2011 International Nuclear Atlantic Conference - INAC 2011
Belo Horizonte,MG, Brazil, October 24-28, 2011
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
ISBN: **978-85-99141-04-5**

ELEMENTAL CONCENTRATIONS IN NORMAL SKIN AND FIBROEPHITELIAL POLIP LESIONS BY SYNCHROTRON RADIATION TOTAL REFLECTION X-RAY FLUORESCENCE TECHINIQUE

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ABSTRACT

In this work, the concentrations of trace elements were measured in acrochordon, a skin lesion also known as skin tag or fibroepithelial polyp, as well as in normal skin from the same patient. The samples were analysed by Synchrotron Radiation Total Reflection X- ray Fluorescence (SRTXRF) in the Synchrotron Light National Laboratory (LNLS) in Campinas/São Paulo-Brazil. The collection of lesion and healthy skin samples, including papillary dermis and epidermis, has involved 17 patients. It was evaluated the presence of P, S, Cl, K, Ca, Cr, Mn, Fe, Cu, Zn, Br and Rb in the paired samples, which were compared, and significant differences were found in some of them.

1. INTRODUCTION

The information on the concentration of elements in normal and lesion using SRTXRF is scarce. Moreover, the values presented in researches done by different methods differ a lot from each other. The concentrations of some chemical elements in diseased human tissues can present differences if compared with healthy ones [1]. It has been seen that elemental content is often related with several skin lesions, and trace elements play a crucial role in a great number of enzymes that are involved in the disease evolution and its treatment [2, 7,9,10]. The choice of the SRTXRF was made by the fact that it allows us to investigate small amounts of tissue with high precision.

The study of fibroephitelial polip lesion was chosen, first, because it is easy to collect samples with low risks to the patients, and second, it is relatively frequent kind of benign skin lesions and, as others skin lesions, still not well understood.

The fibroepithelial polyp, one of the most common benign skin tumors, occur in almost half the population, equally in men and women and its incidence increase with age. Two thirds of people will have some skin tags present by the age of 70 years.
They are small, pedunculated skin-coloured or brown papules that often develop in areas of skin friction around sites of skin folds, particularly around the neck, axila, between the thighs and on the eyelids. The size are usually 2 to 5 mm in diameter [3]. They are more common in obese patients. Some are thought to have a hormonal influence in their growth.
These lesions are benign but may be associated with other disease states, occasionally warranting closer examination of the patient for other signs and symptoms. Many clinicians do not submit these to pathology, but occasionally it's possible to find seborrheic keratoses and melanocytic lesions in these specimens.

2. MATERIALS AND METHODS

2.1. Sample Collection

The paired samples were collected from 17 patients with ages between 32 to 74 years old. None of them had used any medication or cosmetic at least a month before collecting. All the areas examined, located at face and torso, were thoroughly cleaned with 18.2MΩ Milli-Q ultra pure water. Before extracting the lesions, scissors, callipers and scalpels used were decontaminated, biological and chemically. The healthy skin were visually identified and separated from the lesion sample in order to compare its spectra to the lesions.
The samples were washed with Milli-Q ultrapure water and then stored in polystyrene test tubes with cap at -5°C prior to analysis. Subsequently, the samples were lyophilized in free contamination environment at same time to maintain identical drying conditions. The dry weights of the samples measured were between 1.0 ± 0.1 and 18.2 ± 0.1 mg. The lowest values of weight are related to the healthy tissue, the control samples, that were extracted from the edge of lesions.

2.2. Sample Preparation

The digestion procedure of the samples was performed adding Suprapur HNO_3 (65 %) to the samples at rate of 7:1 in mass and warmed in oven at 60^oC for two hours. Immediately afterwards they were diluted with Milli-Q ultrapure water to obtain final masses of 50, 100, 150 or 500 μg, depending on samples masses, and homogenized by strong agitation. Instead of measuring volumes, it was used the precision of a scale, with divisions of 0.1 mg, in order to improve the quality of measurements.
The following step was add aliquots of gallium standard solution to have a final concentration of 2 $\mu g g^{-1}$ in each diluted sample, based on same order of magnitude of zinc concentrations in human skin as reference[4,9]. Finally, droplets of 5 μL of each sample were deposited on lucite sample carriers and then dried in a stove to obtain a regular thin film.

2.3. Spectra Mesurements

The X-ray fluorescence spectra were collected by SRTXRF, a method in which a collimated beam of synchrotron radiation hits, at small angle, the thin film over the lucite plate and is

totally reflected [6,8]. The scattered beam emerging from the plate was measured with a radiation detector. This experiment was carried out at set D09B-XRF bean line of LNLS (National Synchrotron Radiation Laboratory) in Campinas/São Paulo/Brazil. The area under each peak in spectra was calculated with QXAS software from IAEA (International Atomic Energy Agency).
The relative concentration of each element was determined regarding gallium peak of internal calibration. As the samples were diluted, it was necessary to do a mass correction to obtain the absolute concentration of the elements. To detect significant variations among concentrations, groups of normal and fibroepithelial polyp samples were compared using the paired Student's t- test.

3. RESULTS

The principal peaks of the elements were identified in a spectrum as showed in Figure 1.

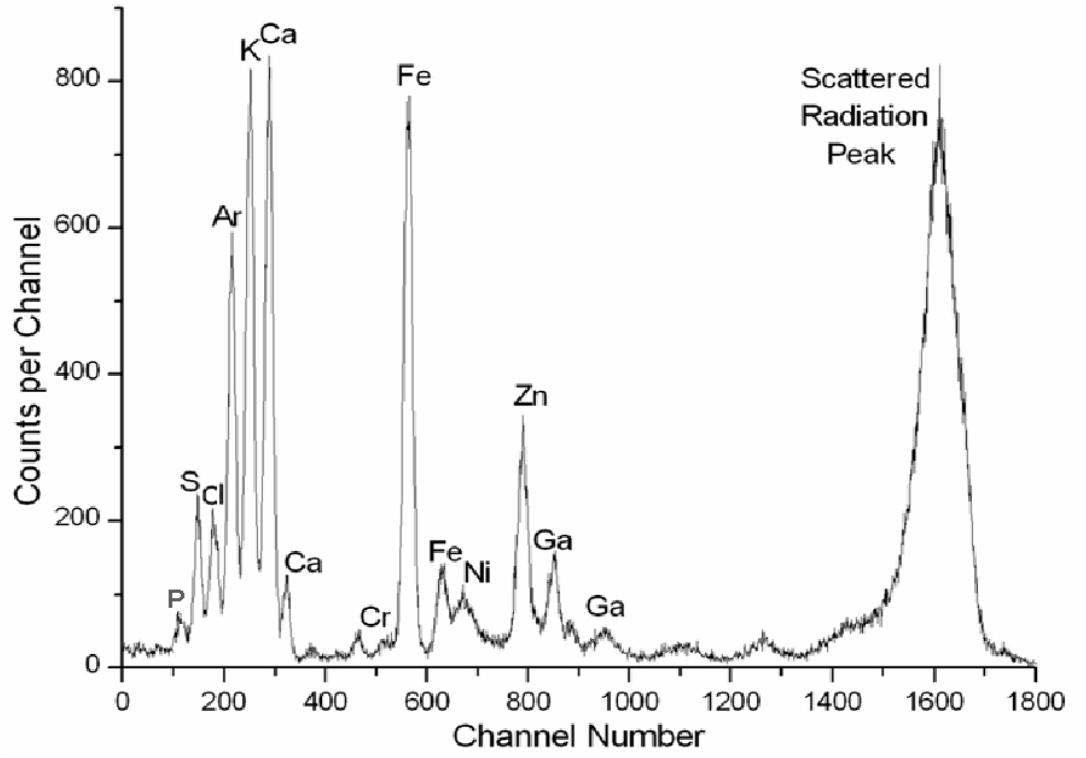

Figure 1. Example of one spectra of the skin samples with peaks identified.

Among the elements analyzed in this experiment, P, S, Cl, K, Ca, Cr, Mn, Fe, Cu, Zn, Br and Rb, a comparison between data of control and lesions samples, by means Student's t-test ($p<0.05$), revealed significant differences in concentration of the elements P, Ca, Fe e Cu. The values of the concentration are showed inside the bars of Figure 2. All values are expressed in $\mu g g^{-1}$. They represent the absolute elements concentrations in samples that

included papillary dermis and epidermis, averaged over 17 patients. The error bars represents the 95% confidence interval calculated trough descriptive statistic tools.

The result of P concentration are the same order of magnitude of the average of values presented by other works, both control and lesion data. The P concentration decreases in seborrheic keratosis and psoriasis skin lesions if compared with healthy tissue [4,11]. But, in this work, on the contrary, its concentration is higher in lesion than in the control samples.

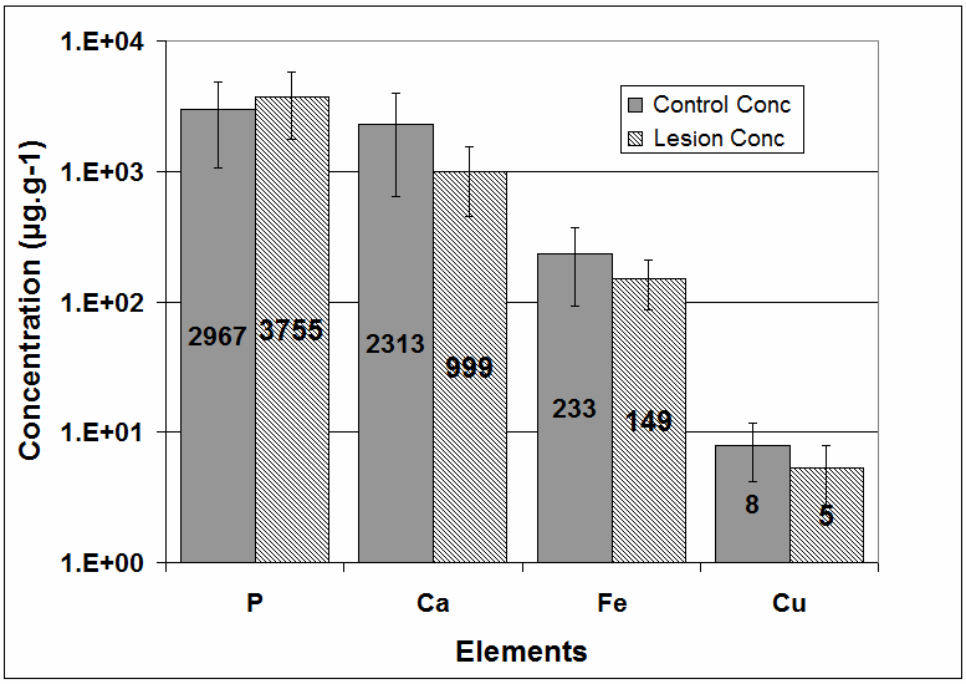

Figure 2. Mean values of elemental concentrations of normal and SK skin samples which presented significant differences (p<0,05 and N=17).

The P compounds, which are involved in vital functions of the organism, are also part of the DNA and RNA structure. The cells use it to store and transport the energy in the form of phosphate and adenosine. Furthermore, it is an important element of the protoplasm and nervous tissue. But the higher values of P concentration has to be more investigated because the low sensitivity of SRTXRF method for this element.

The Ca concentration estimated is compatible with XRF method results and, in relation to normal tissues, it decreases, as here, in almost all lesions broached like kidney, lung, prostate cancers, liver cirrhosis [1] and psoriatic skin [4]. Calcium plays an important role in apoptosis (programmed cell death), and the balance between Ca and Zn has recognisably importance on this.

The lower level of Fe in control samples can be explained by the less amount of blood, rich in iron. Despite differences in absolute values of concentrations, practically the same rate of decrease of Fe was found in seborrheic keratosis skin lesions samples as well as in psoriatic skin lesions [4, 11, 12].

The Cu, which is involved in the synthesis of hemoglobin, melanin, and elastin, an enzyme cofactor, part of some cytochromes in cell respiration, assists in phospholipid synthesis, protein metabolism, vitamin C oxidation and the formation of RNA, showed lower concentration in lesions than in normal skin. This is compatible with results found in psoriasis and malignant skin lesions [4,7], but the opposite of seborrheic keratosis skin lesions [11].

3. CONCLUSIONS

The results of concentrations for normal skin found in this work are far of the values given by others methods like PIXE (proton induced X-ray emission)[4], neutron activation[5], DXS (diagnostic X-ray spectrometry in vivo) and Compton X-ray scattering, but, most of then are of same order of magnitude when TXRF are used[1]. But the presence of the gallium reference peak in SRTXRF technique used in this work, which has his concentration very well known, gives more reliableness to the data. Thus, it can be assured that the SRTXRF technique is adequate to determine traces of the elements P, S, Cl, K, Ca, Ti, Cr, Fe, Ni, Cu e Zn. Beside this, together with statistic tools, it has allowed us to distinguish clearly the P, Fe, Ca and Cu concentrations.

Although this survey has used paired samples, some of them with very small masses, the number of patients investigated made the statistical analysis still inaccurate. But it seems to be worth to carry on our efforts in this research in order to detect variations in others elements concentrations, and, in the future, compare these results to other kind of skin lesions in order to reach a better understanding between elemental content and normal and abnormal physiology.

ACKNOWLEDGMENTS

This work was developed partially at Brazilian Synchrotron Light Laboratory (LNLS - Projects XRF – 9323 and XRF - 9852) with financial support of FAPERJ and CNPq.

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