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ORIGINAL ARTICLE

The Diagnostic Reliability of a Novel 13-Carbon Urea Breath Test in the Identification of an Active Pulmonary Tuberculosis

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ABSTRACT

Background: *The accuracy of diagnostic tests in intervention studies for eradicating Mycobacterium tuberculosis is critical.* ***Objective:*** *This study aims to determine the threshold value for the innovative 13-carbon urea breath test in the diagnosis of pulmonary tuberculosis.* ***Methodology:*** *We conducted a prospective case-control research study to evaluate the accuracy of administering a dosage of 75 mg of C13-urea to a cohort of 200 volunteers, which included 100 individuals in excellent health and 100 individuals suffering from pulmonary tuberculosis. We used a jet nebulizer to deliver the C13-urea and a double-beam infrared spectrophotometer to analyze the exhaled breath.* ***Results:*** *The area under the curve (AUC) was ranging between 0.988 and 1. The test's sensitivity ranged from 98% to 100%, while its specificity ranged from 40% to 100%. We determined the ideal threshold to be 0.288, ensuring 100% sensitivity and specificity.* ***Conclusion:*** *The 13C urea breath test exhibited an extremely high degree of sensitivity in detecting pulmonary tuberculosis. This test offers the possibility to diagnose Mycobacterium tuberculosis in clinical practice with good sensitivity, low cost, and rapid results.*

INTRODUCTION

The Urea Breath Test (UBT) exploits the property of urease production to diagnose *Helicobacter pylori* bacteria, which enables it to quickly break down urea to ammonia and carbon dioxide (CO2). Also, *Mycobacterium tuberculosis* and *Mycobacterium bovis* are known to produce the urease enzyme[1]. The C^{13} urea breath test is considered a secure diagnostic approach, especially in children and pregnant women, because of its non-radioactive nature[2,3]. Also, it is replicable and performed simply in a medical setting. The principal carbon isotopic compounds employed in UBT are C^{14}, an isotope that is radioactive, and C^{13}, a naturally found and non-radioactive isotopes[3,4]. The replacement of the C^{14} component by C^{13} facilitates the secure transfer of breath specimens from medical facilities to laboratories for inspection[5].

A common cost-effective technique for the analysis of C^{13}-labelled CO2 is infrared spectrophotometry. Prior to administering C^{13}-labelled urea, a breath sample of 13 CO2 is taken to establish a baseline measurement. People with specific medical disorders possess a markedly increased likelihood of contracting tuberculosis (TB). The country of Iraq ranks seventh in the region with a substantial TB burden[6]. Tuberculosis (TB) is now commonly linked to Human Immunodeficiency Virus (HIV) infection[7,8]. One of the primary hurdles in the fight against this infectious disease is the absence of dependable diagnostic procedures and devices to track the progress of treatment[9]. Diagnostic errors have a negative impact on tuberculosis (TB), an infectious disease. The detrimental consequences on individuals and the community become clear when instances of tuberculosis (TB) are either neglected or diagnosed late. There is a pressing demand for rapid, on-site diagnostic instruments to address the difficulties in detecting tuberculosis and determining medication sensitivity[10]. The existing approaches reliant on in vitro growing necessitate an extended period, often lasting several weeks. Nucleic acid amplification techniques are unable to distinguish between viable and non-viable cells, and they do not provide information on phenotypic drug resistance[11,12]. Discovering the pathogen metabolic pathways is an attractive alternative to existing methods, which are associated with various limitations and disadvantages. The recognition of metabolic pathways may yield rapid and efficient innovative techniques for tuberculosis (TB) detection, hence improving diagnostic capabilities for HIV patients and adolescents[13]. The metabolic tests possess multiple advantageous characteristics, as they are regarded as harmless and have the ability to expedite point-of-care diagnoses and assess the effectiveness of drugs in the setting of tuberculosis. The application of stable, isotopically labelled materials and infrared

spectrometers in breath analysis can discern metabolic routes specific to infections by identifying labelled byproducts in exhaled breath. We are performing continuous research studies to assess the test's efficacy in healthcare situations[13].

METHODOLOGY

A case-control study was performed at the "National Tuberculosis Centre" in Baghdad, Iraq. The study ran from November 15th, 2022, to February 15th, 2024. The study enrolled two hundred participants, of whom one hundred were in excellent health. We used clinical, radiological, and laboratory examinations, including acid-fast bacilli, gene expert, and cultivation methods, to diagnose the pulmonary tuberculosis participants.

Ethical consideration:

The research obtained Ethical Approval from the "Research Ethics Committee of the Ministry of Higher Education in Iraq, as well as the Institutional Review Board of the Iraqi Ministry of Health." We asked participants to provide informed written consent before initiating any study-related procedures.

Criteria for Inclusion:

The included cases exclusively involved active pulmonary tuberculosis (APTB) and were not associated with HIV infection. The entire group consisted of adults who were 18 years of age or older.

Criteria for Exclusion:

Excluded cases included pulmonary tuberculosis associated with asthma, chronic obstructive pulmonary disease (COPD), and interstitial lung disease (ILD). The patient had a positive result of a urea breath test for *H. pylori* and a positive sputum culture of urease-positive bacteria.

Procedure of the modified Urea breath test:

The research project consisted of two aspects. The initial aspect involved evaluating the accuracy of UBT in individuals in excellent health (the control group) to determine the baseline of delta over baseline (DOB). The second aspect sought to assess the efficacy of UBT in detecting urease production by *Mycobacterium tuberculosis* in individuals having confirmed lung tuberculosis. The kits that consisted of C^{13}-urea lyophilized in an aluminum container seal was used. Each aluminum bags contains 75 milligrams of an active material, which is required to be diluted with three millilitres of sterile distilled water. The solution that is diluted was then nebulized using a Sprint nebulizer.

Prior to initiating the nebulization procedure, the initial samples of breath were collected & preserved using specialized sterile impermeable collection containers. These specimens functioned as the reference standard. The prepared solution was atomised, and the urea was inhaled. Two independent breath specimens were collected, fifteen and thirty minutes after the final inhalation. Analysis of all breath specimens collected within seven days of their initial collection was done. The isotope ratio of 13CO2 to 12CO2 had been determined using a double beam infrared spectrophotometer manufactured via "Delta Analytics GMBH", (Germany). The data was analyzed via "SAS", specifically version 9.2. "The decline curves of DOB were simulated utilizing Origin 5.0 software developed by Microcal Software, located in Northampton, MA."

RESULTS

The mean age of the control group was 44.8±12.4 years, ranging from 22 to 68 years. The mean age of the PTB group was 48.8±15.6 years, ranging from 22 to 79 years. The majority of reported instances of PTB were found in the age groups of 40-49 and 50-59, accounting for 30% and 24% of instances, respectively. The PTB gender distribution consisted of 34 (34%) females and 66 (66%) males. The sex distribution of HC was 32 (32%) female and 68 (68%) males (Table 1).

Table 1: Characteristics of all participants in the study

Characteristics	APTB (N=100)	HC (N=100)	P-value APTB vs HC
Age	48.8±15.6 (22-79)	44.8±12.4 (22-68)	0.053
Age group			
20-29	14	8	0.101
30-39	14	32	
40-49	30	36	
50-59	24	10	
=>60years	18	14	
Sex (M/F)	66/34	68/32	0.832
IGRA (+/-)	100/0	0/100	NA
AFB (+/-)	45/55	NA	NA
Gene Expert (+/-)	100/0	NA	NA
Culture on L-J media (+/-)	84/16	NA	NA

APTB= Active pulmonary tuberculosis; HC= Healthy control; NS= Non significant; NA= Not applicable
*** significant by Pearson chi square**

Out of the 100 patients with PTB, all of them completed the C13 urea breath test. Among these patients, 40 had a mean DOB reading equal to 3 or more, while none of the patients had a DOB measurement beneath zero throughout the 15 minutes. Out of a group of 100 individuals who were in good health, 30 individuals had a recorded DOB reading over zero, while 70 individuals had a recorded DOB value less than zero. Among the 30 healthy individuals who reported a DOB reading greater than zero, 28 of them recorded a DOB of 0.01 after 15 minutes of nebulization. Following a 30-minute period of urea inhalation, all instances of pulmonary tuberculosis (PTB) were reported to have a DOB measurement over zero, whereas 16 cases in the control group recorded a DOB result of 0.01. In relation to the total DOB before and after the nebulization process, all patients with PTB (pulmonary tuberculosis) reported a DOB value greater than zero. Out of these cases, 42 recorded a DOB mean greater than 3. On the other hand, 8 cases of the group serving as the control recorded a DOB reading equal to 0.01 (Table 2).

The mean measurements of the DOB among both PTB patients as well as individuals in good health prior to inhalation of the C13urea solution were documented as zero. Following the procedure of vaporizing, the average DOB values for the HC and PTB were (-1.6005±2.2949), with a range of (-6.5650 - 0.1050), and 4.1950±4.4848, with a range of (0.4710-14.4180), respectively. The average values of DOB for PTB patients at 15 and 30 were 4.8215±5.1889 and 3.5686±3.8484, respectively. By contrast, the values for healthy persons were comparatively lower: -1.2381±1.8392 and -1.9628±3.0717 (Table. 2).

Table 2: DOB (Delta over Baseline) measurements for healthy volunteers and patients with pulmonary tuberculosis

DOB Group		Pulmonary TB		Control		P value
		No	%	No	%	
DOB results after nebulization 15	-2.0---	-	-	22	22.0	0.0001*
	-1.0---	-	-	20	20.0	
	-0.01---	-	-	28	28.0	
	0.01---	16	16.0	28	28.0	
	1.0---	32	32.0	-	-	
	2.0---	12	12.0	2	2.0	
	3.0---	40	40.0	-	-	
	Mean±SD (Range)	4.8215±5.1889 (0.2170-16.0070)		-1.2381±1.8392 (-5.6050 - 2.9740)		0.0001#
DOB results after nebulization 30	-2.0---	-	-	20	20.0	0.0001*
	-1.0---	-	-	16	16.0	
	-0.01---	-	-	48	48.0	
	0.01---	36	36.0	16	16.0	
	1.0---	14	14.0	-	-	
	2.0---	16	16.0	-	-	
	3.0---	34	34.0	-	-	
	Mean±SD (Range)	3.5686±3.8484 (0.3380-12.8300)		-1.9628±3.0717 (-8.3000 - 0.3100)		0.0001#
UBT-TB (DOB mean) before and after nebulization	-2.0---	-	-	20	20.0	0.0001*
	-1.0---	-	-	12	12.0	
	-0.01---	-	-	60	60.0	
	0.01---	18	18.0	8	8.0	
	1.0---	30	30.0	-	-	
	2.0---	10	10.0	-	-	
	3.0---	42	42.0	-	-	
	Mean±SD (Range)	4.1950±4.4848 (0.4710-14.4180)		-1.6005±2.2949 (-6.5650 - 0.1050)		0.0001#

"*Significant difference between percentages using Pearson Chi-square test (χ^2-test) at 0.05 level."

"#Significant difference between two independent means using Students-t-test at 0.05 level."

The median values of the total DOB were substantially higher in patients with active pulmonary tuberculosis (APTB) at (2.21) (Interquartile range [IQR] 1.03 to 4.7) compared to healthy controls (HCs) at (-0.735) (IQR -1.421 to -0.1485). The p-value is less than 0.0001. The interesting observation, 7 cases were reported DOB more than 10. These cases were classified as advanced based on chest x ray classification (figure 1).

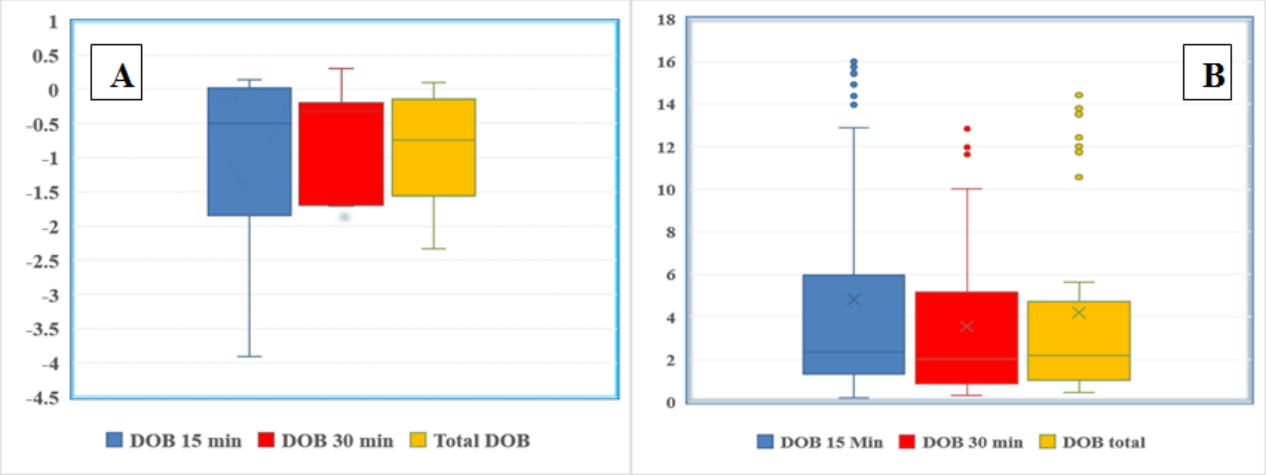

Fig. 1: Delta over baseline levels of both Healthy participants (A) and pulmonary tuberculosis patients (B): "Data are shown as box plots, where the boxes represent the first through third quartiles, the lines within the boxes represent the median, and the lines outside the boxes represent the minimum and maximum values (excluding outliers)."

The area under The area under the receiver operator curve (ROC) for the TB-UBT test at 15 minutes was 0.988 (SE, 0.012), whereas at 30 minutes it was 1 (SE, 0). The area under the ROC curve's for the TB-UBT (Total DOB mean) was 1 (SE, 0). In all instance the P value were less than 0.05(Table 3).

Table 3: Area Under the Curve (AUC) for different point of TB-UBT

Test Result Variables	(AUC)	S.R	P value	95% Confidence Interval	
				Lower Bound	Upper Bound
DOB after nebulization 15	0.988	0.012	0.0001#	0.966	1.000
DOB after nebulization 30	1.000	-	0.0001#	1.000	1.000
UBT-TB (Total DOB mean)	1.000	-	0.0001#	1.000	1.000

DOB = Delta over baseline
AUC= Area Under the Curve
SR = Standard error

The sensitivity, specificity and cut off values was determined by the ROC curve showing in figure 2. The optimal cut off for the "TB-UBT at 15 minutes" was found to be between 0.181 and 0.351. This values demonstrated a sensitivity range of 98% to 100% and a specificity of 98%. The optimal cutoff for the "TB-UBT" with an interval of 30 minutes was determined to be 0.324, with a sensitivity of 100% and a specificity of 100%. The optimal cutoff for UBT-TB (total DOB mean) was determined to be 0.288, having a sensitivity and specificity of 100% (Table 4) (Figure 2).

Table 4: Cutoff, sensitivity and specificity of TB-UBT

Test Result Variables	Positive if Greater Than or Equal To:	Sensitivity%	Specificity%
DOB results after nebulization 15	-.047500	100	70.0
	.116000	100	90.0
	.181000	100	98.0
	.351000	98.0	98.0
DOB results after nebulization 30	-.675000	100	40.0
	-.039000	100	80.0
	.324000	100	100
UBT-TB (Total DOB mean)	-.111500	100	80.0
	.288000	100	100

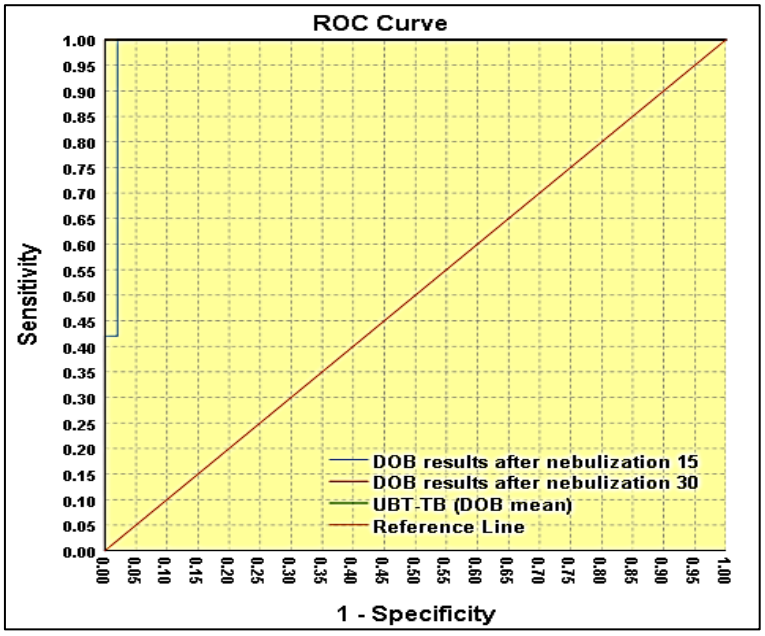

Fig. 2: ROC plots for TB-UBT by comparison between APTB and HC

DISCUSSION

The 13C-UBT has been recognized as a highly suitable diagnostic method for detecting *H.pylori* infection due to its non-invasive nature and high level of accuracy[14]. Nevertheless, the outcomes of 13C-UBT exhibit variations among different groups, depending on factors such as the dosage of 13C-urea and the type of equipment used. Consequently, it is necessary to adjust the cut-off point depending on the communities[15,16]. The proposed threshold values to diagnosis of *H.pylori* for 13C-UBT range from 2.5 to 4.0, whereas the dosage of 13C-urea varies from 75mg to 100mg[17,18].

The diagnosis of *M. tuberculosis* in this study was not determined by a specified cutoff established by the manufacturer. A threshold value was determined through the comparison of APTB versus the HC group. A value of 0.288 DOB was deemed indicative of a positive result for pulmonary TB. The cutoff value of 0.288 DOB was below the manufacturer-designated cutoff of 3.5 DOB that is employed for diagnosing *H. pylori* infections.

The requirement for an elevated threshold in diagnosing *H. pylori* may arise from the existence of urease-producing bacteria in the oral cavity such as *Streptococcus salivarius*. These bacteria might lead to inaccurate positive results when reduced thresholds are employed[19,20]. Another additionally factor contributing to the use of higher cutoff values in diagnosing *H. pylori* is the utilization of a standardized 13C-urea dose, typically above 75 mg, without considering age or body weight. Higher dosages of 13C-urea can affect the DOB results and result in a higher cutoff of 9. Another possible factor might involve the presence of *H. pylori*, as there have been no microbiological or physiological studies conducted to rule out the presence of urease-positive microorganisms. One potential reason for greater threshold readings in patients infected with *H. pylori* could be an amplified impact of the citric acid test meal on gastric emptying and stomach acidity[18]. In

contrast, a standard quantity of citric acid was neither administered nor mixed with a urea solution through the process of nebulization, the nebulization of the urea solution was done through the nose rather than the mouth and the exclusion of the urease-positive pathogen was carried out in APTB. So cutoff value was much lower than the manufacturer-specified cutoff employed for diagnosing *H. pylori* infections.

This study demonstrated multiple strengths. It is the first to determine the optimal cut-off point of 13C-UBT in instances of active pulmonary tuberculosis. Furthermore, a notable advantage is in the utilization of highly reliable diagnostic technologies, such as Gene expert, culture, and x-ray, to accurately diagnose and confirm the presence of *M. tuberculosis* infection. The value of 13C TB-UBT was evaluated, and it was found to have a greater sensitivity in detecting *M. tuberculosis*. This indicates that the new test might be used as an initial and supplementary tool for screening *M. tuberculosis* in high-risk populations. Furthermore, our research confirmed the presence of viable bacteria in the lungs of patients and indicated that a further measure must be considered to address the response of *M. tuberculosis* to antibiotic treatment.

Additionally, our study is subjected to some drawbacks. The validation study had a small sample size. Therefore, more research with a bigger sample size are necessary to fully understand and define the population in the "gray zone" and establish its boundaries. Despite this, we were still able to determine the sensitivity and specificity of 13C-UBT using the optimal threshold. However, using the small sample size is clearly feasible with such a cases diagnosed by gold standard tools and can be considered as borderline of DOB to diagnosis APTB. In addition to above limitations, this study was the first research to determine the accuracy of UBT in diagnosis of *M. tuberculosis* so patient factors may influence optimal threshold values which require to more optimization.

CONCLUSION

In conclusion, the DOB values in PTB patients exceeded those in controls. An appropriate cut-off point of 0.288 for DOB value was identified and validated as the first trial to diagnose APTB using 13C-UBT. The TB-UBT test can serve as an effective first screening technique for detecting *M. tuberculosis* infection in population-based research. This methodology has the capability to not only identify tuberculosis (TB), but also verify its ability to function properly. As a result, it provides an opportunity to assess the seriousness of infections caused by TB and evaluate the alterations in the burden of bacteria after giving anti-mycobacterial treatment. This helps to validate the efficacy of drugs employed for treating tuberculosis.

Declarations:
Consent for publication: Not applicable
Availability of data and material: Data are available upon request.
Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.
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