Effects of diabetes mellitus on salivary secretion and its composition in the human

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Abstract

This study investigated the effects of diabetes mellitus (types I and II) on human salivary gland function compared to healthy age-matched controls. The results have shown that both type I and type II diabetic patients secrete significantly ($p < 0.05$) less resting and stimulated saliva compared to healthy age-matched controls (AMC). It was also found that the diabetic patients have an increased resting and stimulated salivary protein concentration compared to healthy participants. However, the secretory capacity (stimulated minus resting values) was markedly reduced compared to controls. The level of calcium ($Ca^{2+}$) in the saliva of diabetic patients was significantly ($p < 0.05$) elevated compared to the AMC. In contrast, the levels of magnesium ($Mg^{2+}$), zinc ($Zn^{2+}$) and potassium ($K^{+}$) in the saliva of diabetic patients were significantly ($p < 0.05$) reduced compared to the values obtained in AMC. These results indicate that diabetes mellitus can lead to marked dysfunction of the secretory capacity of the salivary glands. In these patients a modified fluid, organic and inorganic salivary secretion may be responsible for the increased susceptibility to oral infections and impaired wound healing described by others in the literature. (Mol Cell Biochem xxx: 1–6, 2004)

Key words: diabetes mellitus, human saliva, protein, calcium, magnesium, zinc, potassium

Introduction

The understanding of salivary function in promoting a healthy oral condition has become a topic of major importance for the nowadays-oral clinicians. In order to understand the role of each salivary component in the oral cavity homeostasis, it is crucial to perceive how its changes or absence may be linked with pathological conditions [1]. The study of salivary function has been overwhelmingly difficult by the enormous natural occurring variability of this fluid when compared to others (such as the plasma) in which the regularity of the composition of plasma has permitted to undoubtedly separate physiological from pathological conditions [2]. However, saliva could offer an excellent alternative to serum as a biological fluid analysed for diagnostic purposes. This would be of great biomedical importance, since saliva is very easy to collect offering a cost-effective approach for screening of large populations, and could represent an alternative for patients whose blood is difficult to obtain or when compliance is a problem [3]. Hitherto, saliva has been proposed for the monitoring of a great number of systemic levels of therapeutical or abuse drugs from digoxin and caffeine to ethanol, cocaine, marijuana or opioids and the number of diagnosis uses for saliva tend to increase rapidly [4]. However, knowledge integration between saliva and oral pathology is far from being complete. Therefore, it is of critical importance to establish which salivation patterns and concentration ranges of each salivary component are to be considered as normal in order for the clinician to diagnose altered salivary phenotypes possibly linked to pathological systemic or oral conditions [5]. Several systemic diseases such as cystic fibrosis [6], HIV infection [7] or auto-immune diseases such as Sjögren...
syndrome [8] among others have also been reported to produce
deteriorative effects on the general health [9]. Diabetes is also
probably the most frequent metabolic disease with salivary
implications. Salivary hypofunction and increased suscepti-
- bility to oral infections such as caries or periodontitis [10] have
long been recognized features of diabetes mellitus, particu-
larly when there has been dehydration and inadequate glucose
blood control [11]. However, there is little knowledge con-
cerning the true effects of diabetes in the salivary parameters
of well-controlled patients and the way that the two types of
disease affect these patients. In this study we have inves-
tigated the effects of type I and II diabetes mellitus on the
quantity and quality of the saliva in well-controlled diabetic
patients compared to healthy age-matched participants.

Materials and methods

Patient selection

This study employed 60 diabetic patients (30 patients with
type I and 30 with type II diabetes). Well-controlled diabetes
was a criterion for patient selection and was assessed from pa-
tient’s clinical records. For both type I and type II diabetic par-
ticipants an equivalent number of healthy age-matched con-
trols (AMC) (e.g. range of 20–30 years for type I and 40–55
years for type II) participants were employed. Four groups
were assigned as type I for diabetic type I, type II for diabetic
Type II, AMC I for age-matched control for type I diabetes
and AMC II for age-matched control for type II diabetes.
Participants were selected as volunteers and provided written
and oral information regarding risks and benefits of the proce-
dures. Each patient signed a consent form. This procedure re-
ceived ethical clearance and approval by local Ethic Commit-
tees at ICSS Portugal and at University of Central Lancashire
in Preston. Saliva was collected at either the Portuguese Di-
abetes Association or a private dental clinic in Lisbon.

Saliva collection

Saliva collection was undertaken between 7 and 8 a.m., and
participants were instructed to be in a fasting state. Both rest-
ing and stimulated whole saliva were collected by established
methods [12]. Briefly, unstimulated saliva was collected by
spitting method. Participants were instructed to swallow the
saliva present in the mouth and a chronometer was started. Four
drops (0.17 g) of citric acid (0.1 M) were applied to tongue
dorsum and participants were instructed to roll their tongue
in order to spread the citric acid. At 1-minute intervals saliva
was collected by spitting into previously weighed propylene
50-mL falcon tubes and more four drops of citric acid were
applied. This procedure was repeated for 10 min. Immedi-
ately after collection, the salivas were weighed and stored at
−80°C until used for analysis procedures. Rates of resting
and stimulated secretions were expressed in g/min.

Salivary ion analysis

Saliva collected was analysed for the concentrations of K+, Na+, Ca2+, Mg2+ and Zn2+. Saliva samples were defrosted
at room temperature and then centrifuged at 6000 rpm for 10
min before being used in order to remove extrinsic contami-
nation elements such as oral epithelial cells, microorganisms
and food debris among others.

For the determination of the salivary ions, saliva was di-
luted at either 1/100 or 1/1000 and either K+, Na+, Ca2+, Mg2+
or Zn2+ concentration was obtained in a Shimadzu AA
670 Atomic Absorbance Spectrophotometer (Japan). Results
were expressed as mg/L. In order to minimize ionic contami-
nation of salivary samples, propylene tubes of saliva collec-
tion were previously decontaminated by immersing there in
60% w/v nitric oxide for a 2-week period before collection.

Salivary analysis of total proteins

Saliva samples were defrosted at room temperature and then
centrifuged at 6000 rpm for 10 min before use. Total pro-	ein concentration expressed as mg/L was determined using
established colorimetric methods [13] with the use of an He-
lions spectrophotometer by reading samples at 720 nm. Bovine
serum albumin was used for calibration purposes.

Statistical analysis

Data are presented either as Mean ± Standard Error of the
Mean (S.E.M). Control and test values were compared using
paired or unpaired Student’s t-test and ANOVA plus post-hoc
tests (SPSS for Windows version 10.0). Values of p < 0.05
were taken as significant while values of p < 0.01 were taken
as highly significant.

Results

Figure 1 shows resting and stimulated flow rates in healthy
age-matched controls and diabetic patients. The results show
that resting and stimulated secretory rates saliva were signif-
ically (p < 0.05) decreased for type I and type II diabetic
patients when compared to AMC. However, the magnitude of