

## A Study on Humoral Immunity and Oral Bacterial Diversity in Patients with *Trichomonas Tenax* Infection

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### ABSTRACT:

#### BACKGROUND:

*Trichomonas tenax* (T tenax) is a protozoan that inhabit the oral cavity of poor oral hygiene. It is found in the dental caries, tartar and periodontal area. It feed on the normal flora of the mouth. It had been found to cause pulmonary infection and respiratory diseases in immunocompromised patients and patients with advanced cancer.

#### OBJECTIVE:

To determine the humoral immune response to *Trichomonas tenax* and studying if there is any relation with specific bacteria.

#### METHODS:

Forty patients who consult Al-Kindy Teaching hospital –maxillofacial and dental department from June-2008 to January -2009. Two gingival swabs were taken from those patients, one examined directly for the presence of *T tenax* by light microscope and other swab was cultured on Blood, Chocolate and MacConkey's agar for isolation of oral bacteria. Blood was collected from patients for estimation of serum IgG, IgA, IgM, C3 and C4 levels by using radial immune diffusion method.

#### RESULTS:

The study group consists of forty individuals, their ages range from 6-65 years. Male more than female (22:18), thirty of them was smoker, 25% of them were positive for T tenax. The types of bacteria that were isolated are a normal flora of the mouth like *Streptococcus viridans*. In spite of the level of Immunoglobulins and complement in both groups lie within normal values, there was a significant increased in serum IgM level and significant decreased in serum IgG, IgA, C3 and C4 level.

#### CONCLUSION:

There was increased in the prevalence of *T tenax* infection due to low social class and low oral hygiene. There was no specific bacteria that was confected with it. Lastly, increased in *T tenax* infection when there is an immune suppression as in advanced cancer patients and on radiotherapy and or chemotherapy.

**KEY WORDS:** trichomonas tenax , immunity, bacteria.

### INTRODUCTION:

*Trichomonas tenax* (*T. buccalis*) is a harmless commensal of the human oral cavity which lives in the mouth of the patients with poor oral hygiene and advanced periodontal diseases, carious tooth cavities and less often in tonsillar crypts<sup>(1)</sup>. Its prevalence in the mouth ranges from 4 to 53%<sup>(2)</sup>. Transmission is through saliva, droplets spray and kissing or use of contaminated dishes and drinking water<sup>(3)</sup>. This trophozoites survive in the body as mouth scavengers that feed primarily on local bacteria that located in the tarter between the teeth,

gingival margin around the gum and tonsillar crypts<sup>(4)</sup>. So the coinfection with bacteria of oropharynx supports the growth of *T. tenax* in the mouth. The typical *T. tenax* infection does not produce any notable symptoms, on occasion bronchopulmonary infection caused by *T. tenax* have been reported mainly in patients with underlying cancer or other lung diseases<sup>(5)</sup>. On the other hand, the use of immune suppressive drugs to treat cancer and to prevent rejection of transplant<sup>(6)</sup> and the global epidemic of Acquired Immune Deficiency Syndrome (AIDS) had led to exacerbation of preexisting parasitic infection, as well as complicated multiple infections of protozoa<sup>(7)</sup>. The development of protective immunity against protozoa required exposure to different antigens of parasite and maturation of immune system.

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Each protozoa had different development of protective immunity, some associated with increased in different classes and subclasses of antibodies (8) and interaction between CD40/ CD40 ligand had a central role in the induction of both humoral and cellular immunity to protozoa (9).

Other protozoa activates B-cells to secrete specific immunoglobulins and IL-10 (10).

So immunocompetant hosts control and eliminate the protozoal infection. However, in patients with defect in cellular immune response (AIDS, Cancer and malnutrition) leads to susceptibility to infection with commensals microorganism (11).

So the objective of this study is to detect the prevalence of this parasite and if there is any coinfection with specific bacteria. The last goal is to determine the human humoral immune response towards this parasite.

**PATIENTS AND METHODS:**

The study population consists of forty individuals who attended Al-Kindy teaching hospital - Maxillofacial department and dental department from June- 2008 to January 2009.

1. Two swabs were taken from gingival and periodontal area, one was examined directly under light microscope for the presence of *T tenax*, and other findings were recorded (*Entamoeba gingivalis*, fungi, pus cells, red blood cells and epithelial cells (12).
2. The other swab was cultured on Blood agar, Chocolate agar and MacConkey agar. Further culturing and tests were done for isolation of specific species of bacteria according to its type (12).
3. Serum were collected from same individuals and stored at -10C<sup>0</sup> till examination was done for immunoglobulins (IgG, IgM and IgA) levels and complement (C3 and C4) level by

single radial immune diffusion method (Biomaghrib-Tunis).

**Statistical analysis:** Student t-test used in analysis the data statistically (13).

**RESULTS:**

The study group consists of forty individuals. The range of ages 6-65 years old (X =35.25), male to female ratio was 22:18. Thirty of them were smokers and we found there is no corelation between the smoking and infection with *T tenax*. Direct examination of their swabs showed only 25% of them was positive for *T tenax* which is significantly difference (p> 0.001) (table1).

Other findings in the direct examination that there were high number of pus cells, red blood cells and epithelial cells in association with *T tenax* infection. While *Entamoeba gingivalis* and fungi could not be detected. All bacteria that could be isolated were normal oral flora that inhabits the oral cavity as shown in table-2- like *Streptococcus viridans* which is present the food of the protozoa (4). There was no significant difference between two groups. There are no specific bacteria that enhance the growth of this protozoa (favorite) that may act as co-infection with it (5).

The humoral immune response was also studied in both groups (*T tenax* positive and negative groups). This includes serum immunoglobulins IgG, IgM ,IgA and level of two complement (C3 and C4). The normal level of immunoglobulins and complement had a wide range according to the kit that used. As shown in table3.

There is significant increased in the level of serum IgM in *T. tenax* positive group and significant decreased in other parameters (IgG, IgA, C3 and C4) in *T. tenax* positive group in spite of the mean values in both groups lies within normal values.

**Table1: The number and percentages of T.tenax positive and negative individuals.**

T tenax negative		T tenax positive		Total
No.	%	No.	%	
30	75	10	25	40
(1)				

(1): P> 0.001

**Table 2: Types of bacteria isolated from gingival swabs.**

Type of bacteria isolated	T tenax positive No.=10		T tenax negative No.=30	
	No.	%	No.	%
Staphylococcus epidermidis	10	100	30	100
	N.S.			
Streptococcus viridans	10	100	30	100
	N.S.			
Neisseria lactamica	1	10	7	23.3
	N.S.			
Moraxella catarrhalis	1	10	7	23.3
	N.S.			
Streptococcus pneumoniae	0	0	1	3.3
	N.S.			
Enterobacter spp.	0	0	2	6.6
	N.S.			
Escherichia coli	0	0	1	3.3
	N.S.			

N.S. = not significance.

**Table 3: The immunoglobulins (IgG, IgM and IgA) and complement components (C3 and C4) immune response in *T tenax* positive and negative groups (Mean  $\pm$  standard error means).**

Immunoglobulins And complement with normal values	<i>T tenax</i> positive No.=10 X $\pm$ SEM	<i>T tenax</i> negative No.=30 X $\pm$ SEM	Level of significance
s.IgG (710-1520) mg/dl	925.05 $\pm$ 8.2	1100.93 $\pm$ 8.05	P>0.001
s.IgM (40-250) mg/dl	167.05 $\pm$ 3.5	92.51 $\pm$ 2.1	P<0.05
s.IgA (90-310) mg/dl	247.13 $\pm$ 8.7	302.86 $\pm$ 3.5	P>0.001
s.C3 (84-193) mg/dl	105.56 $\pm$ 2.2	140.35 $\pm$ 2.5	P>0.001
s.C4 (20-40) mg/dl	27.34 $\pm$ 0.9	35.77 $\pm$ 1.7	P<0.001

### DISCUSSION:

Protozoa, fungi and bacteria of the oral cavity are frequently occurring and connected to clinical adverse effects and still insufficiently known especially immune suppression and cancer patients increased nowadays<sup>(1)</sup>. There is a lack in the literature of publications analyzing and evaluating oral cavity status. First, the prevalence of *T tenax* in our study was 25% while in Basrah in 1993 was 6.7%<sup>(14)</sup>. Other Arabian country like Egypt, these protozoa was not found in studied population<sup>(15)</sup>. The prevalence in Poland was 9.6 %<sup>(16)</sup> and in Czechoslovakia was 1.1%<sup>(17)</sup>. This high rate of infection may be due to type of population who attended this hospital that they were drained from low social class community and poverty that leads to low oral hygiene<sup>(2)</sup>.

Other report showed 20% of studied group had *T tenax* in their mouth<sup>(18)</sup> which is in agreement with our results. Some reports demonstrated that there were mixed infection with *Entamoeba gingivalis* and fungi<sup>(19)</sup> while our results showed only *T tenax* infection. As we know this protozoa feed on the normal flora of the mouth, so it was not able to live unless the presence of other bacteria that may act as co-infection with it<sup>(4)</sup>. The types of bacteria that can be isolated from gingival swabs like *Streptococcus viridans* were the bacteria that inhabit the oral cavity (table2). We found some G-ve bacteria was also isolated like *Escherichia coli*. These G-ve bacteria can be colonized and isolated from elderly, immunodeficient or malnourished patients particularly when they had received antibiotics<sup>(20)</sup>.

Other report found that *Mycobacterium tuberculosis* was co-infected with *T tenax* <sup>(21)</sup> and others found *Peptostreptococci* as co-infected with it in advanced cancer patients with radiotherapy and chemotherapy <sup>(22)</sup>. We could not able to isolate other types of bacteria because of sophisticated techniques and poor facilities available.

It had been reported that *T tenax* caused pulmonary infection in association with *Neisseria lactamica*,  $\alpha$  and  $\beta$  hemolytic *Streptococci* and *Haemophilus Parainfluenza* <sup>(23)</sup> which was the same type of bacteria that was isolated. *T tenax* was first reported to invade the respiratory tract in 1867 <sup>(24)</sup> and necrotic tissues associated with chronic debilitating diseases <sup>(25)</sup>. It was also demonstrated in non necrotic lung parenchyma <sup>(26)</sup>.

The immune system plays an important role in causation of diseases by *T tenax*. It had been reported that *T tenax* empyema and pulmonary infection occurred in immunocompromised patients with advanced cancer who were on radiotherapy and chemotherapy <sup>(19, 20)</sup>. In another case *T tenax* caused pulmonary eosinophilia by hypersensitivity reaction <sup>(27)</sup>. In this study, the patients were consulting dental clinic for dental reasons.

Thus, study humoral immune response is important because it was extracellular protozoa leads to stimulation of humoral immune system.

Thus, studying the serum level of IgG, IgA, IgM, C3 and C4 is important. In spite of the level of those parameters within normal values because the normal levels of these parameters were wide according to the kit that used. It must be established the normal values of our community and compare with it. Despite to that, we found significant increased in the level of serum IgM that indicated resent infection with this protozoa <sup>(28)</sup>.

There were significant decrease in other parameters which indicate that there was a defect in well establishment of the immune system in those patients who consult the dental clinic for other dental reasons.

#### CONCLUSION:

There was increased in the prevalence of *T tenax* infection due to low social class and low oral hygiene. There was no specific bacteria that was confected with it. Lastly, increased in *T tenax* infection when there is an immune suppression as in advanced cancer patients and on radiotherapy and or chemotherapy.

#### RECOMENDATION:

-Determination the level of secretary IgA in the saliva.

-Prevalence of *T tenax* in advanced cancer patients.

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