

Epidemiology Study of Pulmonary Trichomoniasis in Babylon Province

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Abstract: The current study was conducted in the period from September 2011 till may 2012 to investigate the infection of *Trichomonas tenax* in patients had lung inflammation and other Respiratory tract disease in Babylon province.

The total number of examined samples was 653 sputum samples for different ages in addition to 653 saliva samples collected from hospitals, medical centers, and special clinics. The samples were examined by direct smear method and wet preparation method in hospital and medical centers laboratories and the advanced parasites laboratory in the college of the Science / Babylon University.

This study include the relation of age , sex with the infection percentage of Pulmonary trichomoniasis ,The result shows the total infection rate 1.99 % in sputum samples division to 3.07% for male and 1.27% for female, and 7.81% for saliva samples. The static analysis result shows significant difference between male and female infections and significant difference for infection rate in saliva samples.

1. INTRODUCTION

Human is host to three distinct *Trichomonas* species, The genitourinary *Trichomonas vaginalis* , the intestinal *T. hominis* and the oral *T. tenax* can be morphologically, serologically, epidemiologically and culturally distinguished from one another (Lewis *et al.* , 2003) .

T. tenax is an anaerobic commensal of the human oral cavity. There are studies that relate to its prevalence in patients with Marginal Chronic Periodontitis (Sarowaska *et al.*, 2004). *T. tenax* had a pear-shaped, flagellated protozoon with an undulating membrane, has four free anterior flagella, and the fifth one is attached to the cell surface forming an undulating 13 membrane. The parasite size is about 15 µm in length. Parallel to the membrane is disposed in the interior of the cell, a bundle of microtubules called the coasta. Crossing the cytoplasm as an axis and protruding substantially from the rear end, has a structure composed of microtubules called axostyle. A single core with endosome available in the anterior, near the point of insertion of the flagella. *T. tenax* axostyle enclosed glycogen particles, and the open side face the nucleus. *T. tenax* lacks mitochondria, Golgi apparatus is called par basal body and no cyst stage is formed (Jongwutiwes *et al.*, 2000; Duboucher *et al.*, 2000).

Transmission of parasite is through saliva, droplet spray, and kissing or use of contaminated dishes and drinking water (Brook *et al.*, 2007). World widely, its prevalence in the mouth ranges from 4 to 53% (Sarowska *et al.*, 2004).

Usually, *T. tenax* is found as a commensal of the human oral cavity in those with poor oral hygiene. In the case of empyema, anaerobic and aerobic bacteria in addition to the trichomonads are often present and *T. tenax* eats them (Bellanger *et al.*, 2008).

Bellanger *et al.* (2008) study shows *T. tenax* in Pleural liquid and Microscopic analysis of the pleural liquid was positive for flagellated motile parasites identified as *Trichomonas tenax* by their size and morphology ,PCR was carried out on pleural fluid, bronchoalveolar fluid,

Saliva, sputum, and stool. Pleural fluid, sputum, and bronchoalveolarFluid were positive for *T. tenax*.

Species other than *T. tenax* can be responsible for infection of the upper respiratory tract, and their identification is achieved by PCR methods. To illustrate this, *Trichomonas hominis*, regarded as an intestinal commensal, was identified in a purulent pleural fluid (Jongwutiwes *et al.*, 2000) and *T. vaginalis*, generally common in the genitourinary tract, was recently reported in a neonatal respiratory infection (Carter and Whithaus 2008).

In all cases of Bronchopulmonary trichomoniasis infection, bacteria *S. constellatus* isolated from sputum samples and lung tissues and concenter the nutrition source of *T. tenax* parasite and in this way the parasite ability to reproduction increase in the necrosis lung which is the favorable environment for *T. tenax* and that cause elongate the lung cancer, lung abscess, or bronchiectasis or other chronic and acute symptoms (Mallat *et al.*, 2004; Chinche *et al.*, 2005).

2. THE AIMS OF THIS STUDY WERE THE FOLLOWING

- i. To know the prevalence's average of *T. tenax* infection among patients suffering from Respiratory tract diseases.
- ii. Investigate the relationship between age and sex to the pulmonary trichomoniasis infection.

3. MATERIALS & METHODS

In this study 653 sputum samples had collected from patients infected by lung cancer, lung abscess, or bronchiectasis or other chronic and acute lung inflammations, in addition to 653 saliva samples, all the samples examined in laboratories of hospitals in Babylon province by microscopic examination methods:

3.1. Wet Preparation Method

By putting a smear from the sample on clean sterilized slide and use cover slip to get a clear vision and examine in 10x, 40x. Identification of the parasite by its motile and size (Mallat *et al.*, 2004).

3.2. Staining Method

Taking a smear from the sample on clean sterilized slide and fixed it by passing the slide on a flame, then use a several drops from a special stain (Giemsa or Methylene blue stain) for 5min. and wash the slide by Distilled water. The staining method helps us to distinguish the flagellum and the undulating membrane of the parasite (Bafghi *et al.*, 2009).

3.2.1. Preparation of *Myrtus Communis L. Parts Before Extraction*

The areal parts leafs collected from especial arboretum in Babylon province and classified in Biology Department /Science College /Babylon University ,leafs separate from plant and washed ,cleaned , dried well, crashed by mill.

3.2.2. Laboratory animals

25 Swiss white mice Balb/C weight 25 ± 2 gm divided to 5 groups each group include 5 animals were put in iron cages and supplied with especial food in house keeper of Science College /Babylon University and infected by *Trichomonas tenax* in the mouth by scratch the gingival tissue around the tooth by needle and spread the soluble that contain the Trichomonas(30-60 up parasite in 1ml) on gingival tissue, and examined daily to confirm the infections of the animals, also record the signs and symptoms for 30 day to ensure that the trichomoniasis infection reached to the lungs by notice the actions of animals, cough , asphyxia, behavior .

After that we dosed the infected animals with different concentrations (40 , 60 , 80, 100 mg/kg) of *Myrtus communis L.* cold water extracts (1ml) twice a day for ten days by stomach tube .

3.3. Statical Analysis

The results has been counted by Qui- square under significance level ($p \leq 0.05$) to compare between infection rate (Al-Rawi, 1992).

4. RESULTS

The examined of the sputum showed a large number of neutrophils and numerous flagellated, motile organisms with the typical appearance of *T. tenax*, visible on wet preparations and Giemsa staining (fig. 1).

During the mentioned period, 653 specimen of sputum collected from different ages of patients suffering from problems in respiratory tract.

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The results of this study showed the total percentage rate infection of pulmonary trichomoniasis was 1.99% for 653 sample, the maximum infection percentage was 4.16% for age (55-60) year and the minimum rate was 1.63% for age (37-42) year, while no infection recorded in age (1-6) year and (7-12) year, the statistical analysis result shows significant difference in infection rate for different ages (2. 385) , table (1).

Table1. The percentage rate of patients infected by pulmonary trichomoniasis in Babylon province

ages	Examined No.	Infected No.	%
1-6	28	0	0
7-12	44	0	0
13-18	63	2	3.17
19-24	101	2	1.98
25-30	126	3	2.38
31-36	60	2	3.33
37-42	61	1	1.63
43-48	67	1	1.49
49-54	47	1	2.12
55-60	24	1	4.16
61-66	32	0	0
total	653	13	1.99

* Significant difference, $\chi^2 = 2.385$.

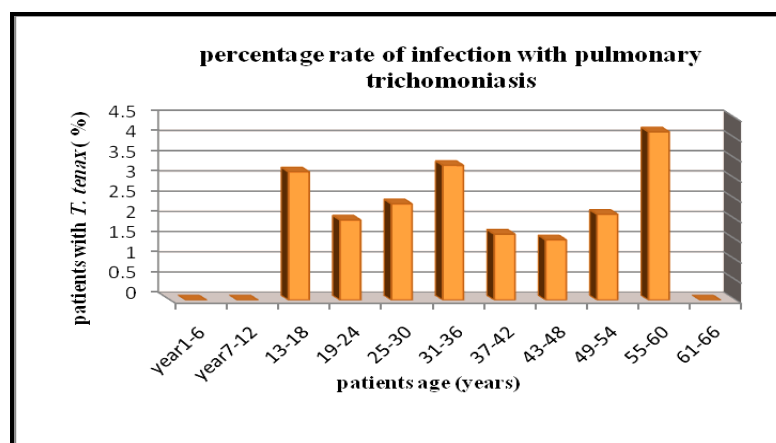


Figure1. The percentage rate of patients infected by pulmonary trichomoniasis

Table (2) shows the number of examined and infected with the total infection percentage rate for pulmonary trichomoniasis male patients according to ages, the total percentage rate was 3.07%, the maximum infection percentage was 11.11% for ages (31-36) year and the minimum percentage was 1.88% for ages (25-30) year, while no infection recorded for ages (1-6) , (7-12) , (43-48), (61-66) year. The statistical analysis result shows significant difference in infection rate for different ages (3. 25).

Table2. The percentage rate of male patients infected by pulmonary trichomoniasis

ages	Examined No.	Infected No.	%
1-6	6	0	0
7-12	20	0	0
13-18	25	1	4
19-24	35	1	2.85
25-30	53	1	1.88
31-36	18	2	11.11
37-42	35	1	2.85
43-48	20	0	0
49-54	27	1	3.70
55-60	15	1	6.66
61-66	6	0	0
total	260	8	3.07

* significant difference, $\chi^2 = 3.25$.

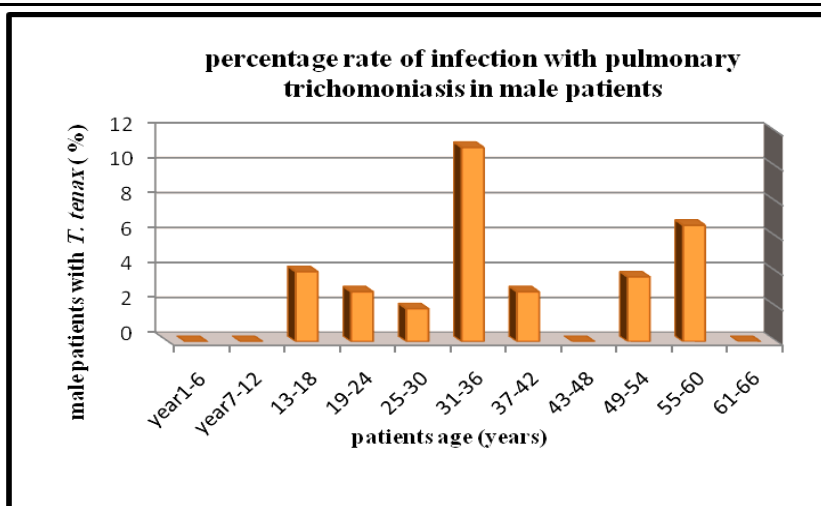


Figure2. Distribution of pulmonary trichomoniasis infections in male patients.

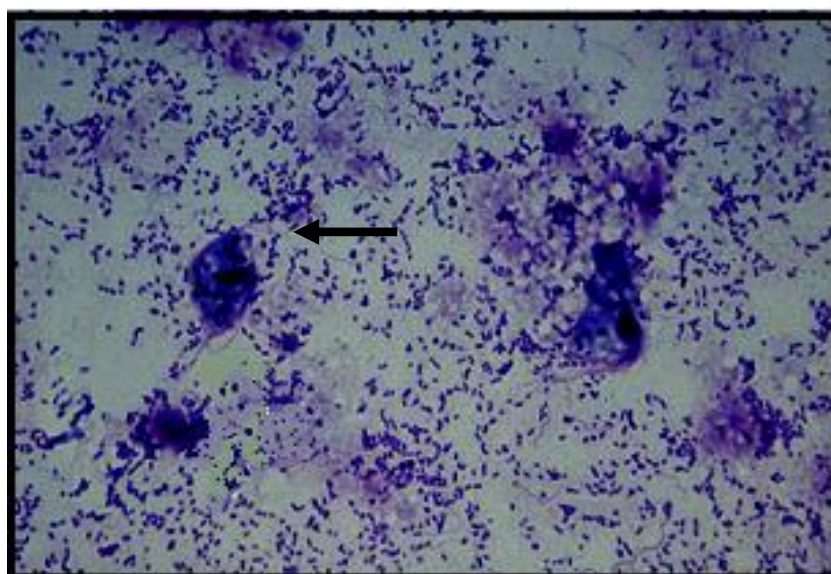


Figure1. *T. tenax* in sputum sample staining by Giemsa stain (40x)

Table (3) shows the number of examined and infected with the total infection percentage rate for female patients according to ages, the total percentage rate was 1.27%, the maximum infection percentage was 2.73% for ages (25-30) year and the minimum percentage was 1.15% for ages (19-24) year (figure 4).

The statistical analysis result shows significant difference in infection rate for different ages (1. 50).

Table3. The percentage rate of female patients infected by pulmonary trichomoniasis

ages	Examined No.	Infected No.	%
1-6	22	0	0
7-12	24	0	0
13-18	38	1	2.63
19-24	66	1	1.51
25-30	73	2	2.73
31-36	42	0	0
37-42	26	0	0
43-48	47	1	2.12
49-54	20	0	0
55-60	9	0	0
61-66	26	0	0
total	393	5	1.27

* significant difference, $\chi^2 = 1. 50$.

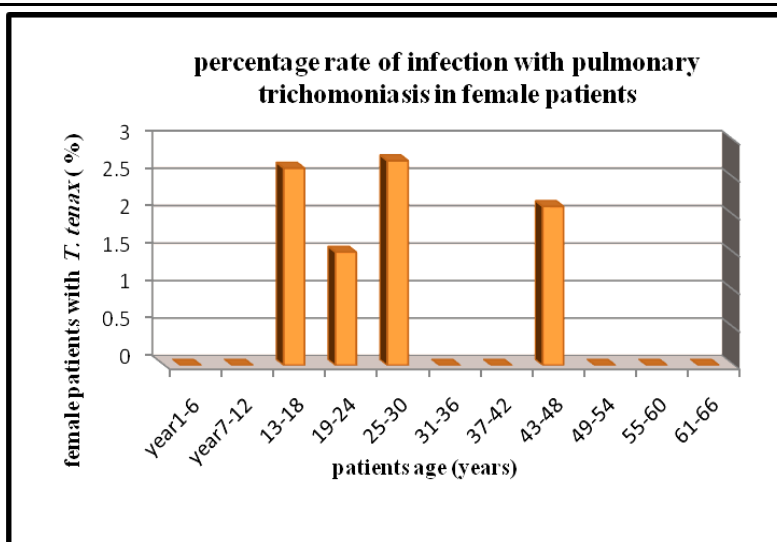


Figure4. Distribution of pulmonary trichomoniasis infections in female patients

In addition, information's recorded for the patients about the teeth cleaning habits, the frequency of cleaning and the instrument of cleaning (toothbrush),653 saliva spearmen examined, figure (2) shows *T. tenax* in saliva samples stained by Methylene blue (40x).

The results show the total infection of *T. tenax* was 7.81%, the maximum percentage was 13.95% for patients how's never used toothbrush, during the examination we found a height rate of *Candida albicans* which characterize by its finger shape and most of patients had a calculus and caries and suffering from a permanent smell in their mouth especially for patients how's never used toothbrush or one in a day, the minimum percentage was 2.73% for patients how's use toothbrush three times in a day, table (4). The statistical analysis result shows significant difference in infection rate for different cleaning habits by using toothbrush (15.609).

Table4. Teeth cleaning habits in relation to prevalence of *T. tenax* in saliva samples

frequency	<i>T. tenax</i>			notes
	Examined No.	Infected No.	%	
non	215	30	13.95	<i>C. albicans</i> , smell, caries, calculus.
1xday	270	16	5.92	<i>C. albicans</i> ,caries , calculus.
2xday	95	3	3.15	caries
3xday	73	2	2.73	non
total	653	51	7.81	

* significant difference, $\chi^2=15.609$

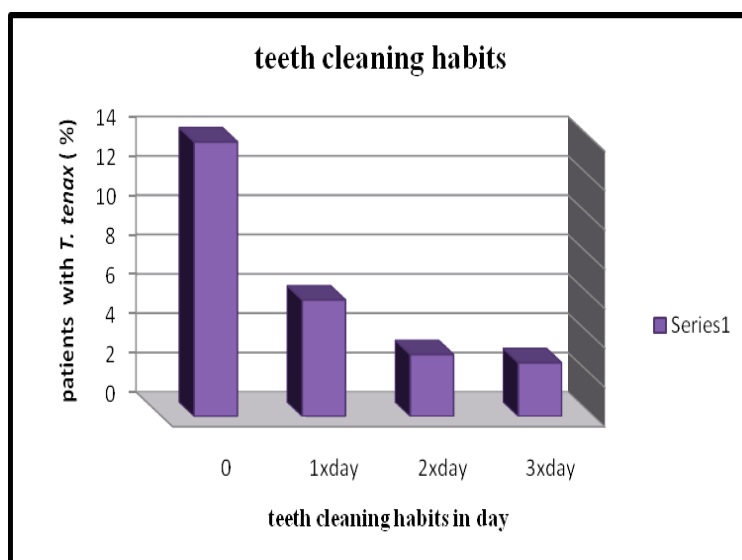


Figure5. Distribution of *T. tenax* infection rates in saliva samples according to teeth cleaning habits.

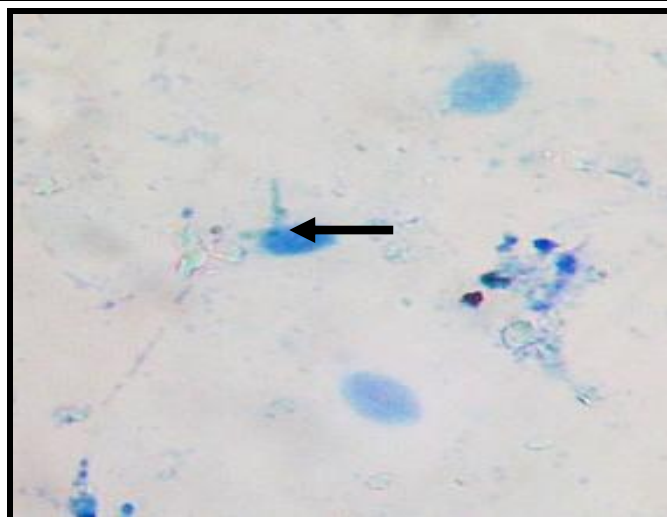


Figure2. *T. tenax* in saliva sample staining by Methylene blue stain (40x).

5. DISCUSSION

The studies of pulmonary trichomoniasis are rare, and few cases of *Trichomonas empyema* have been reported in patients suffering from problems in respiratory tract (lung cancer, lung abscess, or bronchiectasis and other respiratory tract diseases). Since 1942 the first case of pulmonary trichomoniasis recorded by Glaubach and Guller (1942). After that many cases appeared and most of them depending on the clinical diagnosis and symptoms of the patients.

In our study results, the total percentage rate of pulmonary trichomoniasis was 1.99% for 653 patients suffering from problems in respiratory tract and this result is low compared with A Russian study demonstrated pulmonary trichomoniasis in 19 (17%) of 112 patients, mostly in patients with lung cancer, lung abscess, or bronchiectasis (Tumka ,1956), maybe the cause of this low percentage rate due to the weather factor because the temperature decrease for long period in Russia make the respiratory disease and pulmonary inflammation rise compared with these diseases in our country.

In other studies of pulmonary trichomoniasis a single cases recorded in different aeries of the world, in France Bellanger *et al.* (2008) indicate one case of 33-year-old women suffered from chest Paine and lung inflammation, the laboratory examination of sputum and plural fluid show the presence of *Trichomonas tenax* infection.

The results of this study was incompatible with the results of Egyptian study, included 250 individuals ,20 cases among all examined were positive in one or more method (with wet mount preparation, Giemsa-stained smear, and Kupferberg *Trichomonas* broth culture giving, (PCR) assay) for pulmonary trichomoniasis diagnosis, a total prevalence of 12 cases (12%) in (group I) 100 immuno compromised patients with chest complaints, 8 cases (8%) in100 patients with chronic pulmonary diseases (group II), and none in 50 healthy individuals as controls (group III) (Mahmoud and Rahman,2004) , these results were higher in comparison with our study results.

If we examine closely the total infestations rate in the study which mentioned earlier we will find all the rates were higher from the rates that we get in our study in spite of all the hard conditions in our country in a course of time until this time and this proved that Iraq environment is better and most clean from mentioned countries.

The maximum infection percentage was 4.16% for age (55-60) year, this result was compatible with other cases of trichomoniasis infection for advanced ages as in a case recorded in France by mallat *et al.*(2004) for a 58 years old man and in Louisiana, a pulmonary trichomoniasis case recorded for a 59 years old man Hersh (1985), in Taiwan one case recorded for a 55 years old man by Wang *et al.*(2006) , in Germany a case for 45 years old female diagnosed by stratakis *et al.*(1999), in all these cases the patients suffering from respiratory failure, , lung abscess, or bronchiectasis and other respiratory diseases and most of them had Initial chest x-ray showed a moderate amount of right pleural effusion with elevation of the right hemi diaphragm (Wang *et al.*,2006).

The differentiation in the total infection rates due to the differences in the social levels and economic, healthy levels between the examined areas as well as our country exposed to many wars also

economic and medical siege led to prevalent many of parasitic and non parasitic diseases beside to the inconsistency of healthy level, economic, education consciousness level in the mentioned states, the difference of examination methods, the timing of this study from other studies also the size and type of the specimens which played very important rule in the differentiation of the recorded results compared with other studies.

If we compare the total percentage rate between both sex in table (2) and (3) will find the infestation rate for male was 3.07%, higher than female 1.27%, and the maximum infection rate of male 11.11% for ages (31-36) year and the minimum percentage was 1.88% for ages (25-30) year while the maximum infection rate of female 2.73% for ages (25-30) year and the minimum percentage was 1.15% for ages (19-24) year, with significant difference.

Our results was compatible to study of Mahmoud and Rahman (2004) indicate that Pulmonary trichomoniasis was prevalent at age ranged between 31 to 50 years, and in total males (10%) than females (5.5%) with no significant difference.

The decrease of pulmonary trichomoniasis infection in female may be due to the care or interest to themselves more than male and could be other factors lead to increase the infections in male such smoking, alcoholic, the exposure to cold weather than women because they participate in many hard works or jobs in our country, all these factors make the favorable conditions for pulmonary trichomoniasis infection , also many researchers found that the immunity of female more powerful than men to many diseases (Khan,2008).

We collected the samples of saliva according to the habits of teeth cleaning by tooth brush because many of study indicate that the infection of pulmonary trichomoniasis transmute from the mouth to the lungs through the pharynx, respiratory tract and air branches and cause respiratory disease (Mallat *et al.* , 2004 ;Chinche *et al.*, 2005).

The total percentage rate of infection in saliva was 7.81% according to toothbrush habits in a day, the maximum percentage was 13.95% for patients how's never used toothbrush with *C. albicans*, smell, caries, calculus found in these samples and, the minimum percentage was 2.73% for patients how's use toothbrush three times in a day with no fungi or calculus, with significant differences. These results was un compatible with the study of Ozumba *et al.* (2004) he found there is no association between the frequency of cleaning teeth habits by toothbrush and the Trichomonas infections, 52.69% for patients how's use toothbrush once in a day and 47.4% for patients how's use toothbrush twice with no significant differences, other studies indicate that the oral hygiene is very important to avoidance the infection and induced it and a poor oral hygiene is favorable conditions for oral and pulmonary trichomoniasis (Sarowska *et al.*, 2004;Brook *et al.*, 2007).

Maybe the cause of oral Trichomonas infections in patients who brushing teeth once or twice in a day due to the wrong method of tooth cleaning and use a bad quality of toothpaste that available in markets also the un care of eliminate the decay and caries and calculus, all these factors lead to increase the poor hygiene of mouth and as a result cause the infections of pulmonary and oral trichomoniasis.

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