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Stomach Cancer Detection through Exhaled Breath using Biomarkers Analysis

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Normally, when a person is affected by a disease, there is an imbalance in their metabolic system that causes a change in the behaviour of their body.
In the case of a person affected by cancer or gastric diseases, the presence of the specific volatile metabolites has been exposed for them, which are useful as markers and allows the early diagnosis of these diseases, which facilitates the adaptation of treatments for the prevention, diagnosis and cure of patient.
By using of the gas chromatography it is possible to realize a qualitative and quantitative analysis of the composition of breathing in healthy people or with cancer, which allows to identify biomarkers, so it could be a diagnostic method for such diseases, with the advantages to discriminative rapidly, safety way with a non-invasive method for the early detection of the disease, which can be applied for screening tests to be carried out on populations at risk.
This study describes a methodology for the gastric cancer detection through biomarkers in Colombian patients. For the validation of the results obtained, 30 measures were taken from the volunteers, which were stored in Tenax tubes; then they were analysed with a gas chromatography and mass spectrometry equipment. On the other hand, a set of 7 compounds (biomarkers) was found through the analysis of GC-MS, which were related to the metabolism of patients with gastric cancer (GC) and control (C).

1. Introduction

The first work in which a sample is carried through a column, dates from 1951, giving rise to the technique known as gas chromatography. This technique is currently a widely used method for separating volatile and semi-volatile components from a sample (Casas et al., 1994).
Different results have been obtained from the preliminary chemical analysis using GC when trying to diagnose patients with gastric problems, about other conditions and establishing grades of the disease (early and late).
Actually, gastric diseases are complex in different forms, alike to they are also responsible of a high amount of annual deaths (MD Max et al., 2005) (Crew, 2006), outstanding a higher diffusion in the population of South America, besides of a high incidence of the disease in South-Eastern Asia and Eastern Europe.
The main problem is based on the late diagnose. This is due to the asymptomatic tumor, avoiding an early treatment. That is because this study suggests the need of developing new techniques tin order to diagnose the gastric cancer in early stage. In Colombia, gastric cancer is the first cause of death for malign tumors in both genders (Chisato et al., 2008). In reported studies (Martínez et al., 2010), a total of 196.324 deaths were registered in Colombia. Also, gastric cancer took the sixth place as an important national problem, with 4.549 deaths, preceded by other pathologies as: Heart attack, homicides, chronic lung disease, acute respiratory infections and mellitus diabetes.
Clinical endoscopic and histological factors in young patients with Gastric Cancer (GC) and old patients, are an important strategy to make differences that might permit the characterization of the disease in a determined group of patients.
Nowadays, early endoscopy and biopsy tests are the best option to detect and diagnose GC on patients, but they are still procedures that demand time to get results, they are uncomfortable for the patients and very expensive too (Amal et al., 2016).

2. Methods

2.1 Study of the patients

For realising the samples collection process from patients, the security regulation of the hospital was followed, which was previously applied to patients and medical personnel. Each one of patients signed an informed consent before the sample, which was taken respecting the medical rules.
Volunteers of this study, as GC patients and as Control patients (i.e, patients with controlled gastric diseases) were selected from cases found at the Hospital Universitario la Samaritana (HUS) in Bogotá city (Colombia). Endoscopy and biopsy tests were previously made to each one of the patients with Gastric Cancer, because some persons presented important gastric affections, such as: Gastritis, ulcers, secretions, etc. where a pathology report was generated later. On the other hand, a measurement protocol was determined previously, to make the breath tests to gastric cancer and control patients, afterwards to make medical tests.
About the selection of Control cases, it was done considering the age ranks that were also used to classify the Cancer patients. In addition to this, as a selection criteria, characteristics of persons with normal health and/or with other diseases different to cancer were studied, except the diseases transmitted by air, who were treated during the same period by medical personnel at the hospital.
In this study, we make sure that only volunteers older than 18 were part of the measurements collection and no vulnerable individual or group was involved into this study.

2.2 Samples collection

The day before the test (i.e, 10 hours before), patients could not drink, eat and smoke. The data collection was done in the following way: A sampler device called Bio-VOC^{TM} was used to collect the samples and a set of Tenax tubes were coupled to GC-MS for analysis: Two breath samples from each patient (it means, double exhalation) were acquired; so they were stored inside two Tenax TA tubes with hydrophobic material (ORBO^{TM} 420 Tenax TA sorption tubes from Sigma-Aldrich). The collected samples were processed by the deconvolution method, in order to determine the compounds that form the chromatographic signal. These chemical compounds were found by the "Unknown Analysis" software, programmed in automatic mode with a value of 80 (Match factor).

2.3 Biomarkers identification

For the identification of the breath biomarkers associated with gastric cancer, the samples were analysed with a GC/Q-TOF analytical equipment (Agilent G7200AA) for analysing the breath samples, which they were stored on Tenax sorbent tubes (Sigma Aldrich). First, Tenax material was transferred into 20 mL vials that were sealed, tand heated at 100 ^{o}C on a hotplate for desorbing the breath volatiles. We used the Solid Phase Micro-Extraction (SPME) technique to pre-concentrate the volatiles released by the sorbent material, and the volatiles were transferred to be acquired by the SPME fiber into the GC/Q-TOF equipment. The total runtime was 26.25 min. The GC/Q-TOF system was operated in the splitless mode, using the following extraction and chromatographic conditions: Extraction time: 20 min., extraction temperature: 100 ^{o}C. , desorption time: 2 min., desorption temperature: 250 ^{o}C, and oven temperature profile: a) 0 min at 50 ^{o}C b) Ramp of 10 ^{o}C/min until 155 ^{o}C c) Ramp of 20 ^{o}C/min until 270 ^{o}C and d) 10 min at 250 ^{o}C. Then, the deconvolution of the chromatograms acquired by GC/Q-TOF was realized.
In each breath sample were identified up to 653 compounds using the NIST 14 mass spectral library, "QI Macros" software. The tentative breath biomarkers of the GC, were identified applying the statistical t-test using the standard cut-off value= 0.05 (95% confidence interval).
It should note that, as much as possible, the patients and the controls were age and sex matched. Patient's sex (male/female) was considered for disregarding from the initial list of tentative compounds, those that could be affected by external confounding factors. Smoking habits were not considered as a confounding factor in this study because of the reduced number of smoker volunteers that participated in the study.
After realizing these statistical analysis, the following tentative breath biomarkers were identified into each group for a total of 7 biomarkers. For the Gastric Cancer disease were detected: Trans-2,2-Dimetil-3-Deceno, Octadecane, Hexadecane, M-Xylene, Pyridine and for the Control: Eicosane and 1-Cyclohexil-2-Cyclohexilmetil-pentane. Chemical analysis of breath samples revealed that seven biomarkers were statistically different between GC and other gastric diseases, which outstand part of the importance of the study, because most relevant causes of GC on humans can be determine (Amal et al., 2013).

3. Discussion

Gastric cancer is related with several risk factors as family antecedents, because heritage increases the predisposition of an individual to suffer this disease. However, it is not a determinant factor to suffer it.

Besides, studies have demonstrated that cancer developing it is much related to environmental factors, responsible of the mutant genes, present in the environment, which at the same time cause alteration of the genic expression (epigenetic), potentially heritable. It is possible mention the among risk factors that can generate this disease deadly: Gender, excessive consume of salt, smoked foods, smoking habit, abuse of alcohol, infection with Helicobacter pylori, and the origin (country or city), as examples. On the same way there are those factors coming from the occupation, work and the environment (Latino et al., 2016) (Charbote et al., 2014).
Table 1 presents the relation of biomarkers that were obtained as result of the breath analysis for gastric cancer and controls cases:

Table 1: Relation of biomarkers

Group	Compound	CAS Nº
PATIENTS WITH GASTRIC CANCER	trans-2,2-dimetil-3-decene	55499-02-0
	Octadecane	593-45-3
	M-Xylene	108-38-3
	Hexadecane	544-76-3
	Pyridine	110-86-1
CONTROL PATIENTS	1-ciclohexil-2-ciclohexilmetil-pentane	55030-21-2
	Eicosane	112-95-8

After taking as reference the Pankow et al. (2003) and Mohamed et al. (2002) works, during this study no relation was found between medicaments and the biomarkers presence, because no biomarker is product of any of the medicaments that were given to the patients, due to their structure.
A common characteristic of the majority of patients was they were living in the country and used to cook with firewood mainly, which usually exposes to soot, product of the incomplete combustion, characterized by the generation of carbon monoxide, benzene, butadiene, formaldehyde, alike to a high concentration of particles material (PM), recognized by the presence of a high variety of alkanes, alkenes and polycyclic aromatic hydrocarbons (PAHs), as some of the most harmful compounds for human health.
Other relevant factor for the gastric cancer is the occupation, because actually cancer is considered a professional disease, consequence of the possible exposition of the worker to carcinogen agents, normal in the work environment. We have to understand that not all workers are going to suffer it, because it is caused mainly by the exposition level to the same ones (frequency, duration and intensity), to the way how they enter to the organism (inhalation, ingestion or dermic absorption) and to the personal hygienic.

4. Results

4.1 PCA analysis

Figure 1 shows the result of the PCA analysis (Shahdoosti et al., 2016) performed with the values of the abundances of these biomarkers in the volunteers breath. A good classification was obtained between the GC and control groups, with only two control volunteers (C07 and C13) and one gastric cancer patient (G01) misclassified.

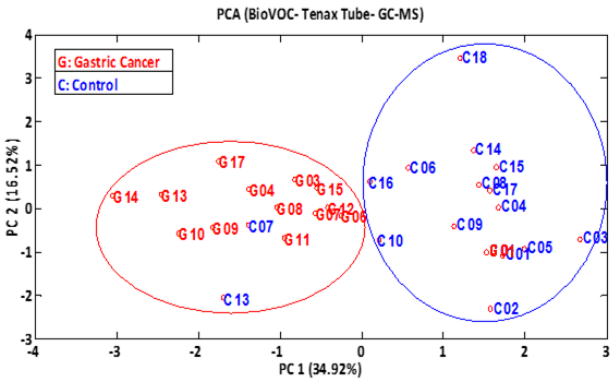

Figure 1. PCA plot (GC and Controls) using BioVOC-Tenax Tube and GC-MS

Only three patients were misclassified by the PCA model based on the GC breath biomarkers identified in this study belong to the control patients C07 (the only control patient diagnosed with ulcer) and C13 (who performed double exhalation because of physical weakness) and to the gastric cancer patient G01 (with very critical health condition), which overall yield 97% accuracy of samples classification, 100% sensitivity and 93% specificity.

4.2 Statistic analysis of biomarkers

The displacement between the graphs about the Y-axis indicates that there is a significant difference between the biomarker values for each group. The highest numerical data are for Gastric Cancer patients, since the peak areas (value obtained from Chromatograph) of these samples contain more of this compound (Monteiro, 2018).

In Figure 2 we can observe the most meaningful biomarker of the group of patients with Cancer, in which the inter quartile ranges between GC and C patients, is differentiable, where each numeric value is projected on each box diagram that corresponds to a measurement.

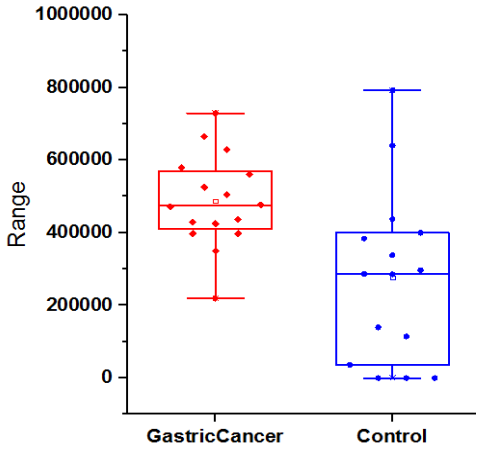

Figure 2. Box diagram. Biomarker: Trans-2,2-Dimetil-3-Decene.

In Figures 3, 4, 5 and 6, box diagrams illustrate the following outstanding biomarkers for the GC group**.** Nevertheless, on figure 3 we observe that measurement number 5, is an atypical value in the GC group, due to the patient was under chemotherapy, what lead to get recovered and this measurement was classified into the C group.

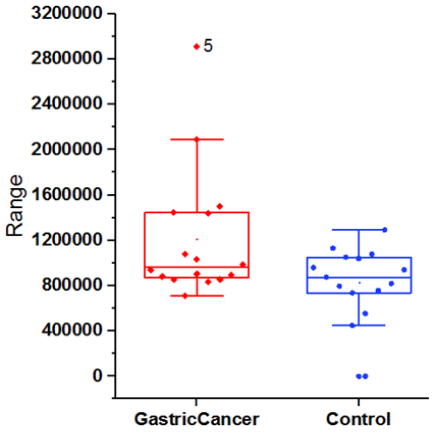

Figure 3. Box diagram. Biomarker: Pyridine.

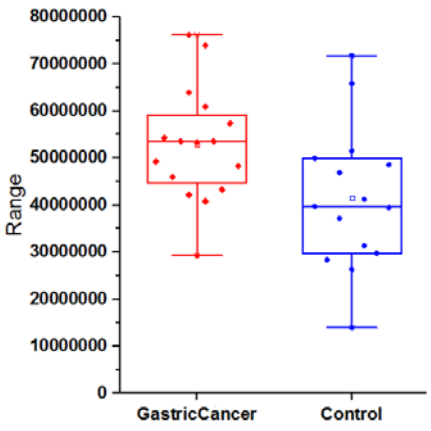

Figure 4. Box diagram. Biomarker: M - Xylene.

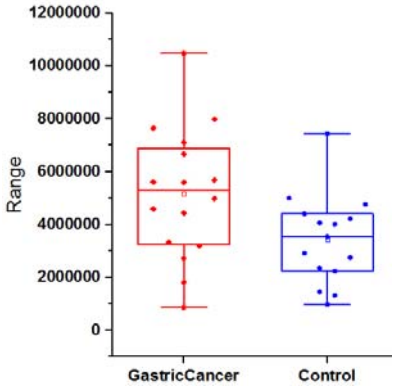

Figure 5. Box diagram. Biomarker: Hexadecane.

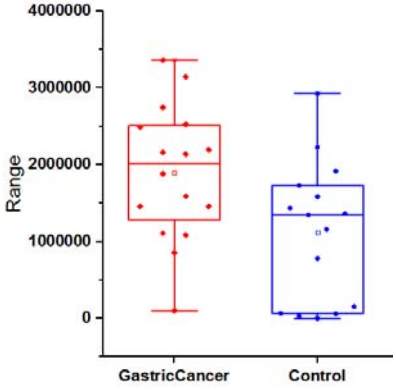

Figure 6. Box diagram. Biomarker: Octadecane.

On the same way, Figures 7 and 8, represent the group of biomarkers identified in the control group.

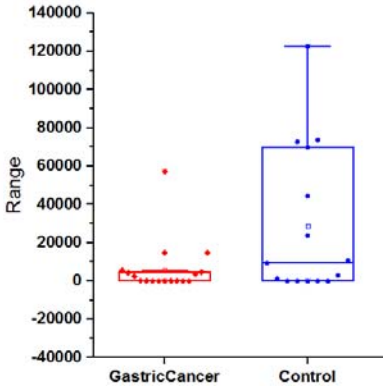

Figure 7. Box diagram. Biomarker: Cyclohexane 1,1'-(2-propyl-1,3-propanediyl) bis-

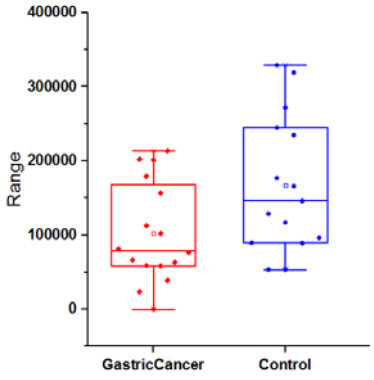

Figure 8. Box diagram. Biomarker: Eicosane.

5. Conclusions

Cancer is a disease with a high mortality, which generates a clinical need that forces the emergence of new techniques for its diagnosis and for certain pathologies that trigger it. Breath tests should be seen as promising techniques complementary to existing ones and never as competitive techniques, each occupying the rightful place.

This method, driven by techniques as powerful as gas chromatography coupled with mass spectrometry, will identify possible biomarkers. volatile that will allow epidemiological studies and investigate diseases with long latency periods, which can be used as appropriate risk indicators and as a preventive technique for the early detection of these diseases.

It would be desirable as future work, that predictive biomarkers could be known in the lower stages of the different pathologies, or when these have not yet been developed and, nevertheless, their future potential is suspected due to a clear genetic component. Therefore, this research address a biomarkers detection methodology that could be used for sensory applications (e.g, Electronic Nose).

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