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Is the antioxidant power of saliva, measured as reducing iron power, only a quantification of salivary uric acid?

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Abstract

Introduction: The main non-enzymatic salivary antioxidant is uric acid. An important and innovative test for the analysis of antioxidants in the oral cavity is the SAT test, based on the determination of iron-reducing power. It is known that uric acid forms stable complexes with ferric ion and therefore it is possible that tests based on the determination of the iron-reducing power might measure only the concentration of uric acid. **Objective:** The aim of this paper is to demonstrate that, in particular, the SAT test quantifies the reducing power of saliva in all its components. **Methods:** It has been quantified uric acid and iron-reducing power in saliva sample from 29 subjects. Iron-reducing power has been morover determined in some samples fortified in vitamin C. **Results:** A strong and significant correlation was found between the uric acid concentration and the iron-reducing power measured ($r = 0.90$, $p < 0.01$), but data are not perfectly overlapping. Iron-reducing power of samples fortified in vitamin C is directly proportional to the addiction. **Conclusions:** The iron-reducing power of saliva is simultaneous influenced by the antioxidants and not only the uric acids. The salivary iron-reducing test (i.e. SAT test) are sensitive both to uric acid and to vitamin C and likely to all salivary reducing agents. Moreover the salivary iron-reducing test are an excellent estimate of the global antioxidant power of saliva and then of the oral cavity.

Keywords: Antioxidant power, Iron power, Salivary uric acid.

INTRODUCTION

Free radicals play a key role in the development of different pathological conditions, therefore, different methods have been developed to measure oxidative stress in body fluids including blood, urine and, more recently, saliva. Free radicals and antioxidant defenses within the oral cavity can play a key role in the development of odontostomatological pathologies [1]. For this reason, many methods have been developed for the analysis of such components (free radicals and antioxidants) in the oral cavity. These methods are based on the most disparate principles and differ in complexity and technique. Some methods are based on the quantification of a given analyte, such as uric acid [2], vitamin C [3,4] or vitamin E [4]. Other methods measure the ability of the saliva to respond to an in vitro induced oxidative insult, for example, the method proposed by Koracevic [5] where the antioxidant power is measured on the basis of the sample's ability to protect the benzoate from the aggression of radicals produced by Fenton reaction. The most classic ABTS assay belongs to this method [6]. Other methods are based on chemoluminescent or voltammetric techniques [6]. Another important category of methods are based on the determination of iron-reducing power, i.e. the ability of a sample to reduce ferric ions to ferrous ions, the principle of the known FRAP method [7].

SAT test, for example, belongs to this category of methods [8]. This method, is based on the ability of salivary antioxidants to reduce, in hydroalcoholic environment, a percentage of ferric ions to ferrous ions. This reduction is proportional to the iron-reducing power.

The presence of the thiocyanate ion, whose complexes with the ferric ion produce a red-colored solution while those with the ferrous ion are colorless, allows the determination of the iron-reducing power by photometric analysis.

This test has the peculiarity of being added with a zirconium salt that binds phosphate ions present in the saliva; these ions permanently bind the ferric ions and therefore cause a discoloration and false positive results.

Saliva is an extremely heterogeneous fluid which contains enzymatic and non-enzymatic antioxidants.

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These last can be easily quantified with SAT test. The main non-enzymatic salivary antioxidants are uric acid, the most abundant [9], followed by vitamin C, and, only in traces, vitamin A and vitamin E [10].

It is known that uric acid forms stable complexes with ferric ion [11] and around the 70% of the non-enzymatic antioxidant in saliva are represented by uric acid, so a doubt that can arise is that tests developed for the determination of iron-reducing power actually measure only the concentration of uric acid. The aim of this paper is to demonstrate that in the case of the SAT test this is not true and SAT test is an innovative test useful for the quantification of the reducing power of saliva.

METHOD

Two different experiments have been performed. Saliva of 29 subjects (29 males, aged between 18-20 years) has been collected after a written and informed consent. In the first experiment saliva has been analyzed with SAT test (H&D srl, Parma, Italy) and with Uric acid AOX FL (Chema Diagnostica, Monsano, Italy), test for the determination of uric acid. This last test is based on the incubation of the sample with ascorbate oxidase in order to avoid the interference due to the ascorbic acid and on the subsequent colorimetric determination of the hydrogen peroxide generated by the action of the uricase enzyme in presence of uric acid. In the second experiment saliva sample was fortified with Vitamin C, standard addition method, and each fortified sample was analyzed with SAT test. SAT test results are expressed as µmol/L (antioxidant used as reference standard is vitamin C) while those related to uric acid in mg/dl.

Statistical analysis

Data were analyzed using linear regression and correlation techniques, such as Pearson's r.

RESULTS

In the first experiment a strong and significant correlation was found between the values of uric acid and the iron-reducing power measured by SAT ($r = 0.90$, $p < 0.01$). Following this result the analysis of the relationship between the two data series was analyzed by means of linear regression analysis. The following were achieved: $R^2 = 0.82$, linearity test (test F) $p < 0.001$, test of significance (t test) of the angular coefficient $p < 0.001$ and of the intercept $p > 0.05$ (Figura 1a).

In the the second experiment, a linear regression analysis was performed, and the following results has been obtained: $R^2 = 0.99$, linearity test (test F) $p < 0.001$, significance test (t test) of the angular coefficient $p < 0.001$ and of the intercept $p < 0.001$ (Figura 1b).

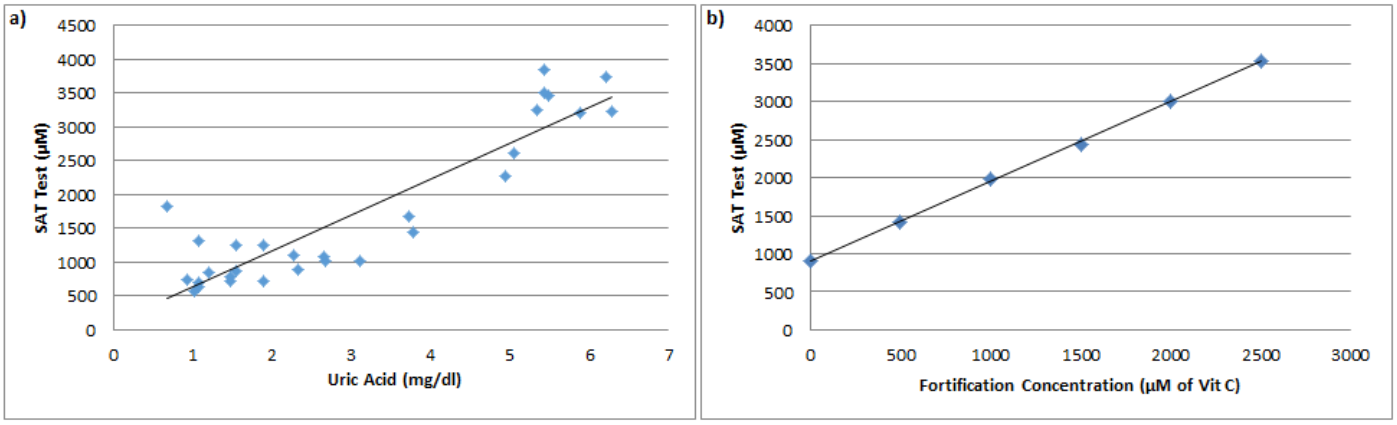

Figure 1: a) results of the SAT and uric acid analysis on 29 saliva samples; **b)** results of the SAT analysis on a fortified saliva sample with Vitamin C.

DISCUSSION

Although the two experiments are conceptually different, the results of both experiments demonstrate that the measure of iron-reducing power of saliva is influenced by both the presence of uric acid and vitamin C, the main antioxidants of saliva.

The first experiment was more complicated because of the impossibility to fortify saliva with uric acid and to recreate a solution of uric acid at physiological concentrations that did not interfere with the SAT test. For this reason, it has been decided to analyze the saliva samples both with the SAT test and with Uric acid AOX FL and compare the data. The comparison was made through the study of the correlation that was strong and significant, therefore the modification of one value also changes the other, therefore the iron-reducing power is strongly influenced by the presence of uric acid. The results of the regression analysis between uric acid concentration and iron-reducing power of saliva are extremely interesting. The relationship between the two quantities is linear but the R^2 is about 0.80, this means that the variation in uric acid concentration contributes to about 80% of the variation of the iron-reducing power of saliva. This particular finding is reflected in literature [9], and proves that the measure of iron-reducing power with SAT test, in particular, is not the measure of uric acid only but the test is simultaneously sensitive to the variations of more antioxidants of the oral cavity and therefore it is an excellent estimate of the salivary global antioxidant power. The second experiment confirms the sensibility of the SAT test for Vitamin C [8]. Unlike uric acid, vitamin C has been used to fortify a saliva sample with increasing concentrations in order to obtain a curve. Figure 1b shows the increase in the signal that is extremely linear with respect to the increase in concentration in Vitamin C.

CONCLUSION

It has been established that the iron-reducing power of saliva is simultaneously influenced by multiple antioxidants and not only the uric acid. Salivary iron-reducing tests, like SAT test, are sensitive both to uric acid and to vitamin C and likely to all salivary reducing agents. Moreover, salivary iron-reducing tests are an excellent estimate of the global antioxidant power of the saliva and thus of the oral cavity.

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Conflict of Interest

None.

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