Original Article

Possibility of Thyroidism Diagnosis by Laser Induced Breakdown Spectroscopy of Human Fingernail

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Abstract:

Introduction: A modern technique for elemental analysis of biological samples is laser induced breakdown spectroscopy (LIBS). This technique is based on emission of excited atoms, ions, and molecules in plasma produced by focusing a high power laser pulses on sample surface. Because of several advantages of LIBS including little or no sample preparation; minimally invasive; fast analysis time and very easy to use, in this study, this method was used for investigating the mineral content of fingernails. As the trace element of nail can be changed by several pathological, physiological, and environmental factors, we analyze the human fingernails to evaluate the possibility of thyroidism diagnosis.
Method: A Q-switched Neodymium-Doped Yttrium Aluminium Garnet (Nd:YAG) laser operating at wavelength of 1064 nm, pulse energy of 50 mJ/pulse, repetition rate of 10 Hz and pulse duration of 6 ns was used in this analysis. Measurements were done on 28 fingernails belonging to 5 hypothyroid, 2 hyperthyroid and 21 normal subjects. For classification of samples into different groups based on thyroid status, a discriminant function analysis (DFA) was used to discriminate among normal and thyroidism groups.
Results: The elements detected in fingernails with the present system were: Al, C, Ca, Fe, H, K, Mg, N, Na, O, Si, Sr, Ti as well as CN molecule. Classification in two groups of normal and patient subjects and also in three groups of normal, hyperthyroid and hypothyroid subjects shows that 100% of original grouped cases were correctly classified. So, efficient discrimination among these groups is demonstrated.
Conclusion: It is shown that laser-induced breakdown spectroscopy (LIBS) could be a possible technique for the analysis of nail and therefore identification of health problems.
Keywords: discriminant analysis; thyroid diseases; hypothyroidism; fingernail.

Please cite this article as follows:
Bahreini M, Tavassoli SH. Possibility of Thyroidism Diagnosis by Laser Induced Breakdown Spectroscopy of Human Fingernail. J Lasers Med Sci 2012; 3(3):127-31

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Introduction

Laser induced breakdown spectroscopy (LIBS) is a modern technique for analysis of biological samples which is based on emission of excited atoms, ions, and molecules in plasma produced by focusing a high power laser pulse on sample surface(1,2). Several advantages of LIBS including little or no sample preparation; minimally invasive; fast analysis time and very easy to use(3), make it very feasible for analysis of biological samples such as teeth(4), hairs(5), cells(6), and bacteria(7). This technique has been used in a previous work in analysis of human fingernails(8).

Analysis of human tissues can be an ideal method for screening a population for identification of diseases. Patriarca et al claimed that determination of trace elements in body fluids and tissues is essential for prevention, diagnosis, and treatment of some related diseases(9). Among human tissues, nail is a favorable biomarker for clinical investigations because it is easy to collect, store, and transport. The component of nail can contain information about metabolic events that occurred during the time of its formation(10,11). Based on the idea that the trace element composition of nail can be changed by several pathological, physiological, and environmental factors(12), it is possible that some disease influences it and nails could serve as an accessible biopsy material. Garland et al. assessed the reproducibility over a 6-year period of 16 trace elements measured in toenails and suggested that a single measurement of these biomarkers reflects long-term exposure(13). Nail clipping test for diagnosis of cystic fibrosis has been done and concluded that this method may be useful in cases in which a sweat test cannot be performed or gives borderline values, or when a patient is far from a diagnostic center(14).

Up to now, the possible correlations between nail minerals and thyroidsm have been rarely investigated. In the present study laser induced breakdown spectroscopy is used for elemental analysis of fingernail of healthy and thyroidism subjects. To investigate the possible classification of healthy and patient groups by LIBS, the subjects are categorized in three groups of healthy, hyperthyroidsm, and hypothyroidsm, by means of a statistical multivariate method named discriminant function analysis (DFA).This method is used to discriminate and classify samples into different groups.

Subjects and Methods

Subjects

The subjects participating in this study were randomly chosen among population of the city of Tehran. They have been informed of the purpose of the study and then, all subjects have given informed consent for participation to this work. The sampling of fingernails included administration of questionnaires containing individual information such as medical history, drug consumptions, and diets.

Here, 28 subjects are categorized into three groups: 21 healthy subjects; 5 hypothyroidism patients and 2 hyperthyroidism patients. Fingernail clippings were taken from free edge of nail plate by stainless steel nail scissors and kept separately in nonreactive plastic envelopes. Then, all of them were stored at room temperature until analysis. In order to eliminate any surface contaminations, specimens were washed by soaking in acetone for 1 minute followed by soaking in distilled water for the same time. Some of them were dirtier and were washed more. Then they were dried at room temperature.

Experiment

The LIBS experimental setup used to analyze fingernails is shown in Figure 1.

Typically, a laser pulse is focused on the nail surface. The intense electric field of the laser pulse creates a micro-plasma on the samples and light emitted out of the plasma is analyzed by a spectrometer. We used a Q-switched Nd:YAG laser operating at wavelength of 1064 nm, pulse energy of 50 mJ/pulse, repetition rate of 10 Hz and pulse duration of 6 ns. The energy of laser pulses can be tuned by using a combination of rotator and Glan–Taylor prism. Laser pulse was focused on the nail samples by a lens with focal length of 8 cm. Plasma emission was collected by two lenses with focal lengths of 4 cm and 1.5 cm and guided by an optical fiber to an Echelle spectrograph with the resolving power of 1700. The spectrograph is equipped by an Intensified Charge-Coupled Device (ICCD) camera for time resolved detection. A part of laser beam is sent to a photodiode by means of a beam splitter and then a signal is send to a delay generator. ICCD is triggered by delay generator, 7 μs after the plasma initiation. Each spectrum recorded in gate width of 20 μs.

Five LIBS experiments were done on each nail sample and the spectrum of each experiment is obtained by accumulation of 10 laser shots. The healthy-patient status of samples was unidentified during experiment. In Figure 2 we can see fingernails after LIBS experiment.

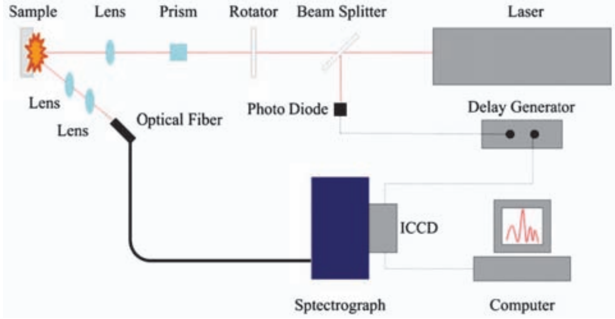

Figure 1. Schematic diagram of the experimental setup for LIBS measurements.

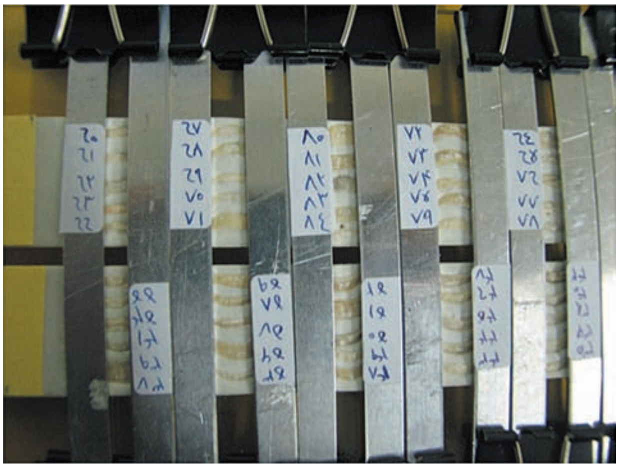

Figure 2. Fingernails after LIBS experiment.

Statistical method: Discriminant function analysis (DFA)

DFA is a multivariate analysis of variance which uses independent variables and form linear combinations to predict and quantify group memberships between distinct groups. In our study, for example, all the spectra acquired from fingernails of healthy subjects should ideally be identical, and thus constitute a group. Fingernail spectra of patient subjects would constitute different groups. DFA uses set of independent variables from each spectrum to predict the group membership of that particular spectrum. Independent variables are intensity of all emissions. The process has three basic steps: construction of discriminant functions, test of significance, and classification. Further detailed information on the statistical procedures can be found in references(15,16).

All the independent variable vectors from all groups were then analyzed simultaneously by a commercial DFA program (SPSS Inc., SPSS v17.0) to construct the canonical discriminant functions which were in turn used to calculate for each data spectrum a discriminant function score for that particular function.

Results and discussion

LIBS spectrum of human fingernail

Characteristic LIBS spectra of human fingernail sample are presented in Figure 3.

The dominant emission lines in the fingernail spectrum were atomic lines of inorganic elements, as have been addressed on the graph. Therteen elements in fingernail spectrum have been identified including

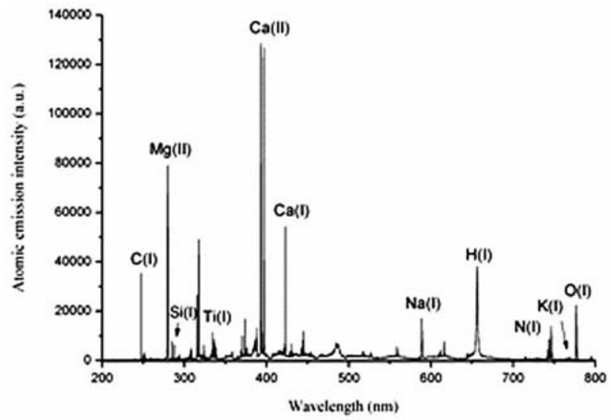

Figure 3. Characteristic LIBS spectra of human fingernail sample.

calcium, magnesium, silicon, sodium, potassium, titanium, strontium, iron, aluminium, carbon, nitrogen, hydrogen, oxygen. It is known that the dominant inorganic elements in nail composition are Ca, Mg, P, Na, K, Fe, Zn, and A1 while other elements like Cu, Cr, Se, Si, Sr, Ti, K, Ni, Pb, V, Co, Cd, Mn, As, Sb, Sn, and Hg can be found in trace levels(17,18).

Classification

A case report of hyperthyroidism based on the LIBS spectra of fingernails was presented in a previous work[8]. In that analysis, Na concentrations of cases with hyperthyroidism were outliers in the box plot. An outlier is an observation that is numerically far from the rest of the data(19). By observing the box plot for potassium level, we can saw that again those cases have outlying levels of potassium in their fingernails. Hyperthyroidism, an illness of the thyroid, is associated with an overproduction of the thyroid hormone. The symptoms of hyperthyroidism are palpitations, heat intolerance, nervousness, insomnia, breathlessness, increased bowel movements, light or absent menstrual periods, fatigue and fast heart rate, which are caused by the effects of too much thyroid hormone on tissues of the body(20). Hypothyroidism is the word used to describe a group of symptoms associated with underactivity of the thyroid gland. The most common method used to detect hyper and hypothyroidism is a blood test. The tests for T3 and T4 measure circulating hormone levels. The TSH test measures the pituitary thyroid-stimulating hormone, which in turn signals the production of T3 and T4.Problems with the blood tests are that circulating hormones may be normal, or even high, yet thyroid function at the cellular level may be deficient. This occurs very frequently. For example, elevated tissue calcium stabilizes cell membranes and impairs the transport of thyroid hormone across cell membranes. In other cases, low potassium impairs thyroid hormone activity at the cellular level. Other metabolic defects may also impair thyroid hormone activity at the cellular level. In the other hand, in some instances, the body may elevate thyroid hormone levels to attempt to compensate for poor transport across cell membranes. In these cases, a person may have some symptoms of thyroid overactivity such as anxiety. Yet he or she may also have some symptoms of hypothyroidism such as fatigue.

Generally, it was observed that there is an agreement between the elevated levels of potassium and sodium in the fingernails and blood test results indicating hyperthyroidism. Potassium deficiency is one of the symptoms of hyperthyroidism(21). On the other hand, the high level of an element in hair and nail may indicate depletion of that element in the body(22). Also, there is relationship between potassium and hypothyroidism(23).

In general, it should be mentioned that only individual cases were investigated for hyperthyroidism, and a more statistical approach is demanded to ascertain whether that results are of a more general nature.

In this analysis, a discrimination between two groups of 21 healthy and 7 patients was done by means of DFA program. The results of this analysis are shown in Table 1.

As it is shown in the Table, 100% of original grouped of healthy and patient subjects are correctly classified. In this classification, all variables from spectra have contributed but the K/Ca and Na/Ca ratios are more effective than other variables.

Also, a discrimination between three groups of 21 healthy, 5 hypothyroidism and 2 hyperthyroidism subjects is done. A plot of three discriminant function scores belonging to classification of three groups is shown in Figure 4.

Table 1. Results of classification in healthy and patient groups.

Classification Results a					
		Category	Predicted Group Membership: healthy	Predicted Group Membership: patient	Total
Original	Count	healthy	21	0	21
		patient	0	7	7
	%	healthy	100.0	0	100.0
		patient	0	100.0	100.0

a. 100.0% of original grouped cases correctly classified.

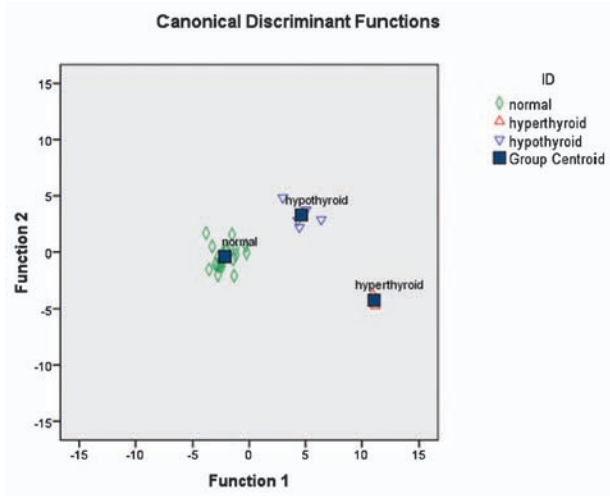

Figure 4. Discriminant function analysis plot showing the first two discriminant function scores of LIBS spectra obtained from fingernails of three different health status (normal, hyperthyroid, and hypothyroid).

In this figure, first two canonical discriminant functions are used for classification. In this analysis 100% of original grouped cases are correctly classified which show that a discrimination between three groups based on fingernail elements is possible. Again, the K/Ca and Na/Ca ratios are more effective than other variables in this classification. However, because the sample size in this analysis is low, the results should be proved by larger sample size.

Conclusion

To investigate whether it is possible to diagnose thyroidism by means of nail analysis, the LIBS spectra of fingernails of 28 subjects have been analyzed. Although fingernails of different health status present similar spectral lines, the small differences in their intensities may contain some information about disease. DFA, a statistical multivariate method, has been used for classification of subjects into healthy and patient groups. The results show that the classification between nail samples of healthy and patient subjects is attainable. Although the optical instruments required for LIBS are so expensive, the price of every experiment is negligible in comparison to the routine blood test. Since the time necessary for every fingernail LIBS experiment is also much lower than the blood test, and also nail is a favorable sample because of simplicity of collection, storage, and transport, this method is so convenient for public screening purposes. The results should always be carefully considered since the number of sample size is too small and should be proved by large sample size. As many physiological factors can effect elemental composition of nail, detailed studies in specific subgroups of subjects is required. Furthermore, the variation of LIBS spectra based on experimental conditions should be carefully examined. These early works point out the possibility of LIBS application in health problems and requiring further work to be accepted as a clinical diagnostic technique.

Acknowledgments

The authors would like to thank every person who participated in this study by giving the fingernail clippings.

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