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Analysing Human Nails Composition by Using Laser Induced Breakdown Spectroscopy

(Analisis Komposisi Kuku Manusia Menggunakan Spektroskopi Pemecahan Teraruh Laser)

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ABSTRACT

This study investigated the elemental composition of pathological and normal nails, according to different age groups and genders. The nail elemental composition was analyzed by using Laser Induced Breakdown Spectroscopy (LIBS) technique. A Q-switched Nd:YAG laser at 1064 nm with output energy of 100 mJ, pulse duration of 6 ns and repetition rate of 1 Hz was fired to the human nails sample. The laser pulse ablated the target sample surface and produced plasma plume with characteristic spectral line comprising sample's elemental composition. The plasma emission spectrum of nail samples were captured by fibre optic detection device and spectrometer equipped with ASEQ Spectra Software for elemental analysis. The elements detected in nails were Mg, Al, Ca, P, Ti, K and Na. Classification of nails into different age groups and genders demonstrate efficient results if compared with Dietary Reference Intakes (DRI). The results indicate that Ca, Al, Ti, P and K composition were higher among female. However, Mg and Na were higher among male. The results also show that Ca and K element decline as the age increase in both genders. The pathological nails' elemental compositions were also noticeably related to the nail disorder condition itself. It is proven that LIBS could be a possible method for analyzing the nails and identification of various nail disorders.

Keywords: Biological sample; elemental composition; laser induced breakdown spectroscopy (LIBS); nail disorder; pathological nail

ABSTRAK

Penyelidikan ini mengkaji komposisi unsur bagi kuku normal dan berpenyakit mengikut perbezaan kumpulan umur dan jantina. Komposisi unsur kuku dianalisis dengan menggunakan teknik Spektroskopi Pemecahan Teraruh Laser (LIBS). Laser Q-suis Nd:YAG 1064 nm dengan keluaran tenaga 100 mJ, tempoh denyutan 6 ns dan kadar pengulangan 1 Hz ditembak pada kuku manusia. Laser yang terkena permukaan sampel akan menghasilkan kepulan plasma yang mempunyai ciri garis spektrum tersendiri dan terdiri daripada komposisi elemen sampel. Spektrum pancaran plasma daripada sampel kuku ditangkap oleh alat gentian optik dan spektrometer dilengkapi perisian ASEQ Spectra untuk analisis unsur. Unsur yang dikesan di dalam kuku adalah Mg, Al, Ca, P, Ti, K dan Na. Pengelasan kuku kepada perbezaan kumpulan umur dan jantina menunjukkan keputusan yang bertepatan dengan Rujukan Pengambilan Makanan. Keputusan menunjukkan bahawa komposisi Ca, Al, Ti, P dan K lebih tinggi dalam kalangan perempuan. Walau bagaimanapun, Mg dan Na lebih tinggi dalam kalangan lelaki. Keputusan juga menunjukkan bahawa unsur Ca dan K berkurangan dengan peningkatan umur bagi kedua-dua jantina. Komposisi unsur kuku berpenyakit pula menunjukkan kaitan yang jelas dengan keadaan masalah kuku itu sendiri. Jadi, terbukti teknik LIBS merupakan kaedah berpotensi untuk menganalisis kuku dan mengenal pasti pelbagai masalah kuku.

Kata kunci: Komposisi unsur; kuku bermasalah; kuku patologi; sampel biologi; teknik spektroskopi pemecahan teraruh laser (LIBS)

INTRODUCTION

LIBS technique demonstrates reduction of measurement time and analysis can be performed even in small-scale sample making it an ideal analyzing technique for biological samples (Priyanka et al. 2014). This technique applies optical contact to the surface of target sample and after that detects elemental composition available in the target sample (Badruzzaman 2012). Laser pulse will ablates the surface of target sample and thus creating high temperature plasma. As the time passes, the excited plasma will cool down. Every element in the plasma will emit its own characteristic spectral line comprising important information about the sample's elemental composition.

Biological samples including blood, sweat, saliva, feces and urine are important in performing diagnostic test. However, biological specimens mentioned above usually involving complex sample management procedures and policies, often stored in the biorepository such as Biobank (Vaught & Henderson 2011). These biological specimens require different optimal temperature (low temperature). Cold storage has many challenges such as sample degradation due to power failure, large amount of waste materials and high maintenance cost of refrigerators (Clement 2009). Elemental compositions in these specimens are easily contaminated and have limitations. For example, the urine test is only up to three days window of detection, saliva test is easily contaminated and the sweat test is expensive.

On the contrary, elements concentration in nail is capable of indicating side effects due to long time exposure to certain type of elements. Nail consists of layers of keratin tissue that grows from the matrix, a part of nail hidden beneath the cuticle and is produced by living skin cells (Farran et al. 2008). In various studies, nail's elemental composition is affected by gender, age, geographical location and lifestyle. Human body tends to eliminate unwanted elements through accumulation in the inside of the nail, making it a potential alternative for diagnostic test (Shadman et al. 2012). In addition, nail is easier to store, collect and transport compared to other biological samples (Bahreini & Tavassoli 2012).

Nail elemental analysis, usually for medical purpose, can be done by several methods such as inductively coupled plasma atomic emission spectroscopy (ICP-OES), proton induced x-ray emission (PIXE), neutron activation analysis, inductively coupled plasma mass spectrometry (ICP-MS) and others (Shadman et al. 2012). Meanwhile, Laser Induced Breakdown Spectroscopy (LIBS) is laser based analytical method that has rapidly grown due to its simplicity and flexibility. Some of LIBS general applications are for analyzing artifact restoration quality, conditions on other planets, quality control of building and glass restoration, human biological sample analysis and remote analysis of deep sea objects (Priyanka et al. 2014; Fortes et al. 2015). Among all of the methods available for elemental analysis, LIBS is the most practical for scientific purpose because it is a simple method that is noninvasive with no or minimal sample preparation.

In this paper, normal and pathological human nails elemental composition are studied by using a LIBS technique in which LIBS spectra of each sample is analyzed and characterized. Since recent studies is lacking in term of basic insight about common nail problems among human such as melanonychia, onycholysis, leukonychia and pitting nails, this technique will provide elemental analysis of pathological and normal human nails for various gender and age range. Although biological samples are hard to deal with in LIBS technique due to its ability to absorb water, this study is an alternative to figure out ways in overcoming these problems. This approach has allowed us to understand the fundamental of LIBS technique and determining the elemental composition of normal and pathological human nails by analyzing their plasma emission spectrum. Digital microscope is used to study the surface topography of nails after ablated by laser pulse to acquire the effects of laser pulse on its surface (craters and spots).

Materials and Methods

SUBJECTS

The subjects in this study were chosen among Malaysian. Information on the individual subjects such as age, gender and health condition was also included. Twelve subjects with normal nails were divided into different age groups (adolescent, young adult, middle adult and older adult) and genders. Four nail samples with diseases such as onycholysis, melanonychia, leukonychia and pitting nail were also obtained. All of the samples were taken from different subjects by using stainless steel nail clipper and stored in separated plastic case with labels to make sure the samples were not mixed up. The samples were stored at room temperature. In order to eliminate contaminations and impurities, samples were washed with acetone for 30 s, followed by distilled water for another 30 s. Acetone was chosen to clean the nails because organic solvents proved to cause less elemental loss compared to aqueous detergents and aqueous acids (Bank et al. 1981). After that the samples were dried at room temperature.

EXPERIMENTAL DETAILS

The experimental setup of LIBS technique used to analyze the samples is shown in Figure 1. A Q-switched Nd:YAG laser operating at wavelength of 1064 nm with pulse energy 100 mJ, pulse duration of 6 ns and repetition rate of 1 Hz was used as a source of energy. Laser pulse was focused on the nail sample to create sparks, ablate the target sample and create plasma. Once the high temperature plasma is created, it cooled down with time. Each element in plasma have its own characteristic spectral line. Since human nail samples tend to bend and move around, a sample holder is needed to make sure that the nails stick flat on the holder. Two stainless steel large clips (wrapped with paper to prevent the laser from striking it) were used to hold the sample because it would be easier for the optical fiber to detect the plasma created. The distance between the sample and laser source was approximately 8 cm (optimum distance) whereas the angle of the fiber optic from the sample was 39 to 45 degrees. Many spectra of each sample were acquired. In order to confirm the spectrum results, the experiment was repeated for a few times with different part of the nail strike by laser pulse. All of the plasma emission was collected by fiber optic and shown in the computer by ASEQ Spectra software for further analysis. The characteristic spectral line was compared with National Institute of Standards and Technology (NIST) database. Matlab R2009a software was used to plot the spectrums and graphs for further comparison.

Results and Discussion

LIBS PLASMA EMISSION SPECTRUMS OF NORMAL NAILS

LIBS plasma emission spectrum of normal nails is shown in Figure 2. The prominent element in normal nails was

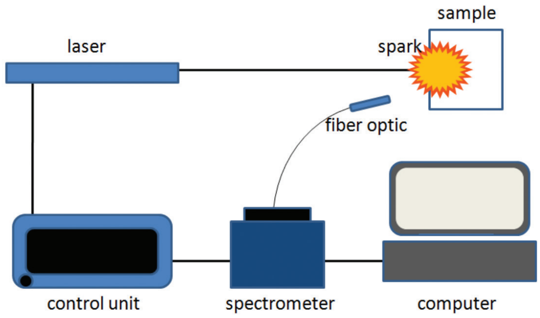

FIGURE 1. Experimental set up of LIBS technique

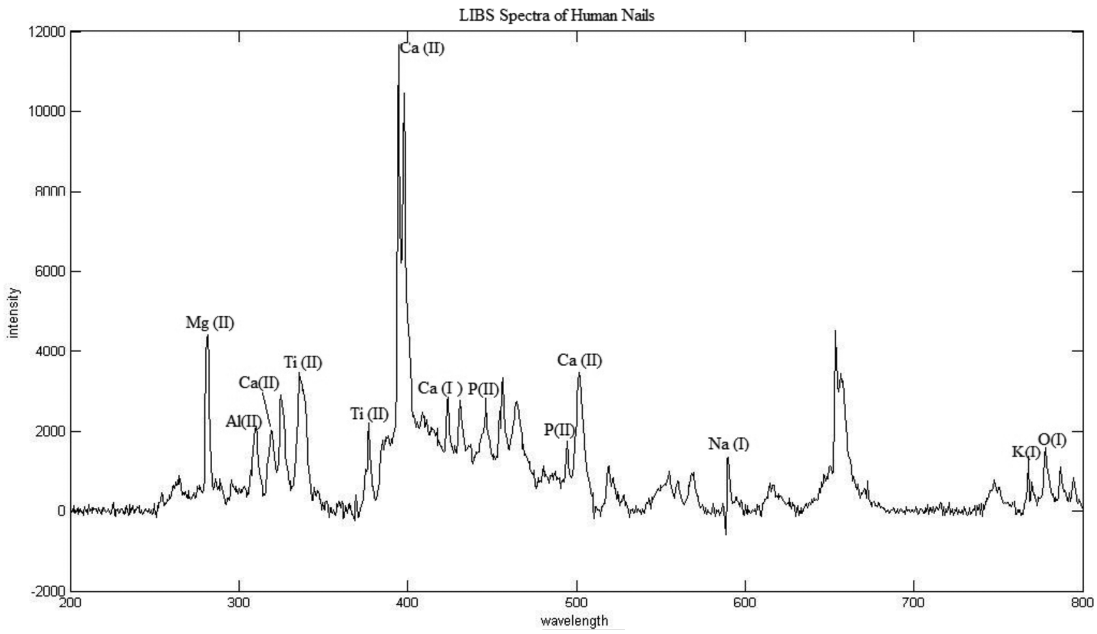

FIGURE 2. LIBS plasma emission spectrum of normal human nail sample

Ca with the wavelengths of 422.672, 317.933, 393.366, 396.846 and 501.997 nm. Other major elements were Mg, P, Al, Ti, Na, K and O. The spectral line depends on the spectroscopic source used and the spectrometer's resolution, whereas the intensities of the spectral lines observed for any element depend on the excitation condition and the light source. The spectral lines of elements observed in this study with their wavelength are shown in Table 1.

ELEMENTAL COMPOSITION ANALYSIS BETWEEN AGE GROUPS AND GENDERS

The elemental composition in nails of different age groups is shown in Figure 3. The intensity of calcium

TABLE 1. Elements detected in normal nail and their respective wavelength

Spectrum	Wavelength (nm)	Spectrum	Wavelength (nm)
Ca (I)	422.672	Al (I)	308.215
Ca (II)	317.933	Al (I)	309.271
Ca (II)	393. 366	Ti (II)	334.94
Ca (II)	396.846	Ti (II)	376.132
Ca (II)	501.997	O (I)	777.194
Mg (II)	280.270	Na (I)	589.592
K (I)	766.489	K (I)	769.896
P (II)	458.804	P (II)	494.41

and potassium start to decline as the age increase. The decrement of calcium level in nail was because the body's calcium absorption ability starts to deteriorate as it aged (Haas 2006). Potassium intensity decrease as the age increase is proven similar to the daily intake requirement. Magnesium, aluminum, phosphorus and sodium showed some changes without exact pattern. Although magnesium levels in nail of young adults was too high and too low for middle adults, consuming a considerable amount of these elements is not harmful (Musso 2009). The amount of magnesium in our body originated from the food that we consume especially from vegetable and seafood (Haas 2006). Moreover, aluminum in human body only depends on foods consumed and preparation of the foods (Haas 2006). This could explain the random intensity level of aluminum in nail shown in Figure 3. High phosphorus intensity level among young adults is usually due to food additives (Mateljan 2006). A sudden increase in the sodium intensity level among older adults is because one of the nail donors has high blood pressure (as notified from the subject's health condition information, 139/91 mmHg). This study also proved that an individual with high blood pressure has lower than average calcium and higher than average magnesium (Haas 2006).

The comparison of elemental composition in nail between female and male is shown in Figure 4. Calcium, phosphorus, potassium and aluminum were higher among female. Higher potassium intake can help lower the chance of getting stroke and symptomatic kidney stones (Higdon 2001). Although the aluminum amount in female' nails was higher, the functions of aluminum in human body were still not fully understood. Besides, the result showed that magnesium and sodium were higher among male. This is because magnesium daily requirement intake is higher for male 14 years and above. However, high level of sodium among male could cause higher blood pressure, leading to heart attack and stroke (Haas 2006).

ELEMENTAL COMPOSITION ANALYSIS OF PATHOLOGICAL NAILS

The comparison of elemental composition in nail between normal and melanonychia nail is shown in Figure 5. Melanonychia nail has higher calcium and lower potassium level compared to normal nail. The sodium level in normal nail was higher because this subject has higher blood pressure (high sodium level in nail). Meanwhile, the intensity level of all elements in onycholysis nail was slightly lower than normal nail. This is because the onycholysis nail was infected by yeast infection, a diploid fungus known as *Candida albicans*, causing it to have less nutrients (Hecker et al. 2015). The comparison of elemental composition in nail between normal and onycholysis nail is shown in Figure 6.

Leukonychia nail showed higher magnesium level compared to normal nail. However, the other elements' level was almost similar in both normal and pathological nails. The comparison of elemental composition between normal and leukonychia nail is shown in Figure 7. For pitting nail, it has higher magnesium, phosphorus and sodium level with lower calcium compared to normal nail. In addition, potassium and aluminum were almost the same in both normal and pathological nails. The elemental composition comparison in nail between normal and pitting nail is shown in Figure 8.

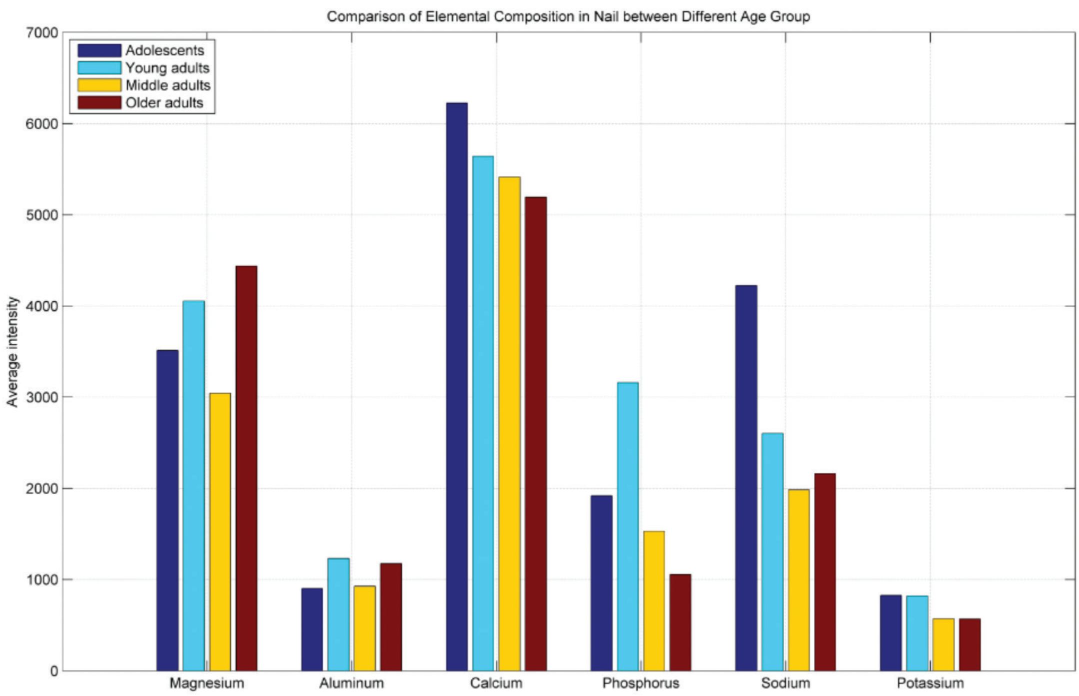

FIGURE 3. Comparison of elemental composition in nail between different age groups

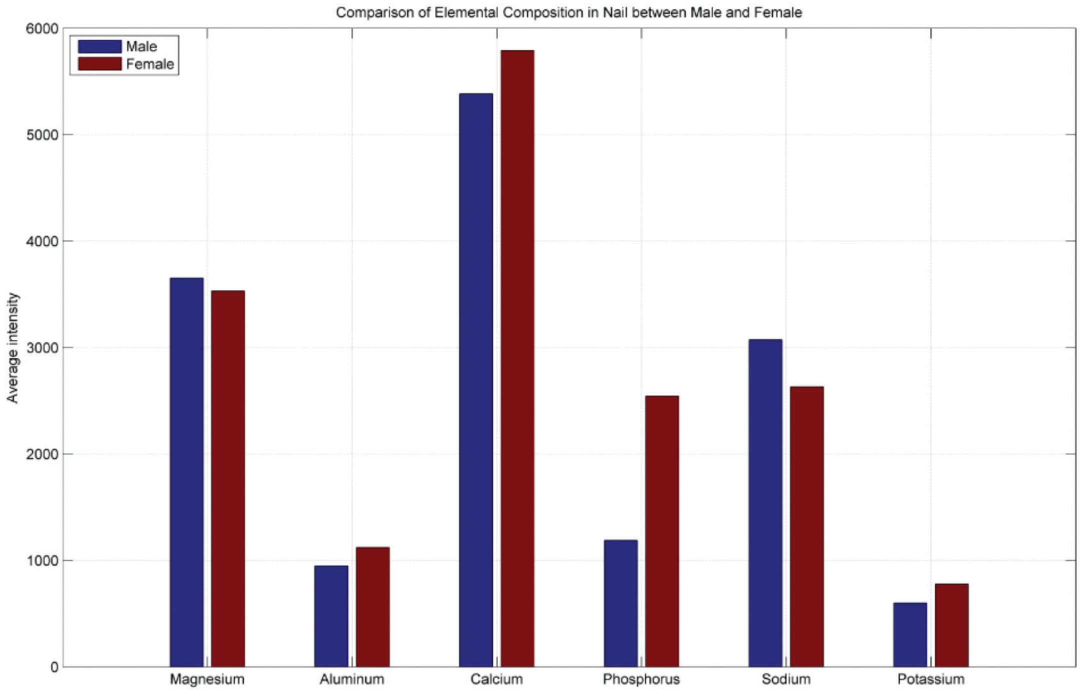

FIGURE 4. Comparison of elemental composition in nail between female and male

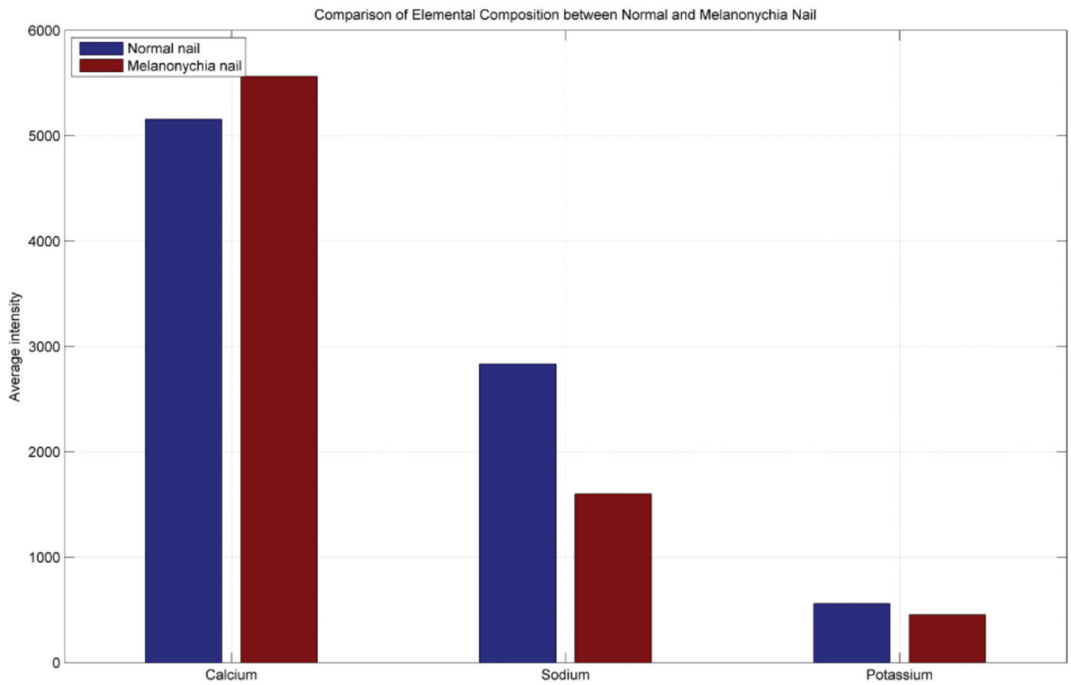

FIGURE 5. Comparison of elemental composition in nail between normal and melanonychia nail

SURFACE TOPOGRAPHY ANALYSIS: DIGITAL MICROSCOPE

The surface topography condition of normal nails after being shot by the laser pulse is shown in Figure 9. The surfaces of these nails were not seriously damaged, but only showing multiple white dent marks. Crates can only be detected on the nail's surface after the hundredth of laser shots. Thus, LIBS is proven as a less destructive technique.

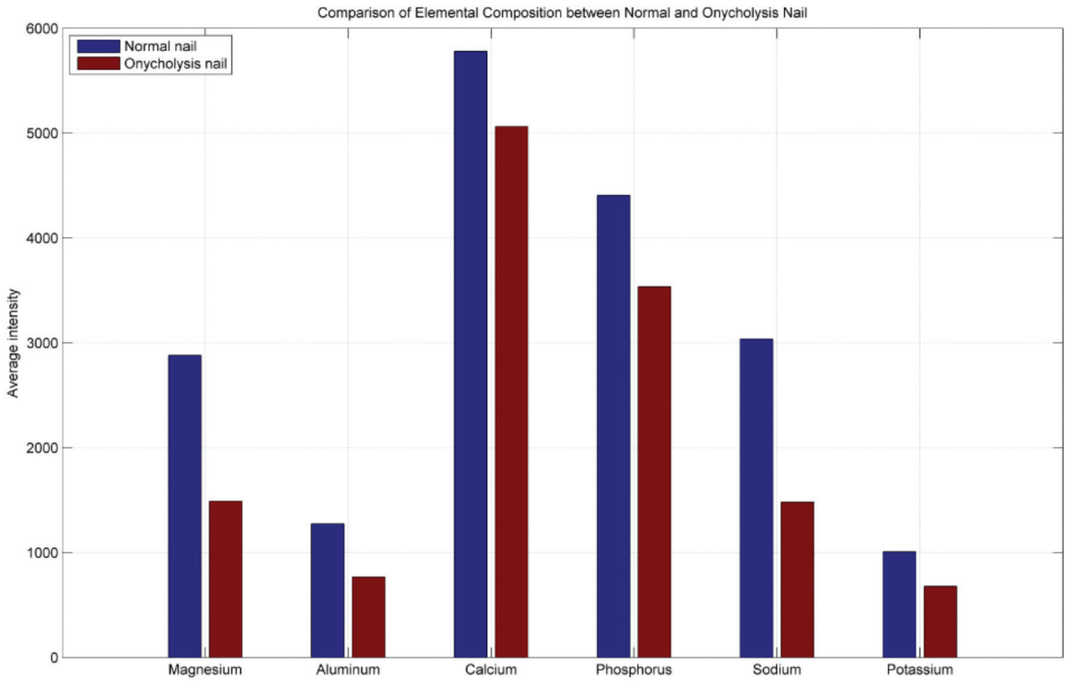

FIGURE 6. Comparison of elemental composition in nail between normal and onycholysis nail

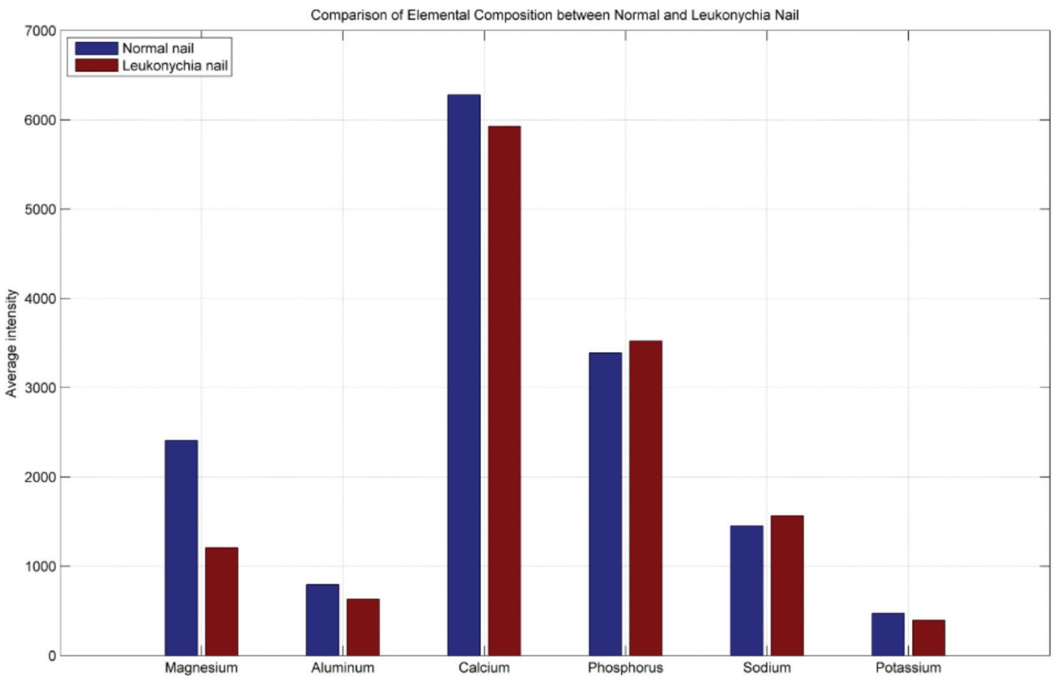

FIGURE 7. Comparison of elemental composition in nail between normal and leukonychia nail

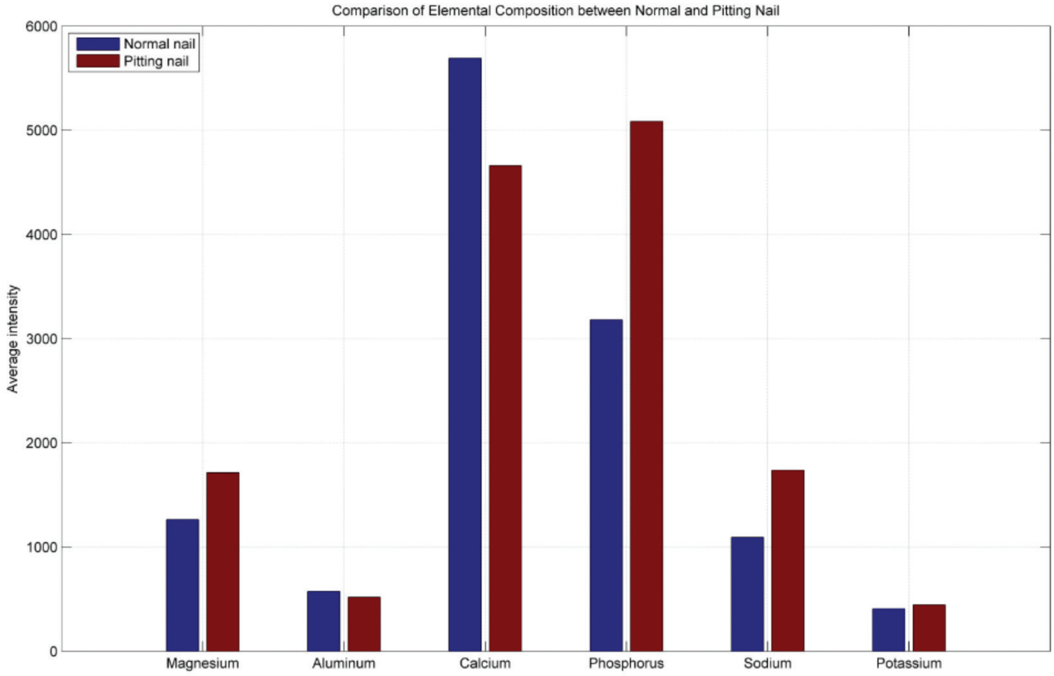

FIGURE 8. Comparison of elemental composition in nail between normal and pitting nail

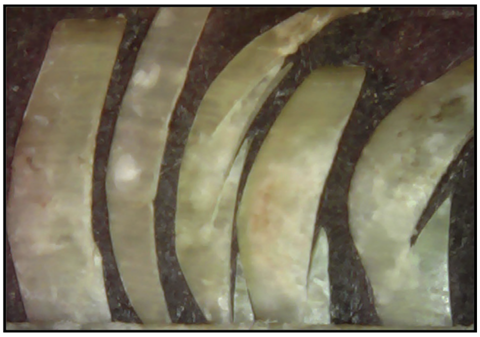

FIGURE 9. Normal nails after being shot by the laser pulse

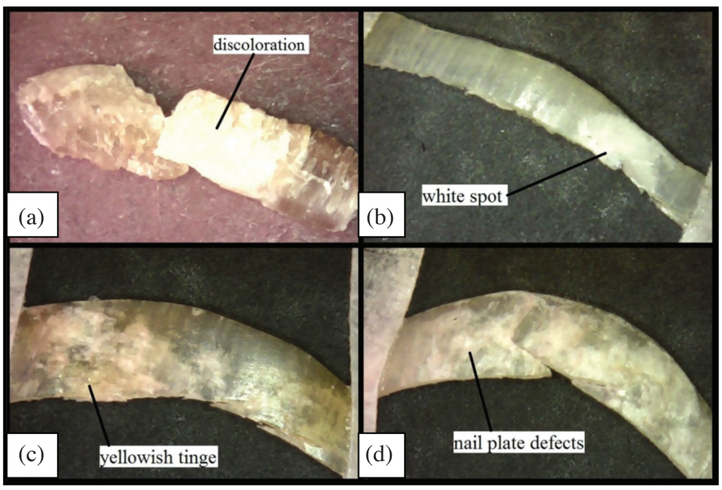

FIGURE 10. (a) Melanonychia nail undergo discoloration, (b) Leukonychia, (c) onycholysis and (d) pitting nails left with several white dents

After being shot by the laser for multiple times, melanonychia nail undergoes some discoloration. The other pathological nails were left with a small amount of white dents because the laser shots were focused only on the diseased part of the nail. None of these nails were seriously damaged, except for melanonychia nail because this type of nail was more brittle. The surface topography condition of pathological nails after being shot by the laser pulse is shown in Figure 10.

CONCLUSION

The LIBS technique has been applied to analyze the elemental composition of both normal and pathological nails. The comparison of normal nails' elemental composition between both genders shows higher calcium, phosphorus, potassium and aluminum among female compared to male. However, both magnesium and sodium are higher among male. Meanwhile, the elemental composition comparison in normal nails based on age group shows decrement of calcium and potassium as the age increase. The other elements, including magnesium, aluminum, phosphorus and sodium show some changes without exact pattern. The pathological nails' elemental compositions obtained were also noticeably related to the nail disorder condition itself, showing prominent differences if compared with normal nails.

The surface topography of nails after ablated by laser pulse shows that the nail's surface was not seriously damaged, proving that LIBS is a less destructive technique. Thus, elemental composition analysis of nails by using the LIBS technique is a highly potential alternative to diagnostic test because of its minimal sample preparation and less time consuming procedure. However, these results only involved a limited number of samples and should be provided with larger number of samples to prove the accuracy of this study.

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