Preliminary study on faeces metabolomics comparison between Vietnamese healthy controls and patients with colorectal cancer using mass spectrometry high resolution

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Introduction

Colorectal cancer (CRC) is a significant global health concern, being the third most common cancer worldwide and the second leading cause of cancer-related deaths. This stressed on the importance of CRC diagnosis at early stage for treatment effectiveness and elongation patients' lifespan. While the gold standard for CRC detection currently is colonoscopy, being invasive, expensive, as well as causing perforation of the colon and post-polypectomy bleeding, a new approach in cancer detection based on metabolic changes, called metabolomics, has been applied with hopefulness. Metabolomics focused on studying composition, regulation of metabolites – low-weight molecules in metabolism processed. Overall, metabolomic studies of CRC has been conducted increasingly in the last decade, both targeted and untargeted approach, reveal biomarkers in groups of amino acids, fatty acids, nucleosides, and phospholipids, etc. For profiling untargeted metabolites in metabolomics studies, liquid chromatography coupled high resolution mass spectrometry (LC-HRMS) is a strong technique to distinguish specifically. In Vietnam, CRC metabolomics studies have lacked important publications. This study chose faeces matrix as sample and LC-HRMS as technique for a preliminary metabolomics study on Vietnamese CRC .

Objectives

This study aimed to investigate the changing of metabolite composition in faeces during CRC development in Vietnamese people. Therefore, promising biomarker would be identified for CRC screening with a clinical meaningfulness.

Materials and Methods

Sample collection
51 faeces samples of CRC patients and 9 faeces sample were collected in 108 central military hospital. All volunteers were aged in the range from 50 to 79 with health BMI.

LCMS analysis
60 mg of a sample was dispersed in 0.6 mL of MeOH, vortexed, ultrasonicated in 30 mins, re-centrifugation (18000.g). The supernatant are diluted for LCMS analysis. The gradient: FA 0.1% and ACN. MS: scan adducts with m/z from 100 to 1500 m/z with scan time of 200 ms.

Data analysis
Progenesis QI for peak alignment, peak picking, and peak recognition based on HMDB and METLIN libraries.
The differential metabolites was defined as follows: CV< 30% (1), p-value < 0.05 (2); fold change > 1.5.

Biomarkers identification

Results

❖ **Basic clinical information of samples:** A (CRC group), B (Health control)

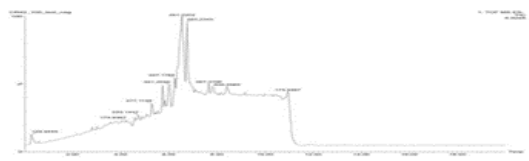
Group	BMI mean	Age mean	Men	Women	Medication	T2D	Total
A	21.78	64.04	27	22	3	8	49
B	24.03	60.11	2	7	0	0	9

❖ **LCMS method optimization:**

Referenced protocol (Cheng et.al)

MeOH: sample (5:1)

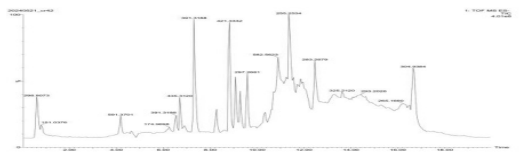
Gradient: FA 0.04% (A) and MeOH (B), 0.4 mL/min.
0-6 min: 95-0% A; 6-10.5 min: 0% A; 10.5-15 min: 95% A



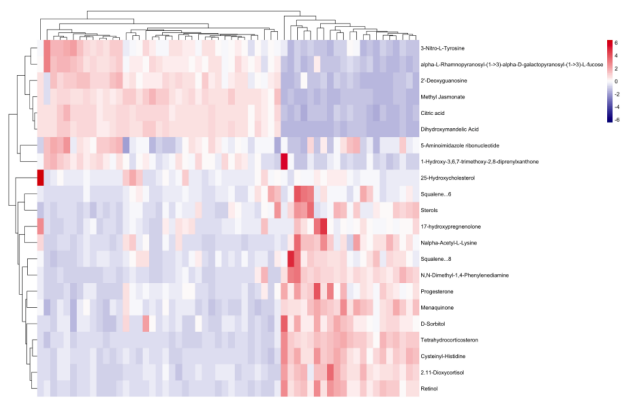
Optimized protocol

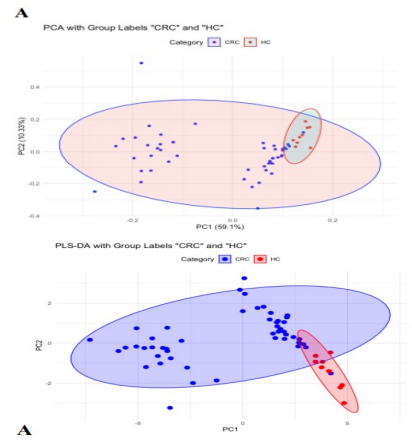
MeOH: sample (5:1)

Gradient: FA 0.1% (A) and ACN (B), 0.3 mL/min.
0-2 min: 80% A, 2-12 min: 80-5% A; 12-17: 5% A, 17-20 min: 80% A

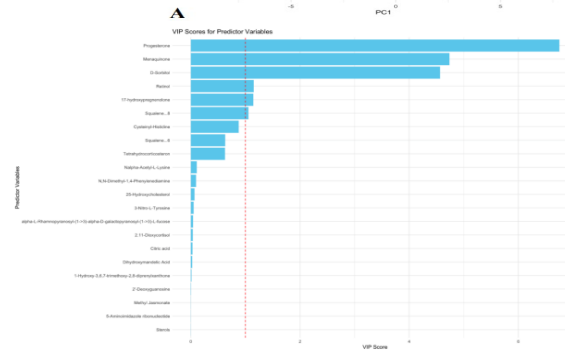


❖ **Data analysis:** Among features detected by Progenesis QI, 22 metabolites were met the set of requirement: CV ≤ 30, p-value ≤ 0.05, fold change ≥ 1.5, showing by the heatmap. Clustering through PCA analysis suggested healthy controls had a similar composition of metabolite. A supervised PLS-DA method for multivariate analysis subsequently conducted, that indicated the metabolic profiles of healthy volunteers were distinct from those of CRC patients in the remaining group.





❖ **Biomarkers identification:**
Variables with VIP scores of PLS-DA greater than 1.0 were considered significant. There were 6 substances among 22 detected metabolites having VIP >1.0, including progesterone, menaquinone, D-sorbitol, retinol, 17-hydroxypregnenolone, and squalene. These biomarkers were classified into categories like steroids, vitamins, steroids, and saccharides. Because those seemed not to be reported before, there is necessary to conduct further studies to ensure these metabolites that were potential in a reality.



Conclusion

- Faeces samples from 49 colorectal cancer (CRC) patients and 9 healthy controls (HC) was analysis using an UPLC-QToF-MS method.
- Initial PCA results indicated overlapping metabolic profiles between the two groups.
- Further analysis using PLS-DA revealed distinct metabolic signatures for CRC and healthy groups.
- Identified 22 metabolites with significant differences (p-value ≤ 0.05, fold change ≥ 1.5, CV < 30%) between the two investigated groups. Notably, 6 biomarkers, including 17-hydroxypregnenolone, menaquinone, squalene, retinol, progesterone, and D-sorbitol, showed potential for CRC diagnosis (VIP score >1).

References

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