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The Role of *Entamoeba gingivalis* and *Trichomonas tenax* in the Microflora of the Oral Cavity and Facial Skin of Human Being

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Abstract

We evaluate correlation between detection of *Trichomonas tenax* and *Entamoeba gingivalis* in oral cavity and patient age.

It was found that:

The presence of *E. gingivalis* and *T. tenax* is not related to the gender of the subjects (p-value ≥ 0.05, the relation is not statistically significant);

The relation was found with the presence of *E. gingivalis* (1 test) with age (p-value=0.004): the presence of *E. gingivalis* finds the most prevalent in the age groups 22-35 years old, 61-75 years old, 76-80 years old. This indicates that the presence of *E. gingivalis* is correlated with the age. The maximal percent of subjects with the presence of *E. gingivalis* was found in the age group 61-75 years old, and 76-80 years old.

Keywords *Entamoeba gingivalis; Trichomonas tenax*; Microflora of oral cavity; Age-related changes of skin

Introduction

It is known that *E. gingivalis* and *T. tenax* belong to the Eukaryotes domain, though different phyla. *T. tenax* belongs to the Metamonada, while *E. gingivalis* belongs to the Amoebozoa [1].

It is known that *E. gingivalis* and *T. tenax* are the basic protozoans that inhabit human oral cavity, mainly in the the root canals, dental plaques [2], the gingival pockets.

Cases of pneumonia [3,4] were caused by these microorganisms have been described. For example, pulmonary trichomoniasis was found in 17% of cases in patients with lung cancer, pulmonary abscess, bronchiectasis [5]. *Entamoeba gingivalis* is also able to colonize of the urogenital tract in women [6].

In experiments *in-vitro*, the proteolytic activity of *T. tenax* was shown to cause disruption of type 1, 3, 4 and 5 collagens. Since collagen is responsible for the elasticity and connectivity of tissues, this activity of microbes may destructively affect them [7]. In subsequent experiments, it was shown that *T. tenax* produces at least two different types of hemolysins. [8]

Another group of authors using electron microscopy showed that oral trichomonas *in-vitro* may also cause damage to mammalian cells similar to that produced by *T. vaginalis*, a well-known pathogen that may cause of diseases of the urogenital tract. *Trichomonas* affects plasmatic membrane of a cell and then may phagocytize the cell's content. Additionally, such damage as membrane foaming and formation of apoptotic bodies has been observed. All these may result in death of a eukaryotic cell [9].

Previously published experiments suggest that the protozoa *T. tenax* and *E. gingivalis* may not be merely commensals, but rather a part of the pathogenic microflora of oral cavity [10]. In the past, microbes were detected almost exclusively by light microscopy and culture. These methods had a number of limitations they do not possess either sufficiently high specificity or sensitivity. Therefore, it seems to be of interest to develop a more accurate and sensitive tests. In this study we use Polymerase Chain Reaction (PCR), which exhibits both high specificity and sensitivity and may reveal even small amounts of microorganisms.

Objective

To evaluate the relationship between age-related skin conditions and detection of *T. tenax* and *E. gingivalis* both in oral cavity and on the surface of the human face.

Materials and Methods

It is well-known that Polymerase Chain Reaction (PCR) method possesses the required properties of high specificity and sensitivity. Based on this method, we developed suitable primers for *E. gingivalis* and *T. tenax.*

The following primers for *E. gingivalis* were developed: 5-gctttcaaaatggctactacttctaag (henceforth, primer 1) and 5-ctccttcttattgtcccatgctt (primer 2); the matrix used was 18 S RNA gene, PCR product was 392 bp. Primers for *T. tenax*: 5–cgctggttgctcagttttaac (primer 1), 5– gtctgcgcggtgactgttgta (primer 2); the matrix was *pms1* gene, PCR product was 318 bp.

The diagnostic system was tested on 175 subjects between 0 and 80 years of age (70 males and 105 females). Samples were taken from the

oral cavity and the facial surface. Test 1 was a sample that was taken from the oral cavity, test 2 was a sample was taken from the external surface of the cheek (facial surface) (Figures 1 and 2, Tables 1 and 2).

Results and Discussion

In order to estimate the relationship between age and detection of *T. tenax* and *E. gingivalis* in the oral cavity and in the facial surface, the subjects were divided into 6 age groups: 0-17, 18-21, 22-35, 36-60, 61-75 and 76-80. Moreover, in each age group male and female were presented separately.

For the data processing, the mathematical statistics methods were used: frequency estimation and Pearson's chi-squared test (to estimate the contingency between indicators). Data processing was performed using the following software: IBM SPSS Statistics 21.0. The results are presented in the Tables 1-8 and Figures 1 and 2.

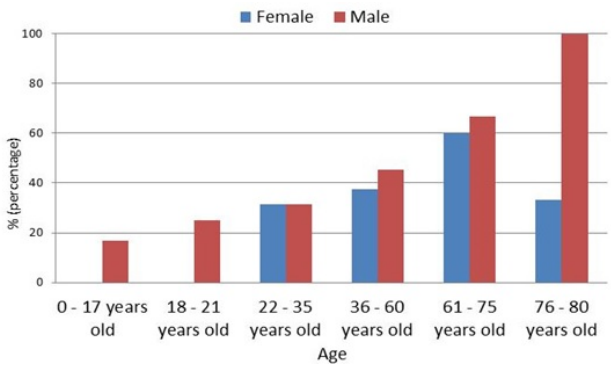

Figure 1: Percentage of subjects infected with *E. gingivalis* by age, test 1 (oral cavity).

1 Experiment				
Gender	**Age**	***E. gingivalis***	**N**	**%**
Female	0-17 years old	-	10	100
	18-21 years old	-	1	100
	22 - 35 years old	-	33	68.8
		+	15	31.3
	36 - 60 years old	-	14	62.5
		+	9	37.5
	61 - 75 years old	-	8	40
		+	12	60
	76 - 80 years old	-	2	66.7
		+	1	33.3
Male	0-17 years old	-	10	83.3
		+	2	16.7
	18-21 years old	-	3	75
		+	1	25
	22 - 35 years old	-	24	68.6
		+	11	31.4
	36 - 60 years old	-	6	54.5
		+	5	45.5
	61 - 75 years old	-	2	33.3
		+	4	66.7
	76 - 80 years old	+	2	100

Table 1: Presence of *E. gingivalis*, depending on the age group, gender (test 1-the oral cavity). N-the number of subjects, %-the percent of subjects of their total number.

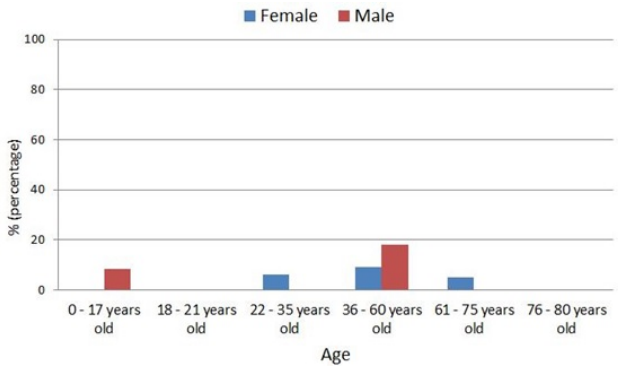

Figure 2: Percentage of subjects infected with *E. gingivalis* by age, test 2 (facial surface).

2 Experiment				
Gender	**Age**	***E. gingivalis***	**N**	**%**
Female	0 - 17 years old	-	10	100
	18 - 21 years old	-	1	100
	22 - 35 years old	-	46	93.9
		+	3	6.1
	36 - 60 years old	-	20	90.9
		+	2	9.1
	61 - 75 years old	-	19	95
		+	1	5
	76 - 80 years old	-	3	100
Male	0 - 17 years old	-	11	91.7
		+	1	8.3
	18 - 21 years old	-	4	100
	22 - 35 years old	-	35	100
	36 - 60 years old	-	9	81.8
		+	2	18.2
	61 - 75 years old	-	6	100
	76 - 80 years old	-	2	100

Table 2: Presence of *E. gingivalis* , depending on the age group, gender (test 2-facial surface). N-the number of subjects, %-the percent of subjects of their total number.

When the sample was taken from the facial surface (test 2), *E. gingivalis* was detected in 9 cases out of 175 (5.14%). *T. tenax* was found in only 2 cases of 175 (1.14%). One case was a 26-year old man with caries, and the other was a 60-years-old female with caries, gingivitis and periodontitis.

When the samples were taken from the oral cavity, *E. gingivalis* was detected in 62 cases out of 175 (35.43%), and *T. tenax* was detected in only 1 case (male, 31 years old with caries) out of 175 (0.57%).

The contingency estimates of the gender and age indicators of the subjects with the presence of *E. gingivalis* and *T. tenax* in the oral cavity were made. The results are shown in the Tables 3 and 4.

1 Experiment					
Variables		***E. gingivalis***		**Chi-square**	**p-value**
		-	+		
Gender	Female	68	37	0.004	0.949
	Male	45	25		
1 Experiment					
Variables		***T. tenax***		**Chi-square**	**p-value**
		-	+		
Gender	Female	105	0	1.509	0.219
	Male	69	1		

Table 3: The results of the investigation of the correlation of the gender with the presence of *E. gingivalis* and *T. tenax* in the oral cavity (test 1).

As can be seen from the Table 3, the correlation with the gender of the presence of *E. gingivalis* and *T. tenax* wasn't revealed (p=0.949 and p=0.219). The groups of males and females do not differ in the presence of Protozoans in the oral cavity.

2 Experiment					
Variables		***E. gingivalis***		**Chi-square**	**p-value**
		-	+		
Gender	Female	99	6	0.176	0.675
	Male	66	3		
2 Experiment					
Variables		***T. tenax***		**Chi-square**	**p-value**
		-	+		
Gender	Female	104	1	0.08	0.772
	Male	69	1		

Table 4: The results of the investigation of the correlation of the gender with the presence of *E. gingivalis* and *T. tenax* on the facial surface (test 2).

According to the results of the 2 test (Table 4), similar conclusions should be made (p=0.675 and p=0.772). The results of the evaluation of the relationship with the age indicator showed the presence of the relationship (in the first test) between the age of the subjects, *E. gingivalis* and *T. tenax*.

1 Experiment					
Variables		***E. gingivalis***		**Chi-square**	**p-value**
		-	+		
Age	0 - 17 years old	20	2	17.36	0.004
	18 - 21 years old	4	1		
	22 - 35 years old	57	26		
	36 - 60 years old	20	14		
	61 - 75 years old	10	16		

	76 - 80 years old	2	3		
1 Experiment					
Variables		***T. tenax***		**Chi-square**	**p-value**
		-	+		
Age	0 - 17 years old	22	0	1.115	0.953
	18 - 21 years old	5	0		
	22 - 35 years old	82	1		
	36 - 60 years old	34	0		
	61 - 75 years old	26	0		
	76 - 80 years old	5	0		

Table 5: The results of the investigation of the correlation of the age groups with the presence of *E. gingivalis* and *T. tenax* in the oral cavity (test 1).

A statistically significant relation between the age of the subjects and the presence of *E. gingivalis* ($p \leq 0.004$) was found (Table 5). This suggests that the frequency of *E. gingivalis* in the oral cavity increases with age (starting from the period of 22-35 years). The following Table 6 presents the results of calculating the Pearson's Chi-square criterion between the age ranges of the subjects and *E. gingivalis* 1 and 2 tests.

Age	**Experiment**	**Result (*E. gingivalis*)**				**Chi-square**	**p-value**
		-		+			
		N	%	N	%		
0 - 17 years old	*E. gingivalis* (1 Experiment)	20	90.9	2	9.1	0.358	0.550
	E. gingivalis (2 Experiment)	21	95.5	1	4.5		
18 - 21 years old	*E. gingivalis* (1 Experiment)	4	80.0	1	20.0	1.110	0.292
	E. gingivalis (2 Experiment)	5	100.0	0	0.0		
22 - 35 years old	*E. gingivalis* (1 Experiment)	57	68.7	26	31.3	22.103	0.001
	E. gingivalis (2 Experiment)	80	96.4	3	3.6		
36 - 60 years old	*E. gingivalis* (1 Experiment)	20	58.8	14	41.2	7.560	0.060
	E. gingivalis (2 Experiment)	30	88.2	4	11.8		
61 - 75 years old	*E. gingivalis* (1 Experiment)	10	38.5	16	61.5	19.600	0.001
	E. gingivalis (2 Experiment)	25	96.2	1	3.8		
76 - 80 years old	*E. gingivalis* (1 Experiment)	2	40.0	3	60.0	4.280	0.038
	E. gingivalis (2 Experiment)	5	100.0	0	0.0		

Table 6: The results of the investigation of the correlation of the age groups with the presence *E. gingivalis* (1 and 2 tests). N-the number of subjects, % is the percent of subjects of their total number.

The significant relations were found between the presence of *E. gingivalis* and the age groups: 22–35 years old, 61–75 years old, and 76–80 years old (Table 6). The risk of the presence *E. gingivalis* in the oral cavity increases since the period of 22-35 years old. The high risk at the age of 61-75, 76-80 years old, where the subjects's percent with the presence of *E. gingivalis* is significantly higher. Significant relations are considered when p is not more than 0.05 (5% precision).

Age	**Experiment**	**Result (*T. Tenax*)**	**Chi-square**	**p-value**

		-		+			
		N	%	N	%		
0 - 17 years old	*T. Tenax* (1 Experiment)	22	100.0	0	0.0	-	-
	T. Tenax (2 Experiment)	22	100.0	0	0.0		
18 - 21 years old	*T. Tenax* (1 Experiment)	5	100.0	0	0.0	-	-
	T. Tenax (2 Experiment)	5	100.0	0	0.0		
22 - 35 years old	*T. Tenax* (1 Experiment)	82	98.8	1	1.2	0.000	1.000
	T. Tenax (2 Experiment)	82	98.8	1	1.2		
36 - 60 years old	*T. Tenax* (1 Experiment)	34	100.0	0	0.0	1.015	0.314
	T. Tenax (2 Experiment)	33	97.1	1	2.9		
61 - 75 years old	*T. Tenax* (1 Experiment)	26	100.0	0	0.0	-	-
	T. Tenax (2 Experiment)	26	100.0	0	0.0		
76 - 80 years old	*T. Tenax* (1 Experiment)	5	100.0	0	0.0	-	-
	T. Tenax (2 Experiment)	5	100,0	0	0.0		

Table 7: The results of the investigation of the correlation of the age groups with the presence *T. Tenax* (1 and 2 tests). N-the number of subjects, %-the percent of subjects of their total number.

The correlation between the age of the subjects and the presence of *T. Tenax* wasn't revealed. The presence of *T. Tenax* is rare in the study group (Table 7).

Gender	**Experiment**	**Result (*E. gingivalis*)**				**Chi-square**	**p-value**
		-		+			
		N	%	N	%		
Female	*E. gingivalis* (1 Experiment)	68	64.8	37	35.2	28.1	0.001
	E. gingivalis (2 Experiment)	99	94.3	6	5.7		
Male	*E. gingivalis* (1 Experiment)	45	64.3	25	35.7	21.6	0.001
	E. gingivalis (2 Experiment)	67	95.7	3	4.3		

Table 8: The results of the investigation of the correlation of the gender and the presence *E. gingivalis* (1 and 2 tests). N-the number of the subjects, %-the percent of subjects of their total number.

The significant differences were found between tests 1 and 2 in the groups of male and female. The *E. gingivalis's* index in 1 test is significantly more common than in 2 test in both groups (p=0.001) (Table 8).

Significant correlations between dental diseases among subjects (caries, gingivitis, periodontitis, stomatitis, periodontitis, etc.), medication intake of metranidazole and the presence of protozoans had not been revealed (p=0.615 and p=0.188).

Conclusions

On the basis of the analysis of the research results, it was found that:

- the presence of *E. gingivalis* and *T. tenax* is not related to the gender of the subjects (p-value ≥ 0.05, the relation is not statistically significant);
- the relation was found with the presence of *E. gingivalis* (1 test) with age (p-value=0.004): the presence of *E. gingivalis* finds the most prevalent in the age groups 22-35 years old, 61-75 years old, 76-80 years old.

This indicates that the presence of *E. gingivalis* is correlated with the age factor. The maximal percent of subjects with the presence of *E. gingivalis* was found in the age group 61-75 years old, and 76-80 years old.

In addition, zero correlation of infection with diseases of the oral cavity indicates that the nature of the colonization of the macroorganism of protozoans is most likely associated with its general parameters, such as age-related changes in immunity, etc.

With age, presence of protozoa in oral cavity increases, which agrees with Mechnikov's theory of microbiological aging and our hypothesis about their possible effect on age-related skin changes [11-13].

Mechnikov gave the convincing experimental evidence of longevity under conditions of the decrease of the microbial load inside the body, using mammals and birds as an example in his books [11,12].

The main conclusion of Mechnikov: the inimical microorganisms both directly cause the destruction of the body, and excrete toxins-poisons, which leads to aging. The use of microorganisms of the microorganism as a substrate for its habitation is inevitable and, of course, in any case negative for it. At the same time, the vital activity of microorganisms leads to multi-level (chemical, physico-chemical, cellular, etc.) degradation of the structures of the human body.

Thus, the following results have been obtained:

- two types of protozoan *T. tenax* and *E. Gingivalis*, localized on the oral mucosa and the external side of the cheek, have been found;
- the correlation of *E. gingivalis* group with age has been shown. That corresponds to our point of view Mechnikov's theory of the microbiological aging of the macroorganism;
- a significant difference in the content of *E. gingivalis* on the oral mucosa and the external side of the cheek was obtained, which may be due to the condition of their appearance under conditions of drift through the tissues.

A small amount of *T. tenax* is most likely determined by phylogenetic differences in the Eukaryotic domain. Differences in the detected amounts of *T. tenax* and *E. Gingivalis* are associated with the manifestation of phylogenetic features in the functioning of these protozoa in macroorganisms.

It is possible that the protozoa that feed by high-molecular compounds, such as, for example, hyaluronic acid, may damage the structure of skin and worsen its appearance. It is likely that disturbances in continuity of the basal membrane discovered by us earlier are also associated with the functioning of these protozoa [13].

The subject of the further research can be an assessment of the effect of eradication of the identified protozoa on manifestations of age-related changes in facial skin.

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