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Breath biomarker for clinical diagnosis and different analysis technique

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ABSTRACT

Disease detection by medical diagnosis has developed rapidly in recent years. Blood and urine are most commonly used in diagnosis as compared to breath. In contrast to blood and urine, breath analysis is easy, specific and highly qualitative. Breath analysis is intended for diagnosis of clinical manifestations of airway inflammations, metabolic disorders and gastroenteric diseases. Breath had analysis by new high quality technical instrument. It's analytical results are qualitative and quantitative as compared to analysis of blood and urine. Mostly Gas Chromatography used in breath analysis with different detectors. Other techniques can also be satisfactorily used in the analysis of breath. This review is describes the breath biomarker use in different disease diagnosis with the help of various analytical technique.

Keyword: Volatile organic compound, Disease, Diagnosis, Gas Chromatography

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INTRODUCTION

Breath analysis is a method to analyze exhaled air from animal or human being. It is used for clinical diagnosis, disease state and exposure to environmental conditions. The exhaled air contains volatile compounds at a concentration related to the blood concentrations. Nearly 200 compounds can be detected in human breath and it is correlated to various diseases.

The actual breath contains mixtures of oxygen, carbon dioxide, water vapor, nitrogen, inert gases, in addition may also contain various elements and more than 1000 trace volatile compounds. Concentrations range from parts per million to parts per trillion. The Volatile Organic Compounds (VOCs) commonly found in normal breath are acetone, ethane and isoprene which are nothing but the metabolic products. [1,2]

Principle involved in VOCs

External chemical enter the body by three routes namely ingestion, inhalation and topical contact. The chemicals absorbed into systemic circulation will distribute in the body or is directly excreted into the feces. This absorbed chemicals are of two types volatile and non volatile. The volatile compounds get exchanged with alveolar air and are excreted in the breath, known as VOCs. Therefore breath analysis detects VOCs. The non volatile compound analysis is done by blood and urine.

The breath analysis can be explained by pharmacokinetic models related to the VOCs. Wallace et al discover a linear pharmacokinetic model of exhaled breath. Here were assume that someone not previously exposed to particular VOCs is suddenly given a high concentration at a constant rate (c air), now the alveolar breath concentration (c alv) is given in equation

$$c_{alv} = f \; c_{air} \; \Sigma \; a_i \; [1 - \exp(-t/\tau_i)] \qquad (1)$$

where f is the fraction of parent compound exhaled at equilibrium; τ_i is residence time in the i th compartment; a_i is the fraction of breath concentration contributed by the i th compartment at equilibrium ($t = \infty$); t is the time of exposure ($t = 0$ at start of exposure); and $\Sigma \; a_i = 1$.

This model was used in the quantitative estimation of VOC in the body or conversely to estimate previous exposure of exhaled breath. It had advantage over previous models, in that it could measure the long term inhalation at low or moderate concentration as compared to instantaneous intake or intermittent exposure at high concentrations. Wallace et al presented equations that can apply to n- compartmental model and at same time it gives linear equation with increasing exposures at low or high concentrations.

Principle of breath analysis explains physiological basis of exchange of gases between air and blood. Gas exchange occurs at the surface of numerous tiny chambers known as alveoli, present at the tip of bronchial air passage. Alveoli's are well adapted to their functions, they are lined with very thin membrane that are loaded with capillaries. That means

there are tiny distances between a red blood cells moving through the capillaries and the air inside the alveolus, the large surface area and tiny distance associated with alveoli afford ready opportunity for volatile organic compounds to diffuse from the air into the blood.

The key challenge in the analysis of the breath are 'separating the alveolar breath', which contains analyte rich air delivered deep in the lungs from the' dead space' air volume of breath contained in the upper airways namely mouth, and pharynx that is uninvolved in gas exchange, and another challenge is separating and identifying the volatile breath components which tend to be present at just picomolar level concentrations [3,4].

Diagnostic uses of Clinical Breath Test
- Breath carbon monoxide test can detect neonatal jaundice
- Breath hydrogen test can detect *disaccharides* deficiency, gastrointestinal transit time, bacterial overgrowth and intestinal statis
- Breath nitric oxide test can be used for asthma therapy
- Breath test can detect heart transplant rejection
- Urea breath test can be used for helicobacter pyloric infection
- Breath analysis monitoring blood sugar level in Diabetic Patients
- Breath analysis with colorimetric array can be used in the diagnosis of lung cancer

Clinical Diagnosis

Many year ago the discovery of VOCs in breath, lead to its use in the diagnosis of diseases, disorder and metabolic studies. Phillips also explained that breath can be analysed without analytical instruments, example in uncontrolled diabetic patients breath smells like rotten apple due to the presence of acetone in their breath, renal failure patient had urine like odor in their breath, musty or fishy reek smell indicate liver disease and lung abscesses patient breath smell like sewer because of the proliferation of anaerobic bacteria [4,5]. New instrumentation for breath analysis was started in 1970, mainly by Gas Chromatography (GC), but the major challenge was correlation of physiological breath's VOCs and breath marker with patient's clinical symptoms [6,7].

Breath test studies have been done by cross section and longitudinal ways. Cross-section studies or predictive marker investigate biomarkers from breath of patient. These biomarkers of breath were comparative control of patients initially which can also be quantitatively analysed. Longitudinal studies for the progression of the disease, can help in therapeutic intervention in therapy. These studies can be applied on Lung disease, Oxidative stress, Gastrointestinal disease, metabolic disorders and various other diseases as given below.

Lung diseases

Lung diseases are Asthma, Chronic Obstructive Pulmonary Disease (COPD), Cystic Fibrosis(CF), bronchiectasis, interstitial lung disease etc. The elevated level of hydrocarbon as

pentane and ethane have been analysed in breath of patients with asthma [8], COPD [9], obstructive sleep apnea [10] and pneumonia [11].

Other than hydrocarbon marker, one more marker in the extensive analysis of different lung disease is Nitric Oxide (NO). The NO marker of breath analysis are used in different lung diseases as Asthma, COPD and CF. The elevated level of exhaled NO in Asthma patient is due to lowered airways [11]. The comparative studies by Dupont et al reported that the exhaled NO in normal persons with respiratory symptoms and asymptomatic positive asthmatic patients with 90% specificity could predict the value at 95% positive of exhaled NO, 15 parts per billion (ppb) was used as normal value for cut off for asthma [12].

Latest information related to the fraction of exhaled NO was at a cut off of 16 ppb, in diagnosis of 90% Asthma with a positive predictive value of 90% [13].

COPD is a disease characterized by shortness of breath and obstruction of airways because of the inflammation.14 COPD are of two types stable and unstable. In stable COPD the exhaled NO is lower in smoking and non smoking asthmatic patient. In unstable COPD patient ,the exhaled NO is higher in smoking ,non smoking and stable COPD patient [15-18].

Phillips et al mentioned VOCs from C4 to C10 alkanes and monomethylated alkane give new types of biomarker for lung cancer in a number of patients with histologically identifiable diseases [19,20].

Lung transplantion rejection can be identified by the breath marker carbonyl sulfide. The increased concentration of carbonyl sulfide in breath is a non invasive sign for the diagnosis [21]. Some breath markers are found in different diseases such as Carbon monoxide (CO) as biomarker of asthmaand CF; H_2O_2 is a breath marker of asthma COPD and CF isoprostanes as breath markers of asthma COPD, and CF34and nitrite/nitrate as breath markers of asthma35 and CF [22-37].

Oxidative Stress

It is the stress generated by chemical reaction of oxidative substance such as free radical which damage the cell wall, protein, genetic material etc.38 It commonly occurs in short critical illness.39 Many of the organs dysfunction because of the critical care required as a result of oxidative stress [40]. The oxidative stress can be measured in blood, urine and breath. A no. of studies of oxidative stresses show significant biomarkers present in breath, which can give quantitative and qualitative results of the status in oxidative stress [41-48].

Breath biomarker are more sensitive than other oxidative stress measurement.48 In oxidative stress , lipid peroxidation generates stable hydrocarbon end product such as ethane and pentane [44]. These hydrocarbons had low solubility in blood and it is excreated into breath quickly as it forms in tissues. This exhaled hydrocarbon are monitored for degree of

oxidative damage in the body [38,45]. The H_2O_2 concentration in breath is a marker for oxidative stress in lung disease and also marker of free radical generated by tissues damage because of cystic fibrosis (CF) [49-52].

Antczak et. al. reported the elevated concentration of H_2O_2, found in the exhaled breath condensate of patients with asthma, COPD, CF, lung cancer and in healthy smokers [50] Phillips et al showed that breath containing methylated alkane complex could be considered as breath marker of oxidative stress [40,42]. They showed that the seperation of VOCs can be done by Gas Chromatography (GC) and detection was done by Mass Spectrography (MS).

Gasroenteric Diseases

The H_2 detection in breath is a sign of malabsorption of Carbohydrate. It is also a diagnosis to a number of disorders related to digestion and absorption, lactase deficiency, starch malabsorption and small bowel bacterial overgrowth [53-55]. Another most useful test in the detection of H. Pylori infection, bacterial overgrowth, lactose and fructose intolerance, bile salt wastage, liver dysfunction and pancreatic insufficiency is UBT test. UBT test function is controlled by H.Pylori bacteria. The bacterial activity metabolite of urea are Nitrogen and CO_2. This CO_2 is absorbed into the blood, then exchanged with alveolar air (exhaled breath). This exhaled CO_2 can be identified by giving Urea containing isotopes of carbon and this isotopic exhaled carbon is detected.

Irving et al studied the kinetics of bicarbonate and made pharmacokinetic model,56-58 This pharmacokinetic model was related to three pools of freely exchangeable bicarbonate, a central vascular pool, a heart-brain-other pool and the muscle pool 58. These three pools have different CO_2 production and output rate.

The isotopic carbon C13 or C14 detected in the breath is isotopical CO_2. The exhaled CO_2 concentration can be diagnosed as infection of H. pylori. This detection can be treated with antibiotic therapy.

There are two types of UBT test for detection of H. Pylori infection, one is 14C UBT which is radio active and 13C UBT which is nonradioactive and stable. The radioactive 14C exposure is hazardous because 1/100th of bone marrow is exposed to upper gastrointestinal series [59]. As compared to 13C UBT test, it is safe and non radioactive therefore it is mostly used in children [60] and pregnant women [61]. This 13C UBT test sensitivity is 90 to100% [62]. Analytically both have nearly the same sensitivity and specificity of 90.2% and 95.8% respectively in H.Pylori [63].

Metabolic disorder

During metabolism, a chemical reaction process breakdown and renewal in the body occurs. The purpose of metabolism is to maintain homeostasis and steady-state condition in

the body. In metabolic disorders imbalance or changes occur at steady state conditions [64,65].

Major metabolic disorder is Diabetes Mellitus, where the body uses glucose as energy source from Ketone molecule which is synthesized by the liver [66,67]. Therefore there is an increased concentration of acetone in the blood. The high level of gaseous acetone in blood is exchanged with alveolar air. Thus, the exhaled breath contain acetone as metabolic break down product and acts as breath marker [68-71] Normal persons exhaled breath contain acetone in range of some 100 ppb. It increases slowly in diabetic patients [72,73]. In comparative studies between normal and Diabetic patients the parameters as age, sex, race and blood glucose concentration are important.

Breath Acetone concentration is quantitatively analysed by GC coupled with Flame ionization detecter[72], ion mobility spectrometry [72,74] and mass spectrometry.

Others

Breath analysis is also applied for condition of respiratory process [75-79] and excreation of drugs [80-83]. Riker JB and Haberman B. studied the detailed respiratory process and analysed exhaled breath by mass spectrometry. They monitored respiratory unstable nonintubated patients, reducing the number of arterial blood gas determinations, monitoring patients who are being weaned off a ventilator; monitoring patients in acute respiratory failure, patients with CO2 retention, to detect rapid decreases in arterial CO2 when these patients are placed on a ventilator and monitoring patients in other cases when there is danger of hyperventilation [84].

Breath analysis also monitors drug of low molecular weights. Drugs and its metabolites can appear in breath because of same or high vapour pressure at body temperature. Therefore drugs or metabolites occur in breath with measurable quantities [85] eg. Disulfiram a drug used in alcohol withdrawal, gets converted into CS2. CS2 is not detected in blood but it is detected in breath [81].

Advantages and limitation of Breath Analysis

- Breath analysis gives exact information of the blood constituent and many biomarkers found in the alveolar breath can be significantly detected in disease or disorders.
- Breath analysis is noninvasive, easy process, which does not have discomfort or embarrassment related with blood and urine analysis.
- Breath analysis is correlated with arterial concentration of analytical constituents which is difficult to detect in blood samples.
- Breath analysis is advantageous over blood and urine analysis with more number of sample collections.

- Breath sample is less complicated mixture than blood and urine, therefore analysis of breath is easiler and faster process.
- Breath analysis gives information mostly related to respiratory system which is not given by other systems.
- Breath analysis shows status of decay of volatile toxic substances in the body.

Limitation

- The major drawback of breath analysis is absence of standard analytical methods, therefore the variation in results.
- Breath analysis require sample collection and preconcentration because concentration of many substances ranges from nmol/L to pmol/L. Preconcentration can be performed by adsorption on sorbent traps, coated fibers or by direct cryofilization. This breath sample collection and preconcentration methods are difficult to standardize than serum.
- Breath analysis have one more problem, it contain more water content in preconcentration sampling and detection of substances.
- Breath analysis are more expensive than regular simple chemical tests which are used in blood and urine.
- The major problem is correlation between breath substances and disease and standardization of VOCs by sampling methods.

Techniques for breath analysis

There are various techniques by which breath can be analyzed as

Gas chromatography

In this system two parts of one Gas by Chromatography (GC) is commonly used in analysis of trace compound in the breath. In GC, samples are injected into the headspace of the chromatographic column which, it travels and is separated by gaseous mobile phase. Separation efficiency depends on the types of GC column. The non-polar compound of GC column as silicone is separated on the basis of the boiling point and the polar component in the Gas Chromatography, column separation depends on polarity of the substances [86]. Various types of detection method are employed with GC for substance which is present in the breath as

i) Mass Spectroscophy (MS)
ii) Flame Ionized Detection (GC-FID)
iii) Ion Mobility Spectroscophy (IMS)
iv) Electrolyzer powdered flame ionization

GC-MS In this MS detection is done on the basis of mass to charge ratio of ionized atoms or molecules quantitatively to analyse the compound. The compound can be identified by the fragmentation pattern and quantified by the formation of daughter ions [87] Now GC-MS is a standard technique in the detection of VOC in breath. Giardina and Olesik show that lung cancer related compound (2- methylheptane, styrene, propylbenzene, decane, undecane). in breath can be detected with help of the low temperature glassy carbon (LTGC) macrofiber to be absorbed on. After the desorption, the VOCs were analysed via GC-MS [88]. Pleil and Lindstrom mentioned GC-MS breath test to assess exposure to halogenated VOCs [89].

GC-FID The GC effluent substances are mixed with hydrogen, air and ignited. Organic compounds burning in the flame produce ions and electrons that can conduct electric potential through the flame. High electrical potential is applied at the flame and a collector is located above the flame. The development of current from pyrolysis of organic compound is measured. FID are more mass sensitive than concentration sensitive, this is an advantage, as for changes in mobile phase, flow rate do not affect the detector responces. FID is mostly used for organic compound detection, it highly sensitive with large response range and low noise. Therefore FID is used in GC for breath test [90,91]. Sanchez et al applied GC-FID for human breath analysis, in that they combined a nonpolar dimethyl polysiloxane column and trifluoropropylmethyl polysiloxane column for selectivity of the VOCs in breath. The detection level of their system are in low ppb range [92]. Phillips and Greenberg assayed VOCs in breath by GC-FID. This method was sensitive, linear, accurate and reproducible for quantitative assay of endogenous isoprene in the breath [93].

Daughtrev et al showed that GC-MS and GC-FID were different in which the relative accuracy was found to be excellent [94].

GC-IMS Ion Mobility Spectrometry was discovered in the late 1960s. The principle method of IMS is to separate ion according to mobility as they travelled through a purified gas in an electric field at atmospheric pressure. This ion travels with varying velocities through the purified gas. The total travel time depends on the drift length, electric field strength, drift gas as air or pure nitrogen, temperature and atmospheric pressure [95]. IMS is a selective detector capable of quantifying substances from mixtures and it is relatively portable and inexpensive. GC-IMS are two different technologies producing a new system that introduces the advantages of the individual technologies. Lord and coworkers investigated presence of ethanol and acetone as biological indicator of human health as well as exposure of VOC by GC-IMS [96].

GC-EFID

Here Common detector GC is used for detection of many range of volatile organic ingredient with new technique namely electrolyzer operated EFID. In that electrolyzer produce oxygen and hydrogen gas mixture for sample seperation and is absorbed in the analytical column by this carrier gas. It has more advantages related to column with different grade type as styrene divinylbenzene and poly dimethylsiloxane absorption film with oxygen up to 180-200°C [97-99].

Proton transfer reaction-Mass spectrometry (PTR-MS)

PTR-MS was discovered by A. Hansel and coworkers for online measurement of complex mixture of trace gas compounds in air with concentration as low as one part per billion [100-105]. In PTR-MS, all VOCs had proton affinity than H2O where each collision proton transfer occurs.

Breath gas analysis by PTR-MS has more advantages for complex mixtures of gas because previous concentration and separation procedure is not required. If compound gets in higher concentration, like NO2, CO2,O2 and H2O, it does not interfere with the measurement, here concentration measurements of very low level upto part per billion and frequent/rapid measurements are possible. PTR-MS characterizes the substances individually according to their mass-to-charge ratio and chemical identification provided by other techniques [106]

Karl et al used PTR-MS technique for measurement of human breath isoprene, here also a correlation between breath isoprene and blood cholesterol level are mentioned that the concentration of isoprene varies with the heart rate [107]. Mayr et al showed the in vivo breath analysis of VOCs released from mouth during eating of ripe and unripe bananas [108]

Boschetti et al could monitor a large number of VOCs with in limited time period at high sensitivity upto part per billion [109]

Amann et al mentioned results of three different studies of VOC emission using PTR-MS(1) analysis of VOC patterns in patients suffering from carbohydrate malabsorption; (2) analysis of intra and intersubject variability of one particular mass; (3) long-time, online monitoring of VOC profiles during sleep combined with polysomnography [106].

This PTR-MS is related only to mass of the product ions but is not a unique marker to identify trace gases because of overlap of mass spectra to different isomers it cannot be resolved [110]. By coupling GC Column to PTR-MS, we can separate complex to different VOCs to single mass ions [111-113]

Selected ion flow tube-Mass spectrometry (SIFT-MS)

SIFT-MS is constructed to allow on-line analyses of the exhaled breath, headspace of aqueous liquid and polluted air. SIFT-MS combines fast flow tube technique with quantitative mass spectrometry. The exhaled breath sample is taken into fast flowing inert gas e.g. helium carrier gas, present as trace gas in the sample and reacts with reagent ions (H3O+, NO+ or O2+) to form specific product ion that identify the compound and is quantified from knowledge of the kinetics of the ion. SIFT-MS has same feature as other analytical techniques, where the sample collected into bag or onto traps are not required, which can compromise the sample,

and time consuming calibration becomes unnecessary. It allows the direct analysis of single exhalations of breath and provides the clinician with immediate results [114].

Spanel et al used SIFT-MS to detect isoprene from breath of 29 healthy volunteers for a period of 6 month during variable time periods of the day [115]. By using SIFT-MS technology, we can detect acetonitrile both in the exhaled breath and the headspace of urine of many cigarette smokers and non smokers [116]. This study showed that detected quantity of acetonitrile depends on the cigarette consumption as compared to the absences in non smokers. Smith et al presented, quantitative study of increasing ammonia from breath after taking a dose of 2g of normal urea in a volunteer, known to be infected with Helicobacter pyroli. In non infected helicobacter pyroli infection, no significant rise in breath ammonia was seen [117].

Chemiluminescence analyzer

This method specially is for asthmatic patient to detect nitric oxide level in breath. It is a more advantageous technique because breath is analyzed directly online to a analyzer or indirectly by sampling of breath in balloon which is analyzed later. Nitric oxide level in breath is measured in part per billion (ppb) [118].

Colorimetric sensor arrays

Colorimetric sensor arrays can be used in the exhaled breath analysis for detection of compounds. It is specially for the lung cancer patient. P. J.Mazzone et al showed that the colorimetric sensor array has 36 spots which contain chemically sensitive compound on disposable cartridges. The changes in color of spots occur due to its contact with volatile active compound which are present in exhaled breath [119].

Flowing After glow Mass Spectrometry (FA-MS)

FA-MS technique have same principle of SIFT-MS in that it exploits ion chemistry coupled with fast flow tube techniques and quantitative mass spectrometry. Mostly FA-MS is specifically used for the on-line determination of the deuterium content of water vapour in exhaled breath and the vapour above aqueous liquids [120].

Differential mobility spectrometer (DMS)

It is micromachined Differential mobility spectroscopy used in breath analysis for identification of many chemicals on low level concentration as part per billion and diagnosis of many diseases. It is a very sophisticated instrument as compared to traditional GC-MS in all its function [121].

CONCLUSION

Rapid non-invasive analyses of breath can now be routinely performed in the clinical environment with minimal stress to the patients, which is a new route to clinical diagnosis and therapeutic monitoring by determination of VOCs or biomarkers by different sampling methods.

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