

# The concentration of anthranilic acid in saliva of orthodontic appliances

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## Abstract

**Purpose:** Anthranilic acid is an important, the aromatic intermediate in the degradation of tryptophan in kynurenine pathway. This compound plays an important role in the regulation of immunological processes as well shows antibacterial activity. The aim of our study was to estimate the concentration of anthranilic acid in saliva of young patients with orthodontic apparatus. We also assessed correlation between saliva anthranilic acid concentrations and time of orthodontic treatment. For the first time we have demonstrated the enhanced concentration of anthranilic acid in saliva of young orthodontic appliances.

**Material and methods:** The study was performed on non-stimulated, mixed saliva of patients with orthodontic appliances. The concentration of anthranilic acid and was determined by high-performance liquid chromatography (HPLC).

**Results:** The concentration of anthranilic acid was significantly higher in orthodontic patients ( $p=0.043$ ) in comparison to healthy volunteers. The mean time of orthodontic treatment was  $15.0 \pm 2.03$  months. We did not observe existence of correlation between anthranilic acid concentration in saliva and time of orthodontic treatment ( $r=-0.250$ ;  $p=0.517$ ).

**Conclusion:** These results might indicate that anthranilic acid can be one of many factors initiating of periodontal disease in orthodontic appliances.

**Key words:** orthodontic appliances, saliva, anthranilic acid.

## Introduction

One of the most interesting compounds, which is supplied with food to organism, is tryptophan (TRP). This essential amino acid is making use of protein biosynthesis in human body. 94% of tryptophan is metabolized via kynurenine pathway [1]. Initially tryptophan is transformed into N-formylkynurenine, which immediately is converted into stable kynurenine (KYN) [2]. Kynurenine is precursor of three metabolites such as anthranilic acid (AA), kynurenic acid (KYNA) and 3-hydroxykynurenine (3-HKYN) [3]. Physiological and pathophysiological properties of KYNA and 3-HKYN are known very well [4,5]. However, the knowledge about biological role of anthranilic acid in organism is seems to be insufficient.

Recently we have demonstrated the presence of anthranilic acid in saliva of diabetic patients with hypertension [6]. In the present literature is not any information about the role of anthranilic acid in oral cavity pathological state.

Saliva, the secretion of small and big salivary glands, presents fluid environment ecosystem contains: 99.5% of water, 0.3% of organic components and 0.2% of inorganic components. The development of analytic methods has allowed to make discoveries about numerous compounds, which are responsible for physiochemical and biological properties of saliva. The saliva composition is changing in different local and systemic diseases and reflects many pathophysiological states [6]. Moreover, the presence of many of these substances may be a marker of pathological changes in oral cavity and also in general disorders. Since the saliva is easy to get and therefore it can be used as a non-invasive diagnostic tool.

The aim of our study was to estimate the concentration of anthranilic acid in orthodontic appliances. We also assessed the correlation between saliva anthranilic acid concentrations and length of orthodontic treatment.

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## Material and methods

Nine patients (5 female + 4 male), aged 12-30, with orthodontic apparatus were included in the study. All patients were clinically stable and free of any illnesses. All patients were treated in Department of Orthodontic Medical University of Białystok.

Thirteen (4 female + 9 male) healthy volunteers, aged 18-25, without orthodontic apparatus served as the control group.

### Saliva sampling and anthranilic acid determination

Samples of non-stimulated, mixed saliva were taken from studied subjects each morning between 7-8 am, 10 min after mouth washing MilliQ water. The saliva samples were immediately treated 2 M HClO<sub>4</sub>. After 15 min of incubation with acid at 4°C, samples were centrifuged 30 min 12000 g and the supernatant was collected in -80°C for measurement of anthranilic acid concentration using HPLC method [7].

### Statistical analysis

The values are expressed as the mean ± SEM; n – represents the number of results. Statistical analysis was done using Student's t-test. P value less than 0.05 was considered statistically significant. Correlations were analyzed using Pearson test.

### Ethics

The Ethics Committee of the Medical University of Białystok accepted the study.

## Results and discussion

The study parameters are summarized in *Tab. 1*. In the control group saliva concentration of anthranilic acid was 3.90±1.89 nM, and increased significantly to a value 9.75±1.77 nM (p=0.043) in the group of patients with orthodontic apparatus. The mean time of orthodontic treatment was 15.0±2.03 months. We did not observe existence of correlation between anthranilic acid concentration in saliva and time of orthodontic treatment (AA=13.015-0.217 x time of orthodontic treatment; r=-0.250; p=0.517) (*Fig. 1*).

To our knowledge, this is the first report, which concerns an effect of orthodontic apparatus on anthranilic acid concentration in saliva of young people. In the current study we have observed an increase of anthranilic acid level in saliva during of orthodontic therapy. However, we did not note existence of correlation between anthranilic acid concentration and time of orthodontic therapy. We have believed that this result depends on small group of subjects taken into consideration.

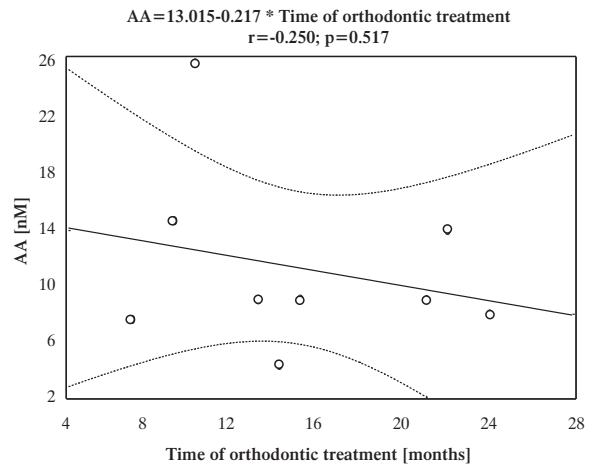
Anthranilic acid is an important, aromatic intermediate in the degradation of tryptophan in kynurenine pathway. For many years it has been known that microorganisms are able to use it as the sole source of carbon and energy aerobically or anaerobically [8]. Now we have known that anthranilic acid plays a key role in the regulation of immunological processes [9,10] and shows antibacterial activity [11].

**Table 1.** Baseline characteristic of orthodontic patients in comparison with control group

	Control group	Orthodontic patients
Age (yrs)	19.85±0.65	20.0±2.17
Male/female	9M/4F	4M/5F
Time of treatment [month]	-	15.0±2.03
AA concentration [nM]	3.90±1.89	9.75±1.77*

\* p<0.05 in comparison to control group

**Figure 1.** The correlation between anthranilic acid concentration and time of orthodontic treatment



Our previous study has shown that there is a strong association between anthranilic acid concentration and anaemia in patients with chronic renal diseases [12]. We have proved that this compound can penetrate cell's membrane. Furthermore, we have noticed the existence of negative correlation between anthranilic acid concentration and number of red cells, haematocrit, and haemoglobin concentration, as well positively relationship between anthranilic acid concentration and osmotic resistance of erythrocytes. In vitro study plays on incubation of healthy subject's erythrocytes with growing concentration of anthranilic acid showed the decrease in resistance of red cells [12]. Its presence in saliva does not indicate the origin of it. On the one hand anthranilic acid can be synthesized by local gingival cells, on the other hand its can penetrate from the blood. Regardless of anthranilic acid origin its presence in saliva is proved in our study.

In conclusion, for the first time we have demonstrated the enhanced concentration of anthranilic acid in saliva of young orthodontic appliances. These results might indicate that anthranilic acid can be one of many factors initiating of periodontal disease in these patients. Thus, further studies are needed to assess whether this increase of anthranilic acid concentration plays an important role in periodontal diseases. Additionally, this study has shown that saliva can be used as a non-invasive diagnostic tool for many pathological state of oral cavity.

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12. Tankiewicz A, Pawlak D, Pawlak K, Szewc D, Mysliwiec M, Buczko W. Anthranilic acid – uraemic toxin damaged red cell's membrane. *Int Urol Nephrol*, 2005; 37: 621-7. Table. 1. Baseline characteristics of orthodontic patients in comparison with control group.