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" *Evaluation Of Super Oxide Dismutase as a marker Of Oxidative Stress In Premature Hair Graying among Youth* "

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ABSTRACT:

Background: Physical attractiveness and youthfulness are highly valued in society, with skin and hair revealing age, gender, race, ethnicity, and health. Hair graying, a noticeable indicator of aging, affects 50% of people by age 50. For Caucasians, Asians, and Africans, premature greying happens before the ages of twenty, twenty-five, and thirty. An important antioxidant enzyme that inhibits superoxide anions and stops oxidative damage is superoxide dismutase (SOD). It has anti-inflammatory properties and can prevent cell alterations. As we age, SOD levels decrease, but free radical production increases. Consuming SOD regularly can maintain a healthy immune system and prevent aging. Natural sources include broccoli, cabbage, Brussels sprouts, wheat grass, and barley grass.

Aim of the work: To estimate (SOD) in premature hair graying and to evaluate its role in the progress of graying hair as a predictive biomarker.

Patient and method: A case-control study design conducted on any young age case in average 25 years old with premature hair graying in the scalp, beard, eyebrow, or eyelashes. A control group of 45 people without premature greying and premature hair greying was clinically diagnosed in 45 cases based on the clinical examination of the lesion were included. Serum SOD levels and other clinical and laboratory tests were performed on all chosen individuals.

Results: Compared to the control group, cases group SOD was considerably lower (P value < 0.001). a significant relationship between patients group SOD and severity. In cases group P=0.011, there was a negative link between SOD and age. There is negative correlation between SOD and duration in cases group (P=0.015)

Conclusion: One frequent and efficient cause of premature hair greying that lowers SOD levels is oxidative stress.

Key words: Premature graying, Superoxide dismutase.

Introduction

Hair length, color, and style are crucial factors influencing individuals' physical appearance and their self-image (Kumar et al., 2018). Hair functions as a significant aesthetic element and a medium for nonverbal communication (Campos & Casado, 2015).

The color and style of hair can profoundly impact a person's physical appearance, thereby affecting their body image. Premature greying (PGH) can have a detrimental effect on a person's self-esteem, and hair greying is frequently linked to ageing (Millien et al., 2022). Although greying, also known as canities or achromotrichia, is a normal aspect of ageing, various ethnic groups experience this phenomena at different times. Grey hair that appears before the age of 20 in Caucasians and before the age of 30 in people of African origin is a characteristic of PGH (Fuentes-Santamaría et al., 2022).

One sign of the natural aging process that typically occurs is grayinghair (canities senilis) that occurs irrespective of gender, racial, ethnicity, and nationality, which is due to certain factors, such as aesthetically unappealing issues, particularly when they are young. Hair graying that results from a complex interplay of normal pigmentation, hypomelanotic, and amelanotic melanosomes is often regarded as a marker of aging (Shin et al., 2015; Zayed et al., 2013). While it is not primarily classified as a genetic disorder with autosomal dominant inheritance, it can manifest as part of various syndromes, including Waardenburg syndrome, Book's syndrome, and progeria (Anggraini et al., 2021).

Additional contributing factors encompass nutritional deficiencies and oxidative stress. It is commonly known that hunger and hair colour changes are related. Significant data is still lacking because there aren't any well-designed systematic studies, despite the fact that a number of micronutrients, including biotin, vitamin B12, zinc, copper, selenium, and iron, have been implicated in the development of premature greying (Almohanna et al., 2019).

Furthermore, smoking has been shown to induce noticeable alterations in hair pigmentation, leading to earlier onset of graying. Chronic tobacco use significantly heightens the risk of premature hair graying, attributed to melanocyte damage caused by free radicals generated from smoking (Saxena et al., 2020).

Melanocytes use a sequence of oxidative processes to synthesise melanin from L-tyrosine, which eventually results in the production of free radicals and reactive oxygen species (ROS), such as hydrogen peroxide (H2O2).

An imbalance between prooxidant reactions and antioxidant defenses results in oxidative stress, which can cause macromolecular and cellular damage (Bafana et al., 2011). Cells use a variety of natural enzymes and tiny antioxidant molecules to reduce ROS. As a lipid-soluble radical scavenger, ubiquinol (coenzyme Q10) shields vital skin proteins as well as mitochondria (Srivastava et al., 2017). Furthermore, CoQ10 maintains the integrity of hair by suppressing the production of certain metalloproteinases (MMPs), including collagenases.

Superoxide dismutase (SOD), one of antioxidant enzymes, is essential forreducing oxidative stress, which converts free radicals into superoxide anions(O2-), which are harmful to cells. It also functions as a prophylactic antioxidant, which means that it might inhibit superoxide anions before they cause cell damage. To show SOD's therapeutic potential and physiologicalimportance, numerous studies have been carried out *(Asz-Sigall et al., 2023).*With aging, SOD levels decline while free radical generation increases.

It has been proposed that taking SOD regularly may help maintain a strong immunesystem, reduce the likelihood of getting sick, and finally stop the aging process. Fortunatly, (SOD) is found naturally in foods including broccoli, cabbage, Brussels sprouts, wheat grass, and barley grass ***(Krishnamurthy & Wadhwani, 2012)***.

Although, ***(Saxena et al., 2020)***, ***(Papaccio et al., 2022)*** studied the role of SOD inmanagement of Premature Hair Graying and suggested that SOD has critical role in evaluation of graying hair, the exact mechanism in youth cases need further estimations.

Aim of the work: To estimate (SOD) in premature hair graying and to evaluate its role in the progress of graying hair as a predictive biomarker.

Patient and method:

Research design: After receiving institutional ethical approval, a case-control research design was used to achieve the study's goals.

Research setting: Tanta Hospital of dermatological and Leprology's dermatological outpatient clinics served as the study's location.

Population and Sampling:

Target population: Any young age case in average 25 years old with premature hair grayingin the scalp, beard, eyebrow, and eyelash.

Study population:

- Cases (n =45): Patients with clinical diagnosis of premature hair graying based on the clinical evaluation of the lesion,
- Control (n=45): Individuals without premature graying.

Inclusion criteria:

- Age about 25 years old, in average (20-30).
- Both sexes.
- Patients with gray hair.

Exclusion criteria:

- Patients with skin depigmentation disorders.
- Patient with thyroid disorders.
- Patient with autoimmune diseases.
- Patient who takes chemotherapy.
- Patient who is exposed to radiation.

Methods:

The following clinical and laboratory tests were performed on each of the chosen patients:

II. I. Detailed history

Alopecic illness, age, sex, medication history, family history of PHG, smoking history, and the existence of a medical condition were all gathered.

III. Written questionnaire:

The onset, location, and number of gray hairs should all be asked about in the questionnaire.

IV. Complete clinical examination:

a. General examination: for exclustion criteria and other clinical signs related to the research topic.

b. Local examination:

The scalp was methodically partitioned into five separate areas: the frontal area, vertex, right temporal area, left temporal area, and occipital area, as demonstrated in Figure 1. In each zone, areas showing the most significant graying were identified through visual assessment. A 1 cm^2 area was outlined with a skin marker, and the hair within this designated space was cut to about 1 mm above the scalp. Afterwards, images of these five parts were captured and shown on a computer monitor to aid in the counting of white and black hairs.

The number of hairs permitted was used to assign a score to each zone according to the percentage of gray hair found in each designated area. The scoring system was outlined as follows: Score 1 for under 10% gray hair per cm^2; Score 2 for 10% to 30% gray hair per cm^2; and Score 3 for over 30% gray hair per cm^2. The Graying Severity Score (GSS) (Singal et al., 2016) for every patient was calculated by adding the scores from the five specified zones. The obtained objective scores were classified into Mild (0–5), Moderate (6–10), and Severe (11–15).

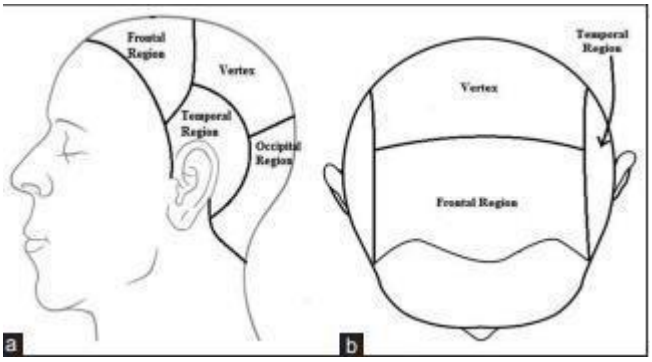

Figure 1: The 5 representative zones of scalp (a) side profile (b) top view

a. Determination SOD level:

The evaluation of the serum SOD was carried out by the use of Human Super Oxidase Dismutase (SOD) ELISA Kit (SunRed Bio Kit company, made in Egypt, Catalogue No: 201- 12-0919).

Ethical considerations:

The Port Said University Faculty of Medicine's Research Ethical Committee approved the procedure. It contained a thorough description of the goals and methodology of the investigation under code **ERN: MED (1/6/2023) s.no (94) DRM818_006.**

Analysis of Statistics:

Statistical assessments were performed with SPSS version 26 (IBM Inc., Chicago, IL, USA). The Shapiro-Wilk test along with histograms was used to evaluate the normality of data distribution. Quantitative parametric variables were reported as means with standard deviations (SD) and were compared between the two groups using an unpaired Student's t-test. For quantitative non-parametric data, findings were shown as medians with interquartile ranges (IQR) and examined using the Mann-Whitney test. Qualitative variables were expressed as frequencies and percentages (%) and analyzed through the Chi-square test or Fisher's exact test as suitable. A two-tailed P value below 0.05 was considered statistically significant.

Results:

Age and sex did not significantly differ between the two groups. Of the patients in the case group, 24 (53.3%) were female and 19 (42.2%) were male. There were 21 (46.7%) females and 26 (57.8%) males in the control group.

Age ranged from 17to 33years with mean (±SD) 25.56 (±4.53) years in cases group and ranged from 13to 33years with mean (±SD) 23.96(±5.36) years in control group. The median (IQR) of age was 25.0 (22.0 – 30.0) in cases groupand was 24.0(18.0 – 29.0) in control group **(Figure 2, and 3).**

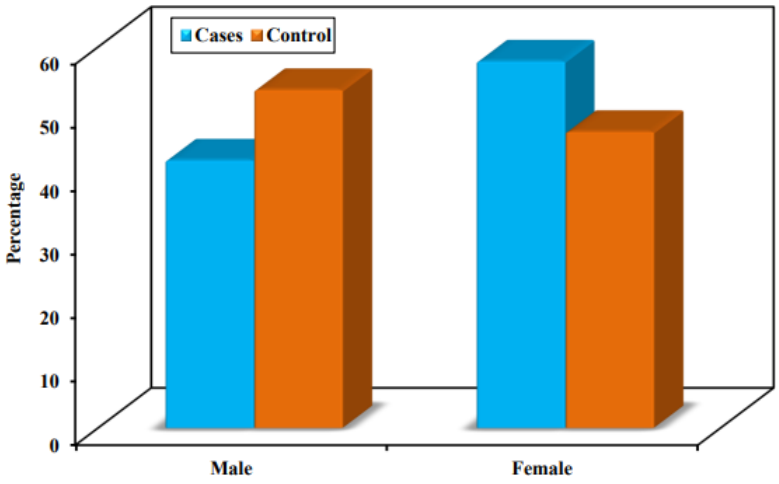

Figure (2): Comparison between the two studied groups according to sex

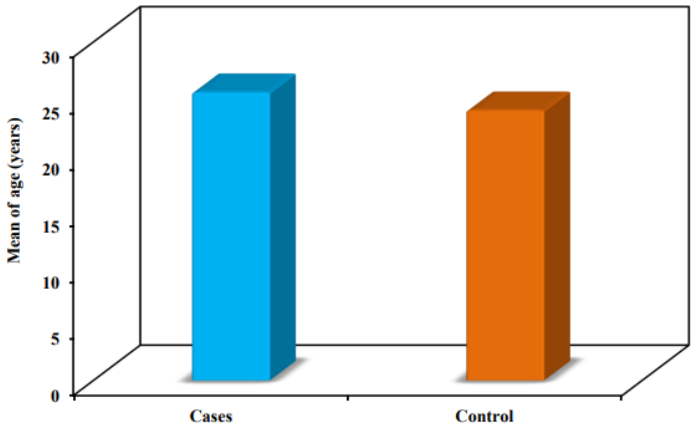

Figure (3): Comparison between the two studied groups according to age (years)

SOD was highly significant lower in cases group than in control group (P value <0.001). SOD ranged from 10.50 to 34.61 with mean (±SD) 20.69 (±5.27) in cases group and ranged from 21.22 to 60.26 with mean (±SD) 33.61 (±9.0) in control group. The median (IQR) of SODwas 19.86 (17.99 – 23.78) in cases group and was 31.82 (27.03 – 40.16) in control group **(Table** 1).

Table (1): Comparison between the two studied groups according to SOD

	Cases (n = 45)	Control (n = 45)	U	p
SOD				
Min. – Max.	10.50 – 34.61	21.22 – 60.26		
Mean ± SD.	20.69 ± 5.27	33.61 ± 9.0	165.500*	<0.001**
Median (IQR)	19.86 (17.99 – 23.78)	31.82 (27.03 – 40.16)		

IQR: Inter quartile range SD: Standard deviation U: Mann Whitney test p: p value for comparing between the two studiedgroups *: Statistically significant at $p \leq 0.05$ **:Statistically highly significant at $p \leq 0.01$

The two groups' histories of systemic illness differed negligibly. There was negligible variation in the medication histories of the two groups. Systemic disease was presentin 43(95.6%) patients in cases group and was present in 40(88.9%) patients in control group. Medications were given to 42(93.3%) patients in cases group and were given to 37(82.2%) patients in control group. 42(93.3%) patients had family history of hair graying **(Figure 4).**

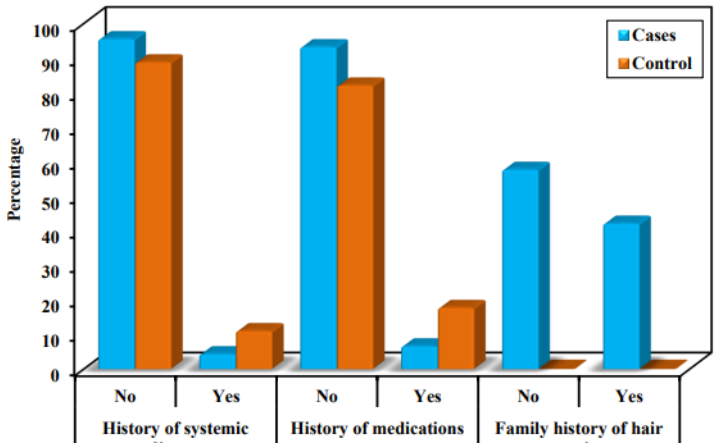

Figure (4): Comparison between the two studied groups according to history of systemicdisease, medications and family history of hair graying

In the mild group, there were 2 patients (66.7%) who were male and 1 patient (33.3%) who were female; in the moderate group, there were 7 patients (30.8%) who were male and 1 patient (33.3%) who was female. Ten patients (41.7%) and fourteen patients (58.0%) were male and female, respectively, in the severe category. Ages ranged from 22 to 32 years old, with a mean (± SD) of 26.67 (±5.03) years for the mild group, 20 to 33 years old, with a mean (± SD) of 26.39 (±4.46) years for the moderate group, and 17 to 33 years old, with a mean (± SD) of 24.79 (±4.59) years for the severe group. The median of age was 26.0) in mild group, was 27.50 in moderate group and was 24.0 in sever group **(Table 2).** Accordingly, age and sex differences across the three groups, mild, moderate and sever, were negligible.

Table (2): Relation between Severity and demographic data in Cases group (n = 45)

	Severity						Test of Sig.	p
	Mild (n = 3)		Moderate (n = 18)		Sever (n = 24)			
	No.	%	No.	%	No.	%		
Sex								
Male	2	66.7	7	38.9	10	41.7	χ^2= 0.919	^{MC}p= 0.721
Female	1	33.3	11	61.1	14	58.3		
Age								
Mean ± SD.	26.67 ± 5.03		26.39 ± 4.46		24.79 ± 4.59		F= 0.727	0.489
Median (Min. – Max.)	26.0 (22.0 – 32.0)		27.50 (20.0 – 33.0)		24.0 (17.0 – 33.0)			

SD: Standard deviation F: F for One way ANOVA test χ 2 : Chi square test MC: Monte Carlo p: p value fo p: p value for comparing between different categories

With a mean (± SD) of 34.18 (±0.70) years, the mild group's SOD varied from 33.38 to 34.61 years, the moderate group's from 20.01 to 27.60 years, and the severe group's from 10.50 to 19.89 years, with a mean (± SD) of 17.17 (±2.86) years. The median of SOD was 34.56 in mild group, was 23.45 in moderate group and was 18.04 in sever group **(Table 3).**

Table (3): Relation between Severity and SOD in cases group (n = 45)

SOD	Severity			H	p
	Mild (n = 3)	Moderate (n = 18)	Sever (n = 24)		
Mean ± SD.	34.18 ± 0.70	23.15 ± 2.18	17.17 ± 2.86	34.513	<0.001**
Median (Min. – Max.)	34.56 (33.38 – 34.61)	23.45 (20.01 – 27.60)	18.04 (10.50 – 19.89)		

SD: Standard deviation H: H for Kruskal Wallis test p: p value for comparing between different categories *: Statistically significant at p ≤ 0.05 **:Statistically highly significant at p ≤ 0.01

There was negative correlation between SOD and age in cases group P = 0.011, rs = - 0.376). likewise, there was negative correlation between SOD and duration in cases group (P = 0.015, rs = - 0.360) **(Table 4).**

Table (4): Correlation of SOD with both age and duration in cases group (n = 45)

	SOD	
	r_s	p
Age (years)	-0.376*	0.011*
Duration (years)	-0.360	0.015*

rs: Spearman coefficient *: Statistically significant at p ≤ 0.05

Table (5): Relation between SOD with the onset and course of hair graying in cases group (n = 45)

	N	SOD		U	p
		Mean ± SD.	Median (Min. – Max.)		
Onset of hair graying					
Gradual	**20**	20.88 ± 5.80	19.43 (10.72 – 34.61)	237.0	0.767
Sudden	**25**	20.55 ± 4.92	20.01 (10.50 – 34.56)		
Course					
Progressive	**34**	21.35 ± 5.42	19.95 (10.72 – 34.61)	145.0	0.277
Stationery	**11**	18.68 ± 4.38	19.49 (10.50 – 24.24)		

U stands for the Mann Whitney test, and p represents the p-value used to compare various groups.

In Table (5), groups did not vary substantially with respect to the beginning of hair graying, or the progression of the condition. In the gradual group, SOD levels varied from 10.72 to 34.61 with a mean (± SD) of 20.88 (±5.80), whereas in the sudden group, they ranged from 10.50 to 34.61 with a mean (± SD) of 20.55 (±4.92). In gradual group, the median SOD was 19.43, and in sudden, it was 20.01. In progressive, SOD ranged from 10.72 to 34.61, with a mean (± SD) of 21.35 (±5.42). In stationery, SOD ranged from 10.50 to 24.24, with a mean (± SD) of 18.68 (±4.38). In progressive, the median SOD was 19.95, and in stationery, it was 19.49.

Discussion:

One sign of the aging process that happens naturally to most people, regardless of gender, race, ethnicity, or nationality is graying hair (canities senilis). Even while it usually doesn't result in health issues, many people find it extremely upsetting for specific reasons, such aesthetic issues, particularly when they are young. Greying hair is caused by a mix of amelanotic, hypomelanotic, and normal pigment melanosomes. The final sign of ageing is white hair (Anastassakis & Anastassakis, 2022).

Senile greying strikes Africans in their mid-40s, Caucasians (whites) in their mid-30s, and Asians in their late 30s. A practical proclamation is that by the time they are 50 years old, 50% of persons typically have grey hair.

Age gray areas differ based on race and ethnicity.Premature graying of the hair is traditionally regarded as occurring in Caucasians (white people), Asians, and Africans, respectively, if it happens before the ages of 20, 25, and 30 **(Adav & Ng, 2023).**

Lipid peroxidation in cells can generate free radicals. In vivo, the activity of antioxidant enzymes and production of reactive oxygen species were both reduced by vitamins C and E. The significance of superoxide dismutase (SOD), as an antioxidant enzyme, lies in its ability to transform free radicals into superoxide anions (O2-), thereby reducing oxidative stress (Jomova, et al., 2023).

It functions as a preventative antioxidant by inhibiting the generation of superoxide anions. Hydrogen peroxide (H2O2) is broken down into oxygen and water (H2O) by the catalase enzyme. Hazardous particles like H2O2 can cause cell death and mutations (Kıran et al., 2023).

Determining the amount of SOD in prematurely greying hair and evaluating its potential as a predictive biomarker for the progression of greying were our objectives.

The case-control study took place at Tanta Hospital of Dermatology and Leprology's outpatient clinics in Tanta. In this work, 90 participants were divided into two groups. Cases group (n=45): young patients with a clinical diagnosis of premature hair graying (PHG) on the scalp, beard, eyebrows, and eyelashes. Control group (n=45): Individuals without premature graying.

The case group had a female majority, while the control group consisted mostly of males. However, there was no substantial gender disparity between the two groups. Saxena et al. 2020 and Acer et al. 2019 obtained no significant difference between the PHG and control groups, in line with the present study's result. Our study, like that of El-Sheikh et al. (2018) and Nath et al. (2020), found no significant age difference between PHG and control groups.

In the cases group, significantly lower SOD levels were detected compared to the control group. The body's antioxidant system, comprised of antioxidants and free radical scavenger enzymes like SOD, safeguards against ongoing damage caused by free radicals. (Mustafa, 2024).

According to the Saxena et al. 2020 study, PHG had lower SOD levels than the control group. Anggraini et al. (2021) reported a similar outcome.

More than half of our PHG cases had a sudden onset of hair graying and a progressivecourse. ***Sharma and Dogra 2018*** reported that in their cases, the progression of premature graying was observed to be slow in most patients. This difference may be due to their patientshaving a lower minimum age than ours.

The most common site in our PHG patients was the scalp, which is consistent with ***Sharma & Dogra 2018***. Another agreement with their study was the distribution; both our study and theirs showed that the distribution was mostly diffuse.

History of systemic disease was insignificantly different between our two studied groups. A Turkish study conducted by ***Akin Belli et al. in 2016*** showed a different result; there was a significant difference between the two groups. This difference was due to the inclusion of the answer "don't remember" in the choices about the history of systemic disease, and this answer was considered as absence in the statistical analysis.

Regarding the history of medications, our study found that there was no significant difference between both groups. In line with our study, the study by ***Akin Belli et al. 2016*** alsoreported similar findings.

Most of the cases we studied were classified as severe in terms of severity. This findingis consistent with the results reported by ***Saxena et al. 2020.*** However, this result contradicts the findings of ***Anggraini et al. 2021*** who reported that the severity degree of gray hair almost the same in 3 groups (mild, moderate, and severe). This discrepancy could be attributed to various factors, including the small sample size used in their studies.

Regarding the relationship between demographic data and severity, our study showed that there was no relation between the severity of PHG and age and sex. However, ***Akin Belli et al. 2016*** reported that age was significantly related to the severity of hair graying. They studied patients within a narrow age range.

Our study showed no significant relationship between severity and clinical history (onset of hair graying, duration in years, and course) in the cases group. However, ***Acer et al. 2019*** found a significant relationship between severity and onset of hair graying in subjects with PHG. This different result may be due to the fact that in their study, the patients were froma small geographic area with some sort of family history element of PHG.

Regarding the relationship between severity and SOD in our case group, there was a highly significant correlation. These results were different from those presented by ***Anggraini in 2021***, where there was no significant relationship between severity and SOD. The observed discrepancy may be attributed to the predominance of patients exhibiting a severe degree of condition. Superoxide dismutase (SOD) levels did not significantly correlate with factors including sex, the commencement of greying hair, or the course of the disease in the case group in the current study. A trend showing a decrease in serum SOD levels in relation to the severity of premature greying was described by Saxena et al. (2020); however, this discovery was not statistically significant. The lack of generally recognised reference ranges for total SOD or its particular isoforms in blood, as demonstrated by the varying normal ranges reported by different researchers, can be blamed for the diverse associations seen across different investigations.

Our research revealed a negative correlation between SOD levels and both age and duration within the case group. Conversely, Anggraini et al. (2021) found that the relationship between oxidative stress and the severity of graying was not significant, potentially due to the majority of their patients being classified with a total gray hair score. Kauser et al. (2011) noted no variation in SOD expression with advancing age; this discrepancy may stem from their study being conducted in vitro using human hair follicle melanocytes sourced from donors of varying ages.

According to the latest knowledge we have in this field up to the date of this study, thereare no previous studies published that addressed the correlation between SOD with age and duration. However, our results in this point were in line with the protective effect of SOD against PHG, as SOD prevented damage to melanocyte DNA by dismutating superoxide ***(Seiberg et al., 2013).***

There is a strong point in our study, as it can be considered the first one focused on therelationship and correlation between SOD and other important parameters in PHG patients. But also, we had some limitations. Firstly, all of our participants were from Egypt, which may require further studies in different Middle Eastern countries. Second, the study's sample size was modest because of the small number of participants, even though it thoroughly evaluated greying severity and SOD analysis.

In conclusion:

One common and efficient mechanism causing premature greying of the hair is oxidative stress, which lowers SOD levels. SOD was highly significant lower in premature hair graying group than in normal population.

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