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Original Article

Fourier-Transform Infrared Spectroscopic Comparison of Normal and Malignant Cervical Tissue

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Abstract

Based on the accumulated data, FTIR spectroscopy seems to be a highly sensitive tool in cervical cancer diagnosis. We, therefore, employed this technique in a screening type of research here in Tehran. The purpose of this study was to look for the spectral pattern differences between normal and malignant cervix samples in Iranian women. Through formal and informal announcements, all gynecology departments in the educational hospitals of Shaheed Beheshti University of Medical Sciences, as well as private clinics were asked to inform us of any volunteers (healthy or patient) for this research. Ectocervical smears were collected from volunteers with a spatula and then centrifuged to provide a small pellet of cells for FTIR analysis. A small amount of the pellet of cells was placed on a BaF_2 window, dried smoothly and placed in the sample holder of the FTIR instrument. For each spectrum, a total of 512 scans at 1 cm^{-1} resolution were co-added. This grouping of spectra clearly showed changes in the course of the pathologic results obtained for different samples. Under study although we were not able to further categorize and correlate the spectral changes with different pathological states, but a specific FTIR spectral region differentaining between healthy and cancerous patients was detected in the 1000-1200 cm^{-1} region of FTIR spectra. Wong's group had also reported that a difference between the FTIR spectra of normal and malignant tissue is possible in this region (Wong *et al*., *Pro. Natl. Acad. Sci. USA 88, 10988-10992*). However, the pattern of our result does not agree with that of Wong. Wong *et al*. have found some rising peaks for cancerous samples, in this spectral region while we have observed some disappearing peaks in this region of malignant samples. Therefore, the main difference between our finding and that of Wong is that a higher stability has been reported for the DNA backbone in Wong's study, while no stable bone was do servable in the cancerous tissues of cervix samples that we studied.

Keywords: Cervix; Screening; Pap smear; FTIR.

Introduction

Fourier-transform infrared (FTIR) spectroscopy is a popular and well-known technique in physics and chemistry for the identification and measurement of compounds in many different matrices. During recent decades, in a modern application of this technique, investigators have utilized this method to study tissue and cellular changes at molecular level (1, 2).

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Basically, this technique measures the vibrational modes of chemical bonds in functional groups within molecules (3). The principle of the technique is that infrared light is applied to tissues resulting in the absorption of energy by individual molecules which then vibrate at the excited energy states, giving rise to absorption spectra reflecting the characteristic vibrational bonds of the functional groups contained in the sample molecules (4). Any changes in the anatomical and/or biochemical characteristics of a given tissue are then expected to be reflected in the recorded spectra (5).

Many researchers have tried to record the spectral characteristics of human tissues in different healthy and impaired situations. It has been documented that this technique can be used to detect malignant, dysplastic, or inflammatory cells in different tissues (6-8). They hope to be able to diagnose different pathological conditions by looking at the spectral changes. The same approach has been used to investigate the interaction of different molecules with living organisms and cells (9).

The infrared spectrum of normal cervical cells is claimed to be quite distinct from those of inflammatory, dysplastic, and cancerous cells (10). In this study, we have investigated the spectral pattern differences between normal and malignant cervix samples collected from some Iranian females.

Experimental

Sampling

Through formal and informal announcements, all gynecology departments in the educational hospitals of Shaheed Beheshti University of Medical Sciences, as well as private clinics were asked to inform us of any volunteers (healthy or patient) for this research. All of the collaborative centers were equipped with sampling eppendorf tubes containing 0.5 ml of storage solution, and icebox. Volunteers were among women who were attending one of the above-mentioned centers from 1999 to 2002 for Pap smear test, and had given their consent to our having access to their medical history for the purposes of this project. Each volunteer had an ectocervical smear collected with a spatula, and spread on a glass slide, fixed and labeled. Then, the remaining of the examining brush used for Pap smear was immersed in the maintaining solution provided in an eppendorf tube and labeled. All the volunteers, demographic data, cytology and histology results, possible treatments, and follow-up were recorded, specifically those needed for correlating the FTIR results with the pathologic findings.

We studied volunteers from the list who had no previous history of excision or other cervical surgery within 6 months and who were not pregnant. 110 volunteers were enrolled to this study, only 4 of whom with cervical cancer. The Pap smears were processed according to standard methods and examined by pathologists at different laboratories blinded to the research proposal. They were then collected from the laboratories and re-examined by our collaborative pathologist. The histological interpretation was used as the gold standard for the comparison of both cytology and FTIR.

FTIR Spectroscopy

The samples for FTIR were stored in icebox and moved to our laboratory within a day, where kept at -70 °C until the spectral analysis was carried out. Before the experiment, samples were kept in -20 °C freezer for some time, and warmed gradually to room temperature. Solutions were then centrifuged to provide a small pellet of cells for FTIR analysis. A small amount of the pellet was placed on a BaF_2 window, dried smoothly and placed in the sample holder of the FTIR instrument.

Infra-red spectra were recorded on a Nicolet's Magna-IR system 550 equipped with Nicolet's OMNIC software. For each spectrum, a total of 512 scans at 1 cm^{-1} resolution were co-added. Spectral parameters of each spectrum, such as frequency shifts and intensity ratios of various infrared bands were determined after proper Fourier self deconvolution and derivatisation were carried out, using the OMNIC software (Band width 0.7 and enhancement 3) and kept for future use. However, for the purpose of this study, FTIR spectra were divided into healthy and malignant based on the Pap smear results. Spectra in each group were then put together and average spectra were generated mathematically.

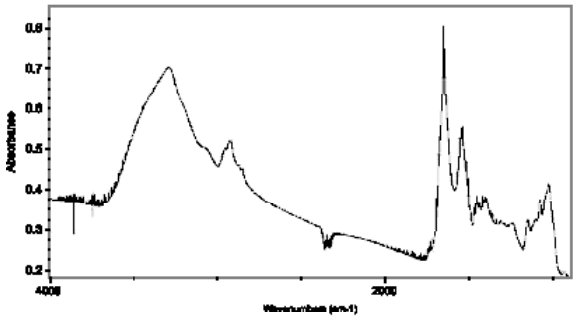

Figure 1. FTIR spectra of cervix samples. Tissue sample was collected from the remainder of pap smear test, and the spectrum was taken with 1 cm^{-1} resolution of 512 scan from 400-4000 cm^{-1}.

The resultant spectra from each category were then compared using computerized software and clear pattern differences within these groups were determined. Where necessary, similar mathematical modifications of specific regions were carried out for differences in visualization purposes.

Results

Figure 1 represents the FTIR spectra of the cervical tissue as was taken from Pap smear samples based on the protocol described in the Method section. The whole spectrum from 400 to 4000 cm^{-1} is shown in this figure.

For a better presentation of the reproducibility of the spectra, the 1600-1700 cm^{-1} region is presented in Figure 2. Figure 2 represents this specific region of FTIR spectra from seven different healthy volunteers. As is shown in this figure, a good reproducibility of FTIR spectra was obtained from the methodology that was used in this project. Figure 3 is shown for the same purpose to focus on the reproducibility of three FTIR spectra of human cervix with pathologically confirmed adenocarcinoma. Again, as is shown in this figure, for those patients with confirmed pathologic illness, the FTIR spectra are reproducible and comparable to each other in the different regions.

Figures 4 and 5 show various patterns of FTIR spectra from different patients. All spectral data collected from different volunteers were categorized into these patterns and grouped together. This grouping of spectra has clearly shown changes in course of the pathologic results. Although we were not able to further categorize and correlate the spectral changes

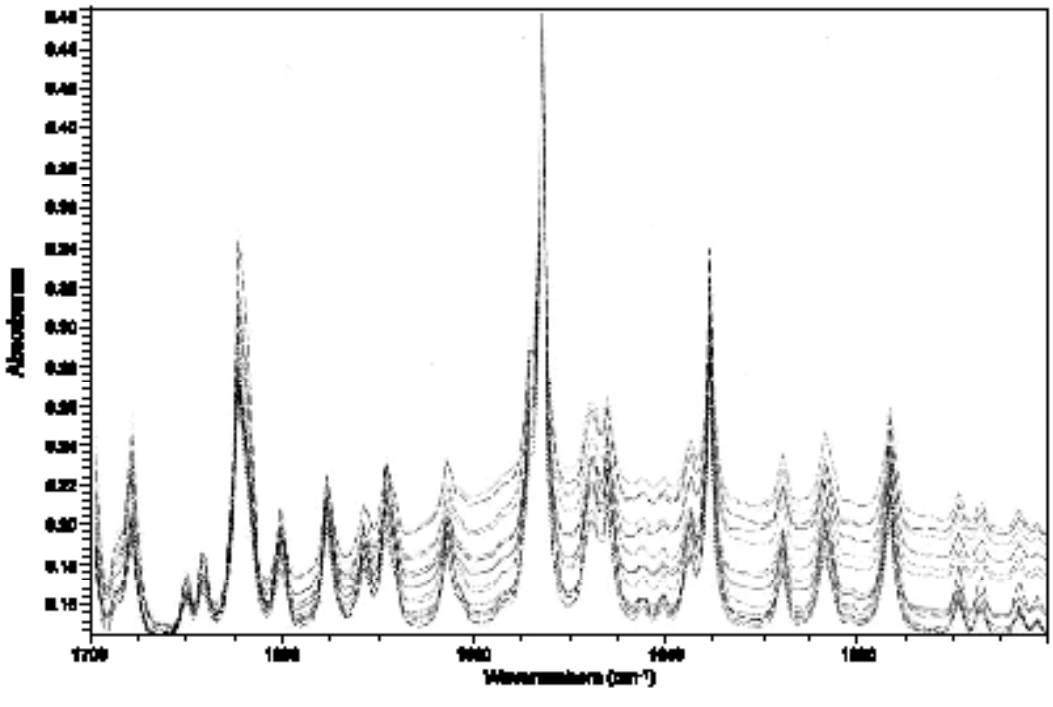

Figure 2. FTIR spectra of seven healthy cervix samples of seven healthy Iranian ladies at the region of 1600-1700 cm^{-1}. Tissue samples were collected from the remainder of pap smear tests, and the spectra were taken with 1 cm^{-1} resolution of 512 scan. This region was selected in this figure for a better representation of the reproducibility of spectra.

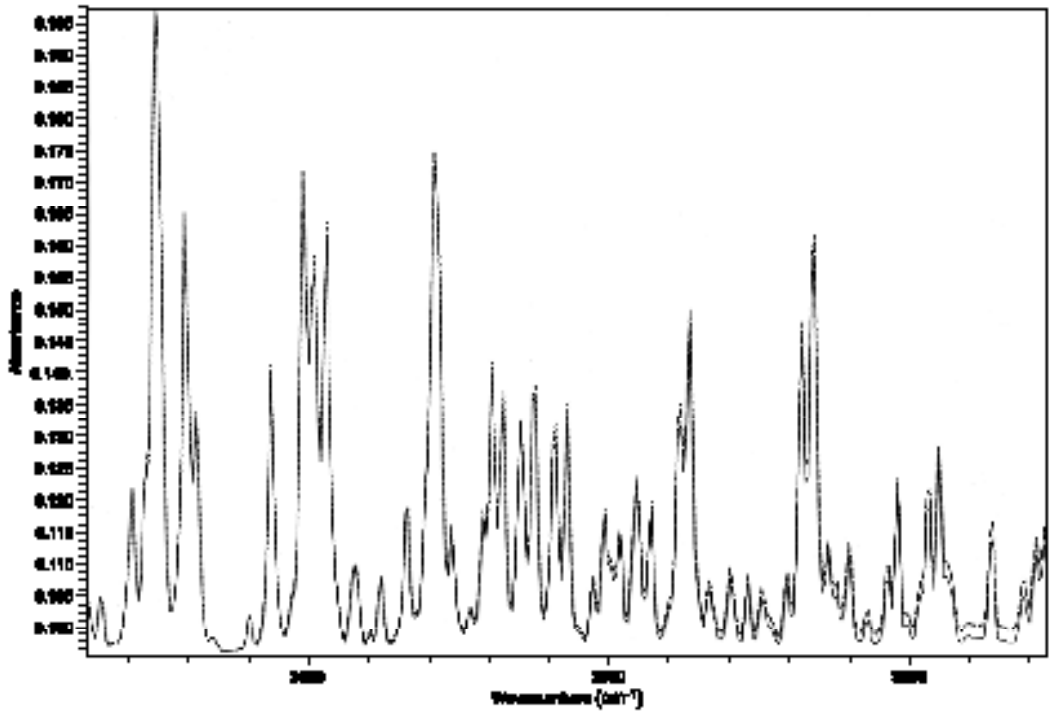

Figure 3. FTIR spectra of cervix samples of three Iranian ladies diagnosed with cervical adeno carcinoma. Tissue samples were collected from the remainder of pap smear tests, and the spectra were taken with 1 cm^{-1} resolution of 512 scan. This region was selected in this figure for a better representation of the reproducibility of spectra.

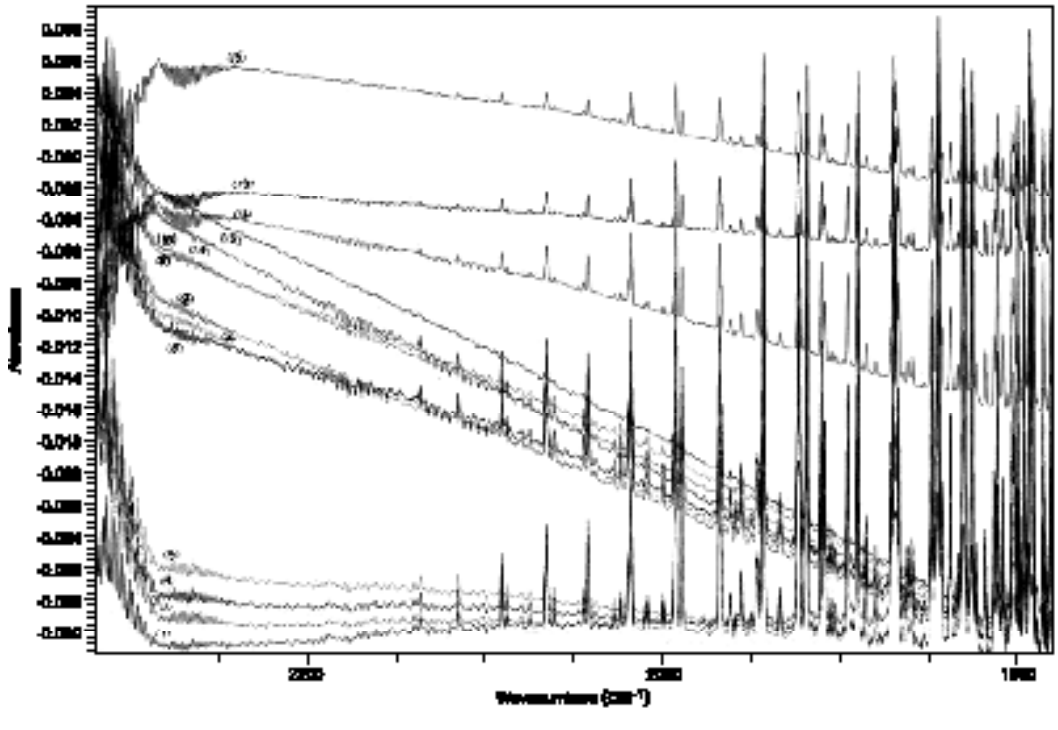

Figure 4. FTIR spectra of healthy and malignant cervix samples from Iranian ladies with 1 cm^{-1} resolution of 512 scan. Focus on this region (1800-2400 cm^{-1}) is shown in this figure to present the variable spectral patterns of different samples. Numbers indicate samples from different patients.

with different pathological states, figure 6 represents a specific FTIR spectral region of difference between normal and cancerous patients. This 1000-1200 cm^{-1} region of FTIR spectra is the clear edge of variation between normal and malignant cervix spectra in our study. In this figure, FTIR spectra of three normal and four cancerous tissues are shown together for better comparison.

Discussion

Many investigators have tried to find a distinguishable FTIR spectral region for the discrimination of cancerous tissues from that of normal ones. The goal was to find a rapid, easy and trustable diagnostic method for the detection of cancer. In many publications, Wong and colleagues have tried to convince the scientific committee of changes between the normal and malignant FTIR spectra, as a result of spectral deconvolution and derivatisation (11, 12). The weakness of their work was the use of a single spectral figure to make conclusions, as well as using variable mathematical modifications of FTIR spectra to achieve this goal.

In an attempt to prove the precision and applicability of this method, Wong's group had examined 301 tissue samples from the pathology department of Ottawa Civic Hospital (13). The result was satisfactory enough and published in 1996. However, lack of reproducibility and knowledge of the mathematical calculation parameters for the resulting spectra has limited its application to only a few articles since then. In any case, the success was satisfactory enough to enable the result to be patented in the USA (14) and market a diagnosis technology of PatScan© in some countries where the routine Pap smear examination was either not available, or too expensive (less than a dollar for PatScan compared to $25 for Pap smear in Canada) (14). The advantage of this technique seems to be its lack of false negative results; false positive results might necessitate further pathological investigations which would benefit the patient.

Most of the recent investigations concerning the FTIR differentiation between cancerous and normal tissues focus on more complicated mathematical calculations of bands related to the cellular DNA, and/or proteins (15). Investigators are focusing on computerized modeling to overcome the problems encountered in previous studies (16).

In the present study, and in an attempt to investigate the critical features of different research protocols, we have studied a limited number of cervical tissues from Iranian ladies. The purpose of this study was to examine the FTIR pattern of these samples, and its potential use for diagnostic purposes. During one year of sampling, we succeeded in collecting only four malignant tissues from Tehran, an area with a population of about 15 million. This is a low sample size for drawing any conclusion on the Iranian population. It may, however, be an indication of low incidence of uterine malignancy among Iranian women. Other collections were normal. We therefore categorized different samples and only compared the FTIR pattern of normal and malignant samples for the purposes of this project. As is shown in the results section, a good reproducibility of FTIR spectra was achieved for each of normal and malignant samples in some specific regions. Among these, only in one region of 1000-1200 cm^{-1}, a discriminative pattern of spectra was distinguishable between normal and malignant samples as is shown in figure 6. Although Wong's group had also reported this region of recognition, a difference between figure 6 and those of Wong's publication is the upset pattern between normal and malignant in our study. Wong has found some rising peaks for the region of about 1100 cm^{-1} (8), while we have noticed some disappearing peaks in this region of malignant samples. The band around 1100 cm^{-1} is mainly correlated to the phosphate symmetric stretching vibration of the DNA backbone (17). Therefore, the main difference between our finding and that of Wong is that a higher stability has been reported for this backbone in his publication, while lack of such a structure is quite clear in the cancerous tissues of cervix samples that we have studied. We were not able to show any other clear differences between our FTIR spectra of different pathological groups as and those of Wong's publications.

We have not been able to present a precise calculation of sensitivity and specificity of the

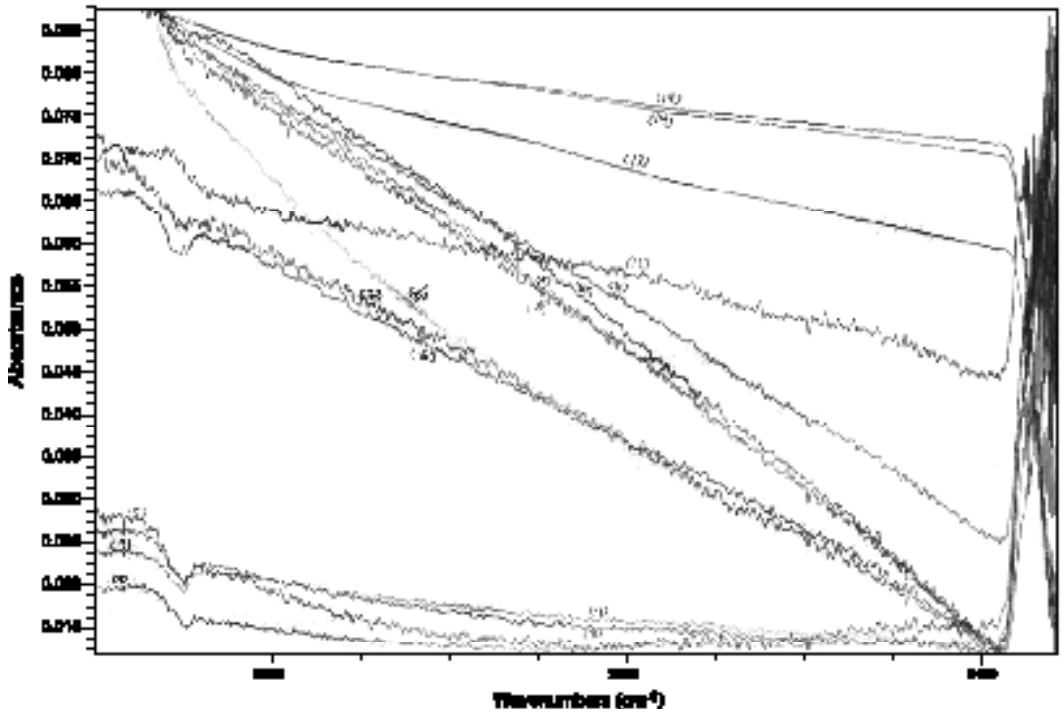

Figure 5. FTIR spectra of healthy and malignant cervix samples from Iranian ladies with 1 cm^{-1} resolution of 512 scan. Focus on this region (2400-3000 cm^{-1}) is shown in this figure to present the variable spectral patterns of different samples. Numbers indicate samples from different patients.

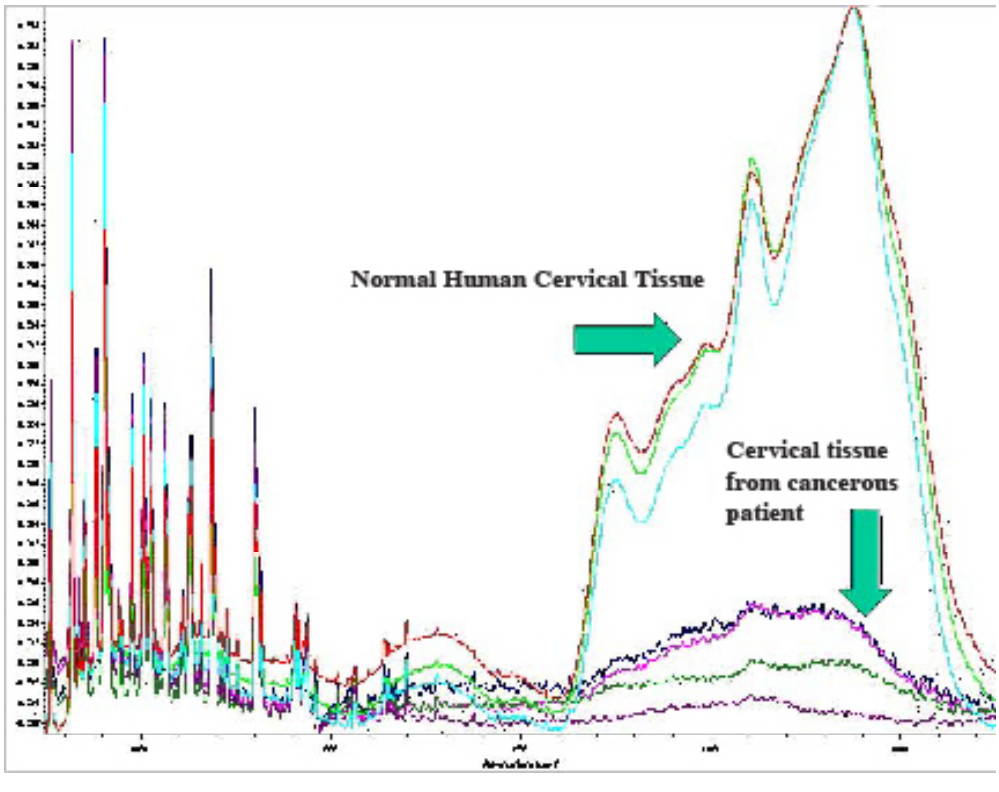

Figure 6. A comparison of the FTIR spectra of healthy and malignant tissues from Iranian population in the region of 1000-1200 cm^{-1}. Spectra of four malignant and three healthy volunteers with 1 cm^{-1} resolution and 512 scan is presented in this figure. A clear edge difference between healthy and malignant tissues is seen in this region

FTIR method compared to our gold standard, due to the small number of samples in each group. However, taking the Pap smear results as the gold standard and adopting the spectroscopic method used by Wong, we have been able to identify differences between FTIR spectral patterns of malignant and normal tissues of Iranian women. We hope further investigations on a bigger number of samples, using more complex mathematical calculations will result in better applicable results in future.

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