Original Article

The Variations of Some Salivary Parameters as Probable Indices of the Hereditary Diabetes

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Abstract

Background: Diabetes has a genetic predisposition and is generally not diagnosed for many years because hyperglycemia develops gradually, without presenting the classic symptoms of diabetes. The aim of this study is to verify whether, in a potentially genetically predisposed population, men and women under the age of 50 years, at the time of the study, not suffering from diabetes can be detected using parameters derived from initial metabolic alteration indices of the possible evolution of pathology. **Methods:** In the hereditary and healthy group, salivary concentration of malondialdehyde, total mucins, and pH were determined. All participants in the two groups had fasting glucose level below 110 mg/dL. The results were statistically analyzed using Pearson correlation test, Mann–Whitney test, and –Student's *t*-test. **Results:** Salivary concentration of malondialdehyde statistically increased in the hereditary group vs the healthy group ($P = 0.0368$) as the mucins ($P \leq 0.005$). The salivary pH decreased but, the values were not statistically significant ($P = 0.085$). Some alteration processes occur without increase in glucose levels, produced by changes in metabolic redox processes along with an increase in the salivary malondialdehyde index of oxidative stress in the body. The modification of the salivary buffer system lowers the pH, whereas increase in salivary mucins alters the value of spinnbarkeit, which measures the capacity of the mucous layer to adhere to the epithelium, causing alterations of the oral mucosa. **Conclusions:** This study shows that it is possible to predict in hereditary predisposition conditions the development of diabetes, and the related dangerous consequences by monitoring two salivary parameters – mucins and malondialdehyde.

Keywords: *Diabetes, genetic predisposition, mucins free radicals*

Background

The onset of diabetes can be attributed to genetic predisposition, viral infection, autoimmunity, and other ethological factors, each of which can be regarded as a common reason for the disease occurrence, which is also commonly linked to exogenous factors such as eating habits, physical activity, and obesity.[1]

The diagnosis of diabetes,[2] if one of the following three criteria are met: (a) typical symptoms of diabetes, associated with a blood sugar level detected at any time of the day, regardless of food consumption, not less than 200 mg/dl; (b) glucose in the blood measured after at least 8 h of fasting equal to or greater than 126 mg/dl; (c), glucose concentration 2 h after 75 g oral glucose load, equal to or greater than 200 mg/dl. The measurement of fasting plasma glucose and oral glucose tolerance test also allow identification of intermediate categories that may precede overt diabetes (prediabetes), and therefore, represent a wake, and we can have (a) altered fasting glucose (IFG), fasting glucose values between 100 and 125 mg/dl; (B) glucose intolerance (IGT), with blood glucose 2 h after an oral glucose load of between 140 and 200 mg/dl.

These parameters, always at the limit of the norm, even in the course of a single day can increase or decrease with no detectable side effects, which is typical of diabetes, mainly cardiovascular, cerebrovascular, and peripheral vascular, usually attributable to the high concentrations of free radicals present in the diabetic patients.[2] In fact, in the diabetic pathology, there is an increase of lipid peroxidation, derived according to some studies,[3] the lack of balance between the production of oxygen radicals and antioxidants in the body. The peroxidation of membrane lipids alters the lipid structure of biological membranes and with it

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the physiological process of the same. Polyunsaturated fatty acids are the main substances structurally altered by lipid peroxidation: the most important byproduct of these biochemical reactions is malondialdehyde, which is identified as the best biological indicator of this process harmful to the body.[4] The evident increase of reactive oxygen species (ROS) accompanied by decrease in antioxidant activity causes deterioration of structural macromolecules (carbohydrates, proteins, lipids, and DNA), which leads to their instability and consequently loss of function.[5] This implies that the biological mechanisms involved in diabetes are negative feedback: the pathology for its intrinsic enzymatic imbalance and/or oxyreduction increases the production of free radicals, which in turn lead to the onset of degenerative processes typical of diabetes. The mechanisms behind the increased oxidative stress in diabetes are not entirely clear. There is evidence to suggest that the increased production of free radicals in the form of superoxide is default of one antioxidant status. The balance in the formation of the compound superoxide is governed by the enzyme superoxide dismutase,[6] in which it is present as a cofactor of zinc whose function is based on its affinity enzyme, as a complex metal-enzyme in humans and animals, diabetes can be the result of a defect in the concentration of these trace elements. The problem of formation of free radicals during excessive production of glucose, more than the limits established by clinical practice but less than those used to define overt diabetic condition has never been addressed. Another relevant aspect of but not sufficiently considered and detected, in pre-diabetes, concerns the oral apparatus, particularly the gums, teeth, and repeated mucosal infections.[7] These damages in diabetes have been brought about by the fact that the buffering capacity of the saliva decreases, lowering the pH, and salivary flow. In a recent study, it has been suggested, as in fact, even the increase in secretory-type mucins, in the saliva, which constitutes the fundamental part of the protective layer of the oral mucosa, results in an alteration of the same protection system.[8] Understanding this process is based on a theoretical hypothesis proposed and discussed in this study: the possible explanation for the alterations of oral apparatus in diabetes, and possibly prediabetes, is another important objective of this work.

Methods

Two groups of 23 volunteers were selected, including 11 males and 12 females. Both groups comprised individuals under the age of 50 years, and underwent analysis of the main blood chemistry parameters (creatinine, transaminase), urine analysis, and blood pressure analysis in the standard condition, and during fasting glucose concentrations of less than 110 mg/dL.

The group called "hereditary," composed of people in whom the possible predisposition to diabetes was linked with the presence of this pathology in one or both parents. Saliva samples were analyzed in the morning and after 2 hours of taking food. People with a history of diseases that can lead to generation of excess free radicals, such as smoking and cardiovascular disease, were excluded from the study. Also excluded were those who were in anamnesis, reported excess alcohol consumption, taking antioxidants such as vitamins or supplements containing curcumin and quercetin polyphenols, and who performed strenuous anaerobic exercises.

The salivary concentration of malondialdehyde were determined using the thiobarbituric acid test. The concentration of total salivary mucins was determined using the alcian blue method[8] briefly described below. A fraction of diluted saliva samples (1:10) was incubated for 30 min in a 1% solution of alcian blue in 50 mM sodium acetate buffer with 25 mM of $MgCl_2$ to pH 5.8 under constant stirring at room temperature. The mucin–dye complex is shown with the addition of a dilute solution 1:2, Aerosol OT (Sigma Chemical Co., St Louis, MO, USA), bis (2-ethylhexyl) sulfosuccinate, sodium salt concentration was determined spectrophotometrically at 605 nm. pH was determined using sticks "Uriscan Roche" for urine analysis and analyzed using an automatic reader (URIYSIS2400 ROCHE). Fasting blood glucose level was assessed using a self-portable meter GlucoMen LX plus (Menarini).

The results of the differences in the values of malondialdehyde concentration in the two group, those relative to males vs females, those in a relationship, and aged over 40 years were analyzed using Mann–Whitney test for execution of the statistical test, those conditions are required.

Requirements

- Two random, independent samples
- Continuous data, i.e., it must, in principle, be possible to distinguish between values at the nth decimal place
- Scale of measurement should be ordinal, interval, or ratio.

The pH values and the total mucins concentration were statistically analyzed with Student's *t*-test.

Results

Table 1 presents the comparison of salivary malondialdehyde concentration for the two groups. The application of the Mann–Whitney test to these results clearly indicates a statistically significant correlation ($P \leq 0.005$) of salivary malondialdehyde concentration in the hereditary group vs the control group, which was a function of increasing age.

Table 2 presents the salivary pH values and the concentration of total salivary mucins. Student's t-test showed that mucins concentration was statistically different ($P \leq 0.005$). pH values using Student's *t*-test does not indicate a difference, but a tendency to decrease ($P = 0.085$).

Table 1: Comparison of salivary malondialdehyde concentration for the two groups

Hereditary group			Control group		
Sex	Age	MDA	Sex	Age	MDA
m	49	4,1	m	49	1,2
m	30	3,9	m	30	1,2
m	30	3,9	m	30	3,5
m	45	4,0	m	45	2,0
m	24	2,4	m	24	2,1
m	36	2,0	m	36	3,2
m	46	4,3	m	46	3,6
m	33	3,6	m	33	2,0
m	44	3,5	m	44	3,4
m	31	2,7	m	31	2,3
m	39	2,7	m	39	1,3
m	43	2,9	m	48	2,7
m	46	3,2	m	49	2
m	47	4,3	m	46	3,2
f	49	4	f	42	2,3
f	37	4	f	37	2,8
f	38	3,3	f	46	3,.5
f	43	3,9	f	30	2,5
f	42	3.2	f	39	3,1,
f	45	4,6	f	42	3,8
f	46	4,4	f	31	2,3
f	38	3,1	f	40	1,3
f	41	2,3	f	44	2,3
f	44	4,1	f	49	1,8
f	36	3,9	f	34	3,4
f	44	3,2	f	46	2,3
f	34	2,2	f	33	2,3
f	35	3,1	f	38	2,5

m=Men, f=Women, MDA concentration nM/ml

Table 2: The values of total salivary mucins and pH

Sex	MUCINS mg/dL	pH	MUCINS mg/dL	pH	Sex
m	30,1	7,4	38,7	6.6	m
m	31,2	7,6	38,7	6,8	m
m	31	6,9	39,8	6,8	m
m	30	7,7	39,6	6.6	m
m	29,9	7,6	40	7	m
m	29,8	7,3	40,5	6,6	m
m	28,9	7,4	39,6	7.0	m
m	30,1	7,0	37,7	6,4	m
m	30,9	7,4	38,8	7	m
m	29,3	7,4	38,8	6,3	m
m	31,4	7,2	39,1	6,9	m
f	30,3	7,4	39,9	7,3	f
f	31,9	7	39,8	6,4	f
f	32,1	7,4	38,6	6,6	f
f	31	6,7	39,1	6,9	f
f	30,3	7,3	40,2	6.1	f
f	30,3	7,8	37,4	6,6	f
f	29,3	7,9	38,9	6,1	f
f	31,3	7,4	38,7	6,2	f
f	30,1	6,7	39,1	6,4	f
f	30,1	7	33,3	6.2	f
f	31,9	7	39,2	7,1	f
f	30,4	7	39,8	5,9	f

m=Men, f=Women

Several studies indicate that hyperglycemia overt diabetes increases oxidative stress through the overproduction of reactive oxygen species, which results in an imbalance between free radicals and the antioxidant defense system of cells.[6] Hyperglycemia can also increase the production of inflammatory cytokines PRO, further impairing the activation of activated protein kinase and increase apoptosis in cultured cardiomyocytes.[7] The results of this study state the increase of malondialdehyde in the possible hereditary group, the same process observed is statistically significant even when there is a hyperglycemic status not due to body's resistance to insulin or its absence but can occur anytime. The homeostasis of the oxidation–reduction of the system, is altered for reasons that are normally associated to hereditary predisposition, and to as incorrect lifestyle. This event results in an impaired glucose increase situation, although less than that concentration which is without a diagnosis of diabetes, as in the case of patients subjected to this study. A similar effect is present when in fact there is uncontrolled type 2 diabetes mellitus in this case.[9] There is a significant positive correlation between malondialdehyde and HbA1c. The result also indicates that in the possible hereditary, there is a statistically significant correlation between increasing age and an increase of malondialdehyde, while in the control group, this phenomenon is present, but in a amount much less. However, it accentuated the phenomenon and an explanation for this can be attributed to Harman's theory[10] or mitochondrial hypothesis expressed already in 1956. Mitochondrial dysfunction has long been considered a major cause of aging and related diseases. Mitochondrial free radical theory of aging postulate that somatic mitochondrial DNA mutations that accumulate in the cell because of excessive production of reactive oxygen species and the damaged macromolecules affect the function of cells and tissues. In fact, studies have shown that the maximum oxidative capacity decreases with age while increasing the production of reactive oxygen species. The hypothesis of Harman has been seriously challenged by recent studies showing that reactive oxygen species evoke metabolic health and longevity perhaps through mechanisms that include autophagy. The purpose of this study is to examine the growing literature on mitochondria from the viewpoint of research on aging and try to identify the priority issues that should be addressed in future research. This theory has been challenged, modified, and extended by many, but there remain two main arguments in support of it: first, there is evidence that an imbalance between antioxidant/oxidant occurs with age, which results in an accumulation of macromolecules that are oxidized and damaged. Second, the accumulation of oxidative damage causes involving the formation of an aged phenotype and degenerated. This second point is the one most questioned that has been recently challenged by several studies.[11,12] The mitochondrial hypothesis justify the data obtained in this study. The results of this study indicate that the increase in the formation of free radicals and the prediabetes condition involves a decrease in salivary pH values. Not statistically significant compared to the control group and a significant increase of the secretory fraction of the salivary mucin has occurred even in overt diabetes alteration of pH, which is one of the most important factors that regulate balance in oral cavity functions of defense and consequences such as mouth ulcers and candidiasis. The decrease in pH along with others such as salivary flow and buffering capacity of saliva lead to change "spinnbarkeit" [see Figure 1], the mucous layer lining the epithelium, which is the clear slippery characteristic elastic consistency the same. The spinnbarkeit saliva reflects the ability of saliva to stick to surfaces inside the mouth, and therefore, represents a protective role in adhesion and lubrication. Therefore, alterations in spinnbarkeit of saliva may result in the loss of adhesiveness or the ability to bind to surfaces that can relate to experimental oral dryness, it is seen that the minimum value in mm (cutoff) must not be less than 11–12, even if the optimal value is approximately 14 mm. The pH of the saliva depends on the bicarbonate buffer system and the concentration of calcium, K, and Na ions, influencing the hydrophilic ability of salivary mucins, which occurs in autoimmune diseases and diabetes. In general, when leading to a lowering of the pH in the saliva (13), (13), even up to values of 5.5–6., also the spinnbarkeit shows a gradual fall. As the pH of saliva decreases, its physiological pH value becomes 7.1, the spinnbarkeit shows a gradual fall. However, the spinnbarkeit of saliva shows a strong decrease when the pH was increased from the baseline, as can be seen in Figure 1. At these pH values, the relative value of spinnbarkeit is below the cutoff. In this study, it is obvious that a decrease of about one unit does not imply a reduction of the spinnbarkeit value below the normal cutoff. Hence, the explanation for the presence of some initial symptoms such as xerostomia reported by some prediabetes patients should be sought along with modification of other parameters such as increase in salivation of secreted mucins. Mucins are important components of saliva structure and are composed primarily of water and mineral salts, as well as the same mucins mainly MUC5B, MUC7, lysozyme, SIgA, along with other components. Figure 2 shows the main functions of these components which form the protective layer of the oral mucosa and larynx. The mucins become the key parameters involved in the presence of prediabetes. In diabetes, as opposed to other autoimmune diseases, there is a considerable increase of these elements, and this increase also presents in the development of oral and laryngeal cancer. The result of this increase is observed in complications to the teeth and oral mucosa, which is evident in diabetic pathology. An explanation of these frequent alterations in diabetes unlike other autoimmune diseases can be obtained, assuming a mechanism not currently checked experimentally, based on the formation in the presence of an overexpression of mucins, MUC5 B and MUC7, of a mucin layer, yet less effective in its protective action than oral epithelium in reality. In fact, it considerably increases the salivary concentration of the type of mucins secreted, MUC5B and MUC7, but not the MUC1 related to epithelial cells, which make provision for the anchoring of the protective layer on the oral mucosa. It is reasonable to assume that this increase in the secreted fraction exceeds that of a MUC1 fraction, which results in mutual interaction between the structures of polysaccharide MUC5B and MUC7 terminals, with formations of bonds. Probably sialic acid in saliva in diabetes is considerably lower than the normal concentrations; in this condition the sialic acid is easily bound in this structure mucin, making it, even more compact, and also, it is highly hydrated. Practically, this situation of overexpression in the formation of mucins, especially MUC5B and MUC7, contributes to the compaction of the bubble type. Basically, it can be assumed that saliva is present in prediabetes not as a relaxed layer adhering to the oral mucosa and/or laryngeal, but as a layer formed from many droplets; consequently, in addition to the possible establishment of the cord oral diseases, there is a strong feeling of dry mouth.

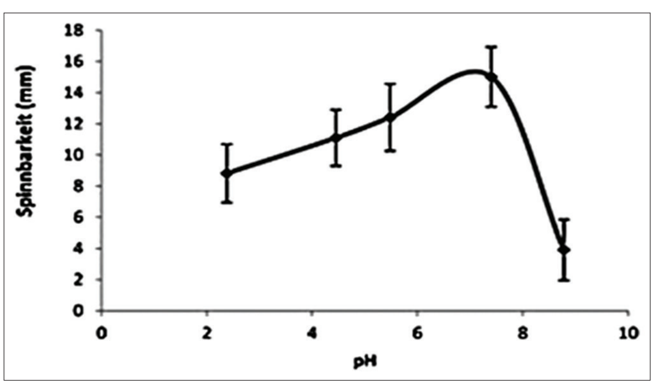

Figure 1: The influence of pH on spinnbarkeit value

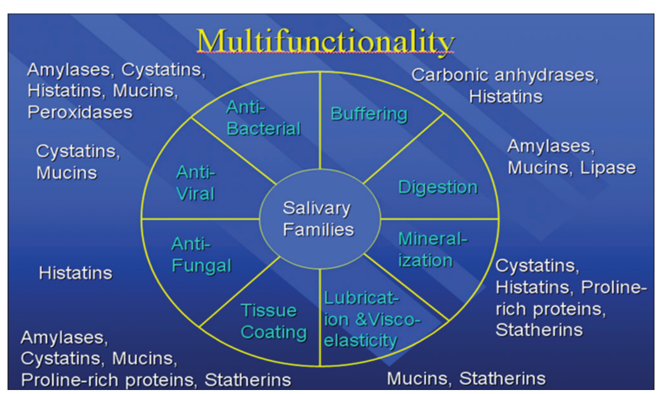

Figure 2: The main functions of the components which form the protective layer of the oral mucosa and larynx

Uncontrolled blood glucose levels in prediabetic patients can cause numerous pathological situations that result in microvascular and macrovascular complications. Prevention of lipid peroxidation may help hinder the development of overt diabetes. These states of affairs may play an important role in the prevention of progress of cardiovascular abnormality for diabetes because monitoring glucose blood balance is very difficult, while it is much easier to use free radicals, which are a more scientifically reliable index. This study proposes that it is important prevent the production of free radicals, and that this result may be obtained, for example with the use of commercial antioxidants preventing high blood glucose levels.

Conclusions

Uncontrolled blood glucose levels in prediabetic patients can cause numerous pathological situations that result in microvascular and macrovascular complications, etc.

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Conflicts of interest

There are no conflicts of interest.

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