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Biomarker of Oxidative Stress in Premature Hair Graying at Young Age

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Abstract

BACKGROUND: Gray hair is a physiological process of aging that occurs in everyone. Premature hair graying (PHG) is the term when early hair gray at an unusual age. The causes of PHG are multifactorial, genetic, nutritional, and environmental, including oxidative stress. Free radicals caused interference with cellular responses that cause direct damage to various proteins and DNA in the long term. The body's defense mechanisms likely antioxidant enzymes, including catalase, glutathione peroxidase (GPx), and superoxide dismutase (SOD) was activated against free radicals.

AIM: We aimed to evaluate markers of oxidative stress and associated with severe graying at young age.

MATERIALS AND METHODS: We recruited consecutive 40 respondents with PHG and healthy controls, male sex and aged <25 years. The severe graying was graded with total of gray hair. The serum samples were collected to detect oxidative stress markers through malondialdehyde (MDA), SOD, catalase, and GPx measurement with enzyme-linked immunosorbent assay.

RESULTS: Serum MDA concentration was higher but not significantly ($p > 0.05$), while serum SOD, catalase, and GPx level, indicators of antioxidant were significantly lower ($p < 0.001$, $p = 0.017$ and $p < 0.001$, respectively) in PHG compared to controls. The correlation between oxidative stress and graying severity was not significant ($p > 0.05$), but the association between onset and severity of graying was significant ($p < 0.001$).

CONCLUSIONS: Respondents with PHG had increased of pro-oxidants and decreased of antioxidants compared than controls. The severity of graying is equivalent to the level of oxidative stress. The supplement of antioxidants is likely recommended in PHG.

Introduction

Graying hair (canities senilis) is one of the markers of the natural aging process that generally occurs regardless of gender, ethnicity, race, and nationality. Although generally, it does not cause medical problems, it is very troubling for many people for certain reasons, such as aesthetic problems, especially at young age [1], [2]. Graying hair occurs due to a mixture of normal pigment, hypomelanotic, and amelanotic melanosome. White hair is terminal sign of aging [3].

The mean age of senile graying for Caucasians (whites) is mid-30s; for Asians of the late 30's; and for Africans of mid-40s. There is a practical declaration which states that at the age of 50, 50% of the population has 50% gray hair (rule of thumb) [4]. The gray of age varies according to race and ethnicity. Hair is conventionally considered premature graying if it occurs under 20, 25, and 30 years old in Caucasians (white), Asia, and Africa, respectively [5].

Lipid peroxidation processes in the cell may produce free radicals. One of the end products of polyunsaturated fatty acids peroxidation is malondialdehyde (MDA). MDA level is generally and widely used as a marker of oxidative stress [6]. *In vivo* and reactive oxygen species (ROS) were suppressed by antioxidant enzymes activity, vitamins C and E [7], [8]. Superoxide dismutase (SOD) is an antioxidant enzyme that is important to inhibit oxidative stress that plays a role in converting free radicals to superoxide anion (O2-). It is also a preventive antioxidant that can inhibit it before superoxide anions cause damage. The catalase enzyme functions as catalysis in degradation process of hydrogen peroxide (H_2O_2) into H_2O and oxygen. H_2O_2 is a dangerous particle that can lead to mutation and cell death. Glutathione peroxidase (GPx), a selenium-dependent enzyme, plays an important role in the reduction of lipids and H_2O_2. If the GPx activity decreases, there will be more H_2O_2 causing direct tissue damage and activate the inflammatory pathway nuclear factor-κB [9]. This study aimed to detect markers of oxidative stress in premature greying.

Materials and Methods

This study was a cross-sectional design with 80 samples of a college student at the Universitas

Sumatera Utara (USU). The protocol of this study has been approved by Medical Ethics Committee USU.

Study subjects

The sample consisted of 40 premature gray hair and 40 normal hair. The inclusion criteria were male, below 25 years old, with gray hair and not have skin pigmentation disorders. Written informed consent was taken from all respondents. The questionnaires, including onset, location, and numbers of graying hair, were collected. The classification of canities with the total number of graying as mild (<10 gray hair); moderate (10–100 gray hair); and severe (>100 gray hair) [2]. The blood samples were collected from antecubital vein to assess stress oxidative biomarkers in both PHG and controls.

The serum was assessed by measuring MDA, SOD, catalase, and GPx levels in the serum through enzyme-linked immunosorbent assay (ELISA) methods, with commercial kits below:

MDA: Human MDA ELISA Kit 96T, catalog number E3696Hu, Bioassay. SOD: EnzyChrom™ SOD. Lot: BJ03A18.

Catalase: Human Catalase Elisa Kit 96T, catalogue number E3053Hu, Bioassay.

GPx: GPx ELISA Kit 96T, catalogue number E3696Hu, Bioassay.

The data were analyzed with Chi-square and Mann–Whitney U-tests with $p < 0.05$ were considered significant.

Results

This study described the characteristic of the subject and correlation parameter oxidative stress with the gray hair severity at young age (the college student at USU).

The distribution of age in cases and controls was compared. The mean age was 20.275 ± 1.99 versus 2.125 ± 2.015, respectively. The location of gray hair was depicted in the parietal areas that were 20 (50%), more commonly involved than other areas. The severity degree of gray hair almost the same in 3 groups (mild, moderate, and severe). The MDA levels were higher in PHG than controls (9.55 ± 11.2 vs. 7.08 ± 5.90; $p = 0.117$). The SOD, catalase, and GPx levels were significantly decreased in PHG groups ($p < 0.001$, $p = 0.017$, and $p < 0.001$, respectively) (Table 1).

This study showed that association onset of graying with gray hair severity was statistically significant ($p < 0.001$). The earlier the onset of gray hair showed a higher degree of gray hair (Table 2).

Table 1: Characteristic of study subjects

Characteristic subjects	Cases (n = 40)	Controls (n = 40)	p
Age			
Mean age (years)	20.275 ± 1.99	2.125 ± 2.015	
Range (years)	19–24	18–24	
≤16 years	28	22	
>16 years	12	18	
Location of PHG			
Frontal	6		
Parietal	20		
Temporal	2		
Occipital	5		
>One location	7		
The number of gray hairs			
<10 (mild)	14		
10–100 (moderate)	12		
>100 (severe)	14		
Parameter oxidative stress			
MDA	9.55 ± 11.2	7.08 ± 5.90	0.117
SOD	1.08 ± 1.10	1.60 ± 0.50	<0.001*
Catalase	74.89 ± 71.0	81.15 ± 107.5	0.017*
GPx	17.81 ± 9.40	27.68 ± 22.60	<0.001*

PHG: Premature hair graying, MDA: Malondialdehyde, SOD: Superoxide dismutase, GPx: Glutathione peroxidase.

Table 2: Association of PHG severity with the onset of greying

Onset of graying (years)	n (%)	Degree of gray hair severity			p
		Mild (n = 14)	Moderate (n = 12)	Severe (n = 14)	
≤16	28 (100)	5	9	14	<0.001*
>16	12 (100)	9	3	9	

PHG: Premature hair graying.

This study showed parameter of MDA, SOD, catalase, and GPx were not significantly with degree of graying in PHG ($p > 0.05$) (Table 3).

Table 3: The correlation of oxidative stress biomarkers with severe PHG

Parameter (Concentration of serum)	Degree of gray hair severity			p
	Mild (n = 14)	Moderate (n = 12)	Severe (n = 14)	
MDA	79.65 ± 76.7	82.32 ± 91.9	63.76 ± 43.5	>0.05
SOD	27.78 ± 20.7	30.67 ± 20.7	25.02 ± 9.1	>0.05
Catalase	1.79 ± 0.6	1.47 ± 0.4	1.53 ± 0.4	>0.05
GPx	11.75 ± 15.6	10.28 ± 11.3	6. 73 ± 4.2	>0.05

PHG: Premature hair graying, MDA: Malondialdehyde, SOD: Superoxide dismutase, GPx: Glutathione peroxidase.

Discussion

Physiological aging and oxidative stress aging in the long term will interfere with cellular responses that can cause aging. Graying is a prominent but little understood feature of aging. Interestingly, the continuous synthesis of melanin in growing hair follicles (anagen) results in high ROS by the formation of H_2O_2 and other chemical agents [10].

Distribution pattern of gray hair is not similar between males and females. The region of temporal and occipital more commonly occurred in males than females. Second, graying usually starts in temporal areas in males, contrast to females in the frontal area [11]. The study of Daulatabad *et al*. (2016) showed involvement of the frontal more likely than temporal region [12] and another study has reported that differences in related pattern with a racial variation. This study is consistent with Jo *et al*. (2012) showed that in

the early-onset group (40 years old) initially, the frontal region was commonly than another site that means the involved scalp region was different depend on onset of graying. This study showed that the association between onset of graying with gray hair severity was statistically significant ($p < 0.001$). The earlier the onset of gray hair showed a higher gray hair degree. In contrast, Jo *et al*. (2012) that the gray rates increased dramatically after 50 years old regardless of age at onset [11].

The generation of free radicals or ROS is highly unstable and capable of damaged cell membranes, DNA, and other proteins caused by oxidative stress, such as superoxide (O2), H_2O_2, and hydroxyl free radicals. Free radicals can cause oxidative stress due to an imbalance between oxidants and antioxidants that have the potential to cause cell damage. Free radicals can increase lipid peroxidation, which breaks down into MDA in the blood. MDA is a marker of cellular defects caused by free radicals [13]. This study described increased levels of MDA pointed toward a high degree of ROS in PHG at young adult. This study showed the catalase, SOD, and GPx levels were significantly lower in the cases than controls ($p = 0.017$, $p < 0.001$, and $p < 0.001$, respectively). This means that the body's response to free radicals designs an antioxidant system to preserve our body from this persistent harm, including antioxidants and free radical scavenger enzymes such as SOD, catalase, and GPx [14]. It is in accordance with the study Saxena *et al*. (2020) demonstrated that serum MDA was significantly higher, while the SOD and rGSH levels lower in PHG compared to controls [15].

The studies demonstrated the role of H_2O_2-mediated oxidative stress in graying hair [16]. Any studies have shown that intrinsic catalase deficiency in gray hair leads to decreased ability to reduce H_2O_2, resulting in higher concentrations in graying hair follicles [17]. Several studies have shown lower levels of catalase in gray hair follicle melanocytes compared to controls, it is consistent with the study of Kauser *et al*. (2011) [14]. Decreased of H_2O_2 concentrations followed with increased tyrosinase activity, whereas high concentrations permanently deactivate the enzyme [18], [19]. It has been demonstrated that many peptides and proteins, likely the reducing enzyme H_2O_2, were damaged structurally and functionally altered by H_2O_2-mediated oxidation, it is a vicious cycle defect of hair follicular melanocytes induced by ROS [13], [18].

Krugluger *et al*. (2011) informed that vitamin B12 and minerals play a role in pigmentation and hair growth [20], while another study showed that premature canities were associated with deficiency calcium and vitamin D3. The pilot study showed decreased vitamin D levels, but serum ferritin was normally at PHG [21], [22].

This study supported a critical role of oxidative stress in the precipitating factors of PHG, especially in college students at USU, Medan. Attempts attempt to slow down PHG with an antioxidant agents needed to investigate.

Conclusions

The serum levels of MDA were higher, while antioxidant markers (SOD, catalase, and GPx) were significantly lower in cases than controls. The oxidative stress measurement is hopefully useful for monitoring the progress of greying hair and as predictive biomarkers for response treatment.

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