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Analysis of Salivary Metabolites via High Performance Liquid Chromatography as a Potential Diagnostic Tool for Breast Cancer

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Abstract: Breast cancer is characterized by abnormal growth of cancer cells in the breast area[1]. This study aims to develop a non-invasive method of detecting and/or monitoring breast cancer. Using a preliminary study on salivary profiling of metabolites of healthy individuals by Chua, Imperial and Guidote[2], the salivary metabolic profile of breast cancer patients and healthy individuals were compared. Saliva of 25 breast cancer patients were collected, prepared and analysed using High Performance Liquid Chromatography. The HPLC profiles generated by the two (healthy and breast cancer) groups were compared. Significant differences in terms of composition (appearance or disappearance of peaks, retention time) and concentration (peak areas) were monitored and analysed in an attempt to identify cancer biomarkers.

Key Words: salivary profiling, biomarker, metabolomics, breast cancer, HPLC,

1. INTRODUCTION

Breast cancer is characterized by rapid growth of cancer cells in the breast area. At present, a study shows that 18.2% of cancer deaths of women worldwide are caused by breast cancer[1]. Different methods used for detection have been very effective but these methods are painful, invasive and also relatively expensive [1].

Saliva is a watery substance that is secreted by the salivary glands[3]. Saliva contains water, protein, salts, mucin and other enzymes that aid the breakdown of carbohydrates such as starches [4]. According to scientific researches, saliva contains metabolites that may change its concentration when an individual is affected by a particular type of disease[5].

The salivary metabolic profile of 24 healthy Filipinas were studied by Chua, Imperial and Guidote [2]. In this study, a method to separate and monitor the components of saliva was developed using High Performance Liquid Chromatography. This baseline study was able to show the relative concentrations, retention times and peaks areas of 42 saliva components.

In this present study, the analytical method developed and baseline study on salivary metabolic profile of healthy Filipinas previously conducted by Chua, Imperial and Guidote was used against which the salivary metabolic profile of 25 Filipina breast cancer patients were compared[2]. Statistical treatment of the data clearly shows an evident pattern that certain salivary components of breast cancer patients are remarkably and consistently very high or extremely low in concentration compared to the concentrations established in the baseline study. Furthermore, certain peaks or salivary components are conspicuously absent or present between the two groups.

1.1 Breast Cancer

Breast cancer is a type of cancer characterized by abnormal growth of cells in the tissues, lobules and ducts on the breast of an individual. The occurrence of the disease is recorded regularly as one of the top causes of cancer deaths in the Philippines[7]. According to Lifestyle Inquirer, the Philippines is reported to have the highest occurrence of breast cancer in Asia and belongs to the top 10 countries in terms of the cases of breast cancer [8]. Breast cancer is prevalent in the Philippines and is confirmed by the Department of Health and the Breast Cancer Society [7]. Studies show that 1 out of 13 Filipino women will develop breast cancer [7].

There are different ways of potential diagnosis for breast cancer. The most popular techniques are breast cancer MRI and breast cancer mammography[1]. Breast cancer MRI uses magnetic field in order to produce an image of the internal organs of the human body as diagnosis for cancer[1]. For mammography, x-ray is used for the examination of the breast area of an individual. Breast ultrasound, lymph node biopsy and CT Scan can also be used for detection. Breast ultrasound uses sound beams in order to produce images of internal organs. Lymph node biopsy uses a lymph node as a sample from a breast cancer patient, placing it under a microscope, in order to detect breast cancer [1].

1.2 Metabolomics

Metabolites are defined as the molecules that are the intermediates and products of a metabolic process or pathway [10]. Metabolites may change in concentration when an individual has cancer [5]. There are two types of metabolites; these are primary and secondary metabolites. Primary metabolites are responsible for the reproduction growth and development of a particular organism [10]. Secondary metabolites are compounds that are not related to the growth, development or reproduction of an organism [10]. Since there are a lot of different diseases which are affecting the metabolites with their specific concentrations, the changes of these compounds are further analyzed for potential diagnosis of different diseases [11].

Metabolomics is a study that compares the metabolites of a biological sample by using different analytical techniques. Examples of the analytical instruments used are GC-MS, HPLC and NMR. Although these analytical instruments are able to pinpoint a large number of metabolites, there are no particular instruments in the present that allows the whole metabolome to be recognized[9].

There are different approaches to metabolomics, the common methods include target analysis, metabolic profiling and metabolic fingerprinting [9]. Target analysis is an approach that uses a specific analytical technique suited for the compounds of interest. The target compounds included for target analysis are a small set of known compounds[9]. Metabolic profiling is the measurement of a large set of the compounds that are present a sample and then classifying them into different types of groups and classes[9]. Metabolic fingerprinting is an approach that aims to reduce the analysis time by identification of metabolites that shows significant difference between the sample and a larger sample population[9].

2. METHODOLOGY

Figures and tables should be referred to in the text. They should be centered as shown below and must be of good resolution. Where equations are used, adequate definition of variables and parameters must be given, as shown in the example below.

$$Wi + \Sigma j\ rij\ = Si\ \forall i \qquad \text{(Eq. 1)}$$

where:

w_i = unused portion of energy source (i)
rij = energy supplied from source (i) to demand (j)
S_i = quantity of energy source (i)

3. RESULTS AND DISCUSSION

Results should be discussed thoroughly but concisely in this section with the aid of figures and tables whenever necessary.

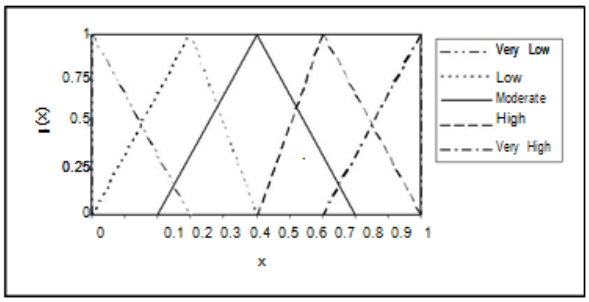

Fig. 1. Captions should be 9 pt. Century, "Tight" Text Wrapping

Text within tables should use 9 pt. Century font, as shown in the example below. Tables should

as much as possible occupy only one column page. Table headings should be re-indicated for catenated tables.

Table 1. Sample table format

Energy Resource	Emission Factor (t CO_2/TJ)	Available Resource (TJ)	Expected Consumption (TJ)	Emission Limit (10^6 t CO_2)
Coal	105	600,000	1,000,000	20
Oil	75	800,000	400,000	20
Natural Gas	55	200,000	600,000	60
Others*	0	>400,000		
Total		>2,000,000	2,000,000	100

Citations should be in this format, APA style (Adamo, 1980; Chen and Hwang, 1992; Tan et al., 2005). They should be listed at the end of the paper in alphabetical order.

4. CONCLUSIONS

This section must summarize the key findings of the study and describe potential areas for further research.

5. ACKNOWLEDGMENTS

Please acknowledge funding sources, collaborators or anyone who has helped in the completion paper at the end of the text.

6. REFERENCES (use APA style for citations)

Berkowitz, R. T., Wadden, T. A., Tershakovec, A. M., & Cronquist, J. L. (2003). Behavior therapy and sibutramine for the treatment of adolescent obesity [Electronic version]. Journal of American Medical Association, 289, 1805-1812.

Carmona, R. H. (2004, March 2). The growing epidemic of childhood obesity. Testimony before the Subcommittee on Competition, Foreign Commerce, and Infrastructure of the U.S. Senate Committee on Commerce, Science, and Transportation. Retrieved October 10, 2004, from http://www.hhs.gov/asl/testify/t040302.html

Crtiser, G. (2003). Fat land: How Americans became the fattest people in the world. Boston: Hougton Mifflin.

Duenwald, M. (2004, January 6). Slim pickingsL Looking beyond ephedra. The New York Times, p. F1. Retrieved October 12, 2004, from LexisNexis.