Research Paper

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Diagnosis of pulmonary tuberculosis in total human exhaled breathe analysis by porphyrin based sensor

Chemistry

KEYWORDS : porphyrin, sensor, pulmonary Tuberculosis, exhaled breathe

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ABSTRACT

BACKGROUND: A rapid non-invasive screening tool is needed to diagnose Tuberculosis.

OBJECTIVES: To assess dependability of porphyrin based sensor array in the total exhaled breathe analysis in pulmonary Tuberculosis (P-TB) cases compared to healthy individual.

METHODS: Total exhaled breathe analysis was conducted on 62 participants: 32 P-TB cases and 30 healthy controls on the colorimetric sensor array that were organized with spots on glass plates of porphyrin derivatives followed by the Red, Green and Blue (RGB) analysis with high resolution scanner before and after direct exposure to total exhaled breathe.

RESULTS: RGB response was noted in the all the porphyrin derivatives under our study after exposure to total exhaled breathe collected from the participants, even when we have taken different higher cut-off of ten RGB difference. 11 (eleven) porphyrin derivatives in our series had shown notable differences in P-TB cases compared to the controls in the selective criteria as follows: Sensitivity: Analyte ICh showed highest, then IEc, IAb; Specificity: Analyte ICa had highest, IIAa, I; Positive Predictive Value: Analyte ICa had highest followed by I; however, positive likelihood ratio was not encouraging in our series. In the binary logistic regression following four analytes showed as fairly strong components in our sensor array: I and Ica.

CONCLUSIONS: For the point-of-care non-invasive diagnosis of active P-TB, porphyrin based sensor array in our research showed promising findings with a provisional signature pattern that need further investigation as an advanced model for diagnosis as well as prognosis of tuberculosis.

Introduction:

The rapid and accurate diagnosis of active pulmonary tuberculosis (P-TB) remains a challenge till date. The mycobacterial infection inside the internal milieu of living cell produces volatile organic compounds typical to TB and comes out in breathe and can be used as a predictive parameter for case detection. The end products of mycobacterial metabolism in human breaths have the potential for the early markers in diagnosis, intervention as well as prognosis. In TB cultures 130 different volatile organic compounds have been consistently detected (derivatives of benzene, naphthalene, and alkanes).[1] Several methods like Spectroscopy, Polymerase Chain Reaction, Bio-optical technology and Immunosensor have detected volatile breath biomarkers for Mycobacteria.[1-7] Exhaled nitric oxide (eNO) levels were also analyzed by validated handheld analyzeron culture-confirmed TB cases with a limited value in the direct diagnosis of tuberculosis.[8,9] The survey of the published literature indicated that desired results with colorimetric sensor array method have not been achieved so far in the studies carried out for lung cancer.[10,11] To the best of our knowledge, we were the first to report colorimetric sensor array studies pertaining to exhaled breath study of TB cases.[12]

The basis of detection by colorimetric method using sensor array is the color change that occurs when the analyte interacts with the sensor element. Porphyrins are naturally occurring colored compounds that exhibit interesting photophysical properties which are sensitive towards the environment and are commonly used in a diverse range of research fields.[13,14] The colorimetric sensor array of porphyrin has been used to detect various types of organic volatile compounds.[15,16] This provided us an impetus to utilize the porphyrin sensor array as a tool to detect the organic volatiles exhaled in breath of healthy and diseased person. It is a known fact that the composition of organic volatile compound exhaled will differ as the metabolism in the internal milieu differs inside living body.[17,18]

It is expected that a unique signature for P-TB patients could be obtained by a proper choice of porphyrin elements that is used to construct the array. If a satisfactory positive predictive value can be obtained even for few porphyrin derivatives, it will open up a new avenue for further studies. Further improvisation can be done by zeroing down on the types of porphyrin compounds to construct future arrays. The interaction properties of the porphyrin can be modulated by variable central metal and peripheral substituent and can be judiciously used to increase the predictive value which is a big advantage for such type of sensor studies. The selectivity in our study with colorimetric sensor was analyzed in pre and post exposure to exhaled breath using red, green and blue (RGB) color model based on the Young–Helmholtz theory of trichromatic color vision and on James Clerk Maxwell's color triangle; utilized earlier in cytology.[19-22]

In this study we have embarked on to assess the potential usefulness of qualitative chromatic analysis of exhaled chemicals by porphyrin based colorimetric sensor array and ultimately to identify unique chemical signature of total exhaled human breath of active Pulmonary Tuberculosis (P-TB) cases in comparison with the healthy volunteers. The final aim would be to establish a standard protocol with a cost-effective screening tool.

Methods

STUDY PARTICIPANTS: The breath analysis was conducted on 62 adult participants during 9-14 July 2012; 32 consecutive P-TB cases presenting at a tertiary care referral state run Sir Thotup Namgyal Memorial (STNM) Hospital; 30 healthy volunteers, free of criteria of P-TB suspect laid down by Revised National Tuberculosis Control Programme guidelines of Government of India (RNTCP), from among the staffs and students of the Sikkim Manipal Institute of Technology and the Sikkim Manipal Institute of Medical Sciences at the Sikkim Manipal University.[23] In both the groups, restrictions on the criteria of gender, ethnicity, smoking or dietary patterns were set aside for participation requisites of inclusion or exclusion criteria.

STUDY INSTRUMENTS: The 'Breath collection chamber' made of glass was innovatively organized in with compatible vacuum pump (Model: Promivac PVUC-50, HP-17.6, RPM 1440) and a high resolution scanner (Model: HP Scanjet G4050). Twenty-four porphyrin derivatives were synthesized and characterized at the laboratory in Sikkim Manipal Institute of Technology, Sikkim, India under strict supervision and were coded. The quality of the porphyrin derivatives were confirmed by thin layer chromatography techniques to ensure ultra-high purity. The elements were diluted with non-interacting petroleum ether solvent and were spotted onto glass plates from solutions (of a few mM) in thin film spots by dots in construction of sensor; after spotting the colorimetric sensor plates were dried under

high vacuum at room temperature for thirty minutes before they were in use in our study.

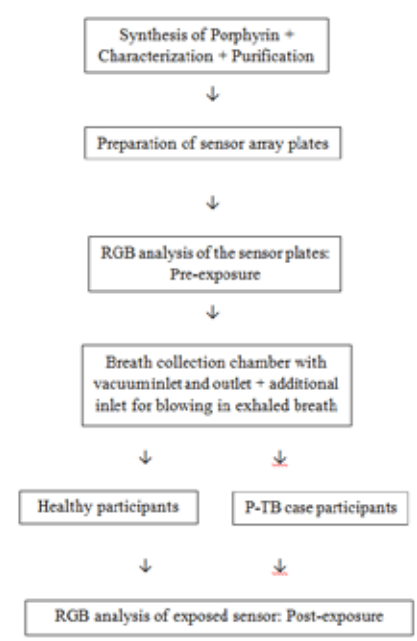
DATA COLLECTION PROCEDURE: The study was approved by Sikkim Manipal Institute of Medical Sciences Ethics Committee (IRB) and Medical Superintendent of the state government run STNM Hospital. P-TB cases were recruited from Chest clinic of the hospital working under RNTCP. Each participant was individually counseled prior to the study that no potential risk was involved in their participation and they had full autonomy to leave the study at any point of time. Each participant provided informed consent before taking part in the study.

Each individual was explained how they will blow into breath collection chamber by inhaling through nose and exhaling through the disposable tubes into the breath collection chamber. The sensor array was initially RGB scanned and the image of each sensor plate was marked as 'pre-exposure' before placing them inside the breath collection chamber. The chamber containing the sensor plate was made free of the natural constituent of air using high vacuum. The participants were then requested to exhale in the chamber with concurrent, careful slow opening of the connecting stopcock to the chamber; equilibration time of ten minutes was provided to ensure uniform interaction of exhaled air with the sensor elements. Then, the sensor plates were removed from the chamber and immediately scanned and the images marked as 'post-exposure'.

To reduce the risk of biohazards, all the precautions were undertaken including use of protection mask by the investigators and single use sterile breathe exhalation tube for the participant that was disposed of using standard operative procedure. Further, the trapped air of one participant was removed from the breathe collection chamber by applying vacuum couple of times before the second participant was approached.

The procedure was well tolerated by all the participants with no reported medical complaint; the time required for the exhaled breathe collection did not exceed five minutes that included the counseling times. In total 15 minutes was required for acquisition of final data. This project was undertaken within the range of PhD doctoral research activities under Sikkim Manipal University. The data have been strictly kept confidential with the investigators and have not been disclosed for the assessment, management or intervention of the cases.

The schematic diagram of the entire experimental process is given in the flowchart below in the flow diagram as follows:



DATA ANALYSIS: The color changes of each spot were converted into RGB numerical values with three numeric values per spot from 0 to 255. The data was analyzed in terms of the difference in RGB spectrum value changes in post-exposure state to total exhaled breath in comparison to pre-exposure in each chemical. The sensitivity, specificity, predictive value and likelihood ratio were assessed for each compound in each participant both control and cases in search of a unique signature pattern between healthy and P-TB cases.[24] In this second phase we did not collect fresh set of data from healthy participants. Instead we utilized the data of breathe analysis of 30 controls from the first phase of our study for distinguishing configurations of RGB vectors of fresh set of breath of P-TB cases for individual porphyrin analyte in this second phase.[25]

Results

Our findings have shown that a colorimetric sensor array is capable of identifying the unique chemical signature of total breathe overlooking the diagnostic, demographic, smoking and dietary patterns. In these spots composed of porphyrin based with array-based chemical sensing interactions, the color changed on contact with total exhaled breath. RGB response was noted in the all the porphyrin derivatives under our study.

Even with single total exhaled breathe analysis the colour responses was noted for individual porphyrin at RGB cut-off 10 or more though all the compounds were not equally responsive. The pre-exposure values of the RGB vectors and their difference with corresponding post-exposure values could distinguish P-TB cases from healthy participants in 11 (eleven) chemicals that showed important ever changes observed as follows (Table1):

Sensitivity: Analyte ICh showed highest (84.37%), then IEc (80.64%), IAb (80.21%), IB (78.12%), IAe (77.08%), IIBa and ICd (70.83%), IIAc (62.50%), I (61.46%), ICa (57.29%). Specificity: Analyte ICa had highest (78.89%), then I (61.11%), IIAb (60.56%), IAb (55.00%).

Positive Predictive Value: Analyte ICa had highest (74.32%), then I (62.77%), IIBa (58.62%), ICd (58.12%), IAe (57.36%), IIAc (56.60%), IAb (55.00%), IEc (54.35%), ICh (50.94%), IB (50.68%).

Positive likelihood ratio: Analyte could reach only up to IAe 1.7, then IIBa 1.60, I with 1.59. In the binary logistic regression following two analytes showed as fairly strong components in our sensor array: I, Ica.

Table 1: Comparison of RGB difference in healthy and P-TB cases

	RGB difference < 10 spells		RGB difference ≥ 10 spells	
Porphyrin Code	P-TB (%)	Healthy (%)	P-TB (%)	Healthy (%)
IAb	10(15.15)	28(31.11)	56(84.85)	62(68.89)
I	26(39.39)	37(41.11)	40(60.61)	53(58.89)
IIBa	04(6.06)	34(37.78)	62(93.94)	56(62.22)
IEc	12(19.05)	21(23.33)	51(80.95)	69(76.67)
ICa	12(18.18)	43(43.48)	54(81.82)	47(56.52)
IAe	10(15.15)	34(37.78)	56(84.85)	56(62.22)
IIAb	71(53.79)	43(43.48)	61(46.21)	47(56.52)
ICd	07(10.61)	37(41.11)	59(89.39)	53(58.89)
IB	13(19.70)	33(36.67)	53(80.30)	57(63.33)
IIAc	08(12.12)	32(33.56)	58(87.88)	58(66.44)
ICh	13(19.70)	26(28.89)	53(80.30)	64(71.11)

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Discussion

There was a discernible variation of observation in second phase compared to the first phase between healthy & P-TB cases in our sensor array. In the first phase notable difference of responses was observed in 10 chemicals compared to 11 in the second phase. In the accuracy parameters, high sensitivity (> 80%) was in 8(eight) analytes viz. IB, IIBa, ICh, IAb, ICd, IEc, IAe, ICf in the first phase compared to 3(three) viz. ICh, IEc, IAb in the second phase; ICa showed both high specificity and positive predictive value (>75%) in both the phases. However, in the binary logistic regression 4(four) analytes were fairly strong components viz. ICa, IIBa, IB, IAb in the first phase compared to 2(two) viz. ICa, I in the second phase.[25]

Exhaled breathe analysis in disease detection has historically taken two major directions. The first approach uses spectroscopy and other methods to recognize the individual volatile and non-volatile organic compounds that are remarkable in the exhaled breath.[7,26] The second path is an advanced procedure with the colorimetric sensor array to find pattern of changes based on the entire blend without qualitative or quantitative detection of specific volatile and nonvolatile components as diseases biomarkers in the exhaled breathe.[10,11,27]

We have pursued the latter method in our research work with the total exhaled breath analysis with array-based chemical sensing, based on interactions of different porphyrin with analytes and can have future clinical application of showing limited provisional signature pattern which is an excellent benchmark for the next phase of research. Each of the indigenously synthesized porphyrin based chemicals in our series provided evident changes after exposure to total exhaled breath even when higher cut-off at ten RGB difference in all the participants presenting with pulmonary tuberculosis (P-TB) cases.

In our sensor array, maximum sensitivity of 86·46 percent could be attained, specificity up to 78.89 percent and positive predictive value up to 77·11 percent. In a breath analysis using gas chromatography and mass spectroscopy in patients hospitalized for suspicion of pulmonary tuberculosis where the pattern recognition analysis identified patients with positive sputum cultures with 82·6 per cent sensitivity.[7] Breath biomarkers were identified by others with 85 percent accuracy in active P-TB with time-slice alveolar gradients, comprising oxidative stress products and volatile metabolites, when sputum culture, microscopy, and chest radiography all positive or negative.[28] Indian scientists are working on human breath to detect early P-TB with an electronic nose technology.[29]

A multicentre research using concentrated breath, automated thermal desorption, gas chromatography, and surface acoustic, noted naphthalene, benzene and alkanes derivatives as biomarkers of P-TB with sensitivity 71·2 per cent, specificity 72 per cent; accuracy was up to 84 per cent in age-matched subgroups. The investigators predicted 13 per cent positive predictive value of P-TB in a prevalence of five per cent.[30]

Upcoming researches are exploiting other methods. The urease breath test for tuberculosis provided the possibility to diagnose TB though biological plausibility is restricted by co-infection with other urease-producing organism.[31] Differential ion mobility spectrometry method assessed VOCs in experimental mycobacterial chronic intestinal infection in animal model with the potential to become a valuable tool.[32]

The strength of our study was that, we synthesized all the compounds with ultra-high purity confirmed by thin layer chromatography and the compounds were diluted with non-interacting petroleum ether solvent. Secondly, the diagnostic accuracy was assessed to compare with studies based on other methods. Thirdly, though conventional five or more RGB difference is judged important and was consistently noted in great majority of the sensor elements, we used higher cut-offs (≥10) for interpretation as we were dealing with human participants. Lastly, in exhaled breathe analysis the mass spectrometer consumes about 45 minutes, sputum smear takes two days, sputum culture up to three months, whereas our sensor system needed 15 minutes time period for the required outcomes with portability, simplicity of use to suggest as a tool to aid early identification of P-TB cases.[29]

We had several limitations. The sensitivity and specificity of any method is of paramount importance conventionally used as parameters in bio-psycho-social sciences. Our study was limited by the possible lack of expected sensitivity and specificity, predictive value and likelihood ratios; possibly small sample size had limited the statistical power. Secondly, we worked in the empirical methods in the resource poor settings. Thirdly, there was likely referral bias, as all the study was conducted in an urban tertiary care chest clinic. Fourthly, due to administrative reasons we included all the P-TB cases irrespective of the status of diagnosis or therapy. However, broader adoption will solve these potential confounders.

Future directions of this pattern recognition analysis will help us reach a new screening test that is highly selective as well as simple, rapid, inexpensive and non-invasive viable method of diagnosis for tuberculosis. The quality of interaction of porphyrin with VOCs can be controlled by suitable changes in porphyrin macrocycle like peripheral substituent and central metal. This might lead to fine tuning of senor array by identifying responsive porphyrin derivatives with better predictive value that has to be further standardized to achieve unique colour 'chemical signature' pattern. For this goal, the data need to be collected in additional phases for higher reliability and rational development of cost effective high accuracy sensor tool with broader adoption. To obviate all the potential biases large multicenter trial are needed to compare P-TB diagnosis with the "gold standard" of sputum culture. Further we will extend our study to persons suffering from other disorders and multiple co-morbidities with the standardized protocol to demarcate healthy from cases in total exhaled breathe.

To sum up, porphyrin array-based chemical sensing showed promising results for the direct diagnosis of pulmonary tuberculosis in total exhaled human breath. It would be worth evaluating colorimetric porphyrin elements based sensor array in direct diagnosis and prognosis of the active P-TB cases. To the horizon of our knowledge, we are yet to find study reported in the published literature from India on the use of porphyrin based sensors for human health and disease. This colorimetric sensing can be visualized as a futuristic model of non-invasive diagnosis to fix the chemicals of breath. Our encouraging results augment reliability to porphyrin based sensors to find novel and reliable non-invasive clinical diagnosis with a far reaching impact to establish new range of self-administered instrument.

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REFERENCE

1. Phillips M, Cataneo RN, Condos R et al. Volatile biomarkers of pulmonary tuberculosis in the breath. Tuberculosis (Edinb) 2007;87(1):44-52. | 2. Phillips M, Basa-Dalay V, Bothamley G et al. Breath biomarkers of active pulmonary tuberculosis. Tuberculosis (Edinb) 2010;90(2):145-151. | 3. Bourzac K. New Breath-Based Diagnostic. An innovative technique for detecting different biomarkers could result in a precise, easy-to-use diagnostic tool. [online] [cited April 18, 2013] Available from: http://www.technologyreview.in/biomedicine/20411/ updated March 14, 2008 | 4. Marczin N, Kharitonov SA, Yacoub MH et al. Disease markers in exhaled breath; p290. [online] [cited October 18, 2011] Available from: http://books.google.co.in/books | 5. Volatile Markers of Pulmonary Tuberculosis in the Breath. [online] [cited October 18, 2011] Available from: http://www.sbir.gov/sbirsearch/detail/229978 | 6. Now there is a breath test for tuberculosis. [online] [cited October 18, 2011] Available from: http://getbetterhealth.com/now-theres-a-breath-test-for-tuberculosis/2010.03.12 | 7. Van Beek SC, Nhung NV, Sy DN et al. Measurement of exhaled nitric oxide as a potential screening tool for pulmonary tuberculosis. Int J Tuberc Lung Dis 2011;15(2):185-192. | 8. Costa C, Bucca C, Massimiliano B et al. Unsuitability of exhaled breath condensate for the detection of Herpes viruses DNA in the respiratory tract. J Virological Methods 2011;173(2):384-386. | 9. Amann A, Corradi M, Mazzone P et al. Lung cancer biomarkers in exhaled breath. Expert Rev Mol Diagn 2011;11(2):207-217. | 10. Mazzone PJ, Hammel J, Dweik R et al. Diagnosis of lung cancer by the analysis of exhaled breath with a colorimetric sensor array. Thorax 2007;62:565-568. | 11. Mazzone PJ, Wang XF, Xu Y et al. Exhaled Breath Analysis with a Colorimetric Sensor Array for the Identification and Characterization of Lung Cancer. J Thorac Oncol 2012;7(1):137-142. | 12. Pal R, Dahal S, Gurung A. Colorimetric sensor: the new horizon in health and disease. Asian Journal Biochemical and Pharmaceutical Research 2013;3(2):92-99. | 13. RGB color model. [online] [cited May 21 2012] Available from: http://en.wikipedia.org/wiki/RGB_color_model retrieved on 28 July 2011 | 14. Xie Y, Hill JP, Charvet R et al. Porphyrin colorimetric indicators in molecular and nano-architectures. J Nanosci Nanotechnol 2007;7(9):2969-2993. | 15. Okamoto K, Chithra P, Richards GJ et al. Self-Assembly of Optical Molecules with Supramolecular Concepts. Int J Mol Sci 2009;10:1950-1966. | 16. Dunbar ADF, Brittle S, Richardson TH et al. Detection of Volatile Organic Compounds Using Porphyrin Derivatives. J Phys Chem B 2010;114 (36):11697-11702. | 17. Janzen MC, Ponder JB, Bailey DP et al. Colorimetric Sensor Arrays for Volatile Organic Compounds. Anal Chem 2006;78:3591-3600. | 18. Cao W, Duan Y. Breath Analysis: Potential for Clinical Diagnosis and Exposure Assessment. Clinical Chemistry 2006;52 (5):800-811. | 19. Boots AW, van Berkel JJ, Dallinga JW et al. The versatile use of exhaled volatile organic compounds in human health and disease. J Breath Res 2012;6(2):027108. | 20. Nunobiki O, Sato M, Taniguchi E et al. Color image analysis of cervical neoplasia using RGB computer color specification. Anal Quant Cytol Histol 2002;24(5):289-294. | 21. Shen H, Lu YD, Zhang YX et al. Quantitative chromatics analysis for computer imaging of cytologic subtypes of lung cancer stained by Papanicolaou stain. Anal Quant Cytol Histol 2000;22(3):263-266. | 22. Kai M, Nunobiki O, Taniguchi E et al. Quantitative and qualitative analysis of stain color using RGB computer color specification. Anal Quant Cytol Histol 1999;21(6):477-480. | 23. Revised National Tuberculosis Control Programme -An Overview. Central TB Division, Ministry of Health & Family Welfare, New Delhi [online] [cited May 21 2012] Available from: https://nrhmmis.nic.in/Notifications/ConcurEval/RNTCP%20presentation%20060209.pdf | 24. RGB verses CMYK Colours [online] [cited April 15 2013] Available from: http://www.minotaurgold.com.au/rgb-verses-cmyk-colours/ | 25. Pal R, Gurung A, Bhutia SD et al. Spotting biomarkers of pulmonary tuberculosis in human exhaled breath using porphyrin based sensor array. International Journal of Bioassays 2013;2(7):1019-1023. | 26. Probert SJ, Ahmed I, Khalid T et al. Volatile Organic Compounds as Diagnostic Biomarkers in Gastrointestinal and Liver Diseases. J Gastrointestin Liver Dis 2009;18(3):337-343. | 27. James D, Scott SM, Ali Z et al Chemical Sensors for Electronic Nose Systems. Microchim Acta 2005;149:1-17. | 28. Jain P, Thaler DS, Maiga M et al. Reporter phage and breath tests: emerging phenotypic assays for diagnosing active tuberculosis, antibiotic resistance, and treatment efficacy. J Infect Dis 2011;204Suppl4:S1142-1150. | 29. Handique M. Breath test by Indian scientists promises faster diagnosis of TB. [online] [cited June 15 2012] Available from: http://www.livemint.com/2011/11/07223240/Breath-test-by-Indian-scientis.html? atype=tp | 30. Phillips M, Basa-Dalay V, Blais J et al. Point-of-care breath test for biomarkers of active pulmonary tuberculosis. Tuberculosis (Edinb) 2012;92(4):314-320. | 31. Maiga M, Abaza A, Bishai WR. Current tuberculosis diagnostic tools & role of urease breath test. Indian J Med Res 2012;135:731-736. | 32. Purkhart R, Köhler H, Liebler-Tenorio E et al. Chronic intestinal Mycobacteria infection: discrimination via VOC analysis in exhaled breath and headspace of feces using differential ion mobility spectrometry. J Breath Res 2011;5(2):027103.