Molecular Characterization of *Trichomonas tenax* Causing Pulmonary Disease

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INTRODUCTION

Trichomonas tenax , is of world-wide distribution in man and monkeys and noted in older works as *Trichomonas buccalis* and *Trichomonas elongate* (1). *T. tenax* was first discovered by Muller in 1773 in an liquid culture of tartar from teeth (2). *T. tenax* could be a pear-shaped, flagellate protozoan with an undulating membrane (3).It is ordinarily individual harmless commen-sally of the human mouth living in tartar around teeth and in carious cavities, in addition as in nasopharyngeal region (4,5).

T. tenax feeds on microorganisms in its surroundings, infection unfold rough secretion, drop spray and kissing, or on contaminated dishes, glasses and hands or mistreatment contaminated tooth-brushes and drinking water (6).

Several studies disclosed that T. tenax has been isolated from samples of dental calculi and sub-gingival plaques of patients with odontology issues chiefly chronic marginal periodontal disease and disease Furthermore; it's been detected in patients with acute lesion periodontal disease (7,8,9).

The prevalence of *T. tenax* in many series ranged from four to fifty three, it's going to exceed the prevalence within the duct of *Trichomonas vaginalis* in adult females (10,11,12).

T. tenax have been found in other diseased organs near the oral cavity including sinusitis, tonsillitis , jaw abscess, cancer of the lingua and oesophagus and in the hypochlorhydric stomach (3).

T. tenax incontestable within the lungs of patients with chronic respiratory organ diseases as carcinoma, respiratory organ abscesses, bronchiectasis, bronchitis and respiratory disease (13).

Diagnosis of pulmonary trichomoniasis relies on spotting of *T. tenax* in sputum , bronchoalveolar lavage, pleural fluid , or pulmonary parenchyma by microscopic examination of wet mount preparation and stained smear (14 ,15).

The microscopic examination of wet mount preparation needs the presence of viable motile Trichomonads (16,17,18) Molecular biological techniques like enzyme chain reaction (PCR) targeting organism's repetitive DNA has proved a reliable diagnostic approach (19,20).

materials and methods

Study area

The study included the collection of samples from Al Hussein Educational Hospital (consultative clinics), Al Zahra specialized Hospital (Specialized Center for respiratory organ Disease), Cancer medicine Hospital and specialized Center for Respiratory and Chest Diseases within the holy governorate of Karbala.

Collection of specimens

The sample distribution included 100 sampling in the descriptor of pulmonary fluid and sputum The patients whose sampling were collected were divided into sections depending on the respiratory disease they were suffering from (lung cancer, pulmonary fibrosis, lung abscess, pneumonia, T.B. , tonsillitis , chronic bronchitis, bronchitis, bronchial asthma , laryngitis).

Taken directly from the patient after the diagnosing of respiratory disease by the specialist doctor and the samples are saved (sputum) in in plastic bottles, special clean and sterile either pulmonary fluid samples are obtained directly from the laboratories of hospital mentioned .

Diagnosis of *Trichomonas tenax* by pcr

-Primers

One sort of primer was employed in this study to diagnose Trichomonas tenax parasites victimization PCR technique, victimization the prefixes utilized by Suharni and zeehaida; 2017 The prefixes were ready by the Korean company Bioneer:

Primer	Sequence		Amplicon
18sRNA gene	F	AGCAGCTGCGGTTCCAT	1054pb
	R	CTTGTTACCACTTCTCCT	

- DNA extraction of sputum

The Quick Genomic polymer Extraction Kit (DSBIO) was utilized by DSBIO (CHINA) to extract the polymer from humor samples. The extraction process was carried out in accordance with instructions issued by the manufacturer

-DNA extraction of pulmonary fluid

The Universal Genomic DNA Extraction kit from DSBIO was used to extract the DNA from the pulmonary fluid samples and the extraction process was carried out according to instructions issued by the manufacturer.

- DNA test

DNA was extracted from the sputum and pulmonary specimens using the Nanodrop Spectrophotometer (THERMO. USA), which detects nucleic acids and measures their concentration. It is detected by determining the concentration of DNA (ng \ µl DNA) with its purity measured through The absorbance reading of the wavelength ranges from (260 / 280nm) .

Preparation of polymerase chain reaction mixture

The polymerase chain reaction was prepared using the prepared equipment from Promega Corporation and according to the instructions of the company.

PCR master mix	**Volume**
DNA template 10-50ng	5µL
Toxo-B1 gene Primary primers Forward primer (10pmol)	1.5µL

Toxo-B1 gene Primary primers Reverse primer (10pmol)	1.5µL
PCR water	12µL
Total	20µL

Pcr thermocycler conditions

The polymerization chine reaction was investigated using a pcr thermocyler, the device is programmed as in the following table:

PCR Step	Repeat cycle	Temperature	Time
Initial denaturation	1	94C	5min
Denaturation	30	93C	5sec
Annealing		55C	30sec
Extension		72C	2min
Final extension	1	72C	5min

Results

In this study, one hundred patients were examined. respiratory organ *Trichomonas* was found in (23%) out of one hundred patients examined (Table 1). The cluster of one hundred patients had forty three females and 57 males. *Trichomonas tenax* was found in (20.93%) females and (24.56%) males (Table 1). *T. tenax* was a lot of oftentimes obtained in males than in females, however the distinction wasn't statistically vital.

The study showed that there was a relationship between parasite and metastasis diseases. variety of cases were diagnosed in folks with completely different metastasis diseases. the best incidence of parasitic infection was in folks with rubor, with fortieth and tenth speech organ and respiratory organ symptom. The results of the applied math analysis showed no vital variations within the incidence of parasitic infection and therefore the calculated worth of Kay (4.574) (Table 1) .

Table (1) frequency of *Trichomonas tenax* by pcr in relation to patient pulmonary disease

Pulmonary Disease	Samples	No	Male	Infected	%	Female	Infected	%	Total	Total %
Lung cancer	Plural f.	10	9	3	33.33	1	0	0	3	30
Pulmonary fibrosis	=	10	6	2	33.33	4	0	0	2	20
Lung abcess	=	10	10	1	10	0	0	0	1	10
Pneumonia	sputum	10	6	1	16.66	4	1	25	2	20
tuberculosis	=	10	5	2	40	5	1	20	3	30
tonsillitis	=	10	3	1	33.33	7	3	42.85	4	40
Bronchitis	=	10	6	2	33.33	4	1	25	3	30
COPD	=	10	5	2	40	5	0	0	2	20
asthma	=	10	2	0	0	8	2	25	2	20
laryngitis	=	10	5	0	0	5	1	20	1	10
total		100	57	14	24.56	43	9	20.93	23	23
X^2		5.908*				4.846*			4.574*	
P value		0.749				0.774			0.870	

The study showed that share the proportion of smokers and other people with metastasis diseases is that the highest percentage of non-smokers, wherever the proportion of twenty seven.45% and 18.36%, severally, and was the best rate of infection in those who smoke and metastasis diseases of females and amounted to thirty seven.5%. The results of the applied math analysis showed no vital variations within the incidence of parasitic infection and therefore the worth of Kay calculated one.164(Table 2).

Table(2)positive cases of T. tenax detected by pcr in relation to smoking

Type	**Sex**						**Total**		**%**
	Fmail			**Mail**					
	No	**Infected**	**%**	**No**	**Infected**	**%**	**No**	**Infected**	
smoking	43	11	25.58	8	3	37.5	51	14	27.45
non	17	3	17.64	32	6	18.75	49	9	18.36
total	60	14	23.33	40	9	22.5	100	23	23
X^2	0.429*			1.290*			1.164*		
P value	0.513			0.256			0.281		

The examination demonstrated that the level of smokers and individuals with respiratory ailments is the most noteworthy level of non-smokers, where the level of 27.45% and 18.36%, separately, and was the most astounding rate of contamination in individuals who smoke and respiratory maladies of females and added up to 37.5%. The aftereffects of the measurable investigation demonstrated no huge contrasts in the rate of parasitic disease and the estimation of Kay ascertained 1.164(Table 2)

The study showed that individuals with metastasis diseases who are suffering from polygenic disease have the best rate of parasitic infection of individuals WHO don't have polygenic disease, and reached thirty two.75% and 9.52%, severally. the best incidence of parasitic infection in females with polygenic disease was thirty eight.09%. all-time low incidence of parasitic infection was in females while not polygenic disease, that reached five.26%. The results of the applied math analysis showed vital variations within the incidence of parasitic infection and therefore the worth of Kay calculated (1.837) Table(3)

Table (3) positive cases of *Trichomonas tenax* detected by pcr in relation to Diabetes

Type	Sex						Total		%
	Mail			Female					
	No	Infected	%	No	Infected	%	No	Infected	
Diabetes	37	11	29.72	21	8	38.09	58	19	32.75
non	23	3	13.04	19	1	5.26	42	4	9.52
total	60	14	23.33	40	9	22.5	100	23	23
X^2	2.208**			6.166*			7.426*		
P value	0.137			0.013			0.006		

Conclusions

1. The study showed a link between *Trichomonas tenax* infection and respiratory organ diseases by victimisation pcr take a look at.

2. The presence of the impact of smoking issue on the incidence of *Trichomonas tenax* infection and each sexes.

3. Diabetes is a crucial issue that will increase the possibility of *Trichomonas tenax* infection.

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