

## Patterns of Serum Antioxidant Enzyme in Young Adult Male with Alopecia

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### Abstract

Alopecia (hair loss) has a social and psychological impact on its sufferers, notably in south eastern Nigeria. The purpose of this study is to determine the relationship between serum antioxidant enzyme levels and alopecia. Serum levels of antioxidant enzymes (superoxide dismutase, glutathione peroxidase, and catalase) and total antioxidant capacity were measured in male patients with alopecia. This study included a total of 230 guys aged 25 to 45. This includes 115 young adult males with alopecia and 115 male controls. Venous blood samples were obtained from all participants in a plain sample container, allowed to clot, centrifuged, and then collected in a separate container for biochemical analysis. Superoxide dismutase, glutathione peroxidase, and catalase were all measured using a spectrophotometer. The generated data was statistically analysed using SPSS version 23. Male individuals with alopecia had significantly reduced serum levels of SOD ( $0.86 \pm 0.16$  u/mg,  $p=0.001$ ), Catalase ( $7.30 \pm 1.04$  u/mg,  $p=0.001$ ), Glutathione peroxidase ( $37.88 \pm 11.38$  u/mg,  $p=0.001$ ), and TAC ( $1.55 \pm 0.2$ ,  $p=0.001$ ) compared to controls. Lower levels of antioxidant enzymes (superoxide dismutase, glutathione peroxidase, and catalase), as well as total antioxidants, seen in young adult males with alopecia may indicate oxidative stress in the development of this disorder in this setting.

**Keywords:** alopecia; antioxidant enzymes; owerri

### Introduction

Commonly known as hair loss, alopecia is an illness that affects people of all ages and has a big impact on one's physical appearance, mental health, and general quality of life [1]. Male hair loss is especially common and can be brought on by a number of things, such as oxidative stress, hormonal imbalances, immunological reactions, genetic predispositions, and environmental causes. The most prevalent type of alopecia in men is androgenetic alopecia (AGA), also known as male pattern baldness. AGA is largely caused by genetic susceptibility, but other important aspects that require more research include oxidative stress and the hormonal control of hair growth and loss [2].

Though more study is required to determine their effectiveness in managing alopecia, antioxidant-based therapies, such as vitamin C and E supplements and polyphenols, have showed promise in lowering oxidative stress-related hair loss. The shortcomings of current treatments emphasize the necessity of a thorough assessment of antioxidant enzyme levels in alopecia patients in order to guide individualized treatment plans [3].

Alopecia also has significant psychosocial effects on those who have it. Depression, social anxiety, and low self-esteem are frequently linked to hair loss, especially in younger men who suffer from early-onset androgenetic alopecia. The psychological toll that alopecia takes emphasizes how crucial it is to manage it using a multidisciplinary approach [4].

The purpose of this study is to assess the levels of antioxidant enzymes (catalase, glutathione peroxidase, and superoxide dismutase) in male alopecia patients. The study aims to find patterns of oxidative imbalance linked to alopecia and their possible relationship to the degree of hair loss by comparing these indicators to those in unaffected people [5]. These discoveries may open the door to better therapeutic approaches and diagnostic procedures, which would ultimately enhance the lives of those who suffer from alopecia.

Although a large percentage of men suffer from alopecia, especially androgenetic alopecia, little is known about the intricate pathophysiology of this disorder. One important but little-studied element in the pathogenesis of alopecia is oxidative stress. Hair follicles' strong metabolic activity and quick cell

turnover make them especially vulnerable to oxidative injury. Follicle dysfunction and early hair loss might result from an excess of reactive oxygen species (ROS) and a lack of antioxidant defence mechanisms. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are examples of antioxidant enzymes that are essential for preventing oxidative stress and preserving the integrity of hair follicles. Nevertheless, not enough research has been done on the connection between oxidative stress, antioxidant enzyme activity, and alopecia. New insights into possible antioxidant-based treatment approaches for hair loss may result from an understanding of these interactions [6]. The relationship between oxidative stress and psychological well-being is another important gap in the literature. Significant psychological suffering, such as low self-esteem and social anxiety, can result from hair loss. Despite these effects, there hasn't been a thorough investigation of the connection between oxidative stress markers and the psychological toll that alopecia takes. Closing this gap may open the door to more comprehensive treatment plans that take into account the condition's psychosocial and physiological aspects [7]. The lack of knowledge about the combined effects of oxidative stress on alopecia makes it difficult to create individualised treatment plans. Individual differences in oxidative profiles among patients are not taken into consideration by current treatment procedures, which are frequently one-size-fits-all [8]. This imprecision highlights the need for focused studies that assess how these variables interact and how they relate to clinical results. This study intends to fill in current information gaps and aid in the creation of more efficient, customised treatment strategies by examining antioxidant enzyme activity in male alopecia patients [9].

One important element in the pathogenesis of alopecia is now being identified as oxidative stress. Since hair follicles are metabolically active structures, the generation of reactive oxygen species (ROS) and antioxidant defence systems must be carefully balanced. Overproduction of reactive oxygen species (ROS) can cause oxidative damage to hair follicle cells, affecting their functionality and accelerating hair loss. By neutralising ROS and preserving cellular homeostasis, antioxidant enzymes including glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) act as buffers against oxidative stress. Studies measuring the amounts of

antioxidant enzymes in male alopecia patients are still rare, nevertheless. Assessing oxidative stress indicators may help identify new treatment targets and offer a fuller understanding of the mechanisms producing alopecia [10]. It is crucial from a clinical standpoint to comprehend how oxidative stress interacts with alopecia in males. Men who have alopecia may experience severe psychological consequences, such as sadness, social anxiety, and low self-esteem. Finding the oxidative causes of this illness is a viable path towards focused therapy. Furthermore, antioxidant-based therapies or dietary changes meant to strengthen the body's defences should be investigated as possible therapeutic approaches if oxidative stress is found to be a contributory factor [11].

Understanding the role of oxidative stress may also help create novel therapeutic approaches that focus on these pathways. By customising therapies to each patient's unique oxidative profile, this research may lay the groundwork for personalised therapy in the treatment of male-pattern baldness [12]. The paucity of comprehensive studies in this field emphasises how crucial it is to carry out investigations that assess antioxidant enzyme activity in male alopecia patients. Evaluating the concentrations of important antioxidant enzymes such glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) may shed light on how oxidative damage contributes to hair follicle dysfunction [13]. This research could help guide a more thorough strategy for managing alopecia. Treatment might be modified to address oxidative stress or imbalances if they are discovered to be important.

Lastly, by adjusting medication to each patient's unique oxidative stress profile, our study contributes to the expanding field of personalised medicine. Clinicians could more effectively choose the best treatments for each patient based on their individual physiological balance by assessing the thyroid, sex hormone, and antioxidant enzyme activity in men with alopecia. Personalised methods may improve therapy effectiveness and reduce adverse effects, providing more accurate alopecia management solutions. An important step towards creating customised treatment plans for alopecia patients and ultimately enhancing therapy results may be the incorporation of hormonal and oxidative stress evaluations into clinical practice.

## Materials and Methods

### Study Area

The study was conducted in Owerri, the capital city of Imo State, located in the southeastern region of Nigeria.

### Advocacy, Mobilization and Pre-Survey Contact

The ethical clearance was obtained from Specialist Hospital Owerri. Informed consent was obtained from eligible study participants.

### Study Population

A total number of 112 male individuals with alopecia were sampled for this study.

### Selection Criteria

#### Inclusion Criteria

Adult young males with alopecia; Participants aged 20–45 years were included to represent adults and middle-aged males. Participants must have lived in Owerri, Imo State, for at least six months to account for environmental factors. Participants must provide informed and written consent to participate in the study. For comparison, healthy male participants without a history of alopecia or any visible signs of hair loss were also recruited as controls. Willingness to complete questionnaires and undergo testing.

#### Exclusion Criteria

Females and individuals under 18 years were excluded.

Individuals with systemic illnesses such as diabetes mellitus, thyroid dysfunction (previously diagnosed), or other endocrine disorders unrelated to alopecia were excluded to avoid confounding variables.

Participants currently taking medications such as thyroid hormone replacement, testosterone, or other hormonal supplements were excluded.

Individuals who have undergone cancer treatment, which may cause hair loss, were not included.

Participants who have experienced significant surgery or acute illness within the last three months were excluded.

Individuals who refuse to provide written informed consent or decline to participate in specific study procedures were not included.

Participants with a history of excessive alcohol consumption or substance abuse were excluded, as these factors can significantly affect hormone levels.

### Study Design

This research adopts a case-control study design to evaluate the levels of antioxidant enzymes in male individuals with alopecia and compare them with healthy controls. The design allows for the identification of potential associations between

antioxidant enzyme levels and alopecia by examining differences between affected individuals and those without the condition.

Anthropometric Data: Body Mass Index (BMI) details, including weight and height measurements.

### Anthropometric Assessment

Anthropometric assessment played a crucial role in this study, focusing on the physical measurements and body composition of participants to evaluate their body mass index (BMI) and its association with Alopecia. Standardized procedures were employed to ensure accuracy, consistency, and reliability in data collection.

Key measurements included height and weight. Height was measured with a stadiometer, ensuring participants stand upright, barefoot, and gaze straight ahead for precise readings. Weight was recorded using a calibrated digital scale, with participants wearing minimal clothing to minimize variability. BMI was calculated using the formula:

$$\frac{\text{Weight (Kg)}}{\text{Height}^2(\text{m}^2)}$$

BMI values were categorized into standard classifications, such as underweight (<18.5 kg/m<sup>2</sup>), normal weight (18.5–24.9 kg/m<sup>2</sup>), overweight (25–29.9 kg/m<sup>2</sup>), and obese (≥30 kg/m<sup>2</sup>), to analyze their association with the presence and severity of Alopecia.

### Study Parameters

The parameters to be determined include; Antioxidant Enzymes (Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx)).

### Sample Collection

A Blood sample was collected as the primary specimen for hormonal assays. Participants avoid strenuous activities and alcohol for 24 hours prior to collection. Written informed consent was obtained. Venous blood (approximately 10mL) was drawn from the antecubital vein under sterile conditions using proper equipment and placed in plain vacutainer tubes for serum separation. The puncture site was cleaned, and pressure applied to prevent hematoma formation. Samples were labeled with participant information and transported to the laboratory for centrifugation at 3000 rpm for 10 minutes to separate serum and storage in cryovials.

### Laboratory Procedure

All reagents were commercially sourced, and the manufacturer's standard operating procedures (SOP) were strictly followed.

The Antioxidant Enzyme (Superoxide Dismutase (SOD) was determined using Nitro Blue Tetrazolium (NBT) Reduction method. The determination of catalase (CAT) activity was carried out using the hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) decomposition method, which relies on the ability of catalase to break down H<sub>2</sub>O<sub>2</sub> into water and oxygen. The rate of H<sub>2</sub>O<sub>2</sub> degradation was monitored spectrophotometrically at 240 nm, providing a quantitative measure of CAT activity in the test samples. The determination of glutathione peroxidase (GPx) activity was performed using an indirect spectrophotometric method that measures the rate of glutathione (GSH) oxidation in the presence of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) as a substrate. GPx catalyzes the reduction of H<sub>2</sub>O<sub>2</sub> by utilizing reduced glutathione (GSH), converting it into oxidized glutathione. The decrease in GSH levels was monitored by coupling the reaction with

glutathione reductase (GR) and measuring the rate of Nicotinamide adenine dinucleotide phosphate NADPH oxidation at 340nm.

### Statistical Analysis

The analysis included data description, group comparisons, and exploration of correlations and potential predictors of Alopecia. All statistical analyses were performed using software such as SPSS, with a significance threshold set at p<0.05. Data will be presented as mean ± standard deviation. Group comparisons will be conducted using Student's t-test, One-Way ANOVA, and Two-Way ANOVA, followed by Tukey's Multiple Comparison Test for post-hoc analysis.

### Results

The results are expressed in tables below.

**Table 1:** The enzymatic antioxidant and TAC in young adult males with alopecia versus controls

Variables (Mean ± SD) (u/mg)	Test Subject (N = 115)	Control Subject (N = 115)	t-Value	P-value
SOD	0.86±0.16	1.02±0.14	-9.14	0.001
Lower 95% C.I	0.83	0.99		
Upper 95% C.I	0.89	1.04		
Catalase	7.30±1.04	8.94±1.27	-12.52	0.001
Lower 95% C.I	7.11	8.71		
Upper 95% C.I	7.5	9.81		
Glutathione peroxidase	37.88±11.38	45.05±11.45	-5.62	0.001
Lower 95% C.I	35.77	42.91		
Upper 95% C.I	39.98	47.18		
TAC	1.55±0.29	2.00±0.20	-14.85	0.001
Lower 95% C.I	1.49	1.97		
Upper 95% C.I	1.6	2.04		

### The enzymatic antioxidant and TAC in young adult males with alopecia versus male controls

SOD was significantly lower (p=0.001) in male individuals with alopecia (0.86±0.16 u/mg) compared to male controls (1.02±0.14u/mg). Catalase was significantly lower (p= 0.001) in male individuals with

alopecia (7.30±1.04u/mg) compared to controls (8.94±1.27u/mg). Glutathione peroxidase was significantly lower (p= 0.001) in male individuals with alopecia (37.88±11.38u/mg) compared to controls (45.05±11.54u/mg). TAC was significantly lower (p= 0.001) in male individuals with alopecia (1.55±0.29) compared to controls (2.00±0.20) (Table 1).

**Table2:** The body mass index and age of young adult males with alopecia compared to controls

Variables (Mean ± SD)	Test Subject (N = 115)	Control Subject (N = 115)	t-Value	P-value
BMI (kg/m <sup>2</sup> )	22.99±2.52	20.94±1.92	8.27	0.001
Lower 95% C. I	22.52	20.58		
Upper 95% C. I	23.45	21.29		
Age (Years)	37.17±9.67	35.48±11.47	1.20	0.22
Lower 95% C. I	35.38	33.36		
Upper 95% C. I	38.96	37.60		

### The body mass index and age of young adult males with alopecia compared to controls

BMI was significantly higher (P=0.001) in male individuals with alopecia (22.99±2.52kg/m<sup>2</sup>) when

compared with controls ( $20.94 \pm 1.92 \text{ kg/m}^2$ ). Age was not significant ( $p=0.22$ ) in male individuals with

alopecia ( $37.17 \pm 9.67$ ) compared to controls ( $35.48 \pm 11.47$ ) (Table 2).

**Table 3:** Correlations of Antioxidants enzymes with BMI & Age

		SOD	CATALASE	GLUTA PEROXIDASE	TAC	BMI	AGE
SOD	r-value	1	-0.045	0.135	0.002	0.095	0.093
	p-value		0.636	0.151	0.985	0.313	0.321
	N	115	115	115	115	115	115
CATALASE	r-value	-0.045	1	0.250**	-0.083	-0.097	0.108
	p-value	0.636		0.007	0.375	0.301	0.253
	N	115	115	115	115	115	115
GLUTA PEROXIDAS	r-value	0.135	0.250**	1	0.071	0.113	-0.002
	p-value	0.151	0.007		0.452	0.229	0.985
	N	115	115	115	115	115	115
TAC	r-value	0.002	-0.083	0.071	1	0.049	-0.056
	p-value	0.985	0.375	0.452		0.603	0.549
	N	115	115	115	115	115	115
BMI	r-value	0.095	-0.097	0.113	0.049	1	-0.038
	p-value	0.313	0.301	0.229	0.603		0.686
	N	115	115	115	115	115	115
AGE	r-value	0.093	0.108	-0.002	-0.056	-0.038	1
	p-value	0.321	0.253	0.985	0.549	0.686	
	N	115	115	115	115	115	115

\*\* . Correlation is significant at the 0.01 level (2-tailed).

### Correlations of Antioxidants enzymes with BMI & Age

There is a significant positive correlation of serum Glutathione Peroxidase level with serum catalase level in male individuals with alopecia ( $r=0.25$ ,  $p=0.07$ ). There was no significant correlation of SOD with catalase ( $r=-0.45$ ,  $p=0.636$ ), glutathione peroxidase, ( $r=0.135$ ,  $p=0.151$ ) TAC, ( $r=0.002$ ,  $p=0.985$ ) BMI ( $r=0.095$ ,  $p=0.313$ ) and age ( $r=0.093$ ,  $p=0.321$ ) in male individuals with alopecia. There was no significant correlation of catalase with TAC ( $r=-0.083$ ,  $p=0.375$ ), BMI ( $r=0.097$ ,  $p=0.301$ ) and age ( $r=0.108$ ,  $p=0.253$ ) in male individuals with alopecia. There was no significant correlation of glutathione peroxidase with TAC ( $r=0.071$ ,  $p=0.452$ ) BMI ( $r=0.113$ ,  $p=0.229$ ) and age ( $r=-0.002$ ,  $p=0.985$ ) in male individuals with alopecia. There was no significant correlation of TAC with BMI ( $r=0.049$ ,  $p=0.603$ ) and age ( $r=-0.056$ ,  $p=0.549$ ) in male individuals with alopecia (table 3).

### Discussion

Male hair loss is especially common and can be brought on by a number of things, such as oxidative stress, hormonal imbalances, immunological reactions, genetic susceptibility, and environmental causes [13]. When comparing male alopecia patients to controls, this study shows that their blood levels of all the antioxidant enzymes (superoxide dismutase,

glutathione peroxidase, catalase, and total antioxidant capacity) are significantly lower. Through their ability to neutralise or scavenge reactive oxygen species (ROS), antioxidant enzymes shield cells from injury [14]. They serve as an essential initial line of defence against oxidative stress, which is linked to ageing and a number of disorders [15]. Because these enzymes cooperate under physiological settings, the body's antioxidant defence will be weakened if any of them are inactivated [16, 17]. An antioxidant enzyme called glutathione peroxidase (GPx) transforms toxic hydrogen peroxide and lipid peroxides into less reactive compounds like lipid alcohols and water [18]. Within cells, particularly hair follicles, this activity aids in preserving the oxidative-antioxidative balance, which is the equilibrium between oxidants and antioxidants. Reduced GPx activity can result in higher hydrogen peroxide and other reactive oxygen species (ROS) levels, which can harm or even kill hair follicle cells, ultimately leading to alopecia [19]. Superoxide radicals are a kind of reactive oxygen species (ROS), and SOD is essential for their breakdown. Superoxide radicals build up when SOD levels are low, causing oxidative stress and imbalance that harms hair follicles and eventually results in alopecia [20].

### Conclusion

Young adult males with alopecia in this study had lower levels of antioxidant enzymes (catalase, glutathione peroxidase, and superoxide dismutase), which may imply oxidative stress in the development of this disorder in this setting.

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