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Analysis of Endometriosis Using Energy Dispersive X-ray Fluorescence Spectrometry

N. EKINCI[1,*], M. INGEÇ[2], B. DOGAN[1], M. AGBABA[1] and E. SENEMTASI[3]

[1]Department of Physics, Science Faculty, Atatürk University, 25240 Erzurum, Turkey
[2]Department of Obstetrics and Gynecology, Medicine Faculty, Atatürk University, 25240 Erzurum, Turkey
[3]Department of Physics, Science and Art Faculty, Agri Ibrahim Çeçen University, Agri, Turkey

*Corresponding author: E-mail: neslihanekinci@yahoo.com

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Energy dispersive X-ray fluorescence method have been used to determine concentrations of the elements present in endometriosis. X-ray spectra have been collected using a Si(Li) detector with Camberra DSA-1000 desktop spectrum analyzer and Am^{241} and Fe^{55} annular radiactive sources. The energy resolution of the spectrometer is 160 eV at 5.9 keV. It has been seen from experimental results that there are general chlorine, potassium and calcium-enrichment. The relative errors for Am^{241} and Fe^{55} radioactive sources were found 2-6 %. Energy dispersive X-ray fluorescence technique has been successfully applied for the determination of elements present in endometriosis.

Key Words: Energy dispersive X-ray fluorescence, Endometriosis, Elemental concentration, Quantitative and qualitative analysis.

INTRODUCTION

Endometriosis is a disease in which tissue similar to the inner lining of uterus, called the endometrium, is present in locations in the body outside of the uterus (Fig.1). The misplaced tissue may be on the ovaries, the surface of the uterus, fallopian tubes, intestines, bladder, bowel or peritoneum (the thin lining of the abdominal cavity). Occasionally endometrium is present in even more distant sites outside of the abdomen, like, for example, the lung or a limb. Endometriosis is a dynamic disease with periods of development, progression and even regression.

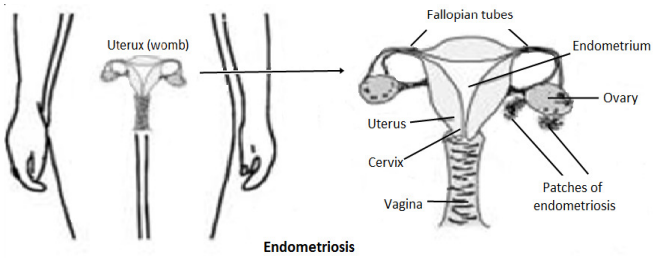

Fig. 1. Locations of endometriosis in the body outside of the uterus

Women with endometriosis may experience a variety of symptoms, though endometriosis can also be asymptomatic. Lower abdominal pain is most common and pain can be particularly intense before and during menstrual periods, as well as during ovulation. Some women have pain throughout the menstrual cycle. Infertility, pain with intercourse, fatigue, allergic diseases and bowel and bladder problems are also common with endometriosis.

Estimates of the prevalence of endometriosis range from 2-4 % women and girls to 10-15 % of in their reproductive years. Endometriosis is present in as many as 30-50 % of women with infertility and 69 % of teenagers with chronic pelvic pain not responsive to antiinflammatory medication or birth control pills. An early age of onset of menstruation and shorter menstrual cycles appear to slightly increase the risk of developing endometriosis.

The cause of endometriosis is still unknown. It is generally accepted, however, that endometriosis is the result of complex series of events that may link genetic susceptibility with environmental factors. The immune and endocrine (hormone) systems are directly involved in the development of endometriosis[1].

In the past several years, a number of studies have been performed on endometriosis tissues. Despite numerous studies on endometriosis, its etiology and pathogenesis have not been fully elucidated[2]. To our best of knowledge, analysis of endometriosis using energy dispersive X-ray fluorescence (EDXRF) have not yet been investigated. In this research paper, we have aimed to analyze endometriosis using Am^{241} and Fe^{55} radioactive annular sources.

During the last decades, the importance of trace elements in such different fields as medicine, biology and environmental science has been well established. Different analytical methods were developed to study the trace elements in biomedical and

environmental samples. A review article has aimed to give a comprehensive insight into both the historical and current thoughts on all aspects of endometriosis including aetiology, diagnosis and medical surgical teatment[3]. In our previous study, we have determinated the elemental concentration Rb, In, Sn, I, Cl, K, Ca in leiomyomata uteri and uterus by energy dispersive X- ray fluorescence spectroscopy[4]. In other study, we have used energy dispersive X-ray fluorescence techniques to analyze gall bladder stones[5]. Besides, samples of malignant and benign human lung tissues have been analyzed by two complementary methods, *i.e.*, particle induced X-ray emission (PIXE) and total reflection X-ray fluorescence (TRXRF)[6]. Energy dispersive X-ray fluorescence (EDXRF) have been successfully used for the determination of lead and other trace elements in the body fluids of industrial workers in Vietnam[7]. Furthermore, the elemental composition of healthy and pathological breast tissues have been investigated[8]. The calibration and application of a facility, based on energy dispersive X-ray fluorescent analysis (EDXRF) using Cd^{109} as an excitation source, for *in vivo* and *in vitro* estimation of Ca, Pb, Sr and Zn in tooth enamel have been described[9]. Also, the correlation between essential trace element concentrations in the placenta and maternal- neonatal characteristics has been investigated[10]. In addition, total reflection X-ray fluorescence (TXRF) procedure have been developed to monitor plasma drug levels and urine elimination in pediatric cancer patients undergoing chemotherapy with platinum-containing drugs[11]. Samples of healthy and carcinoma tissues of colon, breast and uterus on a total of 7 citizens from German population have been analyzed directly by total reflection X-ray fluorescence (TXRF)[12]. Energy dispersive X-ray fluorescence (EDXRF) is one of the fundamental techniques used for multi-element analysis of biological, geological, environmental and archeological materials.

EXPERIMENTAL

The analysis of elements present in endometriosis was carried out by energy dispersive X-ray fluorescence. The experimental system presented in Fig. 2 and used 2π source-detector geometry. For the present study, 5.9 keV photons emitted from ^{55}Fe and 59.5 keV photons emitted from ^{241}Am radioactive sources were used to excite the characteristic X-rays of the elements present in the samples. The main advantages of radioisotope excitation over X-ray tube excitation are the monoenergetic character of radioisotope-emitted X-rays, it is inexpensive and is commercially available. For the other types of excitation modes, *e.g.* X-ray tube, the spectral distribution relationship between scattered and background radiation intensities is more complex owing to the bremsstrahlung continuum. X-ray spectra were collected using a Si(Li) detector with a preamplifier, spectroscopy amplifier, HV bias supply and a multichannel analyzer including the analog to digital converter (ADC). The energy resolution of the detector was 160 eV (full width half maximum) at 5.9 keV. The spectrum acquisition time for endometriosis was 10 h.

Endometriosis samples were taken from a patient from Atatürk University, Education and Research Hospital, Turkey. The endometriosis samples were oven-dried at 300 ºC for 3 h. After drying, these materials were ground and sieved to a mesh size of 200 mm and then mixed for 20 min with cellulose,

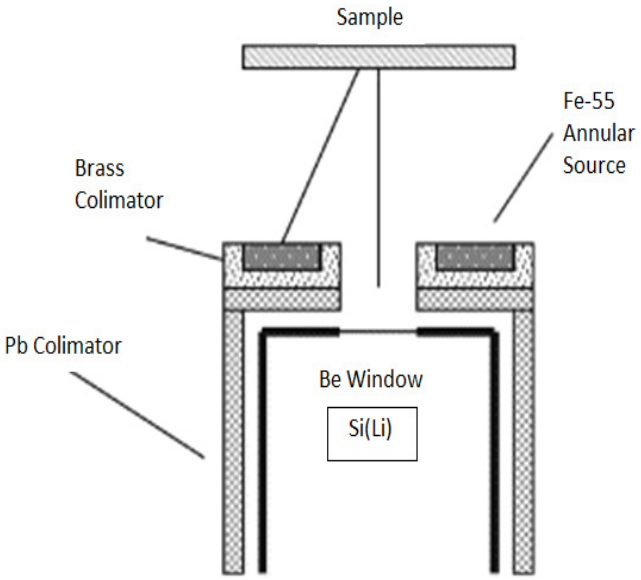

Fig. 2. Experimental set up for energy dispersive X-ray fluorescence analysis of endometriosis

which acts as a binder material. The amount of endometriosis in the mixture consisted of 10 mg. The amount of cellulose in the mixture was kept at a level of 4 mg. The mixture consists of 40 % cellulose and 60 % endometriosis material. A 2 ton hydraulic press was used to compress the sample powder into a thin pellet of 12 mm diameter. The advantage of making these pellets was that interelement enhancement effects in the sample are minimized: The effects of the matrix composition on the measured analyte-line intensity are known as matrix-, interelement-, self-absorption- and absorption-enhancement effects. Whatever absorption-enhancement effects a specified analyte-matrix system may be subject to, they are most severe at and above infinite thickness, decrease in severity as thickness decreases below infinite thickness and essentially disappear in thin samples[13].

To determine the contributions of the background and scattering from sample holder and air, measurements without sample were performed.

The elemental concentrations in endometriosis were determined using the equation:

$$w_i = \frac{N_{ij}}{I_0 G \varepsilon_{ij} \sigma_{ij} \beta_{ij} m} \quad (1)$$

where, w_i is the concentration of the element present in the sample, N_{ij} is the net counts/unit time for the Ith group of X-rays of the jth element, I_0 is the intensity of incident photon, G is a geometric factor, ε_{ij} is the efficiency of the detector for the Ith group of X-rays of the jth element, m is the sample mass in g cm^{-2}, β_{ij} is the self-absorption correction factor for the target material, which accounts for the absorption by the target for the incident photons and emitted characteristic X-rays of the Ith peak of the jth element. σ_{ij} is the X-ray fluorescence cross section of the Ith group of X-rays of the jth element. For example; X-ray fluorescence cross sections for K_a X-rays yields following relationship:

$$\sigma_{Ka} = \sigma_K \varepsilon_K f_{Ka} \quad (2)$$

where, a_k is the K shell photoionization cross section[14], ω_k is the K shell fluorescence yield[15] and f_{Ka} is the fraction of K_a X-rays in the K X-rays[16].

The factor $I_0G\varepsilon_{ij}$ in equation (1) has been evaluated by running, separately, the K X-ray spectra of a number of spectroscopically pure powders of Fe, Zn, Br, Zr, Mo, In, Sn, Cs, Nd, Gd and Er of known concentrations using an annular ^{241}Am source and K, Ca, Ti, V and Cr of known concentrations using an annular ^{55}Fe source.

RESULTS AND DISCUSSION

The spectra obtained from endometriosis samples using annular ^{55}Fe radioactive sources have been presented in Fig. 3. Cl and K were detected by means of the energy dispersive X-ray fluorescence. The sample homogeneity was tested with five sets of measurements for each mixing time 2, 4, 6, 8 and 10 min. The mean concentrations for each mixing time and standard deviation (SD) of the elements observed in the endometriosis are given Table-1. The relative standard deviation in the measurements is estimated to be *ca.* 3.5 %, calculated as the square root of the sum of variances of different parameters used, namely peaks areas (*ca.* 2 %), weighing (*ca.* 2 %) and the systematic errors (*ca.* 2 %).

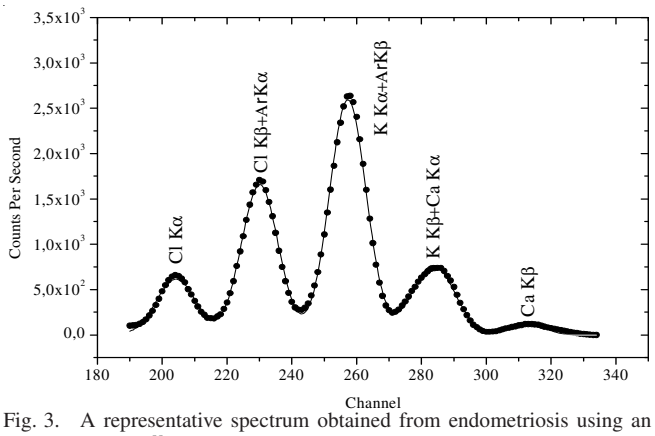

Fig. 3. A representative spectrum obtained from endometriosis using an annular ^{55}Fe source.

TABLE-1
MEAN CONCENTRATIONS AND RELATIVE ERRORS (RE) OF THE ELEMENTS OBSERVED IN THE SAMPLES OF ENDOMETRIOSIS

	Cl	K	Ca
Sample 1	1.963 ± 0.058	0.152 ± 0.009	$5.8 \times 10^{-4} \pm 0.029$
Sample 2	0.890 ± 0.017	0.084 ± 0.005	$8.4 \times 10^{-4} \pm 0.014$
Sample 3	1.493 ± 0.059	0.254 ± 0.017	$7.6 \times 10^{-4} \pm 0.019$
Sample 4	1.263 ± 0.075	0.083 ± 0.004	$6.9 \times 10^{-4} \pm 0.023$
Sample 5	0.957 ± 0.038	0.064 ± 0.002	$2.2 \times 10^{-4} \pm 0.018$
Sample 6	0.370 ± 0.018	0.108 ± 0.006	$3.1 \times 10^{-4} \pm 0.011$
Sample 7	2.366 ± 0.047	0.180 ± 0.008	$1.1 \times 10^{-4} \pm 0.036$

It can be noted that ΔC for calcium was higher in the samples of leiomyomata uteri and uterus. ΔC for calcium was $0.268 \pm 9.38 \times 10^{-3}$. Comparing the results obtained between leiomyomata uteri and uterus from Table-1 is observed that uterus presents a large concentration for the Ca. Leiomyomata uteri presents larger concentrations of Cl and K than those in uterus. The elements Rb, In, Sn and I in leiomyomata uteri present concentrations lesser than those in uterus.

Rb, In, Sn, I, Cl, K and Ca play an essential role in the biological and physiological possess of the human organism. Both deficiencies as well as excesses of these elements may result in a number of disorders in the human body. Rubidium is found in cancerous tissue. The biochemical role of rubidium in tissue is not known.

Potassium, calcium and chloride were found in large amounts in both leiomyomata uteri and uterus. Potassium is necessary for muscle contraction (especially in cardiac fiber), transmission of nerve impulses, synthesis of some proteins and as an enzymic cofactor. Problems associated with low potassium levels include high blood pressure, congestive heart failure, cardiac arrhythmias, palpitations, muscle weakness, hyperthyroidism, elevated blood sugar, mental apathy, depression, fatigue and general weakness, while severe potassium loss can cause death. Excessively high potassium levels result in acute or chronic cystitis (bladder infections) and right-sided ovarian cysts and testicular cancer.

Some studies claim that high chlorine levels increase the risk of bladder cancer and incidence of Hodgkin's disease, colourectal, esophageal and breast cancer. According to these studies, women with breast cancer have 50 to 60 % higher levels of organochlorines (chlorination by products) in their breast tissue compared to women without breast cancer. Chlorine has also been associated with declining sperm counts and male infertility.

Low or high levels of iodine can affect cardiac function. Iodine also affects the thyroid and adrenals. Too much iodine can trigger hyperthyroidism. During pregnancy, iodine can have adverse effects on the baby.

Calcium is now the most promoted nutrient by proponents of conventional, nutritional and alternative medicine, yet at the same time, the assumed need is based purely on the speculation that the body's calcium intake is well below its requirements of the approximately 1 g of calcium in the average 70 kg adult body, almost 98 % is found in bone, 1 % in teeth and the rest is found in blood, extra cellular fluids and within cells where it is a co-factor for a number of enzymes. Calcium promotes blood clotting by activating the protein fibrin along with magnesium helps to regulate heart beat, muscle tone, muscle contraction and nerve conduction. Chronic calcium deficiency is associated with some forms of hypertension, prostate and colourectal cancer, some types of kidney stones, and miscarriage, Birth (heart) defects are seen in children when mother has periodontal disease, sleep disturbances, mental health/depressive disorders, cardiovascular or hemorrhagic diseases. Elevated calcium levels are associated with arthritic and vascular degeneration, calcification of soft tissue, hypertension and stroke, gastrointestinal disturbances, mood and depressive disorders, chronic fatigue, increased alkalinity and general mineral imbalances. High calcium levels interfere with vitamin D and subsequently inhibit the vitamin's cancer-protective effect unless extra amounts of vitamin D are supplemented.

In this paper, the leiomyomata uteri and uterus taken from a patient were analyzed by energy dispersive X-ray fluorescence. This method is a very well suited for the direct analysis of elemental content in biomedical and environmental samples. Absorption effect are minimized, with some advantages such

as simultaneous and multi element character, the possibility of determinations in a wide concentration range from about 1 ppm to 100 % and simple and fast sample preparation. Also, the equipment cost is much lower than for a conventional wavelength dispersive X-ray fluorescence spectrometer, especially when a radioisotope is used instead of an X-ray tube.

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