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Breathe Analysis in Tuberculosis Disease Recognition in New Millennium.

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ABSTRACT

To control the tuberculosis pandemic we need rapid, inexpensive finding tool. To assess the worth of exhaled breath analysis in tuberculosis case detection. A wide-ranging exploration of documents was done in indexed literatures and website-based research reports. Thirty-eight studies were identified on more than 200 potentially relevant articles related to breath analysis on tuberculosis. A broad criterion was formed in the absence of universally accepted method by the researchers on exhaled breathe analysis, irrespective of their criteria for diagnosis of tuberculosis. Wide differences in samples, primary outcome variables, lack of uniformity in criteria for positive diagnosis, and study instruments confounded the outcome variables. These non-invasive breathe tests of tuberculosis and exploring factual and surrogate markers in primary bacterial activity as well as during interventions. Prospective utility of breath analysis by varied methods deserve their proportional weightage. The study reviewed non-judgmentally on the ongoing work in the field of breath analysis that may be worth developing and evaluating as a cost-effective entrant in diagnostic and prognostic algorithms of tuberculosis. Time has come to explore this to the fullest extent for a superior conceptual design of the factors for a futuristic model of non-invasive direct point-of-care diagnostic understanding of the factors influencing diagnosis and prognosis.

INTRODUCTION

Tuberculosis (TB), once on the wane in last half of past century, got fresh upsurge with the HIV/AIDS pandemic. Globally active pulmonary TB is a leading cause of death from infectious disease in 2011; 8.7 million morbidity including 1.1 million cases among people with HIV; 1.4 million mortality, including 430 000 among people who were HIV-positive. TB is one of the top killers of women worldwide, half a million women died from TB in 2011. In 2011, multidrug-resistant TB (MDR-TB) cases were estimated to be 310 000 cases; 60 percent from India, China and the Russian Federation. This scenario has directed us for an urgent need for rapid, cost-effective diagnostic and prognostic tools to halt the impending disaster [1].

Globally acknowledged and practiced diagnostic algorithm of tuberculosis has experienced modest progress in recent times. Sputum microscopy consumes two days, sputum culture up to three months though they remain the basis of laboratory diagnosis. So there is an urgent necessity for novel precisioned diagnostic tools to halt the pandemic of tuberculosis that will be cost-effective, noninvasive, and suitable for use in primary care settings [2,3,4].

The widespread use of rapid, non-invasive, and inexpensive breath diagnosis promises a sea-change in the TB diagnosis process worldwide [5].

In this review, advances of breath analysis by different effective methods in tuberculosis case detection have been detailed with their current progresses and accomplishments.

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METHODS

Study design

This retrospective study design based on systematic review was done on an extensive collection of studies, including meeting presentations, proceedings of conferences and personal communications with the researchers in the field and from different sofy copy and web sources in which exhaled breathe analysis were reported. Through an extensive website-scanned search in peer reviewed journal publications and study reports, Thirty-eight studies were identified from our exploration amongst 200 relevant articles from various institutional libraries of India, and websites on exhaled breathe analysis published since 1990. The studies were identified by searching Pubmed-entrez and abstracts from scientific meetings (1990 – 2012) also. Reviews of citations and reference lists were performed to identify additional studies by the search terms exhaled breathe analysis, biomarkers, diagnosis of airway disease, pulmonary tuberculosis (P-TB) etc. Manual searches were conducted from review articles, cross references and previous meta-analyses, even from personal collections. Where possible, sources were contacted for additional research information unavailable in the public domain or for translations from languages other than English.

Selection criteria

Currently, there is no valid, rapid, cheap breath analysis to diagnose P-TB in the world health scenario. So, we had to develop few criteria to select studies from among peer-reviewed research publications. First, we developed broad criteria to define breathe analysis. Second, we also sought to include all those studies that studied exhaled breathe other than using sensors. Finally, in the absence of universally acceptance of sensors as non-invasive diagnostic method, other methods of diagnosis of breath biomarkers were taken into consideration.

Main outcome variable

Breathe analysis, tuberculosis, biomarkers

RESULTS AND DISCUSSIONS

Data abstraction and analysis

Unearthing of the Biomarkers of tuberculosis

The pulmonary system and the cardiovascular system are carrying products of metabolism from all tissues of human body in a close circuit so that metabolic yields gets opportunity to be exhaled out in breath apart from other excretory channels. Accepting these for granted, researches on breath made the first move. The age old physicians have documented distinct changes in the smell of patients even in non-pulmonary system ailments like diabetes mellitus, liver and kidney disorders. In the seventh decade of last century Nobel laureate Linus Pauling noted more than 200 different endogenous compounds released in different amounts. Currently the researchers have estimated that more than a thousand chemicals are coming out those can provide critical information in real time and deliver numerous advantages over fluid and image-based testing. Researchers in this field have discovered inorganic gases, like hydrogen, nitric oxide, and carbon monoxide, along with innumerable volatile organic compound (VOCs) amid other products of oxidative stress and aerosolized droplets for use as exhaled breath condensate with varied endogenously produced nonvolatile compounds also, such as the dissolved proteins [5,7,8,9,10].

Mycobacterium tuberculosis produces VOCs inside mammalian body and comes out in exhaled breath. The detection of these VOCs in the breath as apparent biomarkers of infection has created interest among researchers in the field of exhaled breath analysis as they are apparently unfamiliar molecules like 2,2-diethyl-1,1-biphenyl or 2-methyl-1(1,1-dimethylethyl)-2-methyl-1,3-propanediyl propanoic acid ester apart from methanol, ethanol, acetone, acetaldehyde and isoprene. Up to 130 different biomarkers were consistently detected; the most abundant were naphthalene, 1-methyl-, 3-heptanone, methylcyclododecane, heptane, 2,2,4,6,6-pentamethyl-, benzene, 1-methyl-4-(1-methylethyl)-,and cyclohexane. Different breath tests assay mycobacterial metabolism viz. Monomethylated alkanes, like dimethylcyclohexane, methylheptane, methylcyclododecane, and tetramethylbenzene, have been identified in pulmonary tuberculosis, antigen 85, urease activity, and even detection by trained rats of disease-specific odor in sputum. Exhaled nitric oxide from tuberculosis patients, though selective in many other diseases, produced equivocal results in case finding [11,12,13,14,15,16,17,18,19,20].

The well-established application exhaled nitric oxide (eNO) levels in the diagnosis and prognosis of other diseases like asthma, had displayed limited value in the direct diagnosis on P-TB cases; researchers hope to employ in screening algorithms of pulmonary TB [21,

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[22, 23].The urease breath test offers the possibility of using exhaled breath samples to prove presence of *Mycobacterium tuberculosis* inside human body with probability of confounding from other urease-producing microbes like *Helicobacter pylori* with high prevalence [24].

Aberrant yet futuristic mindsets

Workers in this field are evolving cost-effective, non-invasive diagnostic technologies based on breath specimens having critical information in real time and advantages over microbiological and imaging techniques. From spectrometry based methods like gas chromatography/mass spectrometry (GC/MS), isotopic ratio mass spectrometer, ultraviolet absorbance spectrometer to Polymerase Chain Reaction (PCR) amplification, immunosensor, bio-optical technology etc. have been on trial to find volatile biomarkers in the breath in concentrations of parts per trillion [14,15,16,17,18,19,20]. Breath analysis with the Metabolomx technology has claimed rapid results with no need of laboratory or trained technologists in a handy device that works over a wide temperature range, unresponsive to humidity and the consumables are inexpensive compared to other diagnostics that need lab processing [5].

Although the viability of breath analysis to detect pulmonary tuberculosis is a proven fact on scientific and chemical basis to notice unique substances, yet current practice of using GC/MS is costly and slow turnaround times; yet other researchers have claimed that they could reach 85 percent accuracy using by 'time-slice alveolar gradients' [7,9,25]. The upcoming concept of electronic nose is encouraging for the diagnoses of P-TB by GC/MS have been identified in breath even at very low concentrations in breath [26]. In a novel ongoing study with a breath scanner that could collect, and concentrate the VOCs in a sample of alveolar breath, and using GC and a surface acoustic wave detector (SAW) identified pulmonary TB with 80 percent accuracy on a preliminary analysis [2]. Indian scientists at are also working on electric nose technology; as per their reported information, the sensitivity of their current tool is low and cost-effectiveness not yet determined [3,4].

A recent multicentre study analyzed concentrated breath with automated thermal desorption, gas chromatography, and surface acoustic identified biomarkers of tuberculosis viz. naphthalene, benzene and alkanes. This study diagnosed P-TB with sensitivity 71.2 percent, and specificity 72 percent; accuracy was calculated up to 84 percent in age-matched subgroups. The researchers predicted that in five percent population prevalence, they can identify P-TB with 13 percent positive predictive value [27]. Differential ion mobility spectrometry method was used for VOC analysis in experimental mycobacterial chronic intestinal infection in animal model with the potential to become a valuable tool for non-invasive assessment [28].

Amongst the different types of porphyrin used in chemical sensing, an India study have shown promising results with satisfactory predictive value with an array-based colorimetric sensor using total exhaled breathe, indigenously synthesized porphyrin and metallo-derivatives along with innovatively modeled breath analysis devices in the normal ambience of the resource poor infrastructure in the state government hospital [29,30].

WHO has officially endorsed a rapid molecular test Xpert MTB/RIF that is capable to diagnose TB and rifampicin resistance within 100 minutes already adopted by 67 low- and middle-income countries; South Africa is the leading one with collaborative efforts from USAID, UNITAID and Bill & Melinda Gates Foundation among others [31,32].

Long way to go

Scientists are on the lookout of cost-effective, non-invasive diagnostic test to from breath analysis to provide critical information in real time and deliver various advantages over fluid and image-based testing. In the insight of clinical epidemiology we need to evaluate the prospective practicability of early diagnosis and prompt treatment of the active pulmonary TB cases by non-invasive diagnostic procedure to fix the VOCs of total exhaled breath. This pattern recognition analysis will help stretch our imagination for the new generation screening test for P-TB with optimum sensitivity, specificity and predictive value when compared to standardized microbiological and clinical parameters to preclude the hidden bias. For this large multicenter trials are needed to compare with the "gold standard" of sputum culture [5, 24, 28, 33, 34, 35].

Numerous confounders hinder the progresses in exhaled breathe research to formulate any standardized procedure that ranges from how the breathe is collected to the multifaceted factors of gaseous exchange to the products coming out from the gastrointestinal system as the physiological parameters such as cardiovascular and pulmonary systems are intimately influenced by the diurnal variation to socio-demographic correlates influencing the physico-chemical properties of breath [6].

The dilemma comes acutely for the detection of TB among people living with HIV with less sensitive sputum microscopy even in cases of active TB; 'Gold standard' culture allows delayed interventions even when advanced laboratory set ups are accessible. TB research and development falls far short of the annual target of US$ 2 billion specified in the Global Plan to Stop TB 2011-2015. Enhancement of diagnostic capacity for TB and MDR-TB is urgently needed to scale-up access to care and treatment of MDR-TB in collaboration [1, 31, 32].

Unlimited 'breathing space'

For the nonstop assessments of patho-physiological states or pharmacodynamics we can imagine breath analysis as the future pathways including affliction of exogenous VOCs inhaled from the environment, using isotopically labeled precursors in the diagnosis and prognosis of a good number of diseases. However, standardization of all the procedures taking into consideration of highest probable number of confounders underlying pulmonary gaseous exchanges including the cardiac output, breathing patterns, diet, addiction, ethnicity etc [6].

Breath testing provide us to reflect beyond 'glass box' of available invasive and non-invasive procedures like patients in critical care units or cost-effective screening in kidney or liver ailments or in cancer with uninterrupted analysis even under stress for improved understanding of physio-chemical processes. Exhaled breathe analysis have inimitable advantages that they can be repeated with lesser considerations of time-place-person in contrast to the routine tests avoiding the invasive methods that need 'universal precautions' or time spells as in collection of the excretory products and, or repetition like imaging techniques or prohibition of administering several tests in extremes of ages or in critical care survivors. The portability dilemma to outreach places without constant power supply in the developing countries is solved with real-time point-of-care testing with a hope in applicability in primary care settings [5, 13, 36].

FDA earlier approved tools for detection of blood alcohol concentration, *Helicobacter pylori* infection, lactose intolerance, end-tidal carbon dioxide. Recently fractional exhaled nitric oxide (FENO) for asthma monitoring was also added to the previous list with the advantages of noninvasiveness, simplicity of repetition, and usage in adult and child populations with severe airflow obstruction where other techniques fail to work [37].

Light at the end of tunnel

Human exhaled breathe provides useful information in health and disease like biometry with hidden data for diseases recognition. Further, our world is moving towards the inexpensive assessing tool and rapid point-of-care diagnostic methods of different diseases. So, for the direct diagnosis of tuberculosis, the breath analysis tools may be worth developing and evaluating as a cost-effective entrant in diagnostic algorithms that may facilitate investigation of individual patients and so enable greater understanding of the factors influencing prognosis for which researchers are working in India and abroad. The author himself is working in a team of researchers in India among them to find out a colorimetric sensor that is highly sensitive and specific and good positive and negative predictive values as well as simple, rapid, inexpensive and non-invasive viable method of diagnosis. To reach at the primary health care levels, we have to improve the portability, simplicity of use and speed of the test device as a tool to aid early identification of tuberculosis. Further we will standardize a protocol to demarcate healthy from cases and also to extend the study to persons suffering from other disorders and co-morbid multiple ailments that may prove otherwise confounding variable. The success in non-invasive clinical diagnosis will have a far reaching impact to establish self-administered instrument. Widespread use of rapid, non-invasive, cost-effective tools in breath analysis opened the vistas in the diagnostic process of tuberculosis globally to provide real time information. In the perceptiveness of clinical epidemiology we have to assess the potential usefulness of exhaled breathe analysis in early diagnosis and prognosis of the active pulmonary TB cases. Further, there is a need to be standardized with similar patho-physiological conditions to reach our goal as a futuristic model of non-invasive diagnostic procedure to fix the VOCs of total exhaled breath. This pattern recognition analysis of VOCs in breath of identified patients with active pulmonary TB infection will help reach a new screening test for pulmonary TB. Further studies have to be undertaken to determine the diagnostic sensitivity, specificity and predictive value when compared to standardized microbiological and clinical indicators of tuberculosis disease to obviate the potential bias in a large multicenter trial even extending to comparison with the "gold standard" of sputum culture [29, 30, 38].

To sum up, developing countries need an urgent rapid, cost-effective diagnostic tools and clinical laboratory tests to detect the tuberculosis. Researches on human exhaled breath has grown out of laboratory bench side to bedside in clinical set ups beyond the respiratory system. The time has come to explore breath analysis to the fullest extent as with the widespread use of rapid, non-invasive, and inexpensive breath diagnosis promises a sea-change in the control of pandemic.

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