MICROSPECTROPHOTOMETRIC QUANTITATION OF IMMUNOHISTOCHEMICAL REACTIONS BY IMAGE ANALYSIS

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ABSTRACT
Immunohistochemistry is a technique by immunological reactions to identify proteins in different biological samples. As a result leave a product in histological preparations, visible under a microscope when using a colour reaction. This is an indicator of the presence of the specific molecule and cellular metabolic activity represents a more or less specific. In the literature have used different methods to estimate the intensity of this reaction trying to get a reproducible value. With our microarray methodology (Cabrini RL *et al.*) we performed a spectral curve for the final product of the reaction of benzidine using as reaction control of a case inmunohistochemistry HER2 positive breast adenocarcinoma. Images were obtained from a single field of the preparation in a Zeiss MPM 800 with an objective x 40/0, 75 different light wavelengths. The maximum absorption was 0.55 optical density at 530nm per pixel. The measurement was performed in 10 cases of carcinomas STA1. The methodology presented in this study showed different values at predetermined fixed areas.

Keywords: Microspectrophotometric, quantitation of immunohistochemical reactions, image analysis.

INTRODUCTION

Immunolabeling techniques are often used as a tool to help with histopathological diagnosis as well as in different scientific research models. These conventional immunological reactions are used for identifying proteins in different kinds of tissue, representing specific cell metabolic activity. The tissue to be studied is placed in contact with the antibodies, to which specific molecules react by bonding to it. Consequently, some cells show up under optical microscope when stained [1]. As a result, they leave an end-product in the histological preparations that is visible under a microscope when stained, which is an indicator of the presence of the specific molecule. The literature reports that different methods have been used to estimate the intensity of this reaction, in an effort to obtain a reproducible value [2,3]. This paper presents an original immunohistochemical quantitation methodology by densitometry and image analysis for studying the different levels of reaction in biological samples.

MATERIALS AND METHODS

At our laboratory and given the possibility of performing simultaneous reactions using a relatively simple methodology[1], we determined a spectral curve for the final product of the benzidine reaction using as reaction control a case immunohistochemistry HER2 positive breast adenocarcinoma (Figure 1). Digital images were obtained from a single field of the preparation using a Zeiss MPM 400 microscope with an objective x 40/0, 75 at different light wavelengths of 475, 500, 550, 600, 650 and 700 nm. Densitometry was used to quantify the mean optical density per pixel at different wavelengths. Densitometric measurements were taken with an image analysis program. The spectral curve was plotted using the absorption maxima for each wavelength. The absorption peak for the immunohistochemical reaction in our sample at 0.55 optical density per pixel was at 530nm. The measurement was performed in 10 cases of human carcinomas under STA1 protein expression (Figure 1).

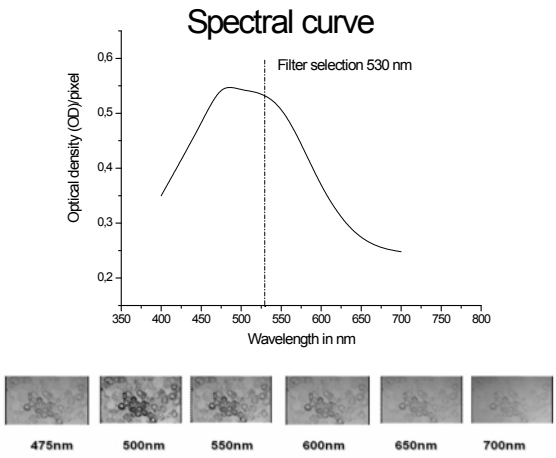

Fig.1. Spectral curve: The lower pictures show the behavior of the tissue under different light wavelengths.

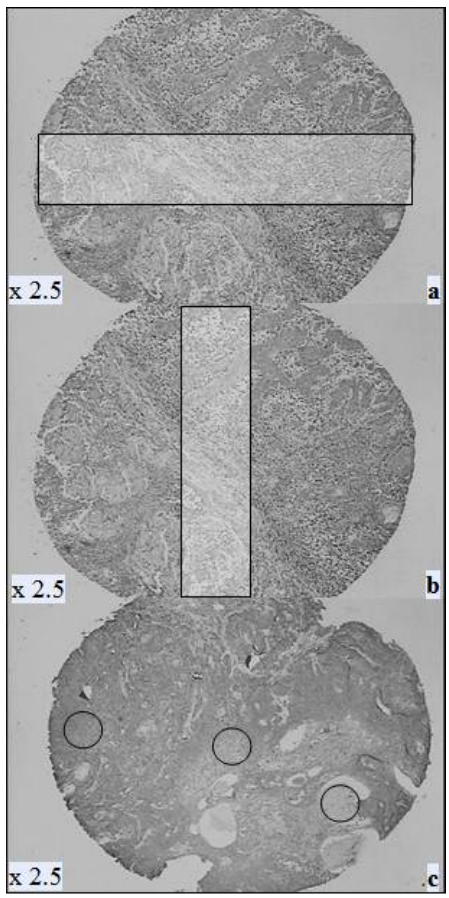

Fig. 2. Microphotographs of squamous cell carcinomas providing examples of the different measurement areas used: a-HAB: Horizontal Analysis Band = 18397μ^2; b-VAB: Vertical Analysis Band = 15086 μ^2; c-CAMaxfIIR: Circular Area of different Intensity of Immunohistochemical Reaction = 1195μ^2; d,e,and

RESULTS AND DISCUSSION

We identified the following areas of densitometric measurement of the target objective x 40/0, 75 and x 5/0, 075 (Figure 2,3).

This paper presents a model for densitometric quantitation of immunoreaction in biological samples. Defined values can be measured using this methodology, which may also be applied to other immunohistochemical responses that have the same final reaction product.In recent years, several groups have presented preliminary immunohistochemical quantitation methodologies.

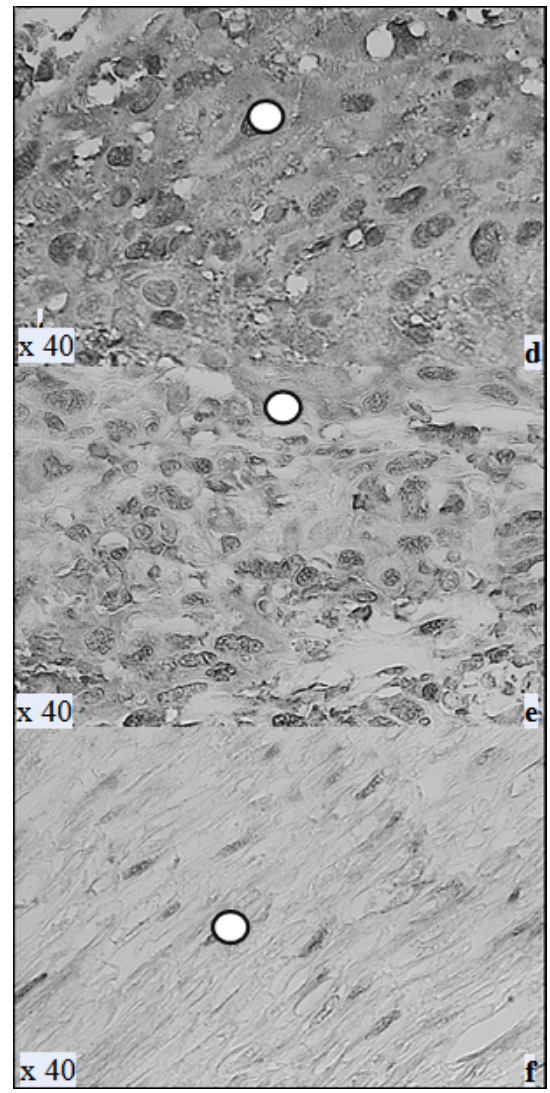

Fig. 3. Microphotographs of squamous cell carcinomas providing examples of the different measurement areas used d- PMaxIIR: Points of different Intensity of

Immunohistochemical Reaction = 113 μ^2 , e- PMedIIR and f- PMinIIR.

Based on our previous experience, microspectrophotometry allowed us to incorporate some concepts of this new method of quantification densitometry by image analysis making a spectral curve used for immunohistochemical reaction. Said procedure allowed us to determine the peak of maximum absorption and thereby determine the wavelength we used for measuring (Figure 1). Another important characteristic of our technique is that quantitation is conducted in fixed measuring areas (Figure 2,3). This is very important for obtaining comparable data (Figure 4). Furthermore, the possibility of selecting areas of measurement is an interesting option when biological samples are not homogeneous or to study a particular areas of tumors.

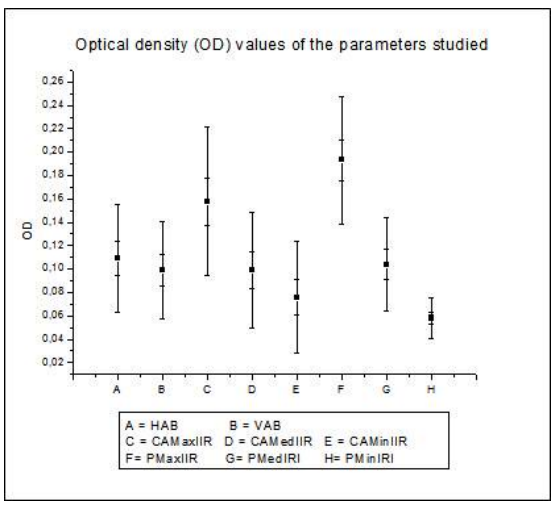

Fig. 4. Values for optical density for 10 SCC of head and neck studied.

CONCLUSIONS

We present an original methodology using densitometry measured by immunohistochemical image analysis of biological samples to obtain comparable results.

ACKNOWLEDGMENT

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