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ORIGINAL ARTICLE

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Assessment of sensitivity of acoustic reflex decay test in diagnosis of patulous eustachian tube

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Abstract

Background It has long been believed that the patulous eustachian tube is a unique but uncommon condition, with an estimated incidence ranging from 0.3 to 6.6% in the general population. Hearing one's own voice and breathing, or autophony and aerophony, are the most common symptoms of PET, along with frequent auditory fullness.

Aim This study is to establish a standardized protocol for the audiological evaluation of patulous Eustachian tube using acoustic reflex decay test during different breathing tasks.

Methods Between January 2022 and December 2022, the ENT department at Suez Canal University Hospitals undertook an observational case-control study. Fifty-two adult patients who present to the Suez Canal University ENT department with complaints of autophony, auditory fullness, or aerophony are diagnosed with patulous eustachian tube dysfunction through endoscopic examination of the tympanic membrane's synchronized movement with respiration.

Results The incidence rate of PET among adults was approximately 3 patients/1000 patients/year. The age of the whole studied group ranged from 20 to 60 years with mean ± SD of 37.4 ± 12.6 years. They included 23 males (44.2%) and 29 females (55.8%). The most common symptom among the studied group was aural fullness (46%), while the least common symptom was aerophony (12%); the most common risk factor among the studied patients was allergy (46.2%), while the least common was radiotherapy (3.9%); 66.7% of the patients with GERD were males, while 66.7% of the patients who lost weight were females; 58.3% of the patients with allergies were females, and also 60% of the patients with thin built were females. In the case group, there is a significant higher change of middle ear compliance in ipsilateral nostril breathing than the mouth breathing and nasal breathing. The sensitivity of the acoustic reflex decay test to diagnose PET with reference to the gold standard test is 88.5% (23/26*100), the specificity is 100% (26/26*100), and the positive predictive value is 100% (23/23*100).

Conclusion The sensitivity of the acoustic reflex decay test to diagnose PET with reference to the gold standard test is high, and the specificity is very high with very good diagnostic accuracy.

Keywords Acoustic reflex decay, PET, Patulous eustachian tube, Diagnostic accuracy

Background

It has long been believed that the patulous eustachian tube (PET) is a rare but uncommon condition with an estimated incidence ranging from 0.3 to 6.6% in the general population [1]. PET is typically more common during weight loss, radiation therapy, pregnancy, allergies,

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or reflux disease; nonetheless, it is usually idiopathic in most cases [2].

Hearing one's own voice and breathing, or autophony and aerophony, are the most common symptoms of PET, along with frequently auditory fullness [3]. When symptoms are severe, they might cause patients to experience severe depressive episodes and suicidal thoughts, among other psychological consequences [4].

Although several approaches have been utilized over time, there is currently no consensus-building procedure for assessing a patent eustachian tube (ET). The pharyngeal opening of the ET is commonly examined using nasal endoscopy; however, diagnostic visualization of the ET is typically very challenging because of the narrow tubular lumen, the opening's eccentricity to the line of visualization, the valve area's concealment, and the presence of secretions [5].

Synchronous movement of tympanic membrane (TM) with respiration during ear endoscopic examination is the gold standard for identifying PET [6]. Long time-base tympanometry is another crucial test for diagnosing the really patulous ET [1]. When the patient inhales deeply, the TM's acoustic immittance is measured (though one nostril). In this test condition, the acoustic immittance is measured to a probe tone using commercial tympanometers set to the "reflex-decay" settings (usually 226 Hz). The immittance changes cyclically as a result of the TM's inner and outward excursions during respiration. It is crucial that the patient not breathe too loudly throughout the test because the microphone in the ear canal will detect this as a signal and produce noise artefact [7].

Other tests that have been documented include nasal audiometry, which involves measuring the audibility in the ear and presenting a sound in the nose [1]. According to reports, this has produced positive outcomes in Japanese literature. Regarding the auditory reflex decay test as a substitute for the gold standard of endoscopic examination during respiration, the available literature presents contradictory data.

The study aims to assess the sensitivity and the specificity of acoustic reflex decay test in diagnosis of patients with patulous Eustachian tube in ENT department at Suez Canal University Hospital.

Methods

An observational case control research was conducted here. The study was carried out from January 2022 to December 2022 at Suez Canal University Hospitals in the ENT department.

Adult patients who present to the Suez Canal University ENT department with complaints of autophony, auditory fullness, or aerophony are diagnosed with patulous eustachian tube dysfunction through endoscopic examination of the tympanic membrane's synchronized movement with respiration.

This study covered patients with a diagnosis of patulous eustachian tube, patients of both sexes, and patients who were at least 18 years old. Patients having tympanic membrane perforations, otitis media, or concomitant cholesteatomas were not included.

This is a convenient sampling method.

The sample size has a total of 52 participants which are recruited using the following equation [8]:

$$n = \left[\frac{z_{\alpha/2}}{\mathrm{E}}\right]^2 * \frac{s_{n(1-\mathrm{S}_n)}}{(\mathrm{P})}$$

Using a 95% CI and the prevalence of bilateral PET was 77% with sensitivity (Sn) was 83.8% (9) and E = 10% the margin of error in addition to adding 10% drop out rate, the total of 52 participants were recruited, and were divided upon 2 groups: 26 participants in each group.

Study procedure

All patients were informed about the study aim and procedures before signing an informed consent before participation.

All patients were subjected to the following procedures:

1) Detailed history taking including the following items

Demographic data: Name, age, gender, and phone number.

Symptoms of patulous eustachian: aerophony, crackling sound, pulsatile tinnitus, auditory fullness, and autophony. Variables that aggravate, like exercise, such as sniffing and putting the head in a dependent position as relieving elements. Environmental allergies, weight loss, thin build, autoimmune diseases, radiation therapy, and gastric reflux are precipitating causes.

Associated comorbidities: diabetes, hypertension, previous ear disease, previous ear surgery, history of ototoxic drugs intake, and special habits of medical importance (smoking).

2) Local examination

Inspection: inspection of the ear for discharge.

Otoscopic examination: The otoscopic exam was performed by gently pulling the auricle upward and backward.

Basic audiological evaluation: Subjects in both groups had full audiological evaluation including pure tone audiometry, speech audiometry, and tympanometry . Both groups did not include patients with a history of middle ear effusion or ET dysfunction, conductive hearing loss (CHL), or high ART.

Endoscopic examination

The endoscopic testing through external auditory canal was performed to diagnose patulous eustachian tube by inspection of synchronous movement of the tympanic membrane with respiration.

1) Equipment

In our clinic we use Clarinet Inventis Middle Ear Analyzer. There is no specific setting for PET test. The machine will be set to the acoustic reflex decay test protocol (allowing for a 15-s recording/10 s analysis window) using 226 Hz probe tone. The acoustic reflex stimulus setting will be conducted on the ipsilateral (affected) ear in cases group and on either ear in control group. The stimulus level was also set to 10 dB above ART at 500 Hz.

2) Test procedure

After the participant was positioned erect in relation to the tympanometer, each person underwent a standard tympanogram. In order to prevent artefact in the recording, the patients were told that they would need to cease breathing for a mere second before beginning the test and then breathe deeply, slowly, and silently.

The test was repeated 3 times in different sittings:

- The first setting was assessed during mouth breathing.
- The second setting was during nostril breathing with closure of the month.
- The third setting was with ipsilateral nostril breathing and closure of the mouth to increase nasopharyngeal pressure.

Patients were closely monitored for compliance with these instructions during testing. Some patients need to repeat the test until they can perform it properly.

Statistical analysis

Version 26 of the SPSS program (Statistical Package for Social Science) was used to computerize and statistically analyze the data that had been gathered. The Kolmogorov Smirnov test was used to determine whether the data had a normal distribution. Tables and graphs were used to display the data when suitable. Frequencies and relative percentages were used to depict qualitative data. As said, the chi square test ($\chi 2$) was employed to look into any associations between the qualitative variables. The mean and standard deviation were used to express quantitative data. The difference between the two groups' quantitative variables parametric and non-parametric was computed using the Mann-Whitney U test. P value < 0.05 denotes a significant difference, whereas $P \geq 0.05$ denotes a non-significant difference.

Results

The incidence rate of PET among adults was approximately 3 patients/1000 patients/year. The incidence rate of PET was calculated based on the flow rate of adults attending ENT clinical at Suez Canal University Hospitals complaining of otological symptoms per year (9900).

This study was conducted on 52 participants divided into 2 groups: 26 patulous eustachian tube cases and 26 controls. The age of the whole studied group ranged from 20 to 60 years with mean ± SD of 37.4 ± 12.6 years. They included 23 males (44.2%) and 29 females (55.8%). The mean age of the cases was 35.3 ± 12.4, while the mean age of the control group was 39.5 ± 12.6. The cases group included 10 males (38.5%) and 16 females (61.5%), while the control group included 13 males (50%) and 13 females (50%). There is no statistically significant difference between cases and controls regarding the age and the gender. However, there is a female predominance in the cases group (61.5%) in comparison to (38.5%) who were males (Table 1).

This chart shows that the most common symptom among the studied group was aural fullness (46%), while the least common symptom was aerophony (12%) (Fig. 1). This chart shows that the most common risk factor among the studied patients was allergy (46.2%), while the least common was radiotherapy (3.9%) (Fig. 2).

This table shows that 66.7% of the patients with GERD were males, while 66.7% of the patients who lost weight were females; 58.3% of the patients with allergy were females, and also 60% of the patients with thin built were females. All the patient with autoimmune disease or underwent radiotherapy were females. There is no significant relationship between the cases risk factors and their gender (Table 2). However, correlation between risk factors and gender must be taken cautiously in the current study and can be more clearer in a larger sample size.

This table shows that the mean values of the results of mouth breathing, nasal breathing, and ipsilateral nose breathing tests were significantly higher in cases

Table 1 Comparison between cases and controls regarding the socio-demographic characteristics ($N = 52$)

		Cases $n = 26$	Control $n = 26$	$N = 52$	P value
Age (years)	Mean ± SD	35.3 ± 12.4	39.5 ± 12.6	37.4 ± 12.6	0.209*
Gender	Male	10 (38.5%)	13 (50%)	23 (44.2%)	0.402#
	Female	16 (61.5%)	13 (50%)	29 (55.8%)	

* Mann-Whitney U test # Chi squared test

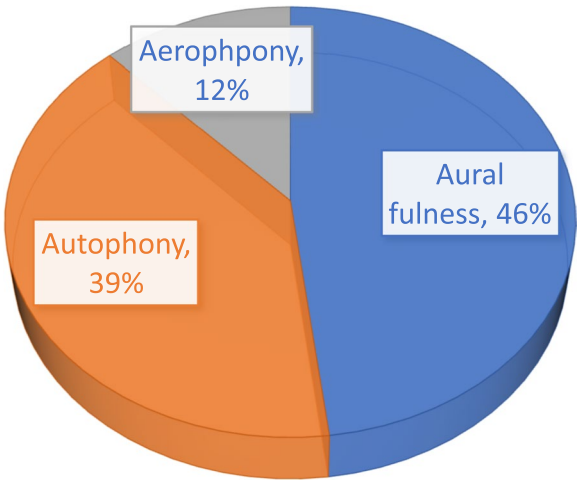

Fig. 1 Pie chart displaying the relative frequency (%) of the symptoms among the studied cases ($n = 26$)

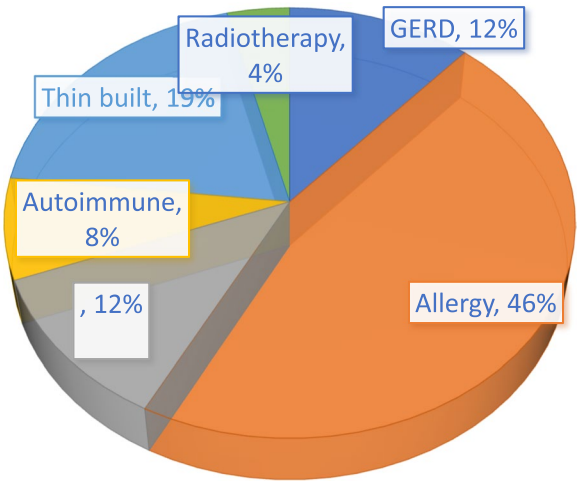

Fig. 2 Pie chart displaying the relative frequency (%) of the risk factors among the studied cases ($n = 26$)

than controls. In the case group, there is a significant higher change of middle ear compliance in ipsilateral nostril breathing than the mouth breathing and nasal breathing. Therefore, the ipsilateral nostril breathing is the most sensitive step to diagnose the PET by acoustic reflex decay test (Table 3).

There were 3 patients among cases had positive acoustic reflex decay test (Fig. 3).

This table shows that the sensitivity of the acoustic reflex decay test to diagnose PET with reference to the gold standard test is 88.5% (23/26*100), the specificity is 100% (26/26*100), and the positive predictive value is 100% (23/23*100), which means that the percentage of the subjects with a positive test and have PET is 100%. The negative predictive value is 89.7% (26/29*100), which means that the percentage of the subjects with a negative test result and do not have the disease is 89.7%. Diagnostic accuracy is (23+26)/52*100= 94.2%. It measures the ability of the test to detect a disease when it is present and detect the absence of a disease when it is absent (Table 4).

Discussion

Regular intermittent opening and shutting of the ET are essential to its effective functioning. The ET is passively compressed while at rest, but while performing actions like chewing, yawning, and swallowing, the paratubal muscles can actively open the ET [9]. Any deviation in the opening and closing muscles is referred to as ET dysfunction (ETD), which is further divided into two categories: patulous dysfunction and obstructive dysfunction [7].

The incidence rate of PET in adults in the current study was roughly 3 individuals per 1000 patients annually. The study by Bance (2019) estimates that the incidence of PET in the general population ranges from 0.3 to 6.6% [1].

Measuring compliance over time (COT) is arguably one of the most crucial tests for the diagnosis of the really patulous ET. When the patient inhales deeply, the TM's acoustic immittance is measured (though one nostril). Under this particular test scenario, the acoustic immittance is evaluated in relation to a probe tone, typically using the "reflex-decay" settings used in commercial tympanometers (usually 226 Hz). The immittance changes cyclically as a result of the TM's inner and outward excursions during respiration [1].

The purpose of the current study was to evaluate the auditory reflex decay test's sensitivity in diagnosing patients with patulous eustachian tubes. Fifty-two individuals were included in this study, split into two groups

Table 2 Relationship between risk factors and gender of the studied cases ($n = 26$)

		GERD ($n = 3$)	Allergy ($n = 12$)	Weight loss ($n = 3$)	Autoimmune ($n = 2$)	Thin built ($n = 5$)	Radiotherapy ($n = 1$)	*P* value
Gender	Male	2 (66.7%)	5 (41.7%)	1 (33.3%)	0	2 (40%)	0	0.704[#]
	Female	1 (33.3%)	7 (58.3%)	2 (66.7%)	2 (100%)	3 (60%)	1 (100%)	

[#] Chi squared test

Table 3 Middle ear change of compliance measured in ml during different breathing tasks in the acoustic reflex decay test

Change of middle ear compliance measured in ml.		Cases $n = 26$	Control $n = 26$	*P* value*
Mouth breathing	Mean ± SD	0.08 ± 0.04	0.01 ± 0.01	< 0.001
Nasal breathing	Mean ± SD	0.14 ± 0.07	0.03 ± 0.04	< 0.001
Ipsilateral nostril breathing	Mean ± SD	0.34 ± 0.12	0.05 ± 0.06	< 0.001

* Mann-Whitney *U* test

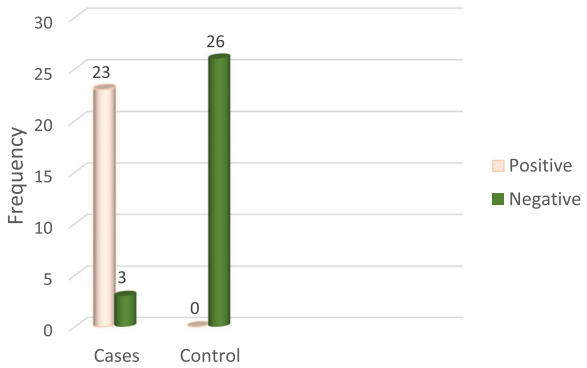

Fig. 3 Bar chart displaying the frequency of the results of the acoustic reflex decay test among cases and controls

Table 4 Accuracy of the acoustic reflex decay test in the diagnosis of PET with reference to the gold standard test among the studied group ($N = 52$)

Acoustic reflex decay test	Gold standard		Total
	Positive	Negative	
Positive	23 (TP)	0 (FP)	23
Negative	3 (FN)	26 (TN)	29
Total	26	26	52

TP true positive, *FP* false positive, *TN* true negative, *FN* false negative

of 26 patulous eustachian tube patients and 26 controls. The average age of the cases was 35.3 ± 12.4 years, and 61.5 percent of the cases were female, compared to 38.5 percent of male cases.

This result was consistent with a study by Ward et al. (2017) that sought to characterize a sizable cohort of patients presenting with patulous Eustachian tubes. Of these, 54% of PET patients were female, and the mean age at which symptoms first appeared was 38.0 ±20.0 years [10]. Similarly, Kawamura et al.'s (2019) prospective assessment of Sen-En Rifu Hospital's medical records revealed 56 patients—21 men and 35 women—with a mean age of 49.3 ± 19.0 years [11]. Moreover, Suzuki et al.'s (2018) study included 76 patients with confirmed PET, 36 of whom were men and 40 of whom were women [12].

According to a study by Dornhoffer et al. (2014), the preponderance of women may have an impact on the correlation between PET and combined oral contraceptive pill use or high levels of estrogen during pregnancy. Increased fat metabolism brought on by elevated estrogen levels results in a decrease in the OF tissues surrounding the Eustachian tube [13].

In terms of symptoms, we discovered that 46.2 percent of patients in the current study experienced auditory fullness, 38.5 percent autophony, and 15.3 percent aerophony. Changes in nasopharyngeal pressure in PET can easily transfer to the middle ear cavity, resulting in autophony, aerophony, and auditory fullness. The mechanism of sampling technique may be one of the causes for the higher prevalence of aural fullness in the current study. A few patients with a PET diagnosis complained of aural fullness when they visited an ENT outpatient clinic and were referred for a basic audiological evaluation. As a screening tool, the acoustic reflex decay was carried out in suspected patients if the evaluation came back normal. Ultimately, the patient exhibiting favorable outcomes was redirected to the ENT clinic for an endoscopic assessment in order to validate the PET diagnosis.

A higher prevalence was noted in the Kawamura et al.'s (2019) study, wherein definitive PET cases exhibited voice autophony, a sense of auditory fullness, and breathing autophony in 93.6 percent, 87.2 percent, and 78.2 percent of cases, respectively. Three of the symptoms were present in 67.9% of the cases [11].

According to Ward et al.'s (2017) study, voice autophony (93%) and breath autophony (92%), auditory fullness (57%), pulsatile tinnitus (17%), and crackling or rumbling sounds were among the common symptoms (14 percent) [10].

According to Suzuki et al.'s (2018) study, 17.1% of PET patients reported having a nasal voice that coincided with the development of PET symptoms such auditory fullness, autophony of the breathing noises, and 91 percent had autophony of their own voice [12].

Excessive pressure and sound transmission from the nasopharynx to the ME through an open ET is what causes auditory complaints. Autophony of voice is frequently the most bothersome auditory sign of PET. This symptom might be particularly annoying for people whose jobs need them to communicate frequently. Aerophony is one of the most important markers to

distinguish PET from SSCDS, whereas autophony is a common symptom between the two conditions [14].

According to the current study, radiotherapy was the least common risk factor among the individuals examined, with allergies accounting for the majority (46.2%). (3.9 percent). Among the patients, 11.5% experienced weight loss and GERD, and 7.6 percent had autoimmune illnesses, while 19.3 percent were thinly developed.

The mucosa and soft tissue of the nasal cavity are affected by allergic rhinitis over time; persistent inflammation results in atrophy of the soft tissue surrounding the ET opening. Additionally, long-term use of decongestants and nasal steroids will hasten this process.

Variable outcomes include environmental allergies (49 percent), weight loss (35 percent), laryngopharyngeal reflux (33 percent), anxiety (31 percent), autoimmune (13 percent), and neuromuscular illness as prevalent comorbidities, according to a study by Ward et al. (2017) (8 percent) [10].

According to Ward et al. (2017), tonic contraction of the TVP muscle was more frequently detected in patients who were experiencing stress or anxiety during nasopharyngoscopy. Since the TVP muscle dilators the eustachian tube, activation of this muscle predisposes to PET [10]. According to Choi et al. (2018), there may be a correlation between the rise in patulous eustachian tubes and the rise in allergy and reflux disorders [15].

About the connection between PET and weight loss, according to Yoshida et al. (2019), patients with PET had connective tissue—including the OF—that was noticeably smaller. Loss of the Ostmann fat pad around the opening of ET may be associated with acute weight loss [16]. This observation is comparable to that of Poe and Pyykkö (2011), who suggested that the loss of adipose tissue within the cartilaginous section of the ET was the cause of patulous ET based on nasopharyngeal endoscopy of the ET lumen [17].

The capacity of a tympanometer to graph the tympanic membrane's COT, sometimes referred to as long time-base tympanometry and often available on the reflex decay testing function in commercial tympanometers, is beneficial for the evaluation of ET function. In order to see the effect on the COT of the tympanic membrane, the patient is given instructions to undertake various breathing exercises throughout the test, such as breathing through the ipsilateral nostril. A PET is indicated by variations in tympanic membrane compliance that are proportionately impacted by intake and exhale [18]. It has been shown that 226 Hz pure tones can be used to identify PET across time through breathing-synchronous middle ear compliance with blockage of the contralateral nostril and forced breathing through the ipsilateral nostril [19, 20].

In the current investigation, we discovered that cases had considerably greater mean values of the middle ear compliance change over time during tests of mouth breathing, nasal breathing, and ipsilateral nostril breathing than controls. This is due to PET's facilitation of easy transmission of nasopharyngeal pressures to the middle ear cavity, which results in cyclic variations in middle ear immittance that are timed to respiration.

According to McGrath and Michaelides (2011), who sought to develop a standardized procedure for the audiologic assessment of the patulous Eustachian tube using a standard clinical tympanometer, they discovered that the positive experimental group had statistically significantly higher values for the tests of mouth breathing, nasal breathing, and ipsilateral nose breathing than the Controls [18].

As per McGrath and Michaelides (2011), who determined that the ipsilateral nostril condition is the specific breathing condition that appears most likely to result in respiratory-synchronous middle ear compliance, we observed that the performance of the acoustic reflex decay test in the ipsilateral nostril breathing has the greatest sensitivity in detecting change of middle ear compliance inconsistence. By remembering the mechanics of air transmission between the nasopharynx and oropharynx, we may explain that. Even in patients diagnosed with PET, the soft palate moves toward the posterior pharyngeal wall during mouth breathing resting conditions, resulting in limited transmission of air pressure from the oropharynx to the nasopharynx and negligible changes in middle ear compliance. While ipsilateral nostril breathing concentrates these pressure changes in the tested side and permits maximum increases in COT, nasal breathing will directly raise nasopharyngeal pressure [18].

In order to ascertain whether the 678 Hz probe tone distinguishes between patulous ET and closed ET more clearly than the 226 Hz probe tone, Pyne et al. (2018) evaluated the results of COT testing in presumed closed and patulous eustachian tubes. They discovered in their ET practice that variations in COT measurements in both tones were higher in cases than control. However, since it lessens the chance of picking up internal noise brought on by variations in nasopharyngeal pressure, utilizing a 678 Hz pure tone is more effective at predicting PET than any other tone that has been utilized previously. Anecdotally, the false-positive rate was too high, and the signals were too tiny to properly interpret with the 226 Hz tone when COT testing was conducted in the eustachian tube clinic [7].

We discovered that both the Acoustic Reflex Decay Test and the gold standard test (endoscope) have a highly significant ability to distinguish between cases and healthy

populations ($P < 0.001$). Additionally, the acoustic reflex decay test has a 94.2% diagnostic accuracy, 100% specificity, 100% positive predictive value, 100% negative predictive value, and 88.5 percent sensitivity when used to detect PET in comparison to the gold standard test.

By comparing the results of the acoustic reflex decay test with the gold standard test, we observed in the current study that the acoustic reflex decay test was unable to diagnose three PET cases (false-negative results). All three of these patients had allergic rhinitis, which can cause alternating nasal obstruction and affect middle ear compliance during different breathing tasks. This is because the test requires an opened nasal pathway in order to be performed accurately [21]. When the endoscopic test (the gold standard) is not conducted during the same session as the acoustic reflex decay test, there is a clear disparity between the results of the test and the results of the gold standard test in patients with allergic rhinitis.

We did not uncover any incidences of false-positive results in the current study.

However, we must keep in mind that the type AD tympanogram may produce false-positive results because of increased middle ear compliance. According to Sogebi et al., this is because the tympanic membrane has higher compliance when there is normal middle ear pressure. This is seen in the discontinuity of the ossicular chain or monomeric membrane [22].

McGrath and Michaelides (2011) demonstrated that the assessment of COT while performing breathing exercises exhibited remarkable sensitivity (83.8%) and specificity (94.7%) for PET (9).

The acoustic reflex decay test is a highly useful diagnostic tool for PET. This is a result of the test's simplicity, speed, non-invasiveness, and sensitivity. It can be added to the PET test battery, particularly in situations where invasive maneuvers are not available or do not provide a precise and coherent picture of ET. The acoustic reflex decay test, however, should only be utilized in dependable patients who are able to complete breathing exercises correctly and whose symptoms are correlated with clinical signs. This is especially true for patients with type (AD) tympanograms. Furthermore, the test can be used to assess patients' progress because the degree of COT measurements decreases after the treatment plan is initiated.

This study was limited by the inability of some patients to perform breathing exercises correctly, particularly the ipsilateral nostril breathing step, the low incidence rate of PET, and the challenge of recruiting patients for the cases group.

Because the auditory reflex decay test has the most sensitivity for identifying changes in middle ear compliance, this study advised performing it during ipsilateral nostril breathing. In addition, if the patient has allergic rhinitis, perform the endoscopic test and the acoustic reflex decay test in the same session. If the patient has a type AD tympanogram, do not use the acoustic reflex decay test to prevent misleading negative results.

Conclusion

When compared to the gold standard test, the acoustic reflex decay test has an 88.5 percent sensitivity, a 100% specificity, and a 94.2 percent diagnostic accuracy for PET diagnosis. Adults had a yearly incidence rate of about 3 individuals per 1000 patients for PET.

Abbreviations

PET	Patulous eustachian tube
ET	Eustachian tube
TM	Tympanic membrane
CHL	Conductive hearing loss
SPSS	Statistical Package for Social Science
ETD	ET dysfunction
COT	Compliance over time

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Authors' contributions

AA was responsible for study conception and design, material preparation, data collection and analysis, and writing of manuscript. AA contributed to the design and implementation of the research; also he involved in the supervision of the work. YM had given his help in scientific writing and contributed to the design of the research. All authors discussed the results and approved final manuscript.

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Availability of data and materials

Data are available in Excel sheet upon request.

Declarations

Ethics approval and consent to participate

Ethical clearance was obtained from the "Research Ethics Committee, Faculty of Medicine, Suez Canal University" at its meeting on (22\6\2022) with reference number 4982. All patients were informed about the study aim and procedures before signing an informed consent before participation.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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