

# MICROSCOPIC SEX-BASED DIFFERENTIATION OF HUMAN SCALP HAIR AMONG UNIVERSITY STUDENTS IN NIGERIA

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## ABSTRACT

Scalp hair is one of the most resilient biological materials frequently encountered in forensic investigations, offering valuable trace evidence, especially when nuclear DNA is degraded or absent. This study examined sex-based differences in microscopic characteristics of scalp hair among a Nigerian population, aiming to provide population-specific forensic reference data. A total of 400 hair samples, comprised of 200 samples each from males and females, were collected from healthy adults aged 18 to 30 years old. Using stereomicroscopy, each sample was assessed for hair shaft diameter, medullary diameter, hair length, density, follicular size, texture, pigmentation, and cuticular integrity. Statistical analysis was performed using t-tests and ANOVA at a significance level of  $p < 0.05$ . The results showed that male hair had significantly larger shaft and medullary diameters and follicle size, while female hair had higher density and longer average length. Differences in hair texture were also observed, with a greater proportion of curly and wavy hair among females. Pigmentation remained consistent in male samples but showed distal fading in females. Cuticle preservation was generally better in males, whereas partial or complete cuticle loss was common in female samples. These findings reveal clear, measurable differences in scalp hair morphology between sexes and underscore the forensic value of microscopic hair analysis. Moreover, the study contributes crucial localized data for forensic investigations in the African context.

**Keywords:** Scalp hair, sex-based differentiation, microscopic, forensic science.

## INTRODUCTION

Human hair is among the most commonly encountered biological materials at crime scenes due to its high durability, widespread presence, and the fact that it sheds naturally and frequently from the scalp. As a result, hair is considered a critical component of trace evidence in forensic science (Laatsch *et al.*, 2014). Structurally, human hair comprises three concentric layers: the medulla (core), the cortex (middle layer), and the cuticle (outermost layer), each of which carries morphological and biochemical signatures that can be used for identification purposes (Franbourg and Leroy, 2005).

In recent years, advancements in hair analysis have reinforced its application in forensic investigations, not only for human identification but also for providing insights into the individual's biological sex, health status, environmental exposure, and even drug use history (Liu *et al.*, 2021; Amiri *et al.*, 2022). While DNA profiling remains the most reliable method for establishing identity, especially through short tandem repeat (STR) analysis, it is not always viable when dealing with telogen-phase hairs or hair shafts lacking viable nuclear material (Zhou *et al.*, 2023). In such cases, microscopic and morphological analyses offer a valuable

alternative or supplementary method for preliminary classification.

Microscopic analysis of hair, particularly through stereomicroscopy, enables the detailed examination of surface features and internal structures without the need for extensive sample preparation. This method is especially effective in identifying features such as shaft profile, pigment distribution, cuticle pattern, and surface texture, all of which may vary across individuals, sexes, and populations (Robertson and Aitken, 1986; Zafarina and Panneerchelvam, 2009). Moreover, stereomicroscopy has the advantage of preserving the sample's integrity for potential downstream molecular analysis.

Despite growing global interest in forensic hair characterization, there remains a significant gap in regional studies, especially in African populations, where morphological patterns may differ due to genetic and environmental influences (Adeyemi *et al.*, 2021). Most reference datasets are derived from European or Asian populations, which may limit the applicability of global forensic standards in African context.

This study aimed to address this gap by conducting a detailed comparative analysis of male and female scalp hair samples, collected from students at Admiralty University of Nigeria. The research investigated the observable differences between the sexes in terms of shaft morphology, pigmentation, surface texture, and cuticle presence using stereomicroscopic technique by focusing on a Nigerian university students.

## MATERIALS AND METHODS

### Study area

This study was conducted at the Admiralty University of Nigeria. It is a Nigerian federal university located along the Ibusa-Ogwo expressway in Delta State (6.16471°N, 6.57544°E). The University has a total population of 716 active students, comprising both male and female, as at the time of study (2022/2023) academic session.

### Sample Collection and Preparation

A total of 400 hair samples, comprised of 200 samples each from males and females, were randomly collected from healthy adult volunteers aged between 18–30 years old. To maintain consistency, scalp hair was selected from the occipital region using sterile tweezers. All samples were cleaned using 70% ethanol to remove surface oils, dirt, and potential contaminants, following protocols established by Wilkins *et al.* (1995). After degreasing, each strand was trimmed to approximately 3cm in length to standardize observation conditions. The hair samples were transferred to the African Biosciences Research Laboratory at Olode, Ibadan, Nigeria, for further analysis.

### Mounting Procedure

At the African Biosciences Research Laboratory, the hair samples were mounted on clean glass slides using a drop of clear nail polish, which served both as a fixative and as a sealant to preserve the sample's morphology. Mounting was performed carefully using fine-tipped tweezers to prevent mechanical damage.

### Microscopic Examination

Each sample was examined using a stereomicroscope (magnification range: 10X to 100X) under both reflected and transmitted lighting conditions, consistent with methods described by Houck (2003) and Khan *et al.* (2014). Reflected light was primarily used to assess surface features such as shaft diameter, colour, and cuticular structure, while transmitted light enhanced the visibility of internal details, such as medullary presence. Images were captured with a high-resolution digital camera for comparative documentation and analysis.

### Measurement of Parameters

**Hair shaft diameter ( $\mu\text{m}$ ):** The thickness or width of an individual hair strand. It was measured using a calibrated optical microscope with a micrometer at 400X magnification. **Medullary diameter and medullary index ( $\mu\text{m}$ ):** The width of the innermost layer of the hair shaft. They were measured using a formula adapted from Khan *et al.* (2014) as:

$$\text{Medullary Index} = \frac{\text{Medullary Diameter}}{\text{Hair Shaft Diameter}}$$

**Hair length (cm):** was measured as the distance from the root end to the tip of the hair shaft using a calibrated optical microscope with a micrometer at 400X magnification. **Hair density (strands/cm<sup>2</sup>)** was calculated as the number of hair strands per square centimeter of scalp from a 1cm<sup>2</sup> scalp impression. **Hair texture** was categorized as straight, wavy, or curly by visual and microscopic inspection. **Hair follicle size ( $\mu\text{m}$ )** was measured as the diameter of the bulb region of plucked hairs under 100X magnification. **Colour** (proximal and distal pigmentation) was determined using visual examination at 100X magnification using a digital magnifying lens. **Cuticle presence and integrity** of the hair shaft's outermost layer were determined using a stereomicroscope at 400X magnification. The cuticle's presence plays a crucial role in hair's overall health and appearance.

### Statistical analysis

All the measurements were expressed as mean  $\pm$  standard deviation. Gender comparisons were tested using t-tests, while ANOVA was applied to assess variations related to hair texture. A p-value of <0.050 was considered statistically significant.

## RESULTS

### Hair microscopic features

Hair microscopic features (Table 1) show that male hair exhibited a significantly ( $p < 0.01$ ) greater average shaft diameter of 85.4  $\mu\text{m}$  than the female hair with 77.9  $\mu\text{m}$ . The medullary diameter was also larger among males (48.6  $\mu\text{m}$ ) than among females (38.2  $\mu\text{m}$ ). Average hair length was longer among female (1.4 cm) than among male (1.1 cm), with this difference being statistically significant ( $p < 0.001$ ). Female participants also exhibited higher hair density (145 strands/cm<sup>2</sup>) compared to males (120

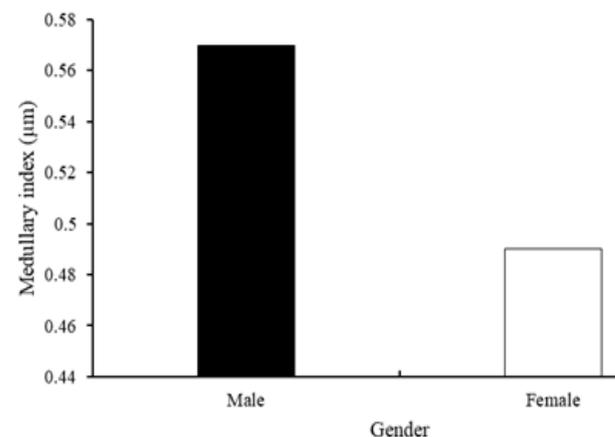
strands/cm<sup>2</sup>), and this difference was statistically significant ( $p < 0.05$ ). Hair texture distribution among males was 40% straight, 30% wavy, and 30% curly. In females, 25% were straight, 40% wavy, and 35% curly. Male average follicular size was significantly ( $p < 0.05$ ) larger (280  $\mu\text{m}$ ) than that of females (260  $\mu\text{m}$ ).

**Table 1:** Comparative Microscopic Hair Features between the Male and Female Subjects

Hair Features	Male (Mean $\pm$ SD)	Female (Mean $\pm$ SD)	t-test	p-value
Hair Shaft Diameter ( $\mu\text{m}$ )	85.4 $\pm$ 6.3	77.9 $\pm$ 5.8	0.005	<0.01
Medullary Diameter ( $\mu\text{m}$ )	48.6 $\pm$ 4.1	38.2 $\pm$ 3.9	0.005	<0.01
Hair Length (cm)	1.1 $\pm$ 1.2	1.4 $\pm$ 2.4	0.005	<0.001
Hair Density (s/cm <sup>2</sup> )	120 $\pm$ 15	145 $\pm$ 18	0.005	<0.05
Hair Texture Distribution	Straight (40%), wavy (30%), Curly (30%)	Straight (25%), Wavy (40%), curly (35%)	0.005	NA
Hair Follicle Size ( $\mu\text{m}$ )	280 $\pm$ 22	260 $\pm$ 19	0.005	<0.05

(s/cm<sup>2</sup>) = strands/cm<sup>2</sup>

As shown in Figure 1, the medullary index was observed to be significantly higher in male samples (0.57  $\mu$ ) than in female samples (0.49  $\mu$ ) (0.005).



**Figure 1:** Medullary index of male and female scalp hair samples

### Color and Cuticle Features

As illustrated in Table 2, male hair retained consistent black pigmentation at both the proximal and distal ends. In contrast, female hair had deep black pigmentation at the proximal end but dark brown coloration at the distal end. In addition, males exhibited intact, overlapping cuticle scales, whereas females showed partial or complete cuticle loss.

**Table 2:** Color and cuticular features of male and Female Scalp Hair Samples

Gender	Proximal Pigmentation	Distal Pigmentation	Cuticle Presence and features
Male	Black	Black	Present as overlapping transparent scales
Female	Deep black	Dark brown	Partial or complete loss of the cuticle

## DISCUSSION

The observed micro-structural differences between male and female hair in this study hold significant implications for forensic identification and anthropological classification. Hair remains one of the most resilient forms of biological evidence due to its resistance to degradation and wide availability at crime scenes (Houck, 2003). While DNA profiling remains the gold standard for human identification, microscopic analysis offers a valuable preliminary tool, particularly in resource-limited settings or in cases where nuclear DNA is degraded (Musa *et al.*, 2023; Franbourg and Leroy, 2005).

The significantly greater hair shaft diameter among the males (85.4  $\mu\text{m}$ ) as compared to that of females (77.9  $\mu\text{m}$ ) is consistent with previous findings that associated male hair with greater thickness due to androgenic influence (Osei-Tutu *et al.*, 2018). Similarly, the wider medullary hair diameter observed among males (48.6  $\mu\text{m}$ ) relative to females (38.2  $\mu\text{m}$ ) supports earlier studies indicating that the medulla is typically more developed in male hair strands (Al-Dulaimy *et al.*, 2020). These features are key differentiators in forensic analysis, especially in the absence of DNA evidence.

Although hair length differences may partly reflect grooming habits, the statistically significant longer hair in females (1.4 cm) aligns with socio-cultural patterns documented in similar studies (Adeyemi *et al.*, 2021). The higher hair density observed among females compared to males could be linked to hormonal factors, as estrogen is known to prolong the anagen (growth) phase of hair follicles (Liu *et al.*, 2021).

Both males and females had higher proportions of curly hair than wavy hair, which could be due to hair texture being genetically determined and varying by population; African hair is predominantly curly due to the elliptical shape of the follicle and irregular distribution of keratin (Musa *et al.*, 2022). Because this study was conducted using samples from Nigerians, the differences noted in this study reflect the influence of genetic diversity within populations.

The significantly high medullary index that was observed in male samples compared to the female samples reflects both the broader medulla and the thicker overall hair shaft observed among males. This is consistent with a previous observation by Musa *et al.* (2021) that male hair tends to have a more developed or continuous medulla, which can be a reliable parameter in sex determination. The larger hair follicular diameter of the males compared to females corresponds with the thicker hair shafts observed and is a marker of androgen-related follicular growth (Schmidt and Klosterhalfen, 2021). This parameter is of forensic importance when evaluating hair root characteristics, particularly in plucked hairs.

The difference in pigmentation at the proximal and distal ends of the female hair suggests possible environmental or cosmetic

influences, as distal hair segments are more exposed to external factors such as UV radiation and hair products (Liu *et al.*, 2023). This aligns with studies that associate colour variations in hair with external factors rather than inherent biological differences (Laatsch *et al.*, 2014). Additionally, the hair cuticular integrity and surface smoothness also emerged as reliable markers of sex differentiation (Musa and Ikhajagbe, 2021; Richard *et al.*, 2014). The better-preserved cuticle integrity observed in the male samples is likely due to increased exposure to chemical treatments and mechanical damage (Zhou *et al.*, 2023). The presence and condition of the cuticle are critical in forensic examinations for determining hair treatment history and external influences.

## Conclusion

This study successfully demonstrated that significant morphological differences exist between male and female scalp hair of Nigerian university students' population, as revealed through detailed stereomicroscopic analysis. Parameters such as hair shaft diameter, medullary diameter, hair density, follicle size, and cuticle integrity varied notably between sexes, with male samples generally exhibiting thicker shafts, wider medullae, and better cuticle preservation, while females showed higher hair density and a greater proportion of curly and wavy textures. These distinctions provide valuable indicators for sex differentiation in forensic investigations, particularly when DNA evidence is absent or degraded.

By establishing baseline reference values and observable patterns in hair structure among males and females, this research not only enhances the forensic utility of hair analysis but also lays the groundwork for future studies incorporating molecular techniques, broader demographic sampling, and longitudinal assessments.

## Conflict of Interest

The authors wish to declare that there is no conflict of interest. All the study subjects consented to the study before their enrolment. All data used in this research were generated as primary data by the researchers. The raw data are with the researchers and can be obtained once the researchers are approached

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