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REVIEW ARTICLE

Breathe Biomarkers in health and disease

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ABSTRACT

Pulmonary system is the joint for countless multifaceted metabolic and respiratory end products transforming the total exhaled breath into a complex and dynamic mixture of a spectrum ranging from simple elements like hydrogen to exceedingly complex organic metabolic end products together with oxidative stress in health and disease. Over last five decades the researches in the field of exhaled breathe have developed and innovatively applied poles apart technologies. These have provided insight with ample evidenced based substantiation that analysis of components of the exhaled breath has the potential to become a valuable device for non-invasive assessment. In the futuristic research models the search for these biomarkers will be the pathfinder in the diagnosis, interventions and prognosis of different communicable as well as non-communicable emerging and re-emerging diseases.

1. INTRODUCTION

Pulmonary system is in close liaison with the cardiovascular system carrying outcome variables of metabolic and respiratory pathways from all over the body. These biomarkers are exhaled in breath as a common exit point from a simple element to intricate volatile and non-volatile chemicals that varies every moment with the change of internal milieu in health and disease. Studies are furthermore inching on finding more and more of these markers in the exhaled breath to provide clinically useful thought processes of prompt point-of-care diagnostic methods of case finding beyond the pulmonary system. The encouraging results are finding out novel and reliable non-invasive clinical tools to extend the work on in the health and disease, covering the entire gamut of communicable and non-communicable diseases putting firm footstep in the new millennium. [1]

Metabolic macromolecular milieu

Endogenous volatile organic compounds (VOCs) are formed from metabolic activities mutually with oxidative stress from womb to tomb. They eventually through different outcome trail of events enter the blood stream and a good number are ultimately excreted via exhalation, skin emission, urine, etc. In 1971, Nobel Prize winner Linus Pauling initiated the research on identification, quantification and pharmacodynamics of potential biomarkers of medical diagnosis and therapy by describing human breath as a complex gas with more than 200 different VOCs. Breath tests later have also been experimented based on the ingestion of isotopically labeled precursors, producing isotopically labeled metabolites viz. carbon dioxide and others. Yet breath sampling has to be standardized to avoid confounding factors that may affect concentrations of analytes; even under resting conditions exhaled breath concentrations of VOCs can strongly be influenced by specific physiological parameters such as cardiac output and breathing patterns, depending on concurrent physico-chemical properties. [2]

2. HISTORY AND GEOGRAPHY

Hippocrates, the father of Medicine, first noted distinctive smells in different diseases. Thereafter, in the nineteenth century, Nebelthau noted acetone in the breath of diabetic patients; later Anstie could isolate ethanol from breath. From the last century measurement of breath

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aldehydes has opened up a powerful window into understanding of the oxidative stress. Currently, the researchers in this field are using two forms of breath- exhaled breath and exhaled breath condensate. The former uses vapour phase molecules, while latter evaluates dissolved components after cooling breath to find varied elements. Breathe include elemental and permanent gases; Nitric Oxide (relatively dependable marker of airway inflammation steadily noted in asthma, allergic rhinitis, eosinophilic bronchitis, COPD etc.); Carbon monoxide (increased in oxidative stress or stimulation by pro-inflammatory cytokines observed in smoking cessation programs, haemolytic jaundice in neonates etc.). Similarly, the aerosolized droplets in the exhaled breath condensate contain endogenously produced hundreds of nonvolatile compounds ranging from the tiniest hydrogen ion to bigger molecules of dissolved proteins (pertinent to inflammation and oxidative injury, environmental exposure to Cobalt, Tungsten etc.). In addition to these, there is a vast assortment of VOCs. These are being evaluated as non-invasive biomarkers of disease including pH such as in hyperlipidemia, lung cancer, bronchial asthma; exhaled aldehydes from the oxidative stress in heart disease, cancer, autoimmune and neurodegenerative disease, some infectious diseases, chronic fatigue syndrome, lifestyle related disorders and habits like smoking. [3, 4, 5]

Enumerating biomarkers: like stars in the sky

Diverse types of VOCs have been consistently detected in exhaled breath; most abundant were Naphthalene, 1-methyl-, 3-heptanone, Methylcyclododecane, Heptane, 2,2,4,6,6-pentamethyl-, Benzene, cyclohexane. Monomethylated alkanes viz. dimethylcyclohexane, methylheptane, methyl-cyclododecane, and tetramethylbenzene have also been identified in the patients suffering from pulmonary tuberculosis. Commercially available analyzers can measure nitric oxide levels in parts per billion and carbon monoxide to the parts per million levels. These breath biomarkers are noted to be quite selective to provide an accurate, rapid, inexpensive and non-invasive diagnostic and prognostic method. In small cell carcinoma the use of colorimetric biosensor has provided encouraging results. In active pulmonary tuberculosis, exhaled breathe tests have been able to differentiate microbiologically positive or negative sputum cases; researchers noted elevated levels in active spells and reduced with therapy; even biomarkers in breath and in sputum cultures were in qualitatively and quantitatively comparable. [4, 6, 7, 8, 9]

Different breath tests assayed products of mycobacterial metabolism viz. exhaled antigen 85, mycobacterial urease activity, and detection by trained rats of disease-specific odor in sputum to provide tell-tale evidence as early markers to assess the efficacy of treatment. Exhaled nitric oxide levels were analyzed on culture-confirmed TB cases with a limited value in the direct diagnosis of pulmonary tuberculosis. [3, 10, 11]

Unrelenting technological progresses throughout the last few decades sprinkling over the new millennium have been able to empower us with much awaited evidence stored in human exhaled breathe to estimate that, more than a thousand constituents with both volatile and non-volatile organic compounds are present in ever-changing qualitative and quantitative proportions in the micro-molecular milieu of human body expressed in breathe. [12]

Recently, the researchers also observed 3481 different compounds in exhaled breath though few were in higher concentrations in inhaled air than in exhaled breath with a "negative alveolar gradient". [13]

3. What is to be done?

Globally, in the transitioning of exhaled breath analysis from 'bench side to bed side', the new entrant is a relatively poles apart though the concept breath sampling has been well accepted as non-invasive and breath samples that can be utilized as often as desired. Incidentally, the mind-set of the health care fraternity is fixed on the microbiological and serological approaches for diagnosis as well as prognosis. This poses hindrances for the practical relevance of breathe analysis in the evidence based medicine.

Exhaled breathe analysis in disease detection has historically taken two major directions. Both the approaches are noninvasive and potentially inexpensive novel advanced techniques. The first approaches use spectroscopy and other related technological methods to hook and recognize the individual elements and volatile and non-volatile compounds in the breath based on broad range of qualitative and quantitative assay methods. They have subsequently put forward some cut off values above or below which the positive and/or negative findings will be affirmed that are remarkable in the exhaled breath. [3, 5, 9, 10, 11, 14]

The subsequent researchers are trying to find pattern of changes using the entire blend of chemicals in the exhaled breathe and exhaled breathe condensate. This second move towards this direction is an unconventional technique with the sensors to find changes in configuration without qualitative or quantitative detection of specific volatile and nonvolatile components as diseases biomarkers. [15, 16, 17]

Researchers at the Innsbruck Medical University, Austria noted that Isoprene, acetone and methanol are appearing in human exhaled breath in significantly lower concentrations in lung cancer patients as compared to healthy volunteers. Further, a comparison of the gas chromatography mass spectrometry (GCMS)-results of lung cancer cases with those of healthy participants revealed differences in concentration in more than 50 analytes. Sensitivity based on presence of (one of) 4 different compounds not arising in exhaled breath of healthy volunteers was 52 per cent with a specificity of cent per cent. Using 15 (or 21) different compounds for distinction, sensitivity was 71 per cent, still with a specificity of cent per cent. The researchers concluded alcohols, aldehydes, ketones and hydrocarbons as potential study markers. [18]

4. Pros and cons in ideas to reality

Serum prostate-specific antigen (PSA) is accepted as one of the finest existing tumour marker for prostate cancer which is globally a common cancer in men folk. Yet, the sensitivity and specificity of PSA varies from 0.78 to 1.00 and 0.06 to 0.66, respectively putting its clinical application under eternal debate. With better understanding of the molecular mechanisms of carcinogenesis and revolutionary advancement of novel sub-branches of molecular biology such as genomics, epigenetic, transcriptomics, proteomics, metabolomics, lipidomics etc., and newer imaging techniques is showing new paths in cancer management. Many newer molecules are being examined recently more than ever like circulating microRNAs (miRNA) as a biomarkers for non-invasive diagnostic procedures in various malignancies. [19]

In search of biomarkers of diseases, recent times have shown collaborative efforts between academic, technical, health, and commercial professionals to find the novel technologies viz. infrared, electrochemical, chemiluminescence, etc. are being successfully employed with precision mass spectrometer technologies with promising results. Researches are ongoing using various approaches like Isotopic ratio mass spectrometer, Ultraviolet absorbance spectrometer, Gas chromatography/ mass spectroscopy, Polymerase Chain Reaction amplification, Immunosensor, Bio-optical technology, Colorimetric responses and other methods to detect volatile organic compounds. The Metabolomics is showing encouraging results as the non-invasive method; some genes involved in fatty acid metabolism including the multi-enzyme protein, fatty acid synthase by increased energy demand in tumor cells can be utilized in tumour diagnosis. [3, 4, 9, 12, 20, 21, 22, 23, 24, 25, 26]

Across the world, exhaled breathe analysis has emerged as a safe non-invasive tool even if repeatedly applied on patients for better analysis even at life threatening situations beyond routine tests at a critical care unit or during surgical interventions; in real-time analysis during an ergometer assessment or during sleep. The downside of the methods of assessment of individual VOCs are that the expense of the system and the expertise needed for interpretation render the technology difficult to use as a point-of-care test. Further, precision related to the breathe collection are challenging; whether real-time sampling of tidal breathing or collection into a sampling bag after forced exhalation or in the selection of participants. Gaseous chemical-sensing devices may be unable to identify specific chemicals or sensitivity as well as specificity may not be up to the acceptable standard. The goals of colorimetric sensors, on the other hand, detect patterns of VOCs that serve as bio-signatures instead of detection of individual compounds to develop into a useful clinical test in the normal ambience of the resource poor infrastructure of the developing countries like India. The specific chemical differences in the breath by other technologies in future could be applied to the refinement of current or developed novel sensor systems by walking on both feet when the pattern recognition in the absence of specific identification has the potential to produce clinically useful results. In the last few decades, porphyrins and metallo- porphyrins is used as sensitive materials in developing the colorimetric sensors. Likewise, the researchers are also on the lookout for a quartz crystal microbalance sensor a respiratory monitoring system. Further research is to investigate structural properties of developed coatings, thermodynamic properties, and influence of other factors (e.g., environmental temperature) on the optimum performance of the sensor. Special attention should be devoted to the examination of possible influence of confounderds known to contain wide range of compounds present at different concentrations present in breath matrix. [4, 27, 28, 29, 30]

Thus the concept of breath testing has propagated from in search of the newer techniques that could have been able to identify data comparable to the fingerprints for specific markers of even specific disease generated from the respiratory bacterial and fungal lower respiratory tract pathogens. Still pre-concentration techniques are needed to analyze target VOCs. Breath testing with an electronic nose has shown promising results in the diagnosis of tuberculosis, and specific volatiles has been demarcated by gas chromatography with mass spectrometry (GC-MS) techniques for breath analysis of Mycobacterium tuberculosis, Pseudomonas aeruginosa and Aspergillus fumigates infections; they found at very low concentrations in breath. Vigilant consideration is necessary to augment the sensitivity and specificity of the techniques to keep the environmental components at alert as the necessary evil in the research on breath; while exhaled breath condensate (EBC) has yet to show a firm footstep. The electronic nose and flow tube-mass spectrometry has to go through rigorous field testing for their practical implementation in the identification of specific microbial biomarkers. That will unquestionably be of immense consequence in this epoch of dangers of infinite emerging and re-emerging diseases. [31]

Differential ion mobility spectrometry (DMS) is an upcoming non-invasive assessment method to detect VOCs in the ppt range that was experimentally applied to assess to discriminate subjects with a mock-up chronic intestinal infection caused by Mycobacteria from non-infected controls in the goat model. Even after adequate consideration of the confounding effects of ambient air surroundings, analysis of VOCs in the exhaled breath and of feces revealed significant differences between chronically infected animals and non-infected healthy controls qualitatively as well as quantitatively. [32]

5. Indian scientists in this field

Indian scientists are working to detect early pulmonary tuberculosis to design a hand-held, battery-powered sensor with an electric nose technology. Further, the sensitivity of their current diagnostic tools is not as much as necessary high to diagnose optimum number of positive cases along with cost-effectiveness. [33, 34]

Amongst the different types of porphyrin used in colorimetric sensors in exhaled breathe analysis few have shown promising results with satisfactory predictive value. In the last few years, other Indian researchers have pursued work with the total exhaled breath analysis with array-based chemical sensing, and based on different porphyrin based chemical-sensing interactions capable of being future clinical application. Each of the indigenously synthesized porphyrin based chemicals in their sensor array provided evident changes after exposure to total exhaled breath of active pulmonary tuberculosis cases even at higher cut-off of ten and more of the red-green-blue (RGB) difference. Further research work is in progress on porphyrin based sensor for the exhaled breath tests in search important surrogate markers in initial phases of the microbial activity and observations after newer antibiotics. All these research activities have generated a wave of awareness about innovative technologies of non-invasive tools for point-of-care diagnosis, intervention and prognosis of different diseases. There is thus ray of hope to provide thoughts in future directions to extend on several other non-communicable diseases and disorders including injury of all forms where every moment counts. All these sensor applications exploited the qualities of analytes in search of a low-cost, disposable, point-of-care and well-defined selectivity for the expected precisioned outcomes. [27, 28, 29, 30 35, 36]

In recent times there is an sincere urge for the evidenced based approach to convert research findings from the bench-side to the bedside even in the in the primary health care delivery system and the significant technical advances have been implemented with newer technological devices has been made user friendly in the new millennium. Several diagnostic methods have established or others are in pipeline to be practised in the routine clinical practice; breathe alcohol concentration (BAC) is in regular use by regulatory authorities in several countries even in some south-east Asian countries. With this appeal of health science, researchers have internalized the eternal symphony of human life in a holistic approach as the medicine is a para-science based on the basic principles of physics, chemistry and mathematics hidden in the harmony of the cell-tissue-organ-systems. Now we hope to take into our custodian and analyze a few thousands of molecules expelled in exhaled breath with an attempt to make out a chemical signature pattern comparable to the image expressed in the biometry whether we are in health or in disease. [1, 37]

6. At the end

To sum up, the exhaled breathe biomarkers are showing promising panorama in the scope of the research on the futuristic approaches of non-invasive diagnostic, intervention and prognostic tools for which we have to go a long way in precision of our ideas and tools. We should look onward for scientifically acceptable specificity, sensitivity, predictive values, and likelihood ratios to demarcate disease from health. We need to join the hands together to bring together all the scientific principles and personnel for a holistic goal where the researchers in different part of the world are working in unity and diversity on the qualitative and quantitative data abstraction of the innumerable prospective disease biomarkers. Future directions of all the studies in this field will be to apply in the diagnosis and prognosis with dynamics of life in the evaluations of exhaled breathe biomarkers. International Association of Breath Research (IABR) has been established to unite researchers in the field with a common platform for exchange of their news, views and abstraction of knowledge; the Journal of Breath Research (JBR) as the official publication of the IABR is relentlessly publishing updated data to help develop this new field.

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