# Crystalloscopic analysis in detection of saliva metabolome specialties in elderly people

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> Abstract. The purpose of this study was to assess the features of saliva crystallization in middle-aged and elderly people. The study included middle-aged (45-60 years old; n=63) and elderly (over 60 years old; n=78) men who signed an informed consent to participate. Crystalloscopic analysis of saliva in order to characterize the metabolic characteristics of the body and the composition of biological fluids. The intrinsic crystallization of mixed saliva was studied using the method of classical crystalloscopy. The structure index, crystallizability, facia destruction degree and the clearity of the marginal protein zone were determined. Each of the parameters was expressed on a 5-point scale. In general, the study made it possible to establish the presence of shifts in the crystallogenic properties of mixed saliva in the older age group (over 60 years old) relative to middle-aged people. Thus, the features of the crystalloscopic picture of biological fluid in elderly subjects included a decrease in the total crystallogenic potential of biological fluid, which was manifested in a decrease in crystallizability and the structural index of dehydrated saliva facies. In addition, the involutive shifts of the crystallograms were represented by an increase in the degree of destruction of facies and the expansion of the marginal zone of micropreparations. The revealed transformations of the crystallogenic properties of the biological medium allow us to speak about age-dependent characteristics of the component composition and physico-chemical parameters of saliva and, therefore, indirectly describe the involutive changes in the metabolome of this biological fluid.

# 1 Introduction

From the point of view of the multilevel integral approach classical for physiology and medicine, health is considered as a derivative function of the adaptive capabilities of the organism [1-3], realized at various levels of organization (from cellular to organizational) and management (psychological, psychophysiological level, local and global neurohumoral mechanisms) of the organism [1, 4-6]. Adaptive capabilities, on the one hand, determine the

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body's response to external influences, including physical training and wellness activities, on the other hand, they themselves depend on a variety of internal and external factors, both unmodified (heredity, lifestyle features in the past, etc.) and modifiable (psychological and physical stresses, lifestyle at the present time etc.) [1-4, 7]. Thus, a comprehensive analysis of indicators related to adaptive capabilities at various levels of organization and management of the body is necessary to determine informative indicators of the remote health monitoring system, as well as adequate selection and evaluation of the result of recreational activities [8, 9].

Biocrystallomics technologies are one of the methodological techniques that integrally describe the physical and chemical properties of biological fluids, in particular, mixed saliva [10, 11]. It is known that the crystallogenic activity of biological media of an organism depends on their component composition and, therefore, allows us to indirectly assess the current state and dynamics of rearrangement of the metabolome of biological fluids in various functional states [11, 12]. On this basis, it is logical to assume that the diagnostic technology under consideration will be informative in monitoring the involutive transformations of the composition of biological fluids [13]. Their noninvasive control can be carried out by analyzing saliva parameters within the framework of the salivadiagnostics methodology, which has been actively developing in recent decades [14-17]. In this regard, the aim of the study was to assess the features of saliva crystallization in middle-aged and elderly people.

#### 2 Material and methods

The study included middle-aged (45-60 years old; n=63) and elderly (over 60 years old; n=78) men who signed an informed consent to participate. The exclusion criteria were: severe somatic diseases in the stage of decompensation or exacerbation, as well as acute diseases that prevented the passage of research procedures in accordance with the protocol or were contraindications to one of the main research methods; known cardiac arrhythmias and conduction that prevent the analysis of heart rate variability; mental disorders that prevent effective communication and the implementation of research procedures.

Crystalloscopic analysis of saliva in order to characterize the metabolic characteristics of the body and the composition of biological fluids. Samples of mixed saliva were collected by spitting into clean, dry test tubes after twice rinsing the oral cavity with distilled water. The intrinsic crystallization of mixed saliva was studied using the method of classical crystalloscopy described earlier [10, 11]. The structure index (SI), crystallizability (Cr), the facia destruction degree (FDD) and the clearity of the marginal protein zone (Mz) were determined. Each of the parameters was expressed on a 5-point scale, according to the previously described criteria [10, 11]. Since there is no data on the reference values of saliva crystalloscopy indicators in the elderly, in this study, the results in the elderly population were compared with the data obtained during the previous study in the middle age group (45-60 years).

Conducting research is regulated by the permission of the local ethics committee of the Lobachevsky University.

The obtained results were analyzed using the Statistica 12 software package (StatSoftInk, USA). After checking the normality of the distribution, a Student's test was used for related samples in order to assess the dynamics of quantitative indicators. P=0.05 was taken as the critical level of significance in all types of analysis.

#### 3 Results

According to all the main biometric indicators, there are shifts in saliva facies in relation to middle-aged subjects.



Fig. 1. The level of crystallization in saliva facias of middle-aged adults and the elderly ("\*" - differences relative to middle aged peoples is statistically valued, p<0.05)

Thus, crystallizability, which is a quantitative characteristic of the result of dehydration structuring of the biological medium and demonstrating its activity, shows a distinct downward trend (by 1.27 times; p<0.05) compared with middle-aged adults (Fig. 1). This may be due to the presence in the saliva of elderly adults of under-oxidized products-substrates of endogenous intoxication, formed, among other things, as a result of free radical processes [18]. The presence in the biological fluid of such compounds capable of acting as pronounced inhibitors of crystallization, and provides conditions for suppressing the formation of organo-mineral aggregates [18, 19], described by visual assessment as crystalline elements [10].

The stated hypothesis is also confirmed by the dynamics of the visuametric criterion characterizing the qualitative side of crystallogenesis - the structure index, which allows taking into account the complexity of the structure of the elements of the dehydrated saliva sample (Fig. 2).

This indicator is also found in older people at lower values, compared with middle-aged adults (1.39 times; p<0.05). These changes indicate that metabolic rearrangements that occur during age-related changes lead not only to a quantitative decrease in the crystallogenic activity of the biological fluid, but also to a simplification of its formed structures. This indicates rather deep shifts in the physico-chemical properties of the biosubstrate [10, 11].

Of particular interest is the assessment of the facia destruction degree – the main criterion for the "correctness" of crystallogenesis, currently considered as an indicator of the degree of changes in the crystallogenic properties of biological fluids [11]. It was found that in middle-aged adults, only moderate signs of destruction of facias elements were recorded in dried saliva samples, whereas in most elderly people, subtotal destruction of the crystal structures of the micropreparation was observed (Fig. 3).



Fig. 2. The structure index in saliva facias in middle-aged adults and the elderly ("\*" - differences relative to middle aged peoples is statistically valued, p<0.05)



**Fig. 3.** The degree of destruction in saliva facias in middle-aged adults and the elderly ("\*" - differences relative to middle aged peoples is statistically valued, p<0.05)

This was reflected in the level of facias destruction, which in middle-aged adults and the elderly is  $1.28\pm0.14$  and  $3.05\pm0.36$  points (p<0.05), respectively, which indirectly indicates the pathological nature of crystallogenesis shifts in the elderly induced by the presence of chronic diseases and the onset of destructive cellular changes.



Fig. 4. The severity of the marginal protein zone of dehydrated micro-preparations of saliva in middle-aged adults and the elderly ("\*" - differences relative to middle aged peoples is statistically valued, p<0.05)

According to the parameter of the severity of the marginal zone (Fig. 4), a higher level was noted in the elderly, which characterizes the expansion of the marginal zone of facies in this group of studied individuals. According to the known molecular mechanisms of structure formation in drying droplets of biological fluids [11, 20-22], protein macromolecules that have preserved their structure, physicochemical properties and conformation, i.e. native, are concentrated in the part of the sample under consideration. In addition, this zone of the micropreparation can be formed due to the aldo-ketone compounds migrating here, which have an inhibitory effect on the structuring of the components of the biological fluid [23]. One of them is malondialdehyde, which is formed as a product of lipid peroxidation, which increases the oxidative stress in the elderly within the framework of their existing oxidative stress. A similar effect can simulate an increase in the amount of native proteins in mixed saliva samples. This is indirectly evidenced by the appearance of numerous chaotic faults in the marginal zone of crystalloscopic facies of the biological environment of elderly people.

# 4 Conclusion

In general, the study made it possible to establish the presence of shifts in the crystallogenic properties of mixed saliva in the older age group (over 60 years old) relative to middle-aged people. Thus, the features of the crystalloscopic picture of biological fluid in elderly subjects included a decrease in the total crystallogenic potential of biological fluid, which was manifested in a decrease in crystallizability and the structural index of dehydrated saliva facies. In addition, the involutive shifts of the crystallograms were represented by an increase in the degree of destruction of facies and the expansion of the marginal zone of micropreparations. The revealed transformations of the crystallogenic properties of the biological medium allow us to speak about age-dependent characteristics of the component composition and physico-chemical parameters of saliva and, therefore, indirectly describe the involutive changes in the metabolome of this biological fluid.

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