

Proteolytic Enzymes and their Inhibitors in the Oral Fluid of Healthy People and Patients with Acute Respiratory Viral Infections

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Research Article

Volume 4 Issue 1

Received Date: October 25, 2019

Published Date: November 08, 2019

DOI: 10.23880/vvoa-16000131

Abstract

The work investigated changes in biochemical parameters of healthy people and patients with symptoms of acute respiratory viral infections. In practically healthy people, the content of the trypsin-like proteinase inhibitor in the oral fluid exceeded the activity of trypsin-like proteinases 1.5 thousand times. In patients with ARVI symptoms, an increase in the content of trypsin-like proteinase inhibitor was observed between the ages of 18 and 30, while in the older age group (over 50), the activity of the inhibitor decreased. There was an increase in the activity of trypsin-like proteinases in the 2nd age group (30-50 years).

Keywords: Oral Fluid; Trypsin-Like Proteinase Inhibitors; SARS

Introduction

The interest in using the secretory fluid of the oral cavity as an object for predicting and diagnosing many diseases and evaluating the effectiveness of therapy is due to the safety and simplicity of its preparation, which, with the wide spread of viral diseases, is of great practical importance [1,2].

The oral secretion (mixed saliva) has a multicomponent biochemical composition and contains a variety of substances of a protein nature, in particular enzymes of different specificity of action. Of these, proteinases (trypsin-like, pepsin, elastase, kallikrein, etc.) are of particular interest [3-5]. They participate in the biochemical stage of protein breakdown, both directly and indirectly through the activation of a number of hydrolytic enzymes. Proteinases play a decisive role in the release of mediators of inflammatory and allergic

reactions-histamine and kinins, which increase the permeability of capillaries, cause itching, swelling, erythema, and increase leukocyte migration [6]. A special role in these processes belongs to proteinase inhibitors, which play a protective reaction in inflammatory and allergic reactions [7].

Of great interest are studies of proteolytic enzymes in viral diseases of the upper respiratory tract, especially with influenza and other acute respiratory viral infections, since the mucous membrane of the nasal cavity first comes into contact with viruses and other environmental factors.

The purpose of this study was to clarify the possibility of using biochemical parameters as prognosis criteria and the effectiveness of treatment for patients with influenza and other acute respiratory viral infections (ARVI) based on determining the total activity of proteolysis enzymes,

trypsin-like proteinases and their inhibitors, and protein content in the oral secretion

Materials and Methods

67 people were examined, of which 46 were practically healthy people (control group) and 21 people with SARS symptoms 3-7 days after the onset of the disease. The average age of the patients was 35 years, of which 23 were men and 46 were women.

The studies were carried out in a solid oral secretion, which determined the activity of trypsin-like proteases by hydrolysis of protamine sulfate, expressed in micromoles of arginine, for 1 min of incubation in 1 ml of oral fluid. The trypsin inhibitor content was determined by the

casein method, in which the inhibitor content was evaluated by inhibition of casein hydrolysis by crystalline trypsin, 1 unit of inhibitor corresponded to 1 μg of crystalline trypsin in 1 ml of oral secretion. The protein content in the oral secretion was determined by the standard method of O. Lowry [8,9].

Results

The results of the studies presented in table. 1, showed that in practically healthy people in the oral fluid (mixed saliva) the content of the trypsin-like proteinase inhibitor averaged 29.76 ± 2.11 units / ml. The spread of trypsin inhibitor values ranged from 15.04 units / ml to 56.06 units / ml.

No. p / p	Age, years	Protein,	IT u / ml * conv. Healthy	AP
		mg / ml	IT u / ml * conv. Healthy	u / ml **
1	18-30	$3,47 \pm 0,25$	$29,30 \pm 1,98$	$0,028 \pm 0,0023$
		(n=38)	(n=25)	(n=4)
2	31-50	$2,92 \pm 0,27$	$29,92 \pm 2,15$	$0,024 \pm 0,0019$
		(n=8)	(n=8)	(n=9)
3	>51	$3,82 \pm 0,26$	$32,15 \pm 2,31$	$0,012 \pm 0,0010$
		(n=3)	(n=3)	(n=8)

Table 1: The activity of trypsin-like proteinase and its inhibitor in the oral fluid of healthy people (age distribution).

Notes: IT is the content of trypsin inhibitor in the sample in units / ml. * 1 unit an inhibitor corresponds to 1 μg of crystalline trypsin; AP - enzyme activity - trypsin-like proteinase in units / ml. For ** 1 unit activity take the amount of enzyme causing the formation of 1 micromole of arginine per 1 minute of incubation.

The activity of trypsin-like proteinases was insignificant and ranged from 0.0001 units / ml to 0.040 units / ml, on average 0.019 ± 0.0016 units / ml

(micromoles of arginine per 1 minute of incubation in 1 ml of oral fluid). The protein content in the oral fluid averaged 3.35 ± 0.24 mg / ml.

No. p / p	Age, Years	Protein,	IT	AP
		mg / ml	u / ml *	u / ml **
1	18-30	$2,79 \pm 0,22$	$41,00 \pm 3,51$	$0,020 \pm 0,0017$
		(n=6)	(n=6)	(n=3)
2	31-50	$3,73 \pm 0,23$	$32,45 \pm 2,32$	$0,037 \pm 0,0028$
		(n=13)	(n=12)	(n=13)
3	>51	$4,67 \pm 0,34$	$26,44 \pm 1,87$	$0,023 \pm 0,0019$
		(n=3)	(n=1)	(n=3)

Table 2: Trypsin-like proteinase activity, inhibitor and protein content in the oral fluid of patients with symptoms of SARS (age distribution).

Notes: IT - content of trypsin inhibitor in the sample in units / ml; * 1 unit an inhibitor corresponds to 1 μg of crystalline trypsin; AP - enzyme activity - trypsin-like proteinase in units / ml. For ** 1 unit activity take the amount of enzyme causing the formation of 1 micromole of arginine per 1 minute of incubation.

In the oral fluid of patients with acute respiratory viral infections, the content of trypsin-like proteinase inhibitor

increased and averaged 35.11 ± 2.87 units / ml (Table 2). The spread in the content of trypsin-like proteinase

inhibitor ranged from 16.27 units / ml to 62.60 units / ml. The activity of trypsin-like proteinases in patients also increased and amounted to an average of 0.033 ± 0.0027 units / ml. Fluctuations in the activity of trypsin-like proteinases ranged from 0.003 units / ml to 0.126 units / ml. Sampling of oral fluid (mixed saliva) in patients with

ARVI was carried out on 3-7 days after the onset of the disease. It can be assumed that there was an increase in the activity of an inhibitor of trypsin-like proteinases, associated with the development of the inflammatory process caused by viruses. It was necessary to determine the content of trypsin inhibitor in the oral fluid in the first 24 hours after the onset of the disease, which was difficult, because patients went to the clinic 3-5 days after the onset of the disease, when they themselves could not cope with the symptoms of acute respiratory viral infections (high body temperature, runny nose, cough, etc.). The protein content in patients with SARS averaged 3.57 ± 0.29 mg / ml and did not differ significantly from the control group.

Patients were divided into three age groups. The results of the study showed that the contents of the trypsin-like proteinase inhibitor in the oral fluid of

practically healthy people aged 18 to 50 were almost the same and amounted to 29.30 ± 1.98 units / ml. In the group of patients of the 3rd age group (from 51 years of age and older), the inhibitor content increased to 32.15 ± 2.31 units / ml, and the proteinase activity decreased to 0.012 ± 0.001 units / ml.

In patients with signs of acute respiratory viral infections in the 1st age group (from 18 to 30 years), the content of trypsin-like proteinase inhibitor increased 1.5 times and amounted to 41.0 ± 3.51 units / ml, while in the 3rd age group group inhibitor content decreased to 26.44 ± 1.87 units / ml. There was an increase in the activity of trypsin-like proteinase (TP) in the 2nd age group (31-50 years) to 0.037 ± 0.0028 units / ml. In the 1st and 3rd group, TP activity practically did not change $0.020 - 0.023$ units / ml.

At the next stage of the study, we analyzed the presence of proteolytic enzymes and their inhibitors in the oral fluid of patients, divided by gender.

The results of the study showed that in practically healthy women and men (Table 3), the content of trypsin inhibitor in the oral fluid did not differ significantly.

No. p / p	Floor, m / f	Protein, mg / ml	IT, u / ml *	AP, u / ml **
		conv. healthy	conv. healthy	conv. healthy
1.	men	$3,95 \pm 0,38$ (n=12)	$27,91 \pm 1,19$ (n=11)	$0,020 \pm 0,0017$ (n=6)
2.	women	$3,10 \pm 0,19$ (n=28)	$30,54 \pm 2,38$ (n=26)	$0,018 \pm 0,0015$ (n=17)

Table 3: Trypsin-like proteinase activity, inhibitor and protein content in the oral fluid of healthy people (sex distribution).

Notes: IT - content of trypsin inhibitor in the sample in units / ml; * 1 unit an inhibitor corresponds to 1 μ g of crystalline trypsin; AP - enzyme activity - trypsin-like proteinase in units / ml. For ** 1 unit activity take the amount of enzyme causing the formation of 1 micromole of arginine per 1 minute of incubation.

Table 4 presents the results of differences in the content of TP inhibitor and its activity in men and women with symptoms of acute respiratory viral infections. In

male patients, the amount of TP inhibitor was higher than in women, while TP activity and protein content were slightly higher in women.

No. p / p	Floor	Protein, mg / ml	IT, u / ml *	AP, u / ml **
		sick	sick	sick
1	men	$3,33 \pm 0,20$ (n=8)	$35,69 \pm 2,78$ (n=7)	$0,033 \pm 0,0029$ (n=7)
2	women	$3,72 \pm 0,33$ (n=8)	$33,73 \pm 2,21$ (n=11)	$0,037 \pm 0,0031$ (n=10)

Table 4: Trypsin-like proteinase activity, inhibitor and protein content in the oral fluid of patients with symptoms of SARS (distribution by gender).

Notes: IT - content of trypsin inhibitor in the sample in units/ml; * 1 unit an inhibitor corresponds to 1 μ g of crystalline trypsin; AP - enzyme activity - trypsin-like proteinase in units/ml. For ** 1 unit activity take the amount of enzyme causing the formation of 1 micromole of arginine per 1 minute of incubation.

Thus, in practically healthy people, the content of trypsin-like proteinase inhibitor in the oral fluid exceeded 1.5 thousand times the activity of trypsin-like proteinases. In patients with ARVI symptoms, an increase in the content of trypsin-like proteinase inhibitor was observed between the ages of 18 and 30 years, while in the older age group (over 50), the activity of the inhibitor decreased. An increase in the activity of trypsin-like proteinases was observed in the 2nd age group (30-50 years).

Conclusion

In practically healthy people, the content of the trypsin-like proteinase inhibitor in the oral fluid (mixed saliva) was on average 29.76 ± 2.11 u/ml, while in patients with SARS symptoms the inhibitor content increased on average to 35.11 ± 2.87 units/ml. The activity of trypsin-like proteinases in healthy people was low and averaged 0.019 ± 0.0016 units/ml; in patients with SARS symptoms, activity increased on average to 0.033 ± 0.0027 units/ml.

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