]. Glutathione (GSH) is a non-enzymatic antioxidant in animals, fungi, plants, and some bacteria and archaea. However, the GSH is capable of protecting against injury to important cellular components triggered by ROS such as free radicals, lipid peroxides, peroxides, and heavy metals [32]. It is a tripeptide that has a gamma (γ-) peptide bond which links the cysteine's amine group to the carboxyl group of the glutamate side-chain and the cysteine's carboxyl group is linked by a normal peptide linkage to a glycine. Reducing agents, thiol groups, are present at a concentration of around 5 mM in animal cells. Glutathione serves as an electron donor to reduce the disulfide bonds formed in cytoplasmic proteins to cysteines. In the process, glutathione is transformed to its oxidized form, glutathione disulfide. At once, when glutathione oxidised it can be reduced back by glutathione reductase (GR), using NADPH as an electron donor [33]. Often, when intended to measure the cellular oxidative status, the ratio of reduced glutathione to oxidised glutathione within cells is used [34], [35]. Upon exposed to oxidants and when trying to neutralized their effects, GSH get reduced to glutathione disulfide and then reduced to GSH by glutathione reductase [31], [36]. Moreover, it was also reported that reduction in GSH concentration is an indirect biomarker for OS in living systems [37], [38] Uric acid (UA) is the highest antioxidant product in human blood. UA is an antioxidant oxypurine produced from xanthine in the presence of enzyme xanthine oxidase, and is an intermediary product of purine metabolism [2]. UA molecules along with albumin account for 85% of antioxidant capacity in plasma, it serves in terminating the free radical chain reactions by interacting to the reactive oxygen species [36]. Özkaya *et al*. [39] reported an unfamiliar case which started with the topical use of a mercury containing cosmetic product. Erythema, allergic contact dermatitis (ACD), moderate infiltration and scaling of both eyelids, and itching (symptoms lasting 4 months) were also documented by Travassos *et al*. [40] in a Belgian woman who used a nickel (Ni) contained eye pencil. When she had stopped the use of this eye pencil, the symptoms disappeared, but after she started to use it again they returned. [41]. Amry *et al*. [42] reported severe eye keratitis due to the use of kohl, which contained high levels of cadmium (Cd) (6259 mg/kg). Also, trace amounts of Hg and thallium (Tl) have been investigated in a 21-year-old woman. Weldon *et al*. [43] detected an elevated level of Hg in the urine (mean 146.7 μg/L; range 0 – 1,170 μg/L; while the nontoxic concentration is up to 20 μg/L as per CDC, 1990) among 330 users of lightening-cream from Texas (USA). Increased lead (Pb) amounts in the blood were investigated in adults, they got through using of cosmetic preparations containing this heavy metal at the southern region of Iraq by Al Naama *et al*. [44]. Up to the authors' knowledge, the evaluation of non-enzymatic concentration antioxidants and MDA in particular makeup and non-makeup users have not yet been explored. This study focused to find the lipid peroxidation status by measuring MDA levels in serum, and the levels of non-enzymatic antioxidants (GSH, vit. C, and uric acid) in female students who use makeup against female students who did not.

2. Materials and methods

2.1 Subjects and blood sampling

This study involved 69 healthy female volunteers (students) from the Technical Institute of Baquba. They were included in the study after giving informed consent. The eligibility of any participant was affirmed via a questionnaire form, which excludes the students who are at the menstrual cycle, pregnancy, used supplements with antioxidants in the past three weeks, and those who received transfused blood within the last month. Those conditions may alter the concentrations of parameters intended to be measured. The students' characteristics are listed in Table 1. To whom accepted to participate and were eligible, 10 mL of venous whole blood was drawn via additive-free gel tube from June to November 2017. The participants were grouped into two groups, 38 non-makeup users (as control) and 31 makeup users (as cases). Moreover, the makeup users group was also divided into 3 subgroups A, B, and C based on the duration of makeup use as ≤ 12 months, between 12-24 months and more than 24 months, respectively. The separation of serum was done via centrifugation at (3000 x*g*) for 10 minutes at room temperature (25 °C) and then kept at (4 °C) before an immediate investigation made on the same day.

Table 1. Descriptive characteristics of makeup users and controls.

	Makeup users (*n* = 31)			Controls (*n* = 38)
	A (*n* = 14)	B (*n* = 10)	C (*n* = 7)	
Age (years)	19.64±0.92	20.0±1.24	20.85±3.48	20.03±1.87
BMI (kg/cm^2)	23.3±1.73	22.7±2.38	22.6±2.56	23.7±1.66
Hb (g/ dL)	15.2±0.52	14.8±0.64	15.0±0.27	14.6±0.77
Normality testing	ND	ND	ND	ND
Variance	Equal	Equal	Equal	Equal
HoV	Homogenous	Homogenous	Homogenous	Homogenous
DMA (months)	< 12	12 - 24	> 24	NA

Abbreviations: BMI (body mass index); Hb (hemoglobin concentration); ND (normally distributed, according to *Kolmogrove Smirnov*), variance (according to *Levene's* test), DMA (duration of makeup application); HoV (homogeneity of variance, according to *Levene*'s statistics), NA (not applicable).

2.2 Biochemical analysis

All glutathione (GSH), ascorbic acid (vit. C), uric acid and MDA concentrations were measured spectrophotometrically. Moreover, Sedlak and Lindsay method [45] and Omaye *et al*. method [46] were used to determine the GSH concentration (by reducing the GSH's thiol group to form a colored product) and vit. C, respectively. Also, the uricase enzymatic method of Burtis *et al*. [47] was used to estimate uric acid (UA) level according to the manufactured kit by Spinreact (Girona, Spain). Lipid peroxide concentration of serum was determined by lipoproteins precipitation by trichloroacetic acid (TCA) and boiling with thiobarbituric acid which reacts with MDA to get the pink color following the method of Wierusz-Wysocka et al. [48].

2.3. Statistical analyses

The results of this study were expressed as mean±SD. IBM SPSS version 21.0 was used to perform the data analysis, while the use and interpretations of data was carried out in accordance to Morgan *et al*. [49]. The significant differences between the two nominated student groups were carried out using *t*-test. In addition, ANOVA test was used to compare the significant differences between makeup users' subgroups. Basically, *p*-value less than 0.05 was considered significant whereas < 0.001 was considered as highly significant.

3. Results and discussion

Serum samples of both groups (control and makeup users) were tested to determine the concentrations of glutathione (GSH), vitamin C, uric acid (UA) and MDA. The allocated mean concentrations data of these parameters and the groups' differences are given in Fig. 1. The GSH, vit. C and UA concentrations in makeup users were significantly (p< 0.000) lower than in non-makeup users as 2.44±0.0.89 vs. 3.46±0.41 μmol.L^{-1}, 69.49±10.89 vs. 94.78±8.27 μmol.L^{-1}, and 250.45±13.99 vs. 282.87±9.23 μmol.L^{-1}, respectively. In addition, MDA concentrations in makeup user students revealed a significant (p< 0.000) increase than those in non-makeup users as 4.00±0.40 vs. 2.72±0.60 nmol.mL^{-1}.

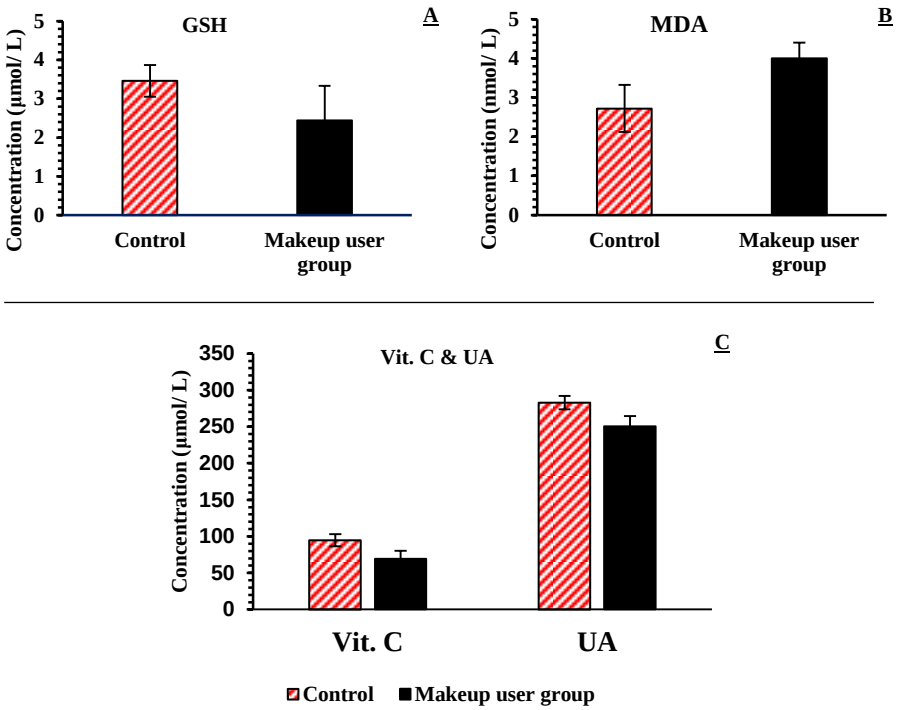

Figure 1. Level of GSH (A), vit. C, UA (C) and MDA (B) in the serum of control and makeup users groups. All four tested parameters were significant at ($p < 0.05$).

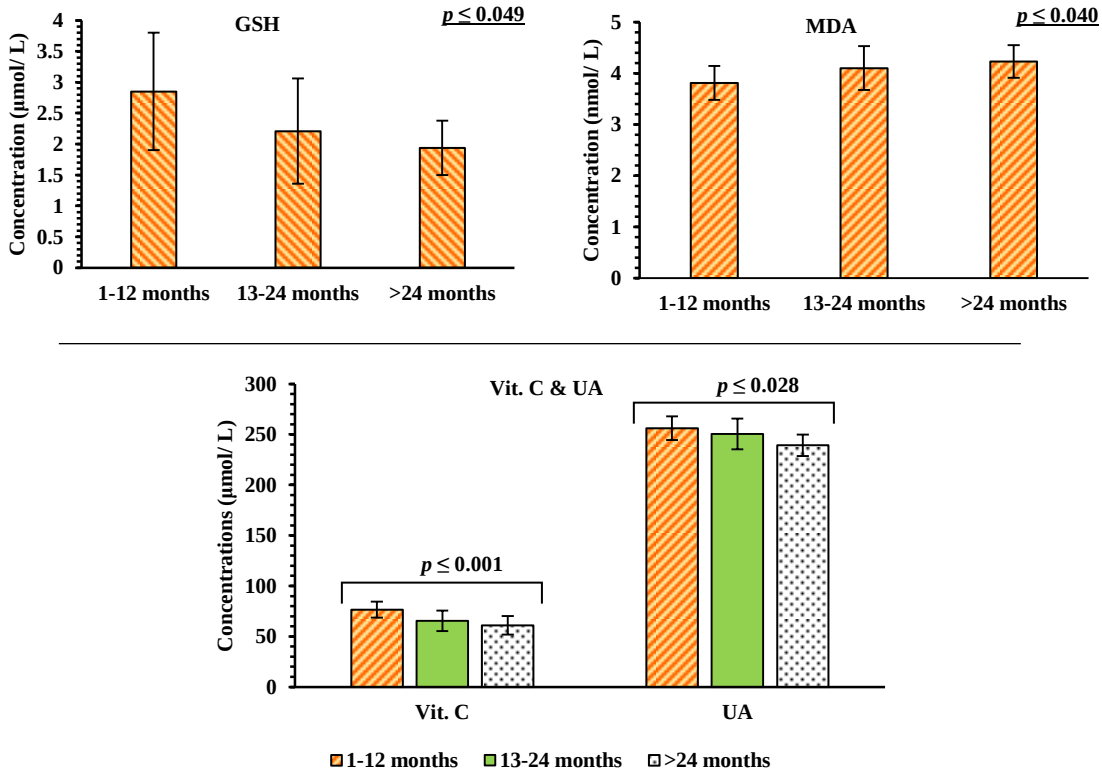

Figure 2. Levels of GSH, vit. C, UA and MDA in the serum of makeup users. The three groups A, B, and C were sorted according to the duration of makeup. All the four tested parameters were significant at ($p < 0.05$).

As stated above, makeup users group was divided into 3 subgroups A, B and C according to the applied duration of makeup as follows; (A ≤ 12 months, B between 12 – 24 months, and C more than 24 months). In this respect, the analysed findings revealed a significant ($p< 0.049$) reduction in GSH concentrations. This is because of subgroup A has a highest level (2.85±0.95 μmol.L^{-1}) followed by subgroup B (2.21±0.85 μmol.L^{-1}) and the lowest level observed with subgroup C (1.94±0.44 μmol.L^{-1}). Vit. C and UA concentrations were also showing a significant ($p< 0.001$, < 0.028, respectively) diminishment among subgroups, subgroup A (vit. C: 76.58±8.01 μmol.L^{-1}, UA: 256.09±11.65 μmol.L^{-1}), subgroup B (vit. C: 65.46±10.10 μmol.L^{-1}, UA: 250.40±15.25 μmol.L^{-1}), and subgroup C (vit. C: 61.07±9.15 μmol.L^{-1}, UA: 239.23±10.67 μmol.L^{-1}). In an increasing manner, the MDA concentrations were determined as (subgroup A: 3.81±0.33, subgroup B: 4.10±0.43, subgroup C: 4.23±0.32 nmol.L^{-1}) as depicted in Fig. 2. The results of Fig. 2 show that makeup application in makeup users group causes a reduction in GSH, vit. C and UA of the serum if compared to non-users. This is specifically obvious as a consequence to increasing the exposure period of makeup. This in turn denotes a reduced potential for buffering OS in plasma of makeup users. Moreover, the use of makeup including an exposure to heavy metals has reduced the ability to neutralise overproduction of ROS due to an increased OS. In this respect, a clear relation between GSH levels and OS is obtained and induced by an exposure to heavy metals. The reduction of vitamin C, uric acid and GSH in makeup users might be attributed to an exposure to heavy metals present in makeup products. This is specifically causing a series of reactions that neutrailised ROS and passively impacting the tested parameters. A good agreement of high consistency is also observed between the recent study and the findings of Vinodhini and Narayanan [50] regarding vit. C. In this regard, Vinodhini and Narayanan [50] studied the impact of toxic heavy metals on antioxidant in fish and noticed a noticeable drop in vit C level. Moreover, Uric acid (UA) results agreed with Glantzounis *et al*. (29) and [51]. Specifically, they found a decrease in UA concentrations along with increased lipid peroxidation and OS. GSH levels match to the findings of Jozefczak *et al*. [52]. MDA results corroborate to those of Aflanie *et al*. [53]. An elevation in MDA concentration among makeup users indicates the lipid peroxidation resultant from possible toxicity of heavy metals found in makeup products, which lead to OS. More importantly, this study comes with the findings of that makeup application has a statistically significant effect on non-enzymatic antioxidants levels throughout the human body by reducing their plasma levels (vitamin C, uric acid and GSH), in addition to its effect in lipid peroxidation induction.

Conclusions

Makeup is widely used to flourish the appearance and improve the face expressions. It has been approved that makeup items contain heavy metals that are toxic to biological systems and lead in several cases to oxidative damage. To systematically explore this influence, this study aimed to determine the concentrations of lipid peroxidation biomarker (MDA) and non-enzymatic antioxidants (vitamin C, uric acid and GSH) in serum of female students applying makeup products. This in turn indicated the alteration of serum concentrations of non-enzymatic antioxidants and OS due to heavy metal exposure in female students who apply makeup. The toxicity resultant from heavy metals contained in makeup items produces ROSs that may be related to OS in makeup products appliers. Specifically, the study outputs affirmed a significant reduction in GSH, vit. C, and UA concentrations besides an elevation of MDA concentrations in makeup-users group as compared to makeup non-users. Furthermore, a considerable difference in all tested parameters was statistically noticed based on a simple comparison between three makeup users' subgroups. Further research is also required to evaluate the effect of heavy metals contained in makeup products on the oxidative status throughout the body. This might consider some limitations such as the size of the sample, accurate concentrations of heavy metals and the authorization state of makeup manufacturers.

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Conflict of interest

The authors declare that they have no conflict of interest.

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