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OBSERVATION ON HAIR SHAFTS OF SOME ROYAL MUMMIES IN THE EGYPTIAN MUSEUMS

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Abstract
Archaeological studies always seek for understanding history and draw right pictures about the life in the past in the different civilizations. Consequently, varieties of sciences are applied in these studies to extract the historical information. Hence this study investigated hair samples that were taken from four royal mummies with the aim of obtaining reliable information about the materials used in embalming, health status of these mummies, their age, and hair color that affects its composition. A variety of analytical techniques were applied in this study; digital microscope, polarizing optical microscope, environmental scanning electron microscope coupled with energy-dispersive X-ray unit, Fourier transform infrared spectroscopy, gas chromatography, and Raman spectroscopy analysis. The results showed the significance of hair in biomonitoring health status and determining age. Besides, it was proved that mastic resin has been used in embalming as early as the Second Intermediate Period especially for royal mummies.

***Keywords**: Hair; Royal mummies; SEM-EDS; FTIR; GC; Raman spectroscopy.*

Introduction

Hair is a valuable tool for archaeological and forensic studies; it provides reliable information about the person's age, sex, colour, growth rate, geographical region, and habits including different cosmetic treatment, smoking, and consuming alcohols. Moreover, hair examination determine environmental exposure, drugs, toxicants, and pesticides. Furthermore, hair composition can be used as a bio indicator for the disease history and health status of the individual; however, morphological abnormalities of hair reflect disorders. Besides, the protein composition of hair is affected by composition and concentrations of the blood explaining nutritional deficiencies; since diet influences the ratio of sulphur proteins that determine the hair fibre curvature [1-7].

Hair is a fibre with an oval or a circular cross-section. It is composed of three concentric layers: first, the cuticle which is the outer thin layer covered by scales; second, the cortex which is the main constituent of the hair made up of differently shaped cells characterize the hair type, third, the medulla which is the keratin fibres [5]. Added to, chemically the human hair is a complex compound of proteins, lipids, and pigments. The hair shaft is stiff which helps to preserve hair from damage. Morphological changes in hair strand can be observed and evaluated in cuticle layer considering the most external part of the hair, thus it is directly subjected to external impacts and weathering [8]. Elemental composition of hair has a

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significant correlation regarding metabolic disorders, mal-nutrition, diseases, environmental exposure [9-12].

Egyptian Museum in Cairo contains one of the largest collections of mummies in the world, and the only collection of royal mummies. In general, mummies are considered one of the historical information sources regarding the early Egyptians' life span, diseases, and mummification procedures and materials [13].

Four mummies were chosen for this study:

1. Seqenenre Taa II mummy which is registered under number JE 26209(b) CG 61051 and dating to the seventeenth Dynasty. Examination of his body revealed that he was about thirty years old on hid death time and that he met a violent death; the skull is covered with different wounds and Taa' brain still remains inside it.
2. Yuya mummy, the father of Tiye, wife of Amenhotep III, is one of the best-preserved mummies from ancient Egypt that is dated to the eighteenth Dynasty. It is registered under number JE 95229 CG 61190; its wrappings are in a good condition and covered with strips of linen immersed in a solution of thin plaster.
3. Thutmosis IV mummy which is registered under number JE 34559(b) CG 61073 and is dating back to the nineteenth Dynasty. It is of an emaciated man, slightly bald, who died at thirty years of age. It is believed that he was suffering from a disease that may be contributed to his death.
4. Unknow man E mummy registered in the museum under number JE 26218(b) CG 61098 According to the museum records, the mummy is dating to the twentieth Dynasty, was wrapped in a sheepskin, and was possibly of Prince Pentewere, a son of Ramesses III, who had been involved in a conspiracy against his father. The age at the time of his death was in the early twenties. His hair is plaited and has a cheese-like material [13, 14].

In contrast to bone and teeth (the most analysed and studied human tissues in archaeology), the hair didn't get the same attention in the archaeological studies. Therefore, this paper investigated hair samples collected from four royal mummies using multidisciplinary analytical techniques with the aim of deducing information regarding the morphological changes of the hair surface and consequently their causes, the status of conservation, as well as identifying the resinous materials covering the hair surface that were used in embalming. Besides, draw attention to the significance of examining mummies' hair in determining their age, health status, and other historical information.

Experimental Part

Materials

Hair samples were collected from four different royal mummies (Fig. 1); Seqenenre Taa II, Yuya, Thutmosis IV, anonymous mummy which is assumed it belongs to Prince Pentewere, a son of Ramesses III. These mummies of different ages and preserved in the Egyptian museum. Worth to mention that the hair sample no. 2 from Yuya mummy was not sufficient to do the multidisciplinary investigations, as a result only microscopic examination, elemental analysis, and Raman analysis were undertaken on this sample.

Methods

This investigation was done by different analytical methods:

Microscopic examination

Different types of microscopes were used to examine the morphology of the hair samples collected from four royal mummies:

a. Dnt DigiMicro Mobile USB/TFT portable digital microscope 500X, 5.0 megapixel was used for examining the selected samples.

b. Leica DM750P Polarizing Optical microscope equipped with reflection kit.

c. The hair surface of the four samples was observed with environmental scanning electron microscope (ESEM), Quanta 250FEG, by fixing the specimens on stubs with double-sided cellophane tape.

d. The elemental composition of hair samples was analyzed using an EDAX Ametek Octane Pro.

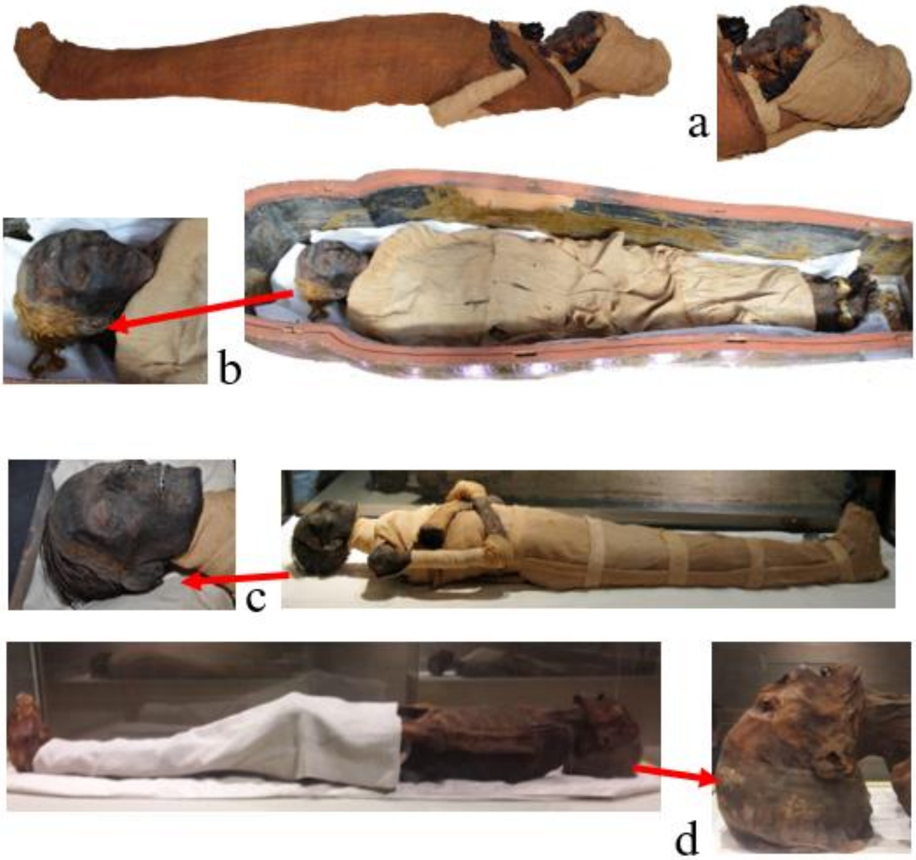

Fig. 1. Royal mummies from whose hair samples were taken;
a. Seqenenre Taa II mummy; b. Yuya mummy; c. Thutmose IV;
d. the anonymous mummy

FTIR Spectroscopy

The chemical characterization of hair samples was analyzed using FTIR spectrophotometer (Nicolet 380). The FTIR spectrum of the hair samples was obtained in the transmission mode with TGS detector and by using KBr method and represents (2mm/s) co-added scans at the spectral region in 4,000–400cm^{-1} range with a 4cm^{-1} resolution.

Gas chromatography

Sample preparation for GC analysis followed the method presented by Cirimele *et al* [15] and modified by Rusevska and Zdravkovski [16]. 200 mg of each hair sample was cut into small pieces and then manually milled in a marble mortar with pestle. They manually milled with adding few droplets of methanol (organic solvent) to swell the hair and make it easier to break, which improve the extraction step. Then, the milled hair samples extracted in 1 mL methanol at 45°C for 24. The obtained extracts were centrifuged at 8000rmp for 15 minutes, then concentrated to 50μL in an oven at 60ºC for 30 minutes, and then transferred to a vial of which 1μL was injected into the GC.

GC analysis of hair samples extraction was performed on a YL6500 GC quipped with an OV-17 capillary column. The solutions of the hair samples were injected into the system in split mode (1:10) and were analyzed under the following conditions: The column temperature was initially at 100ºC at 7ºC/min maintained for 1.0min., then raised to 200ºC at 10ºC/min and finally raised to 300ºC at 20ºC/min maintained for 20min. The flow of the carrier gas was held at 0.6mL/min. The injection temperature was 250ºC and the detector temperature was set at 270ºC.

Raman spectroscopy

Raman spectroscopy analysis of natural organic materials acquired by visible green wavelengths that only Raman allows a proper collection of their spectrum, since it totally avoids their fluorescence emission. So, Raman spectroscopy was performed by using a LabRAM HR Evolution Raman spectrometer (HORIBA Jobin Yvon Technology, France). The instrument is equipped with a diode laser with three laser sources wavelength: 325nm, 532nm and 785nm. In order to take possible sample inhomogeneity into account, for each sample analyzed by laser green Raman spectrum were recorded using a diode laser of 532cm^{-1} wavelength. The laser was focused on the grains of resin using objective lenses 50× and 100× to allowing a resolution down to 1 mm. All spectra were recorded in the wavelength region 100 and 2000cm^{-1} for at least accumulations time of 5.0s and with laser intensity of ND filter 1%.

Results and discussion

Microscopic examination

All samples exhibited the preserved scales generally especially in SEM micrographs. Observation of the surface morphology of sample no.1 taken from Seqenenre Tao II mummy revealed presence of a resinous material covered the hair shaft (Fig. 2a, b, c, e and g), and a high degree of degradation and damage (Fig. 2d, f, h and i).

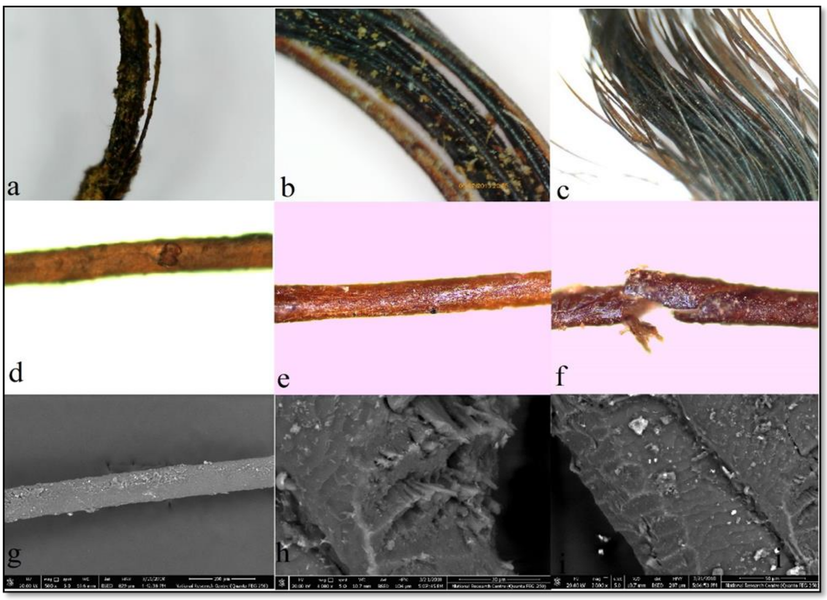

Fig. 2. the results of microscopic examination of Seqenenre Taa II hair shaft:
a, b and c. Digital microscope images; d, e and f. Polarizing optical microscope images;
g, h and i. Micrographs of ESEM

The damage of the hair shaft is evident in the form of transverse cracking as shown in polarizing microscope image (Fig. 2f). Moreover, scanning electron microscopic image (Fig. 2h) show that the shaft was infested by lice and suffered degradation which was detected in the end of the fiber. In figure 2i, it is also possible to verify the presence of damage on the cuticle layer of hair shaft, such as voids, erosion and little cracks, indicated by arrows.

The microscopic results of sample no. 2 belonging to Yuya mummy show the color of the hair indicating the age of Yuya, with traces of a resinous material that doesn't seem to adhere well to the hair shaft (Fig. 3a) as appears clearly in figure 3; polarized and SEM images. The hair shaft exhibited weakness of the cuticle due to the extensive erosion (Fig. 3b and c), presence of a longitudinal split near the end of the shaft (Fig. 3d) and disappearing the scales in some areas (Fig. 3b). Longitudinal tunnel along the hair shaft could be obviously seen in figure 3e, f and g with a couple of voids (Fig. 3h). Moreover, the hair shaft was partially transversely broken (Fig. 3i).

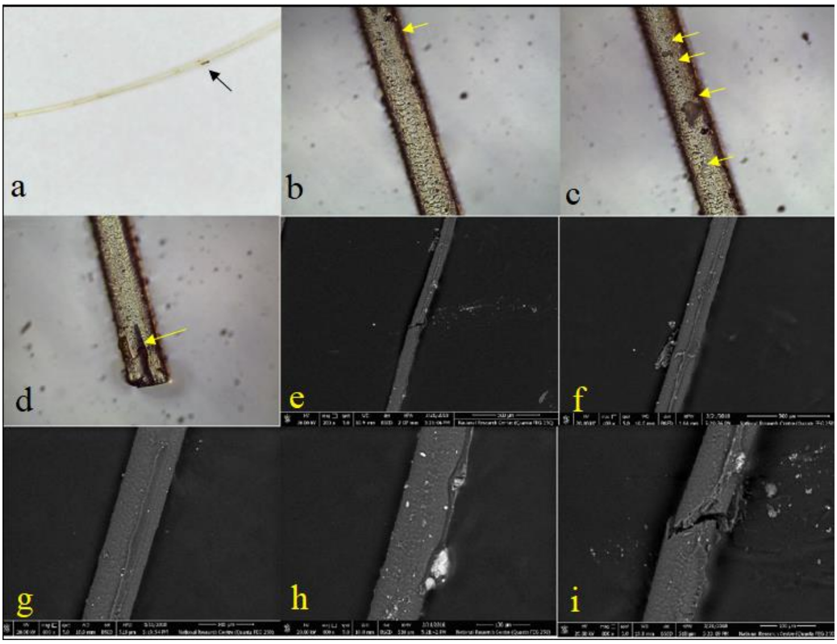

Fig. 3. the results of microscopic examination of Yuya hair shaft:
a. Digital microscope image; b, c and d. Polarized optical microscope images; e, f, g, h and i. Micrographs of ESEM

The hair sample no. 3 of Thutmosis IV was extensively covered with a resinous material as shown in figure 4a-e. Clean, transverse fractures across the hair shaft, accompanied with a localized absence of cuticle cells, appeared in figure 4c, d and i as well as a longitudinal grooving on a hair shaft (Fig. 4f and h) suggesting some kind of structural abnormalities or disorders of hair shaft [1, 17-20] which can be consistent with the fact of Thutmosis IV's illness [13].

Microscopic examination shows resinous deposits on the hair sample no.4 (Fig. 5a-d). Significant changes in the morphology of hair shafts can be observed. the main damage occurred was deformation of surface morphology and the longitudinal fractures (Fig. 5f). Besides that, the gradual erosion from the endocuticle, tearing segments of several scales were spotted in SEM micrographs (Fig. 5f-i). These changes can be caused by plaiting [21].

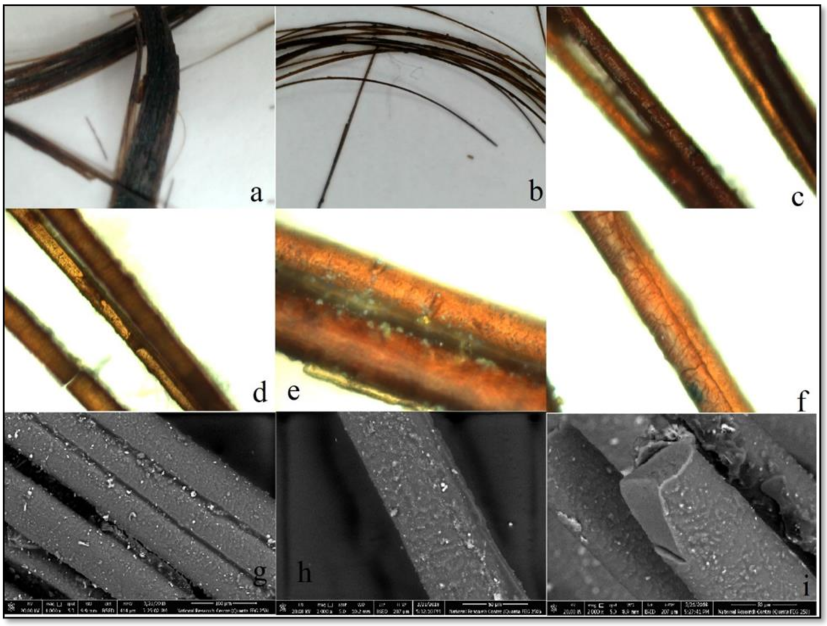

Fig. 4. the results of microscopic examination of Thutmosis IV hair shaft:
a and b. Digital microscope image; c, d, e and f. Polarizing optical microscope images;
g, h and i. Micrographs of ESEM

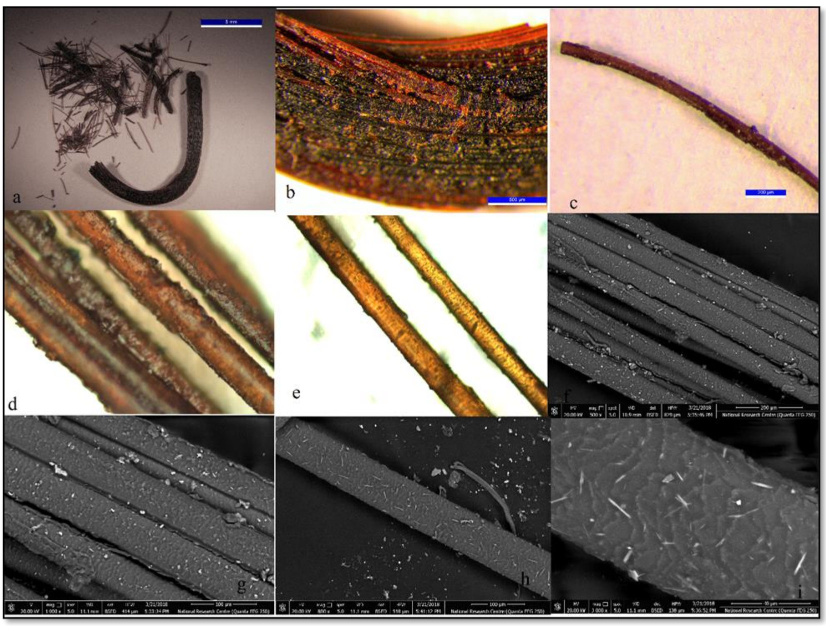

Fig. 5. the results of microscopic examination of the anonymous mummy hair shaft:
a, b and c. Digital microscope image; d and e. Polarizing optical microscope images;
f, g, h and i. Micrographs of ESEM

Elemental analysis

Energy-dispersive X-ray microanalysis (EDX) coupled with electron microscope was performed to get quantitative data on elements' presence and their concentrations in the hair samples. Spectra obtained from hair samples showed that sulphur was the main element detected by EDX microanalysis in all samples. Peaks for Sodium, Aluminium, Silica, phosphorous, potassium, and calcium were also found in the hair samples, but the existence and the intensity of these peaks was quite variable (Table 2). Changes in sulphur concentration between the four samples can be observ ed. In general, the sulphur content average of men's hair varied between 4.6 and 5.4 percent [22]. So, the amount of sulphur was found to decrease drastically in sample no.1 (Seqenenre Taa II mummy) suggesting protein disintegration in this hair sample. In contrast, sample no. 2 (Yuya mummy) shows increase in the concentration of sulphur content in spite the hair deterioration which can be interpreted by the hair color of the mummy, reddish-blond hair [13], since the colored hair contains more sulphur than black hair [22]. The sulphur concentration in sample no. 3 (Thutmosis IV) is less than normal average which indicated sulphur-deficient hair as a marker of hair abnormality and confirm the microscopic findings [20]. As for sample no. 4, the sulphur content implies to the normal average with taken in consideration the hair deterioration.

Sample no. 1 exhibited an elevated level of potassium in relation to sodium indicated that Seqenenre Taa II was severely malnourished, because the sodium/potassium ratio was less than 3.4±1.9 [9]. Moreover, calcium was only detected in sample no. 4 (the unknown mummy). Furthermore, this sample shows more level of sodium comparing to calcium indicating the mummy's age which can be around 20 years old [5, 6] and this finding go along with the mummy's description that it belongs to a young man. Besides, the elemental composition of sample no. 4 confirmed that this man was not poisoned as hypothesised by D' Fouquet [14] Surprising, Zinc was absent in the hair elemental composition of all samples and this finding can be in agreement with Mansilla *et al* [23] whose results confirm the low percentage of zinc and potassium elements in mummies.

Table 1. Elemental analysis results of hair samples taken from the royal mummies by SEM-EDS

Element	Seqenenre Taa II	Yuya	ThutmoseIV	Anonymous mummy
C	65.14±2.423	66.42±0.99	54.75±1.14	59.13±3.14
N	4.89±1.20	4.49±0.20	9.81±2.47	5.19±1.53
O	24.41±1.59	22.87±0.31	30.08±0.78	28.03±3.02
Na	0.83±0.02	0.81±0.02	1.42±0.03	1.86±0.56
Al	1.28±0.20	0.52±0.05	——	0.49±0.10
Si	0.84±0.03	——	0.55±0.01	——
P	0. 45±0.01	——	——	——
S	2.98±0.55	4.89±0.73	3.57±0.75	4.33±1.37
K	1.01±0.35	——	——	——
Ca	——	——	——	1.14±0.22

FTIR Spectroscopy

The degradation mechanism of protein bonds can be examined by infrared spectroscopy that provides details about the structure of proteins [24]. FTIR was used to determine the chemical composition of hair samples of four royal mummies and their structural changes. The FTIR absorbance bands of hair samples were analysed using the Essential FTIR software.

From figure 6, spectrum of sample no. 1 (Seqenenre Taa II mummy) revealed drastic decrease in amide A band at 3417cm^{-1} and absence of the band at 3072 associated with amide B which indicate occurring an extensive degradation. Strong bands found at 2853 and 2921cm^{-1}

arising from amino acid bands of CH_2 and CH_3 symmetric and asymmetric modes, respectively. This finding support SEM results that show damage in the cuticle and cortex layers in this sample [25]. The band at 1704cm^{-1} stands for unidentified conformational structure. Moreover, the IR spectrum demonstrated a shoulder band at 1633cm^{-1} assigned to amide I of β-sheet structure [26, 27] and dramatic decrease in the intensity of amide II band at 1543cm^{-1} as well as absence of amide III band. Also, a shift in the band of CH_2 at 1458cm^{-1} can be noticed.

In contrast of such, IR spectra of sample 3 and 4 show the characteristic bands of typical amino acids of hair; amide A at 3419cm^{-1}, a weak shoulder around 3065cm^{-1} assigned to amide B, amide I at 1650cm^{-1}, amide II at 1536cm^{-1} with a weak broad shoulder at 1230cm^{-1} attributed to amide III band while this band disappeared in sample no. 1.

All samples exhibited a weak absorption around 1370-1380cm^{-1} characteristic of a sterol compound [28, 29]. Added to, the bands in the region at 1200-1000cm^{-1} associated with oxidation of cystine that provide evidence of the chemical changes due to oxidative damage to the fiber [30, 31]. The band at 1040cm^{-1} in sample 3 and 4 indicate that the disulphide bond S-S had been cleaved then oxidized to cysteic acid [31]. Also, the band at 1188cm^{-1} in sample no.1 and around 1171-1177cm^{-1} in samples 3 and 4 assigned to sulfonate, S-O symmetric stretch [30].

Comparing the hair samples 1, 3 and 4, we can notice the decrease in amide A intensity and appearance the shoulder around 1630cm^{-1} attributed to amide I in sample no. 1 suggesting changes in the keratin protein associated with transformation of the secondary structure from α-helix to β-sheet arrangement in amide I and II [31] indicating uncoiling of the collagen triple helix [32]. Hence, denaturation occurrence can be deduced in sample no. 1. Besides, oxidation of hair samples can be detected in the IR spectra arising from environmental weathering [3].

Interestingly, the findings of SEM-EDX and FTIR that confirmed the presence of lice and intensive decay in the hair sample no. 1 with malnutrition may support the opinion that advocate the hypothesis of the survival of Seqenenre Taa II from the battlefield and his assassination on his sickbed; since there are two types of weapons were used in his injuries and some of his wounds had healing signs [13, 33].

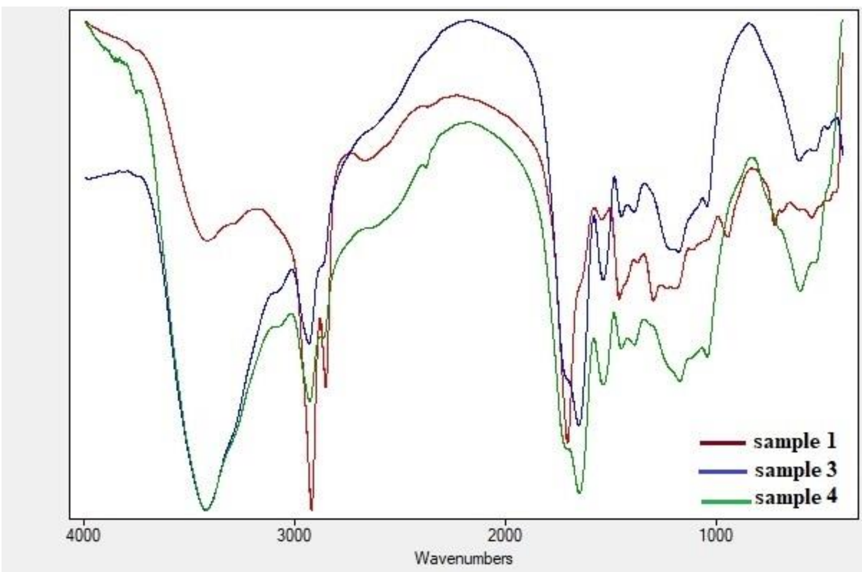

Fig. 6. FTIR spectrum of the hair samples taken from royal mummies:
red line present sample 1 (Seqenenre Taa II); blue line of sample 3 (Thutmosis IV);
green line express sample 4 (the anonymous mummy)

Gas Chromatography

GC technique was used for determining the unsaturated fatty acids in the hair samples. The results showed that sample no. 1 (Fig. 7a) contains small amounts of oleic acid (C-18:1), linoleic acid (C-18:2) and linolenic acid (C-18:3), whereas sample no. 3 (Fig. 7b) contains small amounts of palmitic acid (C-16) and stearic acid (C-18) and a higher amount of oleic acid (C-18:1) and linolenic acid (C-18:3) than sample no.1. As for sample no. 4 (Fig. 7c), it contains small amounts of capric acid (C-10) and palmitic acid (C-16) with traces of lauric (C-12) and myristic acid (C-14), in addition to stearic acid (C-18) and high amount of oleic acid (C-18:1), linoleic acid (C-18:2) and linolenic acid (C-18:3). Studies concerning distribution of unsaturated fatty acids in hair showed evidence that the cuticle layers included many unsaturated fatty acids, [34] and each cuticle cell and part of each scale edge is covered with fatty acids that are mainly palmitic, stearic and other fatty acids including some oleic acid. [21] A direct relationship between the amount of unsaturated fatty acids and the existence of the cuticle layer can be deduced. Therefore, the reported evidence suggests that occurrence damage of the cuticle layer in sample no.1 is more than in sample no.3 and 4 which confirm the results of SEM and FTIR.

Raman spectroscopy

Raman results of the collected hair samples are shown in figure 8. Raman spectrum of hair sample of Seqnenre Tao II (sample no.1) indicated that the hair was coated by mastic resin.[35] Since, the Raman spectrum of the sample has a peak of stretching mode ν(C=O) at 1704cm^{-1}, strong peak of ν(C=C) in the region 1607 and 1636cm^{-1}. The band at 1636cm^{-1} exhibits a prominent peak which identified for the mastic resin. Also, the spectrum shows week peak of δ(CH=CH) in the range 1201-1214cm^{-1}, opening aromatic ring of ν(C–C) at 1001cm^{-1}, and week peak of δ(C–C–O) at 564cm^{-1}. Worth mentions the decreasing in the δ(CH=CH) at 1214cm^{-1} due to the oxidation of unsaturated bonds and the oxidation of aromatic ring in the mastic resin. Also, the disappear of Raman spectrum of the bonds ν(C=C) at 1624 cm^{-1}, δ(CH_2) and δ(CH_3) at 1458cm^{-1}, and ν(C-C-OH) at 858cm^{-1}, all of these due to the molecular changes in the mastic resin which should be happened as a result of aging and rapid oxidation of triterpenoids.

Upon the oxidation process and the accumulation of oxidation products, a broadening band which is a prominent shoulder at 1707cm^{-1} is ascribed due to an increase in carbonyl and carboxyl groups. Raman spectrum also shows increase at 1802cm^{-1}, this increase may be related to the accumulation of molecular modifications within aged samples. Hence, the oxidation of unsaturated bonds causes less variation in the chemical composition of the resin with aging, specifically within the fingerprint region of carbonyl C–O stretching band, small bands of δ(CH=CH) and ν(C–H) [36].

Raman spectrum of hair (Fig. 8) sample of Yuya (sample 4) indicated that the hair coated by traces of mastic resin. In the spectrum, the sample has a peak of stretching mode ν(C=O) at 1715cm^{-1} and a strong peak of ν(C=C) in the region 1627cm^{-1}. It shows a week peak of δ(CH=CH) at 1215cm^{-1}, opening aromatic ring of ν(C–C) in the range of 808-1013cm^{-1}, and a week peak of δ(C–C–O) at 527cm^{-1}. Raman spectrum has peaks of mastic as week peaks of δ(CH=CH) at 1215 cm^{-1} which can indicate presence of mastic resin.

According to Raman spectrum of hair sample no. 3 (Thutmosis IV), the hair was coated by mastic resin. This sample has a broadening band appear in the peak of stretching mode ν(C=O) at 1710cm^{-1} and strong peak of ν(C=C) in the region 1606cm^{-1}. Besides, the spectrum shows a week peak of δ(CH=CH) in the range of 1200cm^{-1}, opening ring of ν(C–C) at 1096cm^{-1}, and a week peak of δ(C–C–O) at 562cm^{-1}.

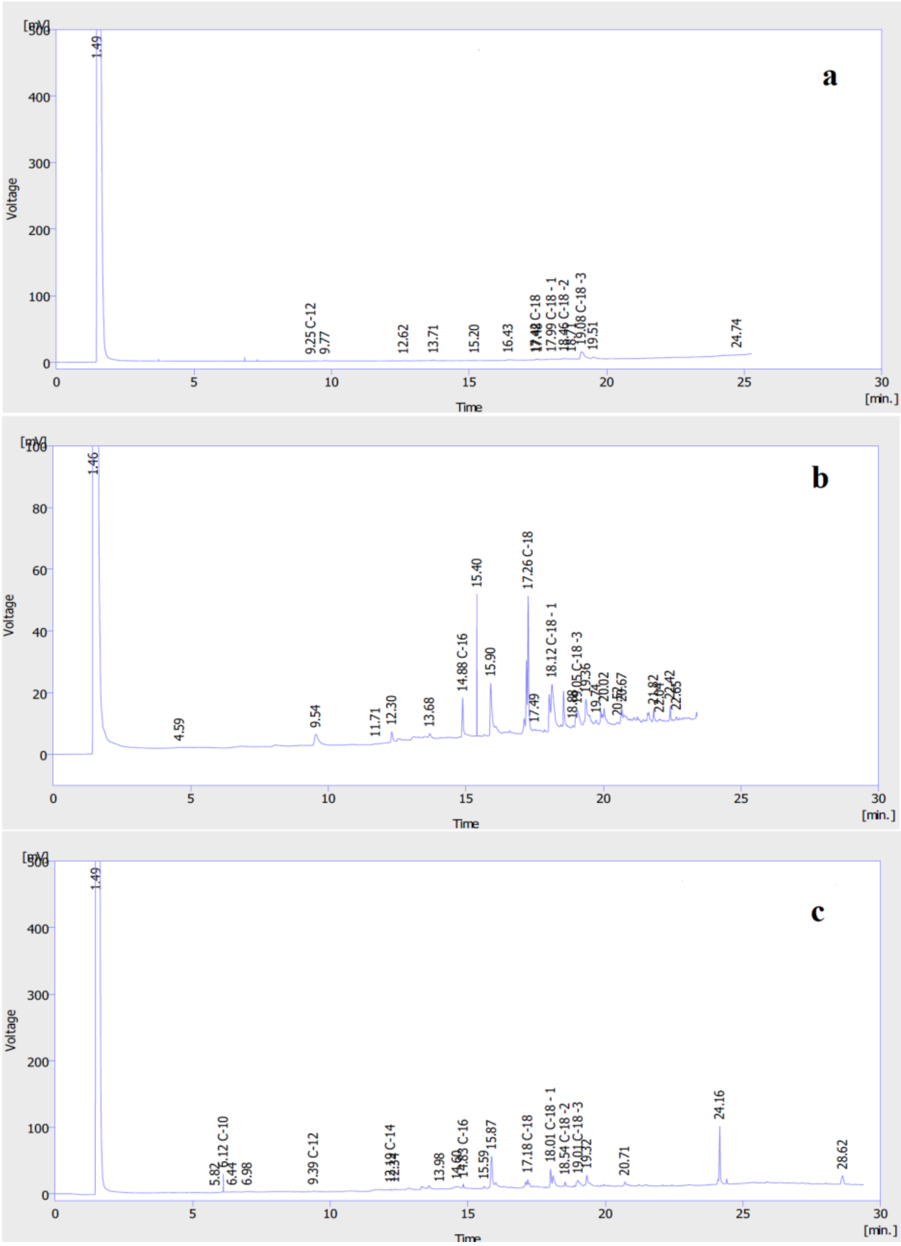

Fig. 7. Gas chromatography analysis of lipids in thee collected hair samples:
a. sample 1; b. sample 3; c. sample 4

Raman spectrum of hair sample no. 4 (man E) showed almost the same results as sample no. 1 and 3 concerning the resinous material coating the hair which is identified as mastic resin. A broadening band appear in the peak of stretching mode ν(C=O) at $1704cm^{-1}$, a week peak of δ(CH=CH) at $1208cm^{-1}$, opening ring of ν(C–C) at $990cm^{-1}$, and a week peak of δ(C–C–O) at $562cm^{-1}$.

Within the context, small but significant changes in Raman spectra of mastic are detected in the analyzed hair samples due to the molecular changes that can happened because of aging and oxidation process. These molecular changes appear as the spectra disappear or decrease ascribed to the rapid oxidation of triterpenoids, while others increase in intensity suggesting presence of accumulation of the molecular modifications or oxidation products within the samples.

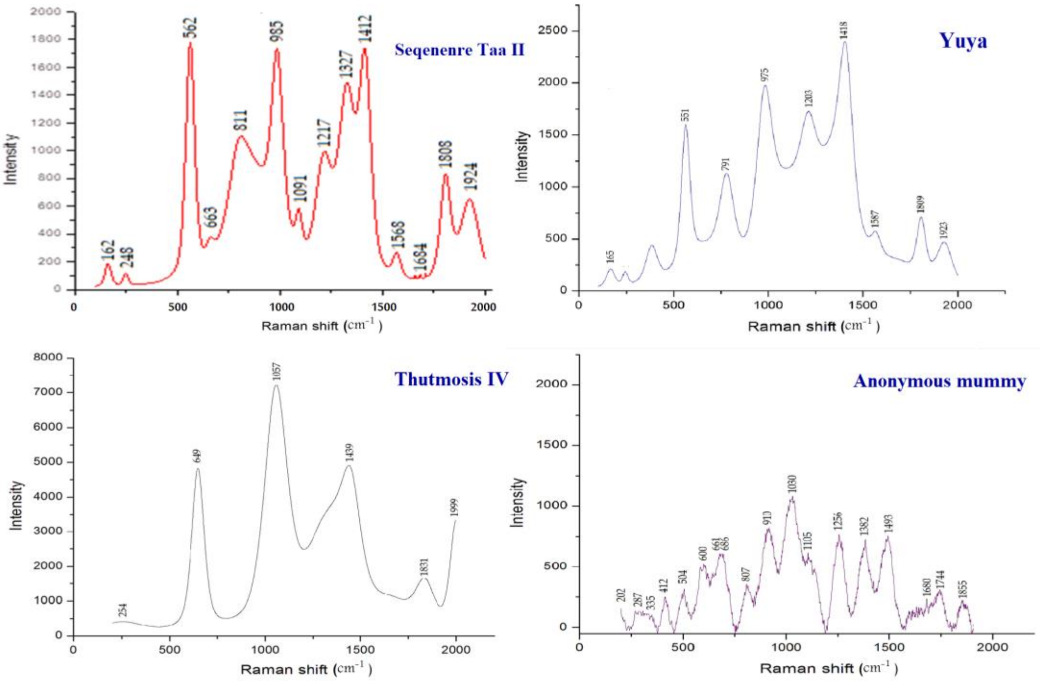

Fig. 8. Raman spectrum of the collected hair samples of four royal mummies

The Egyptians used mastic in embalming. It may have a religious significance even when used for embalming [37]. Although, there are several studies proving the presence of mastic resin among mummification materials [38-41], the results of this study lead to the suggestion that the hair has been coated with the resinous materials used in coating the entire body. Added to, mastic resin was used during mummification process notably mummification of royal mummies as early as the Second Intermediate Period.

Conclusions

The present paper investigated hair samples of four royal mummies by applying various analytical techniques with the aim of obtaining information that concern the resinous materials covering the hair of royal mummies during their embalming. Besides, determine or confirm their age, health status.

Based on ESEM examination, the mummies hair has been covered with a resinous material after mummification process, perhaps with the whole body, which was identified by Raman analysis as mastic resin. So, this finding thus supports the conclusion that mastic resin was used during mummification process notably mummification of royal mummies as early as the Second Intermediate Period.

Concerning the hair of Seqenenre Taa II, the results support the probability of Seqenenre Taa II survival from the battlefield and his assassination on his sickbed. Moreover, the increasing of Sulphur concentration in Yuya hair was a result of its blonde color which affected the adherence between mastic resin and the hair. Furthermore, diagnosis of Thutmosis IV hair shaft revealed signs of structural abnormalities or disorders that can be related to his illness.

The main results of the anonymous mummy hair indicate that his age on death time was about twenty years and proved that this man was not poisoned as hypothesised before.

The mummies hair is a valuable tool for extracting historical information concerning these mummies.

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