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RESEARCH ARTICLE

Microscopic Hair Shaft Analysis in Iraqi Population

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Abstract

Hair is a biological evidence contributing important role at a crime scene. Microscopic hair analysis can help to identify of a suspect in forensic investigation. The principle of this study was to find diagnostic aspects of the hair shaft from the Arab Iraqi population through compound light and scanning electron microscopy examination and analysis of the shaft diameter, medullary index, ovoid body and medulla type for human scalp hair shaft in forensic analysis in the crime scene investigation. The investigation under compound light microscope included 76 out of 100 samples because of 24 hairs samples were deep black view and photos showed no data. The shaft diameter range of hair samples was 34.75 to 115µm.The medulla type classified into four groups as absent type (78%), continuous (13 %), fragment (8%) and interrupted type (1%). The medullary index of human hair was observed in ranging from 0.083-0.189. The ovoid bodies in hair shaft samples were absent (80%) and present (20%) of all samples. In this study reported that no significant differences in shaft diameter, medullary index, medulla type and ovoid body among male and female groups. The scanning electron microscope results indicated that high amount of hair shaft damage and the difference between the individuals in outer cuticle scale pattern showed that all samples were imbricate type, while chipped, lifting, jagged and smooth reported 17%, 8%, 4%and 1%, respectively.

Keywords: *Hair shaft, Microscopic analysis, SEM, Iraq.*

Introduction

Hair is among the most frequently encountered contact trace evidence in forensic science. Each hair arises below the surface of the skin from a root which is enclosed in a follicle. The hair shaft consists of the innermost medulla (only found in thick hairs), a middle layer known as the cortex and the outermost cuticle [1]. Hair examiners have recognized that in some cases the microscopic characteristics of an individual's hair sample may have changed with time and could possibly lead to a false exclusion of a victim or suspect.

Microscopic analysis of the human hair is of substantial importance in the forensic sciences field [2]. The characteristics of hair can be used to separate a suspect's hair from the victim. This question of separation and identification is always the key issue with any biological evidence, in addition to that hair analysis play a crucial role in criminal investigations related to wildlife, ethnicity of population and medical aspects [3].

Hair microscopic analysis might include information is better than nothing, if this technique were validated; investigators might have some information about gender, ethnicity and age to narrow down a suspect grouping [4]. However, microscopic hair analysis is rapid and inexpensive and could be used to reduce time-consuming for the investigation of abundant hair evidence [5]. Electron scanning microscopy (SEM) can be used to assess and demonstrate the subtle structural damage to the hair shaft.

SEM is a powerful tool for forensic analysis of trace physical evidence [6], revealing easily comparable images and it is indispensable for examination of various tissues, which permit considerable magnification [7]. We performed the microscopic analysis of the hair shaft that included comparative imaging of the compound microscope and scanning electron microscope, in order to examine and compare hair shaft samples to obtain the maximum morphological characteristics of human hair.

Material and Methods

Sample Collection

The human scalp hairs were collected from 100 unrelated individuals in subsequent three generations from different south regions of Arabic Iraqi, aged 15-52 years old from both males and females. All individuals were agreed by signing on the consent document of participation. The procedure for sample collection included combing of the scalp region. Each sample was involved 10 catagenic or telogenic phase scalp hair and kept in the envelope labeled with the code number.

Compound Light Microscope Examination of hair shaft

The hair samples were washed with distilled water and alcohol 70%, twice to remove suspended impurities and dried on a filter paper, then fixed on a glass slide contains on a drop of mounting media (DPX) and put a cover slip, sealing around the cover slip by using nail polish It's ready to examine under light microscope under 40X, then analyzed by using OMAX ToupView software version: 3.7.7149 [8].

Scanning Electron Microscopy (SEM) of Hair Shaft

The hair samples were cleaned by placing it in Petri dishes contain distilled water and sterile cotton dipped in ethanol left for 5 min. Hairs left to be air dried by using filter paper in room temperature, then hair sample fixed and stick on adhesive tape two faced, the upper face stick on hair and the lower face stick on metal slide, then immediately the imaging examined in scanning electron microscope (FEI, Holland) [9].

Statistical Analysis

The statistical analysis in this study included Shapiro- Wilk test and t-test to find a significant difference in the average of hair diameter in hair samples. The Chi-Square test is used to find the significant difference in ovoid body and medulla type in hairs samples. The SPSS software is used to carry out this test.

Results and Discussion

Hair Shaft under Compound Light Microscope

A total of one hundred scalp hair shaft samples which have been collected from the Iraqi population for observed the hair microscopic characteristics between individuals as forensic evidence. The characteristics of hair included in this study were shaft diameter, medulla diameter and medulla index as well as observation of medulla type and ovoid body. The study included 76 samples and 24 samples were excluded because the reminder samples inconclusive appeared black and not have any clear microscopic characteristics when examined by light compound microscope as shown in Figure (1).

Figure 1: Human scalp hair shaft under light compound microscope 40X showed that hair features are unclear

Hair Shaft Diameter

In this study showed that the shaft diameter of hair samples between 34.75 to 115µm.The diameter of a human scalp hair does not have a standard value due to several factors affected on a diameter range of hair such as inheritance factors, hair color (black hair is thicker than red hair) and the age (diameter of a hair is increased gradually with the age from babies into adult). As a person grows up, their hair becomes thicker and stronger reported that in normal females, on the back of the head had the thickest diameter, followed by the sides, top, and front in decreasing order [10]. The shaft diameter range in female scalp hair was 34.75 to

115µm (means of 76.05µm and standard deviation ± 17.55), while the shaft diameter range in male scalp hair was 35.67 to 107.65µm (means of 73.70µm and standard deviation ±19.77) showed in Table (1). The statistical analysis showed that no statistical difference in the shaft diameter between the male and female (*P*-value 0.870). This study is agreement with Kang study that was found that inter- and intra-individual variations in the hair. Minimum shaft diameter reported 13.52µ while the maximum 38.071µ with a mean value of 25 hairs is 27.975µ ± 6.573. 13.52µ [11].The previous study of Birch population measured hair shaft diameters of hair samples taken from 294 women, which reported that mean hair diameter increased from age 22-30 years old increasing the age. There was considerable variation in mean diameters among individuals [12].

Tab 1: The significant difference in the averages of shaft diameter between males and females

Gender	No.	Mean (µm)±SD	*p*. value
Male	54	76.05±17.55	0.870
Female	46	73.70±19.77	0.131

Medulla type and Medulla Index

The comparison microscopic process involves the side-by-side analysis of hair samples. This work allowed for a direct comparison of the microscopic characteristics. In this study, medulla type appeared the nature of opaque and translucent forms of the medulla from the proximal end to the distal end of the hair shaft [13]. The medulla type was divided into four groups as shown in Figure (2): continuous, fragment, interrupted and absent [14].

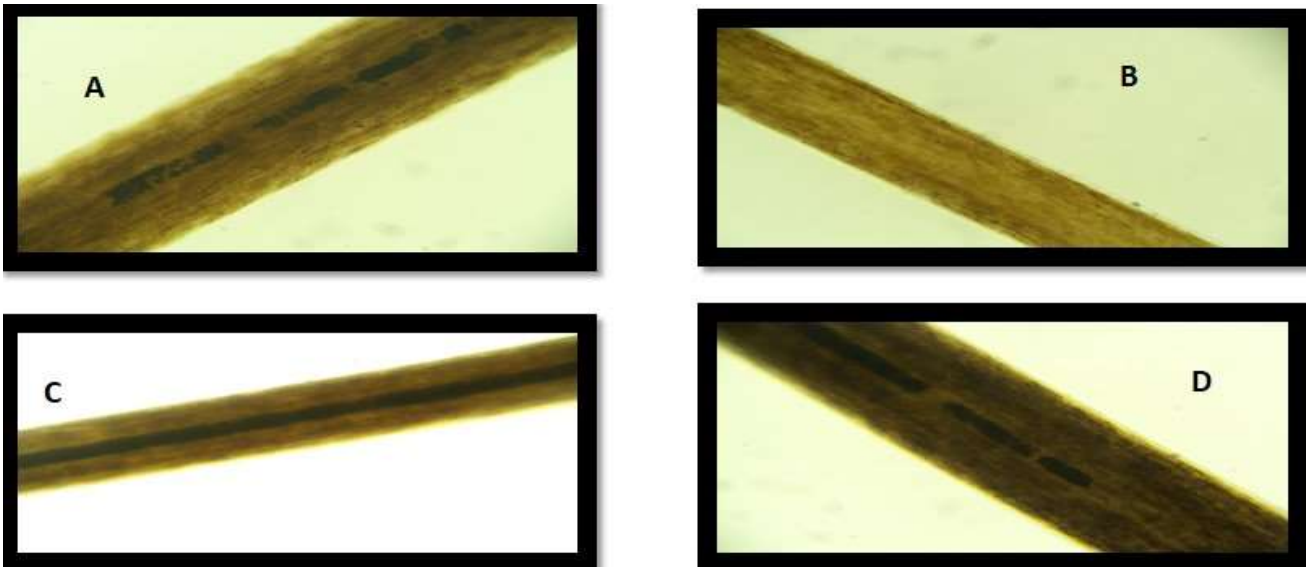

Figure 2: Light microscopic comparison (40X) of scalp human hair for four different samples refers to the four types of medulla: (A) fragment (B) absent (C) continuous (D) Interrupted

The results of this study showed the fragment medulla type were 4 samples in male group and 2 in female group, continuous typewere5 samples in male group and 5 in female group, absent in 35 male and 25 female samples and only one female sample showed the interrupted type of the medulla as shown in Table (2). The statical analysis was shown that P-value of medulla type (0.54) > 0.05, therefore there no significant difference between male and female groups. The study of De La Mettrie has reported that variations of medulla types were not only from one population to another, but also vary in different age group of the same population [15]. The Kaur study showed that there is no one particular type of medulla of all the hair of one person. It may vary from individual to individual and even from hair to hair of the same person. This finding may indicate that the presence of the medulla maybe related to the stage of the growth of a particular hair and further work is being done in this direction [16]. The medullary index of hair is used to differentiate animal hair from human hair in cases of forensic examination. Medullary index is articulated as a ratio of the shaft diameter to the medulla diameter. In human the medulla ratio is usually less than 1/3 while in animals the medulla will make up more than 1/2 of the total diameter [17].

Table 2: Classification of medulla types between male and female of hair samples

Gender	Absent	Continuous	Fragment	Interrupted	Total
Female count	25	5	2	1	32
Male count	35	5	4	-	44
Total count	59	10	6	1	76

The diameter medulla is a useful parameter for differentiation among the human and animal hair in forensic applications. In this study observed that the medulla diameter of human hair between 7-20µm. In the present study, it was observed that the medullary index of human hair was from 0.083- 0.189. This study agreement with Saferstein study showed that the medullary index of human hair ranged from 0.1 - 0.25.

The Kshirsagar study showed a difference in the medullary index between animal and human as well as, that observed the medullary index was less than 0.25 in human hair and more than 0.44 in animal hair.

Thus, the medullary index is the most significant and hence the most useful parameter to distinguish between human and animal hair [18].

Ovoid Body

Ovoid bodies are part of the cortex and as an oval structure. All samples were analyzed for the presence or absence of ovoid bodies. In this study showed that 10 male's samples have ovoid bodies and only five samples contain ovoid bodies in female as shown in Figure (3), but the thirty-four of remaining male hair samples showed that lacked the ovoid bodies. In the female's hair twenty-seven samples have been lacking ovoid bodies as showed in Table (3). Ovoid bodies are highly impenetrable defined margin oval structure and present in non-dispersive form. Analysis was done by using compound light microscope documentation and software.

Table 3: Gender and ovoid body Cross tabulation of hair samples

Gender	Absent	Present	Total
Female count	27	5	32
Male count	34	10	44
Total count	61	15	76

The statistical analysis of ovoid bodies showed that *P*- value (0.432) > 0.05, therefore, there is no relationship between the gender and the ovoid body number. The result of this study disagreement with study of Khan using thirty hair samples for analysis of presence or absence ovoid bodies from different sites of scalp hair of Asian population showed that twenty-five hair samples contain ovoid bodies and absent in only five samples. The results showed that in Awan caste, these structures were present in twenty-seven samples and absent in only three samples. Whereas in Butt and Gujjar castes, ovoid bodies were present in all thirty samples and absence was not recorded. In case of Rajput caste, ovoid bodies showed the presence in twenty-five samples and absent in only five samples [17].

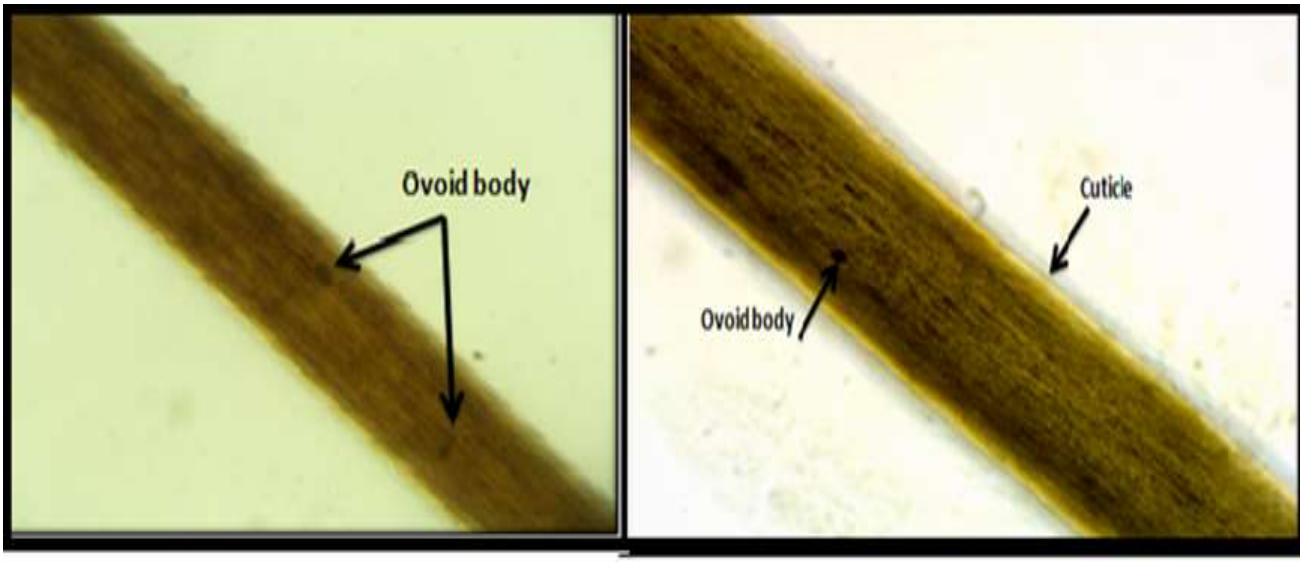

Figure 3: Light compound microscope 40X of human hair shaft that showed ovoid body

Scanning Electron Microscope Analysis of Hair Shaft

SEM micrographs have been identified in thirty samples of individuals aged 15- 52 years old and both male and female to observe the differences of the cuticle scale pattern among the persons in the Iraqi populations. Cuticle scales may be contributing to the identification purpose in forensic examination.

These cuticle scales can be viewed directly via SEM showed all samples were imbricate type, but 17% were chipped, 8% were lifting and 1% were smooth. Scanning electron microscope micrograph of 22-year-old man showed that the cuticle scale as an imbricate (flattened) type under the magnification power 4970X as shown in Figure (4). Some cuticle scale has broken and another has jagged-like edges as shown in Figure (5). This study agrees with Paul study that showed the image of a hair from a 23-year-old female Asian permanently dyed hair, the surface of the hair appeared different the cuticle scales of this hair represent the trademark jagged appearance.

This is attributed to the effects of oxidative permanent dyeing and this observation suggests that chemical damage is not uniform along the surface of the hair [19]. There was sample showed appeared cleared appearance and smooth of the outer layer of cuticle as shown in Figure (6). The SEM image of the hair shaft sample aged25 year's old female showed the surface topography of this hair shaft that the cuticle scale appears broken off in random location in a long hair shaft as shown in this study in the Figure (7).

This study of the hair shaft from 19years old male showed that the cuticle scale chipped-like edges as shown in Figure (8). Scanning electron microscope of another hair shaft from 23 years old showed that the outer layer of cuticle is lifting in Figure (9). The results in this study similar to Paul the Paul study that using scanning electron microscope to difference between treated and untreated hair for both male and female from Asian ,African and Caucasian individuals showed the results of these studies that many difference and damages in the scale outer cuticle for example, in the sample from 22 years old African male showing that the hair approximately 70μm in diameter and the cuticle scale are spaced approximately 8μm and another sample that showed a cosmetically treated hair SEM image for an 18 years old African male. The only form of cosmetic treatment claimed to have used in the application of a moisturizer known as a pink lotion this type of moisturizer is a popular product amongst African or persons of African origin because it protects the hair from dryness and brittleness as a result of blow dying, hot curling, or combing the product is specially formulated to maintain the hairs natural moisture.

This is to be expected as semi-permanent dying involving no chemical reaction with the chemical structure of the hair, only a diffusion of colored molecules from solution into the hair cortex SEM image of normal or damaged hair limitedly shows exocuticle layers, which may be one of the natural factors for determination of hair texture. The scale pattern provides distinguishing characteristics between animal and human hairs. The scales of an animal's hair show many distinctions such as coronal (crown-like) and spinous patterns, whereas in the case of humans the scale patterns are of the 'imbricate' type (flattened) [20].

The damaged hair is impossible to be structurally restored to the original state because grown hair is a mixture of lifeless compounds like a corneous tissue. These differences allowed them to be distinguished from one another between these samples. Generally, the cuticle surface of these hairs is inherently different to the surface topography of the untreated hair.

The edges of the cuticle scales are severely jagged in appearance with pieces of the cuticle seemingly chipped away in most places. In some locations of the cuticle scale edge it is difficult to ascertain whether the pieces have been torn off or debris has adhered to the hair fiber furthermost white areas of the cuticle layer as indicated on the micrograph are in fact regions where the cuticle cells has been up lifted further from the surface exposing the underlying layer [19].

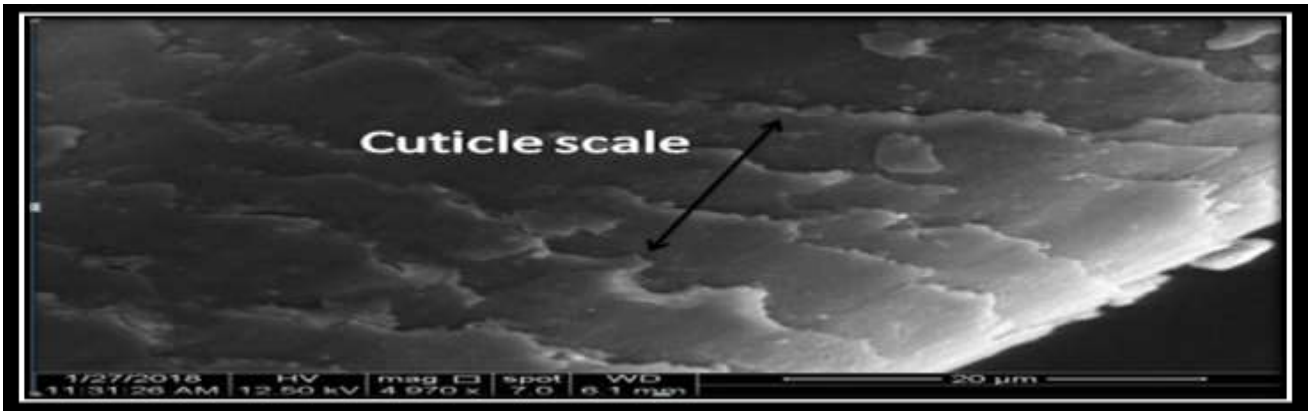

Figure 4: Scanning electron microscope of human male hair scalp showed flattened cuticle scale

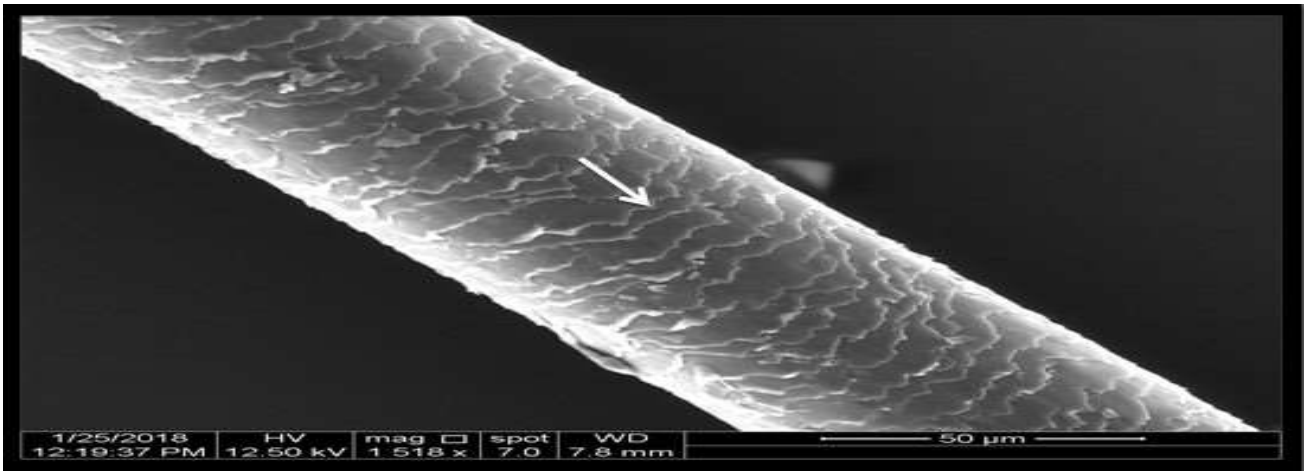

Figure 5: Scanning electron microscope of male human scalp hair showed that cuticle Jagged

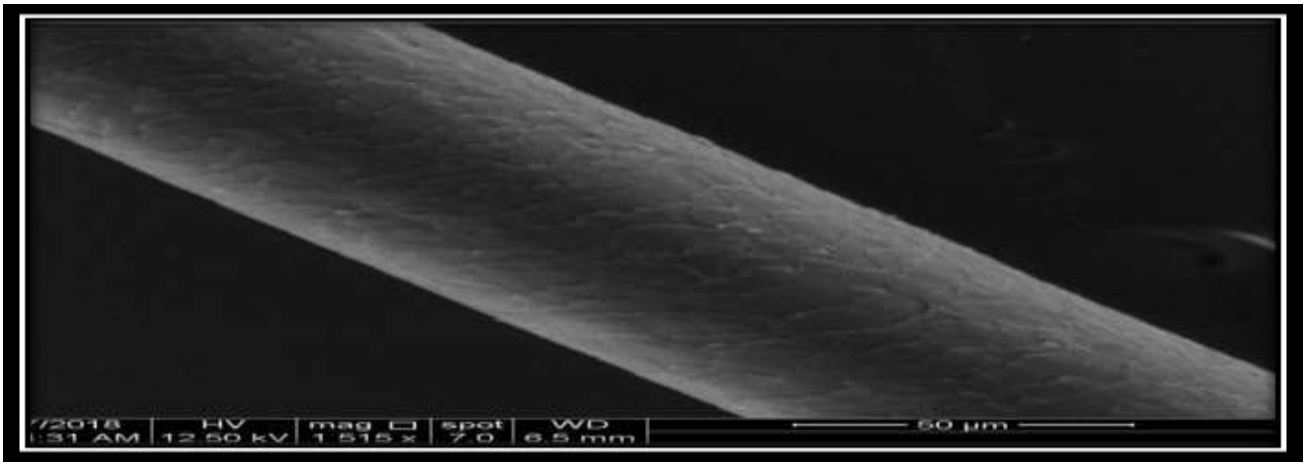

Figure 6: Scanning electron microscope of human scalp hair shaft showed smooth cuticle scale

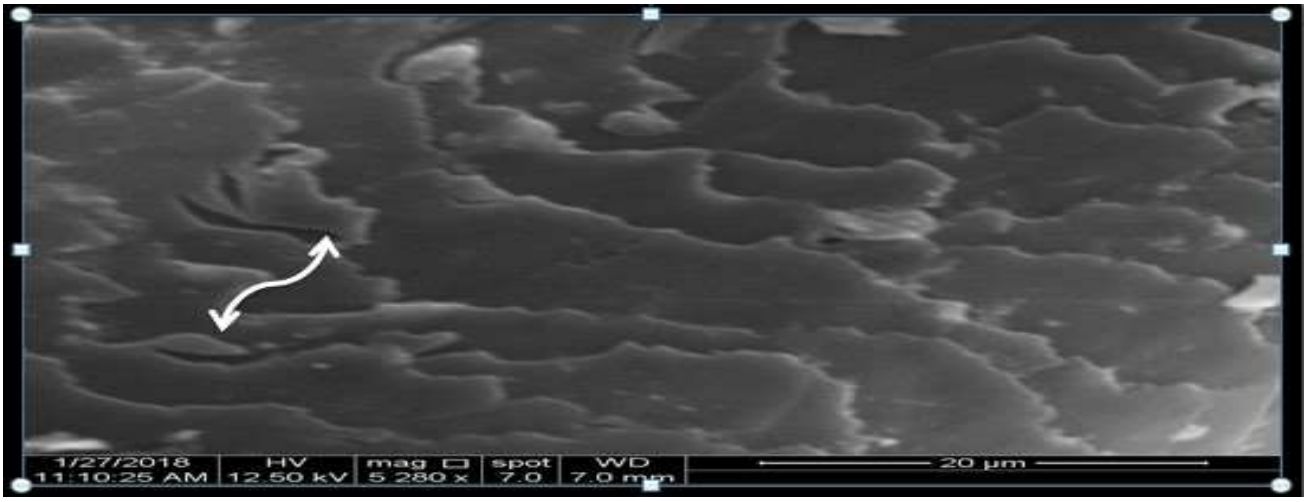

Figure 7: Scanning electron microscope of female human scalp hair showed that breaking of the cuticle scale

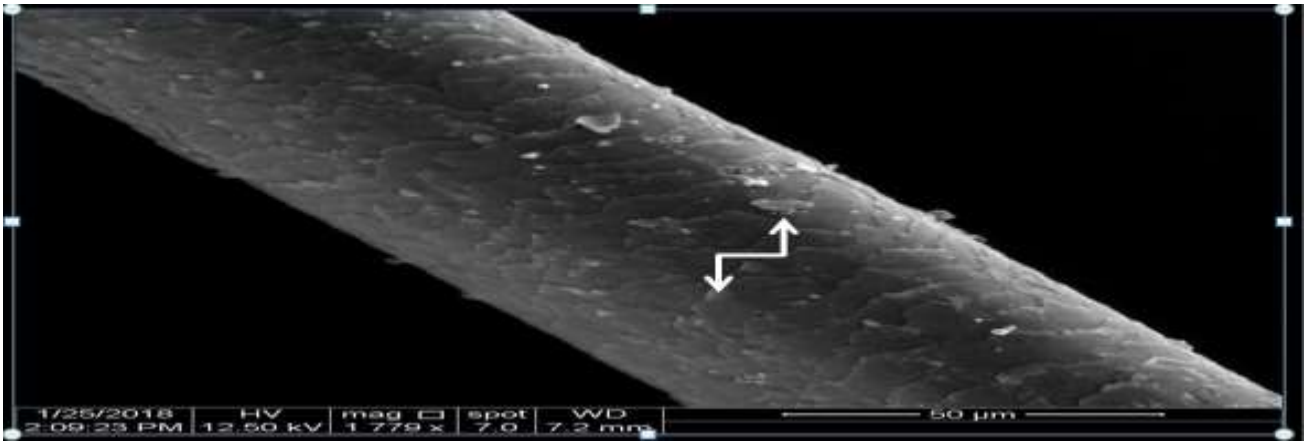

Figure 8: Scanning electron microscope of female human scalp hair showed the cuticle scale chipped- like edges

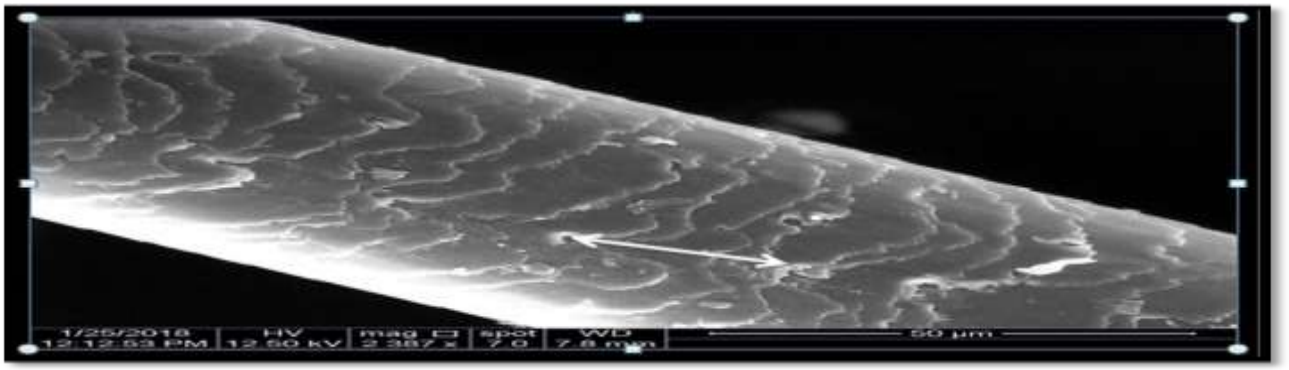

Figure 9: Scanning electron microscope of male human scalp hair showed that lifting of cuticle layer

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