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Review Article

Novel Diagnostic Biomarkers in Liver Disease-a Metabolomic Review

Ahmed I*[1], Niaz Z[2], Cheema S[3], Kassem W[4] and Manuele F[5]

[1]*Department of Medicine, Aldara Hospital and Medical Centre, KSA*

[2]*Department of Medicine, Mayo Hospital, Pakistan*

[3]*Department of Surgery, International Medical Centre, KSA*

[4]*Department of Medicine, Aldara Hospital and Medical Centre, KSA*

[5]*Department of Medicine, University of Genova, Italy*

***Corresponding author:** Iftikhar Ahmed, Consultant Gastroenterologist Aldara Hospital and Medical Centre, Riyadh KSA and Associate Professor in Al-Faisal University, Yardville Compound | P.O. Box 1105 Riyadh 11431, Kingdome of Saudi Arabia.

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Abstract

Disease of the liver are complex and pose significant challenges to physician due to diagnostic difficulty requiring invasive techniques such as liver biopsy. Changes in the odour of human excretions could provide diagnostic insight into pathophysiological imbalance. Identifying underlying volatiles metabolites responsible for these odorous changes can be correlated with the pathological process within the body. Advances in the technology have enabled us to interpret the volatile signature of these changes in the odour. This has opened a promising area to lay the foundations of a rapid, non-invasive and point of care diagnostic tool. This review explores the diagnostic potential of volatile organic metabolites as novel biomarkers and extends the discussion on the clinical applications of these biomarkers in liver disorders.

Keywords: Non-invasive biomarkers; Volatile organic metabolites; Liver disorders, Metabolomics; Gas-chromatography-mass- spectrometer

Abbrevations: VOMs: Volatile Organic Metabolites; GC-MS: Gas chromatography Mass spectrometer; GI: Gastrointestinal; SAMe: S-Adenosylmethionine

Introduction

The liver is one of the vital organs in the human body which plays several critical roles in maintaining human's health and well-being. Its main functions are metabolism of nutrients and excretion of toxic substances, additionally it performs an array of functions that help support immunity, digestion and vitamin storage [1]. The liver is intertwined with nearly every system in the body hence prone to a variety of pathologies. Diseases of the liver are complex and require extensive and often invasive investigations including liver biopsy which remain the currently most reliable test if not the "gold standard' [2]. Liver disorders place a heavy economic burden on healthcare resources and have widespread effect on the individuals, encompassing physical and psychological morbidity and mortality, and poor quality of life [3-5]. There has been an increasing clinical interest at uncovering non-invasive diagnostic tools and scientific research into these has yielded multiple scoring systems, formulae, and imaging modalities to diagnose liver disease and to monitor the disease activity [6,7]. More recently, investigations of volatile metabolites in numbers of research studies have focused on the utilization of these non-invasive biomarkers in the diagnosis of liver disease [8,9].

New developments in the analytical techniques has enabled the detection and interpretation of changes in the volatile organic metabolites (VOMs) to assist diagnosis in liver disorders [10]. VOMs are the chemicals that are products and intermediates of metabolism and may be altered during the diseases process. Changes in the signature of VOMs could potentially provide diagnostic information about health and disease. There are growing number of studies which have reported the differences in VOMs profiles of healthy controls vs. patients with underlying disorders [11-14]. VOMs profiles have been used to segregate different types of liver diseases including stages of liver fibrosis and cirrhosis. The correlation of VOMs with microbiota is interesting and supports the

hypothesis of gut microbial dysbiosis in the etiology of liver disease. The aim of this paper is to review the diagnostic potential of these volatile biomarkers in different types of liver diseases.

A few complex aetio pathological mechanisms are involved in Liver disorders and clinical presentation may range from an asymptomatic state to complete liver failure depending upon the type and severity of disease [15,16]. Due to vital role of liver in various complex metabolic and synthetic functions, any pathological insult to hepatic parenchyma leads to altered concentration of toxic metabolites in the systemic circulation [17]. Some of these metabolites may be exhaled through the lungs giving rise to malodorous breath. Others may be excreted in other biological fluids such as sweat, urine and feces leading to altered odour of these biological excretions [18]. For example, sulphur containing compounds such as dimethyl sulphide, hydrogen sulphide and mercaptans were shown to be increased both in blood as well as in alveolar breath due to incomplete metabolism of sulphur containing amino acids in liver disease. Some of these metabolites may give a characteristic smell to breath; which has a sweet, musty, or even slightly faecal aroma, termed fetor hepaticus [19,20]

Analysis of VOMs in Breath

Impairment in several metabolic processes in liver was proposed as a cause of these metabolic compounds. Initial studies focused on methionine metabolisms, which in healthy individuals, produced S-adenosylmethionine (SAMe) which regulates hepatocyte growth, differentiation and death. Alteration in the metabolisms of methionine was Studies by Kinsell et al. [21] and later by Chen et al. [22] and found increased levels of sulphur containing compounds in the breath of liver patients in comparison to healthy individuals when both were fed methionine. Similarly, low biosynthesis of SAMe, as a result of impaired methionine metabolism, was also suggested to play a causative role in liver cirrhosis [23]. However, a recent systematic review was unable to demonstrate any significant benefit of SAMe replacement in patients with alcoholic liver disease [24].

Modern and sophisticated analytical techniques completely revolutionized the metabolomic analysis making it possible to detect even very slight alteration in the very small volatile molecules in the breath based on their molecular masses. Consequently, studies from Kaji et al. [25], Tangerman et al. [26] and Hisamura et al. [27] demonstrated the higher levels of these sulphur containing volatiles in the breath of patients with liver disease by using modern analytical methods. More recently Van den Velde and colleagues [28] analyzed the breath from 50 patients with established liver cirrhosis by using GS-MS techniques. In this small study, they found that dimethyl sulphide; acetone; 2-pentanone and 2-butanone were significantly higher in alveolar breath of patients with hepatic cirrhosis. The altered concentration of these metabolites was able to discriminate the cirrhotic group from normal individuals with a sensitivity of 100% and specificity of 70%.

In another study from Netzer et al. [29], a group of four breath markers were identified with the use of ion molecular reaction-mass spectrometry (IMR-MS). Their study group consisted of patients with alcoholic fatty liver disease (AFLD), non-alcoholic fatty liver disease (NAFLD), cirrhosis and healthy controls. Among the detected markers, acetaldehyde, Mx103, isoprene, Mx67 and Mx60 (where Mx indicate unannotated compound mass) were found to discriminate between the diseased groups from healthy control. It is important to note that none of these studies was able to demonstrate whether a disease might have a unique breath volatile pattern.

Several studies support the crucial involvement of oxidative stress in the pathogenesis of liver disease including alcoholic and non-alcoholic hepatotoxicity, infections, iron overload and autoimmune liver damage [30,31]. The peroxidation of polyunsaturated fatty acids, such as linoleic acid and linolenic acid, which are cell membrane components, induces the formation of volatile alkanes that are excreted in the breath. These straight chain aliphatic hydrocarbons have been advocated as non-invasive markers of free-radical induced lipid peroxidation in humans [32,33]. Exhaled hydrocarbons especially breath ethane and pentane, appear to be better correlated with alcohol induced hepatic injury than to other aetiologias.

For example, Letteron et al. [34] measured the ethane levels in the breath of patients with alcoholic and non-alcoholic hepatitis and found it significantly higher in alcohol abuser than other groups, albeit a weak correlation with level of alcohol use, histological scoring or other complications. This stronger correlation of breath ethane with alcohol might be due to increased induction of cytochrome P450 by alcohol, leading to increased production of oxygen radicals. In contrast to alcoholic induced liver injury where alkanes were the predominant volatiles in breath, in non-alcoholic fatty liver disease (NAFLD), which is more prevalent in obese patients, ethanol levels were found to be raised in exhaled breath of patient even in the absence of ethanol ingestion [35].

The hypothesis of increased endogenous production of ethanol in obese individuals was supported by preliminary animal models followed by human studies showing the role of intestinal microbiota in the production of ethanol in obese patients [36,37]. However, it remains unclear whether increased intestinal permeability with secondary endotoxin-mediated damage, in addition to increased endogenous ethanol, contributes to the development of steatohepatitis in obese individuals [38,39]. A study by Khalid et al [40] studied the breath VOMs profiles of patients with various liver disorders and concluded that patient with different stages of liver disease due to alcohol can be differentiated based on the presence or absence of VOMs fingerprints. Of note in this study, the presence of hepatic encephalopathy was correctly identified in 90.9% of the alcoholic cirrhosis cohort. In a recent study, Fernandez et al. investigated the breath VOMs of patient with liver cirrhosis before and after liver transplant in comparison with healthy control. They found a group of seven volatiles which were elevated in the breath of patients with cirrhosis versus controls. Following liver transplant, five VOMs out of these (limonene, methanol, 2-pentanone, 2-butanone and carbon disulfide) showed statistically significant decrease in abundance [41].

Analysis of VOMs in Blood

In addition to breath analysis, studies have also reported the analysis of VOMs from blood, which are more representative of the internal environment of biological activities. Goldberg et al. [42] analyzed the serum VOMs of patients with hepatic cirrhosis by using a direct injection capillary column gas chromatography method. They found raised levels of 3-methylbutanal in chronic encephalopathy, which correlated well with severity of the disease. In contrast, Marshall et al. [43] found no difference in the level of 3-methylbutanal in cirrhotic patients when compared to healthy controls. This aldehyde results from breakdown of leucine by bacteria. However, when researchers fed leucine to cirrhotic patients, no change in the clinical conditions were observed even when the value of 3-methylbutanal rose to 700% above the base level. More recently, a small study published by Ruyi et al. [44] reported the analysis of VOCs in blood of patients with liver cancer. By using the GC-MS technique, they found hexanal; 1-octen-3-ol and octane as possible biomarkers of liver cancer with good sensitivity and specificity. However, further studies are needed to evaluate these markers in more details and their correlation with liver cancer.

Conclusion

In short, quantification of VOCs in alveolar breath and blood as markers for diagnosis and routine monitoring of organic disorders is receiving growing medical interests and appear to fulfil the demand and desire for a means of non-invasive investigation of disease due to its ease of sample collection, repeatability, reproducibility and acceptance by the patients group especially children and younger adults. Investigation of human breath samples with various analytical methods has shown a correlation between patterns of VOCs and the occurrence of certain diseases. Although it has been demonstrated that modern analytical instruments allow the determination of many compounds found in human breath both in normal and anomalous concentrations, but due to technical problems of sampling and analysis, and lack of normalization and standardization, huge variations exist between results of different studies. Consequently, VOCs analysis of breath has yet to be introduced into clinical practice.

Among the more frequently used breath tests are glucose-hydrogen breath test for small bowel bacterial overgrowth, hydrogen breath test for lactose intolerance, and the urea breath test to detect Helicobacter pylori infection in the stomach. Breath testing remains an under-valued tool for assessing various gastrointestinal and liver diseases which deserve further attention. This provides an important platform to explore the role of dysbiosis in IBD and other GI disorders pathogenesis and development of novel therapeutic targets. In future, further understanding of faecal VOMs may lead to the development of a rapid and simple point of care diagnosis and monitoring of liver disorders.

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