Volatilomic patterns of gastric juice and their potential for diagnosis of gastric cancer

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Introduction

Volatilomics is an effective and economic approach for non-invasive disease diagnosis. This study centers upon the volatilomic signatures of gastric juice obtained from gastric cancer patients and non-gastric cancer controls. Due to its anatomical location, gastric juice can be considered as a promising reservoir of potential gastric cancer markers. Since sampling of gastric juice is invasive, this fluid received a limited attention in volatilomics.

The main goal of this study was to characterize the chemical patterns formed by volatile organic compounds (VOCs) released by gastric juice via gas chromatography-mass spectrometry (GC-MS) and evaluate if the cancer-related alterations could be employed for gastric cancer detection.

Experimental

Subjects: The study cohort included 35 patients (13 females, 22 males; 33-85 years of age, median age 62 years) diagnosed with gastric cancer and 58 controls (36 females, 22 males; 21-80 years of age, median age 56 years) from different populations (Ukraine, Brazil and Colombia).

Sampling: Samples were collected during upper endoscopy by sucking gastric content (10-12 ml, min 3 ml) into 20 ml glass vials. In parallel, blank sample containing 10-12 ml of distilled water was taken per participant to identify contaminants. The samples were stored and transported at -80 °C (dry ice).

Olympus SSU-2 suction pump

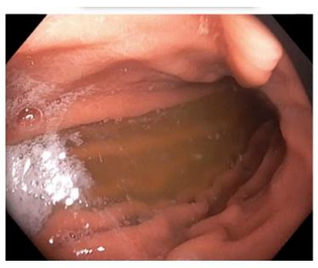

Figure 1: Sample collection

GC-MS analysis: Headspace solid phase micro extraction (HS-SPME) coupled with GC-MS (Agilent 8890/7079B GC-MS system, Agilent, USA) was used to identify the VOCs released by gastric juice samples collected from gastric cancer patients and healthy controls.

Figure 2: GC-MS analysis steps

Method development and optimization: Extraction temperature (optimum 70 °C), incubation time (optimum 60 min), extraction time (optimum 3 min).

Results and Discussion

- The typical profile of GC-MS chromatogram identified several VOCs emitted from gastric juice samples.
- A total of 1181 distinct compounds were found in the headspace of gastric juice samples from gastric cancer patients and control subjects.
- Only 13% of these species found in the samples from gastric cancer patients exhibited incidence above 20%; whereas, in the samples taken from controls, this percentage amounted to 10%.
- Six omnipresent VOCs, occurring in at least 80% of the samples: hexanal, benzaldehyde, ethyl acetate, acetone, ethanol, and pyridine.
- The volatiles 2-propanol, propofol, hexafluoroisopropanol, and their metabolites are related to the hospital environment or the treatment site ambient air.
- The relative distribution of VOCs in cancer and control groups under study was similar (Figure 3) with aldehydes as the dominant class (17 vs 18), followed by hydrocarbons (13 vs 14), alcohols (13 in both), ketones (15 vs 11), aromatics (7 in both), terpenes (3 vs 5), heterocyclics (4 in both), and phenols (2 vs 3).

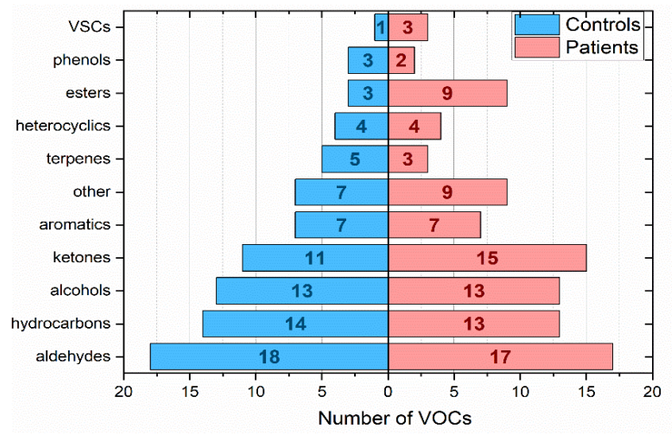

Figure 3: Distribution of VOCs according to their chemical classes in gastric cancer patients and control subjects

- Only exception was esters – represented by 9 species in samples from patients, whereas only by 3 in controls.
- While comparing the peak abundance of VOCs with occurrence above 30%, nine compounds out of eleven (Figure 4) appeared at significantly higher levels in the patient group than in the controls (Mann-Whitney U test, $p<0.05$).

CAS	Name	p-value	Median abundance [arbitrary units]		
			change	patients	controls
104-76-7	1-Hexanol, 2-ethyl-	0.002	↑	5180530	3624770
78-93-3	2-Butanone	0.011	↓	529966	622452
78-84-2	Propanal, 2-methyl-	0.021	↑	974808	434873
71-23-8	1-Propanol	0.018	↑	1487737	690900
590-86-3	Butanal, 3-methyl-	0.008	↑	3964787	1062987
106-42-3	p-Xylene	0.03	↓	184537	633572
108-95-2	Phenol	0.0006	↑	28583957	13899645
122-78-1	Benzeneacetaldehyde	0.0002	↑	1311759	183311
107-87-9	2-Pentanone	6.8E-05	↑	3006432	275563
4748-78-1	Benzaldehyde, 4-ethyl-	0.02	↑	2026474	126892
110-43-0	2-Heptanone	0.002	↑	1017930	243667

Figure 4: Compounds exhibiting differences in gastric cancer patients and controls

- These are two ketones (2-pentanone, 2-heptanone), four aldehydes (2-methylpropanal, 3-methyl-butanal, benzeneacetaldehyde, 4-ethyl-benzaldehyde), two alcohols (1-propanol, 2-ethyl-1-hexanol), and phenol.
- 2-butanone and p-Xylene showed lower abundance in patients than in controls.

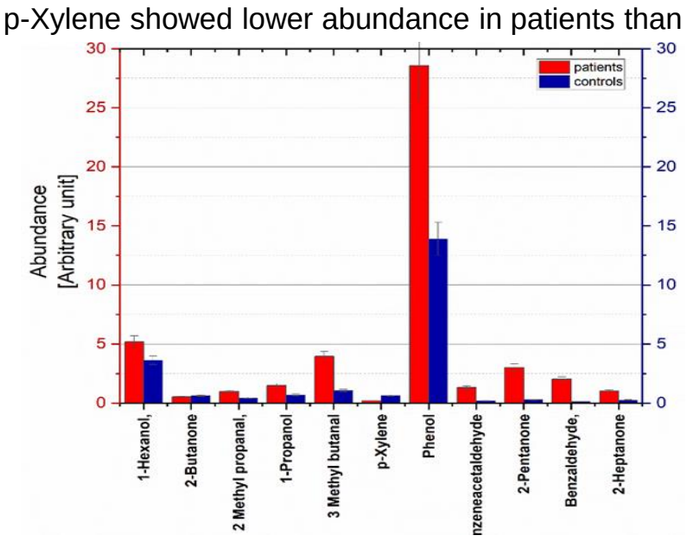

Figure 5: VOCs exhibiting differences in the gastric juice headspace between gastric cancer patients and controls

Conclusions

- Preliminary results obtained within this study suggest that gastric juice is promising fluid providing information on potential biomarkers of gastric cancer.
- The VOCs identified from gastric juice can assist in developing non-invasive tests for the diagnosis of gastric cancer.
- To the best of our knowledge, this is the first study reporting the composition of gastric juice volatilome/chemical signatures in different human populations.

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