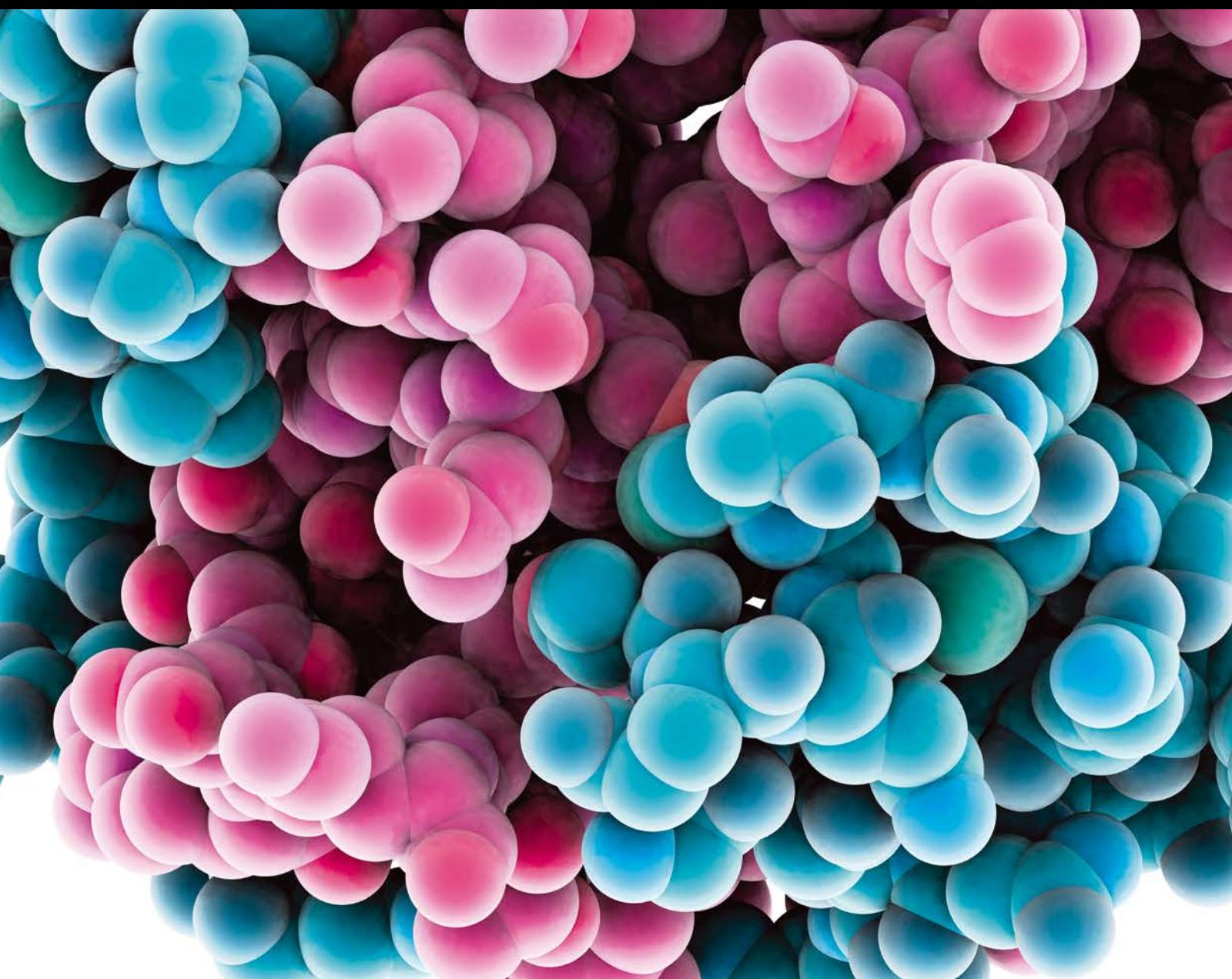


Dentistry and Diabetes: The Influence of Diabetes in Oral Diseases and Dental Treatments

Guest Editors: Eugenio Velasco-Ortega, Rafael Arcesio Delgado-Ruiz, Jose López-López, and Gustavo Avila-Ortiz



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Editorial

Dentistry and Diabetes: The Influence of Diabetes in Oral Diseases and Dental Treatments

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Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin that it produces. The number of people with diabetes is increasing. These trends highlight the urgency for a better understanding of diabetes as well as for improving the dental care of patients with diabetes. Patients with diabetes have increased frequency of periodontitis, tooth loss, and xerostomia, and diabetes has been considered a risk condition for oral surgery and dental implants with the fact that it is associated with delayed wound healing, prevalence of microvascular disease, and impaired response to infection.

J. Gonzalez-Serrano et al., in a systematic review, showed that a higher prevalence of oral mucosal disorders was found in patients with diabetes mellitus (DM) compared to non-DM patients. This increased prevalence of oral disorders in DM groups may be due to an inadequate metabolic control of DM or a slow healing process.

The results of an experimental study in animals about the oxidative damage caused to the salivary glands in streptozotocin-induced diabetes are presented by M. Knaš et al. and demonstrated that the unstimulated salivary flow in DM rats was reduced in the 2nd week, while the stimulated flow was decreased throughout the duration of the experiment versus control.

R. M. López-Pintor et al., in a systemic review, showed a decreased salivary flow in DM patients in relation to non-DM patients. The reasons for these problems could be due to damage to the gland parenchyma, alterations in the microcirculation to the salivary glands, dehydration, and disturbances in glycemic control.

J. Matczuk et al. observed that the high fat diet regimen had caused significant changes in the salivary glands lipid composition, especially in regard to phospholipids (PH) and triacylglycerol (TG) in rats. The observed reduction in PH concentration is an interesting phenomenon frequently signifying the atrophy and malfunctions in the saliva secreting organs. On the other hand, the increased accumulation of TG in the glands may be an important clinical manifestation of metabolic syndrome and type 2 diabetes mellitus.

The importance of periodontitis in diabetes is assessed by Q. Wang et al. that presented a comparison of experimental periodontitis induced by *Porphyromonas gingivalis* in diabetic mice.

Given the increasing number of dental patients with diabetes worldwide, there is a necessity for a better understanding of mechanisms of the oral manifestations of diabetes and its comprehensive treatment to prevent possible complications.

Eugenio Velasco-Ortega
Rafael Arcesio Delgado-Ruiz
Jose López-López

Research Article

Comparison of Experimental Diabetic Periodontitis Induced by *Porphyromonas gingivalis* in Mice

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Periodontitis is one of the severe complications in diabetic patients and gingival epithelium plays an initial role on the onset and progression of this disease. However the potential mechanism is yet sufficiently understood. Meanwhile, the research on the correlational experimental animal models was also insufficient. Here, we established periodontitis with type 2 diabetes in *db/db* and Tallyho/JngJ (TH) mice and periodontitis with type 1 diabetes in streptozotocin induced diabetes C57BL/6J (STZ-C57) mice by oral infection of periodontal pathogen *Porphyromonas gingivalis* W50. We demonstrated that periodontal infected mice with high blood glucose levels showed dramatically more alveolar bone loss than their counterparts, in which infected *db/db* mice exhibited the most bone defects. No contrary impact could be observed between this periodontal infection and onset and severity of diabetes. The expressions of PTPN2 were inhibited whereas the expression of JAK1, STAT1, and STAT3 increased dramatically in gingival epithelia and the serum TNF- α also significantly increased in the mice with diabetic periodontitis. Our results indicated that the variations of inflammation-related protein expressions in gingival epithelia might lead to the phenotype differences in the mice with diabetic periodontitis.

1. Introduction

Periodontitis, a worldwide complication of diabetes (known as diabetic periodontitis), is diagnosed by destruction of periodontal cementum, ligament, and alveolar bone [1]. Both types of diabetes in clinic, type 1 diabetes (T1D) and type 2 diabetes (T2D), exhibit dramatically higher risk and severity of periodontitis [2–4]. In recent years, researches are focused on the bidirectional communication between diabetes and periodontitis [5]. However, the underlying mechanism and the potential therapy are yet understood. Because of the complexity of clinical trials, establishing reliable animal models is particularly necessary.

In diabetic periodontitis, significantly increased inflammatory cytokines could be found in serum and gingival cervical fluid [6]. Previous studies have confirmed immune and inflammatory responses in the gingival epithelium play an initial role on the onset and development of diabetic periodontitis [7, 8]. Protein tyrosine phosphatase nonreceptor type 2 (PTPN2) is an intracellular tyrosine-specific phosphatase, which is expressed in epithelial cells, fibroblasts, or endothelial cells [9]. The biological function of PTPN2 is believed to vary in response to proinflammatory stimuli such as interferon-gamma (IFN- γ), tumor necrosis factor (TNF- α), hyperosmotic stress, or hyperglycemia [10, 11]. Moreover, proteins on Janus family kinase (JAK)/signal

transducer and activator of transcription (STAT) pathway, an important signaling in inflammation, have recently been found to be targets of dephosphorylation by PTPN2 [12]. Inactivation of those substrates by dephosphorylation lead to the negative regulation of signaling pathways involved in inflammatory responses induced by the proinflammatory cytokines like TNF- α , which is also increased in serum and gingival cervical fluid of patients with periodontitis [13].

Mice are usually used as the experimental animal models for the research of human disease, because of its unique host response [14]. A large number of researchers have studied the experimental mice models of chronic periodontitis and diabetes disease, respectively [15, 16]. But the research about the establishment and characteristics of the experimental diabetic periodontitis mice models is yet insufficient. In this study, we established three mice models of experimental diabetic periodontitis and compared characteristics in metabolism and periodontal inflammation. The levels of PTPN2 expression and key factors on JAK/STAT pathway were analyzed to reveal the potential biological reasons behind the differences.

2. Materials and Methods

2.1. Experimental Animals. Twenty 4-week male *db/db* mice, 20 male Tallyho/JngJ (TH) mice, and 40 male C57BL/6J (C57) mice were fed commercial mouse food (finely ground autoclaved low-fat diet) and housed under a controlled environment (temperature around 22°C and relative humidity 45–55% with a 12-12-hour light-dark cycle) for the entire research process. At 6 week, 20 C57 mice were received streptozotocin (STZ, 55 mg/kg body weight; Sigma-Aldrich, St. Louis, MO, USA) for 5 days successively by intraperitoneal injection to establish diabetic mice (STZ-C57). Animal treatment was approved by the Ethics Committee of Animal Welfare (WCCSIRB-2015-133).

2.2. Study Design. All mice were randomly divided to infection and shame-infection group. *Porphyromonas gingivalis* (*P. gingivalis*) W50 was acquired from the State Key Laboratory of Oral Diseases, Sichuan University and cultured anaerobically in blood agar (Oxoid, Oxoid Ltd., Hampshire, England) with hemin/menadione (Sigma-Aldrich).

At 6 weeks, the mice in the infection groups were inoculated with *P. gingivalis* as the following methods [17]: 10⁹ colony-forming units of *P. gingivalis* were diffused in 100 μ L phosphate buffered saline (PBS) with 2% carboxymethylcelulose and then orally inoculated three times every second day. The mice in shame-infection groups were infected with the equal volume of PBS (100 μ L). All mice were killed at 18 week.

2.3. Measurement of Fasting Blood Glucose and Body Weight Levels. Fasting blood glucose was determined every 2 weeks in tails blood collected from tail veins of the mice after an eight-hour fast using the glucose meter (OneTouch; LifeScan, Milpitas, CA, USA). Body weight was also determined every 2 weeks during the experimental process.

2.4. Determination of Serum Tumor Necrosis Factor-Alpha (TNF- α) Levels. At sacrifice, the serum tumor necrosis factor-alpha (TNF- α) levels were determined in tails blood collected as above in triplicate by ELISA kit (CUSABIO; Sino-American Biotechnology, Wuhan, China) according to manufacturer's instructions.

2.5. Quantification of Alveolar Bone Loss. Level of alveolar bone loss was determined using SEM (scanning electronic microscope; Zeiss EVO, CRAIC Technologies Inc., Kirchdorf, Germany) [18]. The alveolar bone loss level was measured by the average area (mm^2) bordered as the cementoenamel junction, the mesial and distal line angles, and the alveolar bone crest on the lingual sides of the mandibular second molars [19]. Three evaluators calculated the data of same samples in a blinded, random fashion.

2.6. Immunohistochemical Analysis of Gingival Epithelium. Maxillas of *db/db* and C57 mice were dissected after sacrifice and were made into section slides and stained with immunohistochemical (IHC) as presented previously [20]. In short, both sides of maxillas were decalcified in 10% EDTA solution (BioRad, BioRad Laboratories, Hercules, CA) for 14 days and embedded in paraffin. The paraffin-embedded tissues were cut into thin sections (4 μm) and stained with immunohistochemical. The primary antibodies, anti-PTPN2 (1:50), anti-JAK1 (1:100), anti-STAT1 (1:100), and anti-STAT3 (1:100), and the secondary antibodies (1:1000) were all from Santa Cruz Biotechnology (Santa Cruz, Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Five slides were applied for each sample at 10 intervals. The staining pictures were captured by an optical microscope (Nikon 80i, Nikon Ltd., Tokyo, Japan).

2.7. Histological Examination of Pancreas Tissue. Pancreas tissues of four types mice were dissected, rinsed in PBS, and fixed in 4% paraformaldehyde (Sigma-Aldrich) for 24 hours. The paraffin-embedded tissue samples were cut into a series of 4 μm section slides and stained with hematoxylin and eosin (Sigma-Aldrich). The morphological characteristics of pancreas tissues were observed in the images captured by an optical microscope (Nikon 80i).

2.8. Statistical Analysis. Data were shown as mean \pm standard error of the mean (SEM) and analyzed with Student *t*-test when comparing two groups or one-way analysis of variance (ANOVA) test followed by SNK-*q* multiple comparisons when comparing three or more groups using SPSS software (SPSS 17.0, Chicago, IL, USA). A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Different Metabolic Characters Were Shown in Four Types of Mice. The fasting blood glucose and body weight levels of all mice were determined every 2 weeks and changes over time are presented (Figure 1). Within the mice with *P. gingivalis* infection, *db/db* mice exhibited hyperglycemia

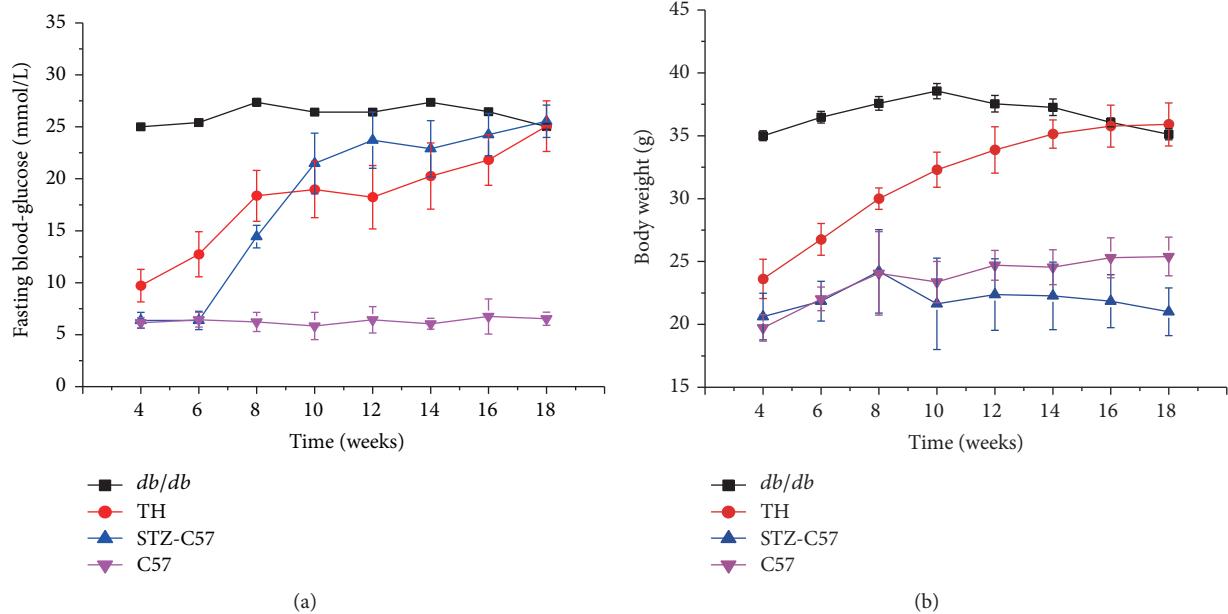


FIGURE 1: Fasting blood glucose (a) and body weight (b) levels in the infection groups of different mice. Values for each time point are expressed as mean \pm SEM.

from the beginning of research and the fasting blood glucose was maintained at a high value as averagely 26 mmol/L during the experimental period. TH mice increased spontaneously and exhibited high glucose at 8 weeks old and then elevated continuously to a final value as approximately 25 mmol/L at sacrifice. STZ-C57 mice exhibited hyperglycemia 2 weeks after STZ injection at 8 weeks old and continued to increase from weeks 8–10. The level maintained steady as evenly 25 mmol/L from weeks 10–18, while the fasting blood glucose level of C57 mice maintained normal from 6 mmol/L to 7 mmol/L for the entire experimental period. The body weight level of *db/db* mice increased spontaneously from weeks 6–10 but decreased gradually after 10 weeks. Conversely, the body weight level of TH mice increased gradually during the research. The body weight level of C57 mice was steady, but the level of STZ-C57 mice in the infection groups decreased.

3.2. *P. gingivalis* Infection Did Not Affect the Fasting Blood Glucose Level among the Four Types of Mice. As shown in Figure 2, comparing infection groups with shame-infection groups, no statistical difference was observed in the fasting glucose levels among the four types of mice ($P > 0.05$), which indicated that *P. gingivalis* infection did not affect the metabolic feature in the mice. However, the body weight of the *db/db* infected mice exhibited a gradual decrease since weeks 10, while the shame-infection group continued to increase. At sacrifice the body weight of infection group was dramatically higher than that of shame-infection group in *db/db* mice ($P < 0.01$).

3.3. High Glucose Aggravated the Alveolar Bone Loss after *P. gingivalis* Infection among the Four Types of Mice. The level of

alveolar bone loss was significantly higher in injection groups compared to shame-injection groups (Figure 3). Within the infection groups, *db/db* mice exhibited more alveolar bone loss than those of TH mice, STZ-C57 mice, and C57 mice (*db/db* mice versus TH mice, STZ-C57 mice, and C57 mice: $P < 0.01$). Similar results could be found in the shame-infection groups (*db/db* mice versus TH mice, STZ-C57 mice, and C57 mice: $P < 0.05$).

3.4. Different Serum TNF- α Level Were Found among the Four Types of Mice. As shown in Figure 4, the levels of serum TNF- α after sacrifice were higher in the infection groups compared to the shame-infection groups (C57 mice: $P < 0.01$; STZ-C57 mice: $P < 0.05$; TH mice: $P < 0.05$; *db/db* mice: $P < 0.05$). Within the infection groups, C57 mice exhibited less serum TNF- α levels than those of diabetic mice (STZ-C57, TH, *db/db* mice versus C57 mice: $P < 0.01$). Similar results could be observed in the shame-infected groups (STZ-C57, TH, *db/db* mice versus C57 mice: $P < 0.01$). No statistical difference was found in the serum TNF- α levels among diabetic mice in neither the infection nor the shame-infection groups ($P > 0.05$).

3.5. Alterations of Inflammation-Related Proteins Were Found in Gingival Epithelial Tissues. To further illuminate the underlying mechanism of the alveolar bone loss in these mice models, we evaluated the protein expression of PTPN2, JAK1, STAT1, and STAT3 in gingival epithelium at sacrifice (Figure 5). C57 mice share the same genetic background with *db/db* mice and are often used as controls to study the diseases mechanism. The results demonstrated that high glucose seriously reduced PTPN2 expression in gingival epithelia of diabetic periodontitis mice. JAK1, STAT1,

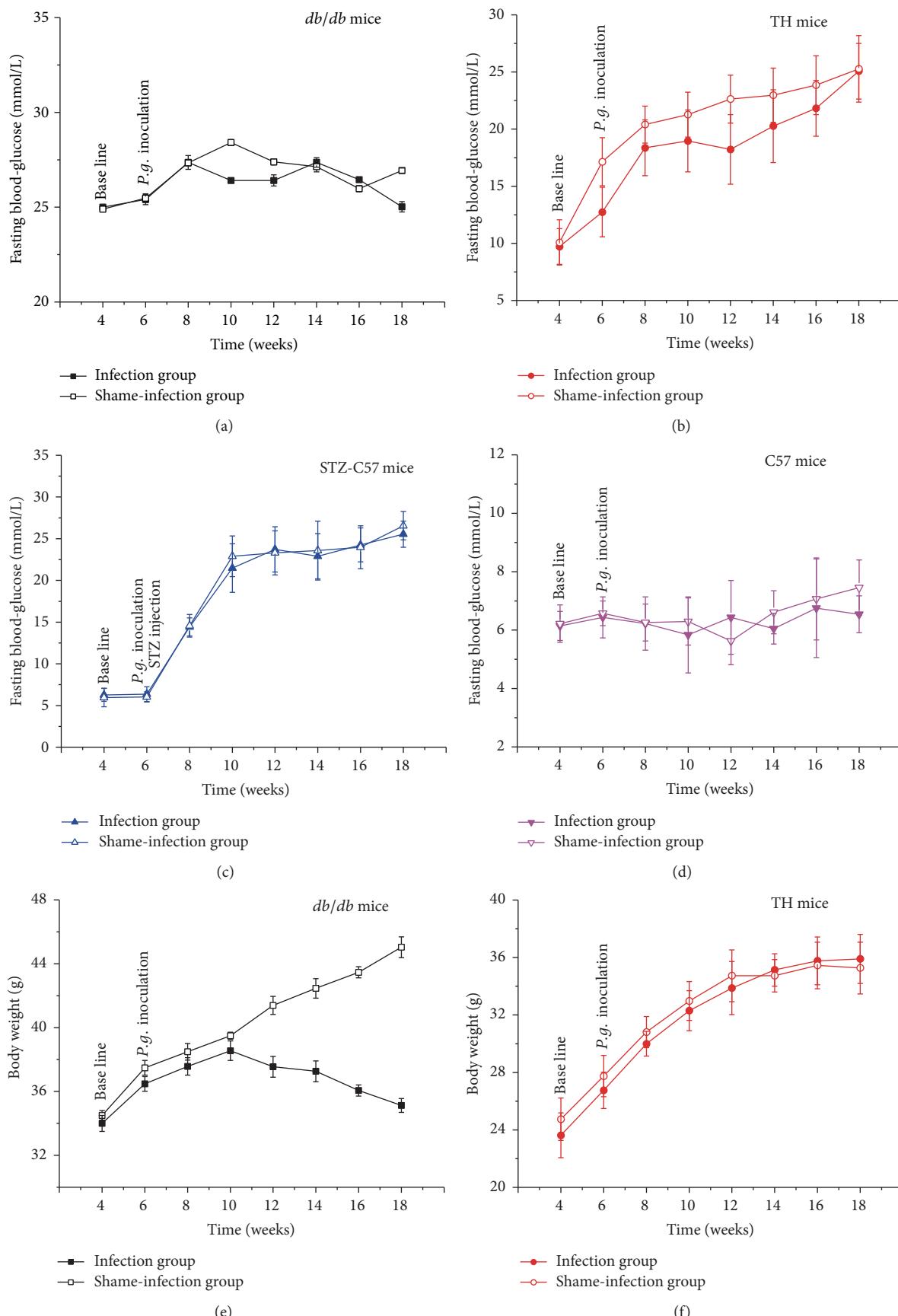


FIGURE 2: Continued.

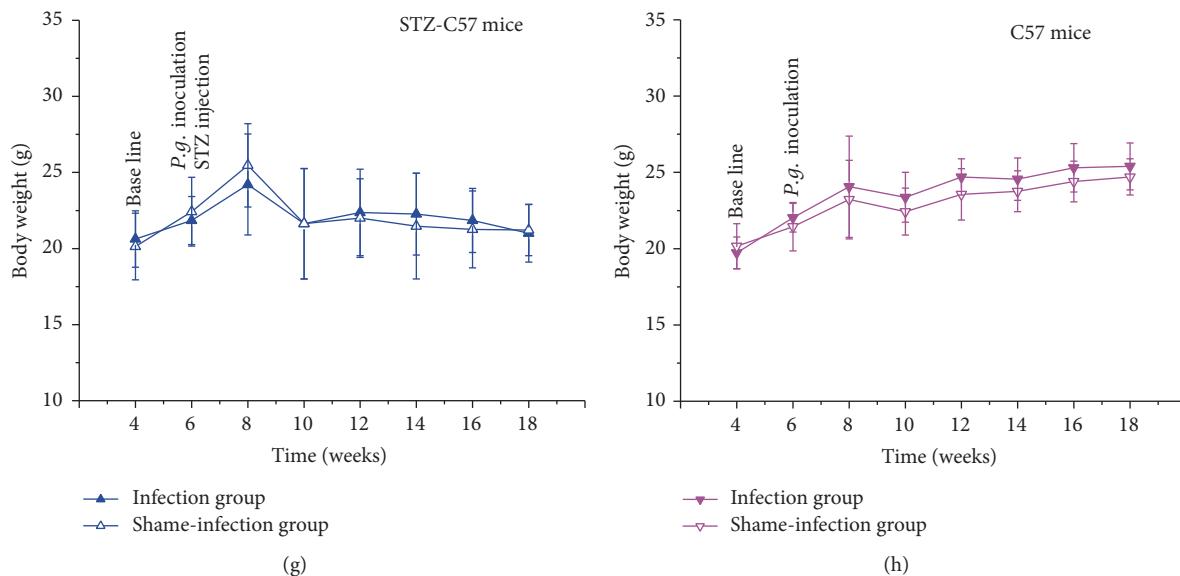


FIGURE 2: Comparison of fasting blood glucose and body weight levels in different mice: (a, e) *db/db* mice; (b, f) TH mice; (c, g) STZ-C57 mice; (d, h) C57 mice. No statistical difference ($P > 0.05$) detected for the fasting glucose levels between infection and shame-infection groups in four types of mice. At sacrifice the body weights of infected *db/db* were significantly higher than their controls ($P < 0.01$). No significant difference was found in the body weight between infection and shame-infection groups in TH, STZ-C57, and C57 mice. Values for each time point are expressed as mean \pm SEM.

and STAT3 expression levels were dramatically elevated in infection groups of both *db/db* and C57 mice. Moreover, infected *db/db* mice exhibited the lowest PTPN2 expression compared to their shame-infection controls and C57 mice.

3.6. Pancreas Morphology of T1D and T2D Was Observed in the Four Types of Mice. As shown in Figure 6, in C57 mice, round or oval islets cells clusters with clear border were located in the central part of islets and contained a large number of islets β cells with round nuclei. In *db/db* mice and TH mice, the volume of islets cells clusters shrank slightly; vessels enlarge mildly, which were the histological appearances of type 2 diabetes, while in STZ-C57 mice, the volume of islets clusters decreased dramatically. Vacuolar degeneration, necrosis, or disappearance in islets β cells and proliferous fibrotic tissue between islets β cells could be observed; thus the islets appeared empty. STZ-C57 mice showed the histological appearance of type 1 diabetes.

4. Discussion

In this study, three mice models of diabetes periodontitis were established by *P. gingivalis* oral inoculation and the metabolic and periodontal features were compared. We found no contrary impact could be observed between this periodontal infection and onset and severity of diabetes in both types 1 and 2 diabetic mice. Variations of PTPN2 and JAK/STAT pathway in gingival epithelia and different amount of serum TNF- α may lead to the different inflammation response to periodontal pathogen.

Currently, chemical induced and transgenic mice are two main types of diabetic models. *Db/db* mice had a long

history of utilization as T2D spontaneous model. They shared the same genetic background with C57, which often used as the genetic controls to explore disease mechanisms [21]. TH mice are relatively new model for T2D characterized by glucose intolerance and hyperglycemia and show metabolic abnormalities [22]. Chemical induced diabetic mice were generally induced by streptozotocin (STZ), which destroy pancreatic β cells, resulting in irreversible insulin-dependent diabetes mellitus (T1D) [23].

Oral infection of periodontal pathogens has been generally used to establish periodontitis in experimental mice because of limited oral space [24]. *P. gingivalis* is widely implicated as an crucial etiological agent in the pathogenesis of periodontitis [25]. The selection of bacteria with different virulence is important in making sure of the alveolar bone loss [26]. *P. gingivalis* W50 is more aggressive in experimental mice for severe periodontal tissue damage because of its high virulence, compared to other strains such as *P. gingivalis* 381 and W83 [27, 28]. This research implied that *P. gingivalis* W50 could cause significant periodontal tissue destruction and alveolar bone loss in all infected mice, indicating that oral infection of W50 is an available method to establish periodontitis mice models with both types 1 and 2 diabetes.

The alveolar bone loss levels positively correlated with the severity of periodontal inflammation [29]. In our study, we could observe that all the diabetes mice exhibited alveolar bone loss and *db/db* mice exhibited more compared to other diabetes mice in both the infection and shame-infection groups. It proved that three types of diabetes mice could all exhibited the characteristic of diabetes periodontitis and *db/db* mice showed more severe periodontal infection than TH and STZ-C57 mice.

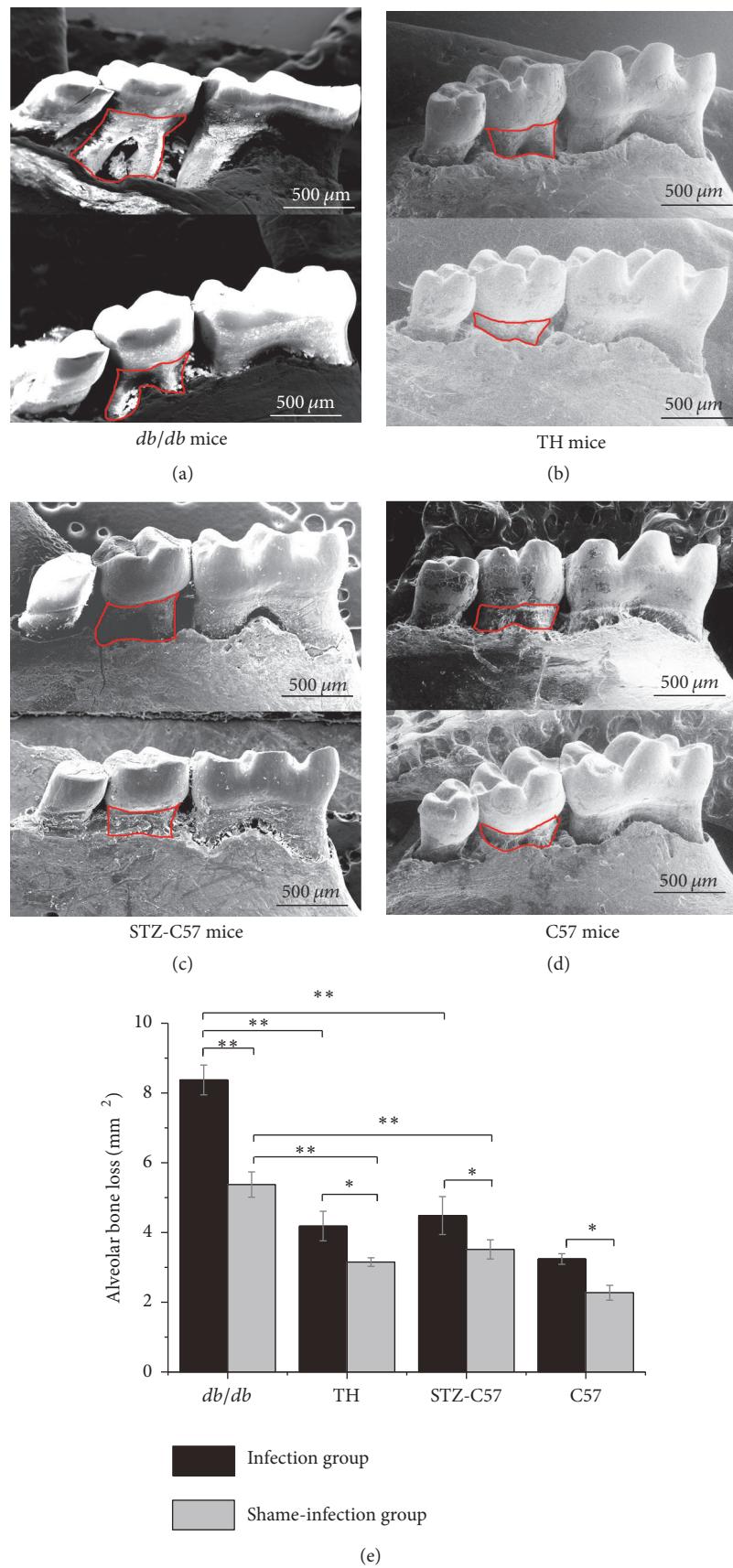


FIGURE 3: SEM images of mandibular jaws from mice in all groups: (a) db/db mice; (b) TH mice; (c) STZ-C57 mice; (d) C57 mice. Area within the red line was calculated as bone loss on the lingual sides of the mandibular second molars. (e) Comparison of alveolar bone loss level in different mouse models (*P < 0.05; **P < 0.01).

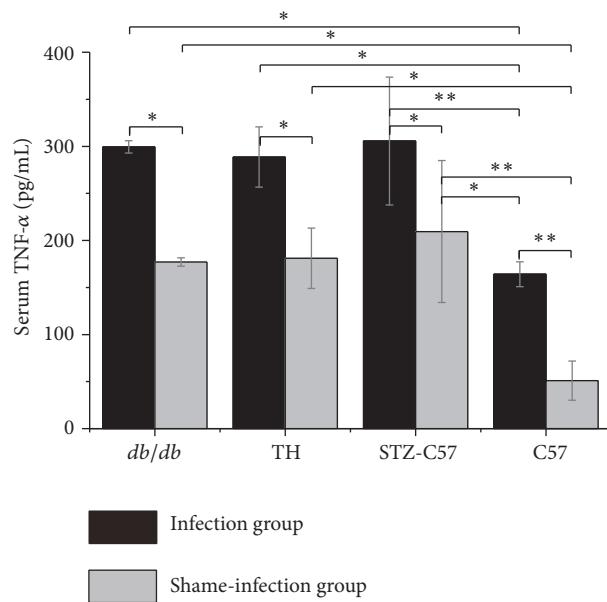


FIGURE 4: Serum TNF- α level at time of sacrifice determined by ELISA (* $P < 0.05$; ** $P < 0.01$).

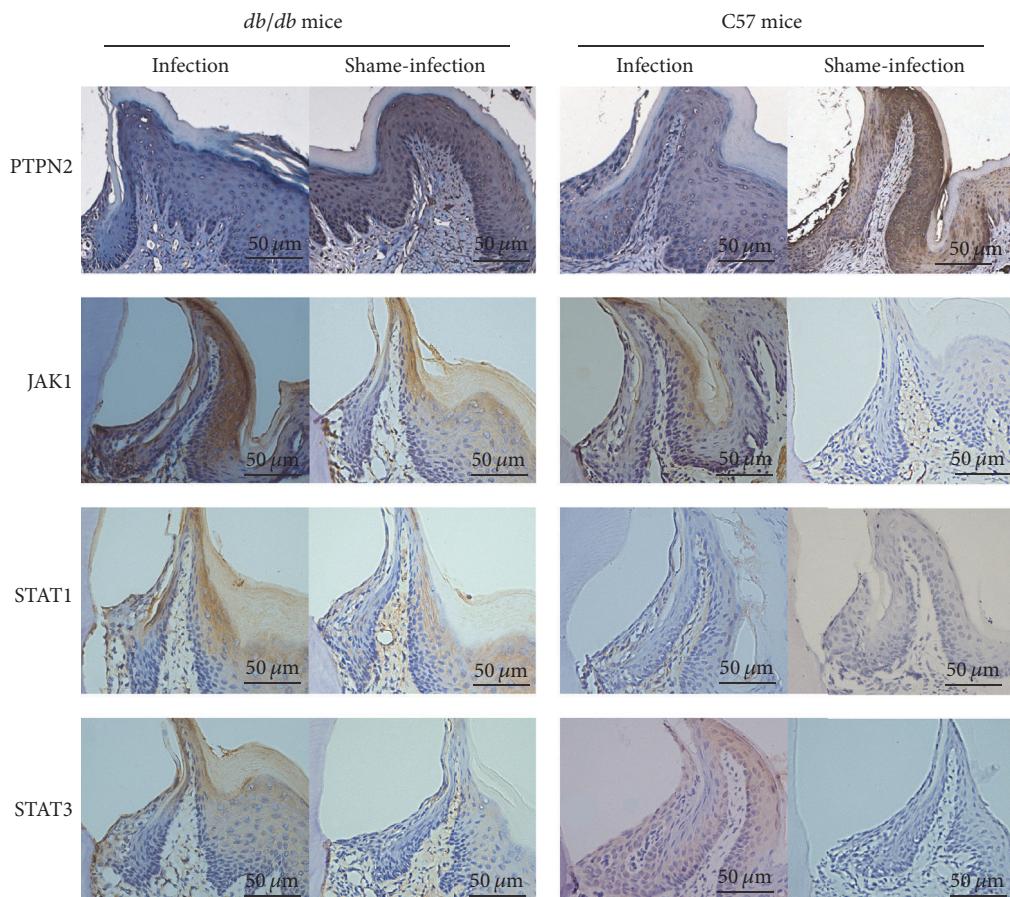


FIGURE 5: Protein expression of PTPN2, JAK1, STAT1, and STAT3 in the gingival epithelium of *db/db* and C57 mice was shown in the IHC staining images. Attenuated expression of PTPN2 and elevated expression of JAK1, STAT1, and STAT3 were seen in infection groups compared to shame-infection groups in both *db/db* and C57 mice.

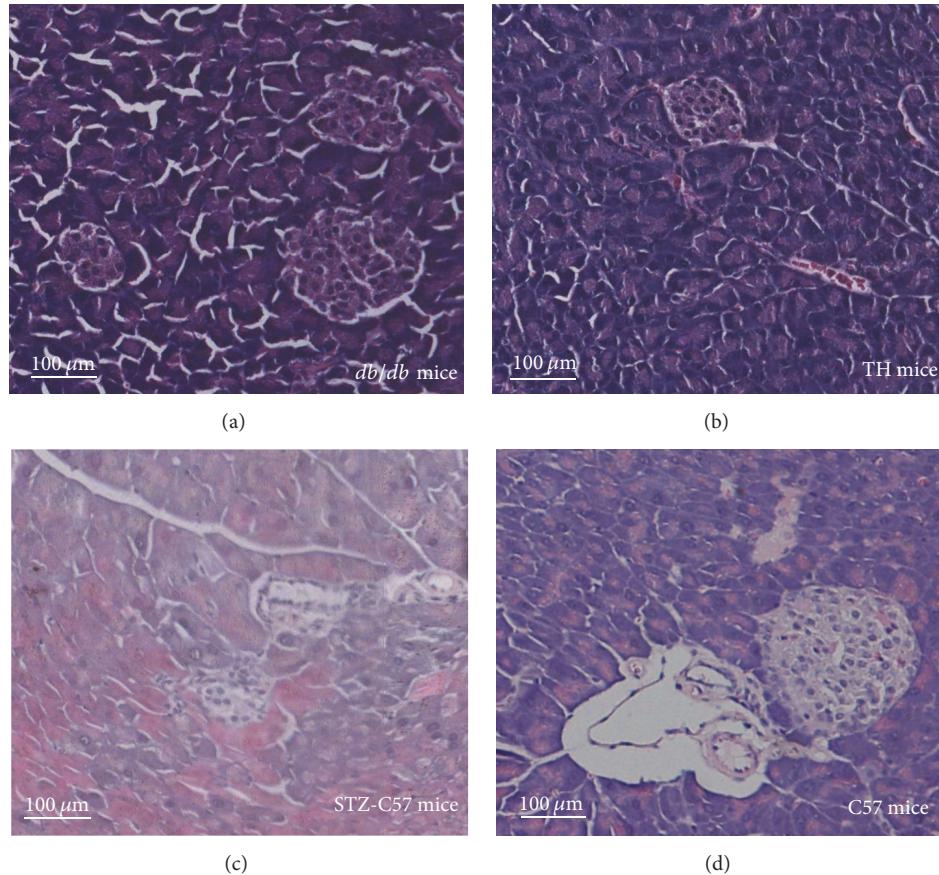


FIGURE 6: Pancreas morphology featured T1D and T2D in the four types of mice were observed in HE staining images.

On the infection groups of all mice, no effect of periodontal infection on the fasting blood glucose was observed. However, the reverse change was found in the body weight of *db/db* mice between the infection and shame-infection groups. As *db/db* mice exhibited more severe periodontal damage, leading to the more difficulty in feeding than other mice with periodontitis, we supposed that the reverse change was elicited by the more periodontal damage, resulting in less food-intake. In all, the results revealed that periodontal infection could not affect the risk and severity of diabetes in both type 1 diabetes and type 2 diabetes mice.

Inflammation plays a central role between the pathogenesis and diabetic periodontitis. Prior research has proved that the serum TNF- α levels positively correlate with fasting blood glucose level, which can not only destroy pancreatic β cells and reduce the sensitivity of insulin but also active the signaling pathways like JAK/STAT [30, 31]. In our study, we observed that the serum TNF- α levels of diabetic mice were higher compared to their normal controls, and the diabetic periodontitis mice showed higher serum TNF- α levels than those of C57 mice in the infection group. However, the differences among three types of diabetes mice were not statistically significant. The results indicated that diabetes mice models could all represent the degree of diabetes periodontitis inflammation.

Recent reports have found PTPN2 is a regulator of inflammatory response [9]. Its crosstalk with inflammatory pathways such as JAK/STAT has been found in immune cells like astrocytes and macrophages [32]. Researches about human macrophages also demonstrated the communication between PTPN2 and JAK/STAT signaling [33]. A recent research implied that the expression of PTPN2 decreased in gingival epithelial under condition of periodontal disease [19]. Based on this research, we found the PTPN2 expression in the gingival epithelium negatively correlated with the severity of periodontal destruction and hyperglycemia. Moreover, the expression of JAK1, STAT1, and STAT3 increased in the gingival epithelium of diabetic periodontitis. JAK/STAT pathway is important in the progress of chronic human inflammatory diseases including diabetes. The main proteins in this pathway (JAK1, STAT1, and STAT3) are proven to be enhanced in periodontal inflammation [12]. Our result demonstrated that the interaction between JAK/STAT pathway and PTPN2 may contribute to the development of diabetic periodontitis. However, further comprehensive studies are needed to clarify the accurate mechanisms.

Taken together, severe periodontitis with T2D could be observed in *db/db*, while mild T2D periodontitis was found in TH mice. STZ-C57 mice exhibited characteristics of T1D. The changes of inflammation-related protein expressions

in gingival epithelia might lead to the differences in the mouse models. These researches may provide the evidence of diabetic periodontitis for further study.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Prevalence of Oral Mucosal Disorders in Diabetes Mellitus Patients Compared with a Control Group

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Chronic hyperglycemia is associated with impaired wound healing and higher susceptibility to infections. It is unclear whether patients with diabetes mellitus (DM) present more oral mucosal disorders compared to control groups. The objectives were to compare (a) the prevalence rates of oral mucosal disorders in the DM and non-DM population and (b) the prevalence rates of specific disorders in the DM and non-DM population. Full-text articles were included if they met the following inclusion criteria: (a) they must be original articles from scientific journals, (b) they must be only cross-sectional studies in English, (c) the prevalence of oral mucosal disorders in DM patients must be evaluated, (d) results must be compared with a healthy control group, and (e) oral mucosal disorders must be specified in DM and non-DM group. All studies showed higher prevalence of oral mucosal disorders in DM patients in relation to non-DM population: 45–88% in type 2 DM patients compared to 38.3–45% in non-DM groups and 44.7% in type 1 DM patients compared to 25% in non-DM population. Tongue alterations and denture stomatitis were the most frequent significant disorders observed. The quality assessment following the Joanna Briggs Institute (JBI) Prevalence Critical Appraisal Tool showed the low quality of the existing studies.

1. Introduction

DM is an endocrine disease characterized by a deficit in the production of insulin with consequent alteration of the process of assimilation, metabolism, and balance of blood glucose concentration [1]. It is expected that the number of people with DM worldwide will increase from 171 million in 2000 to 366 million in 2030 [2] or to 642 million in 2040 [1]. Basically, there are two types of DM: type 1 DM (T1DM) and type 2 DM (T2DM) [3].

DM frequently predisposes to oral complications [4]. DM has been associated with higher prevalence and severity of periodontal disease [5], fungal infections [6], alterations in salivary flow rates, and composition or dental caries [7, 8].

An association of diabetes as a risk factor for oral diseases has been discussed in several studies [9, 10]. Some studies found a possible association between DM and potentially malignant disorders such as leukoplakia, erythroplakia, or

lichen planus [11–13]. Other studies have observed higher prevalence of tongue alterations [14] or oral manifestations of candidiasis, including rhomboid glossitis, denture stomatitis, or angular cheilitis [15]. Meanwhile, other studies had neither representative samples nor comparison of DM patients with a control group [16].

Considerable debate exists surrounding the issue, if the presence of oral mucosal disorders is greater in DM than in non-DM patients. No systematic review has been performed up to now. Given the lack of systematic knowledge, we have conducted the first systematic review concerning the prevalence of oral mucosal disorders in DM compared to non-DM patients.

The main objectives of this review were (a) to compare the prevalence rates of oral mucosal disorders in DM and non-DM population and (b) to compare the prevalence rates of specific disorders in DM and non-DM population.

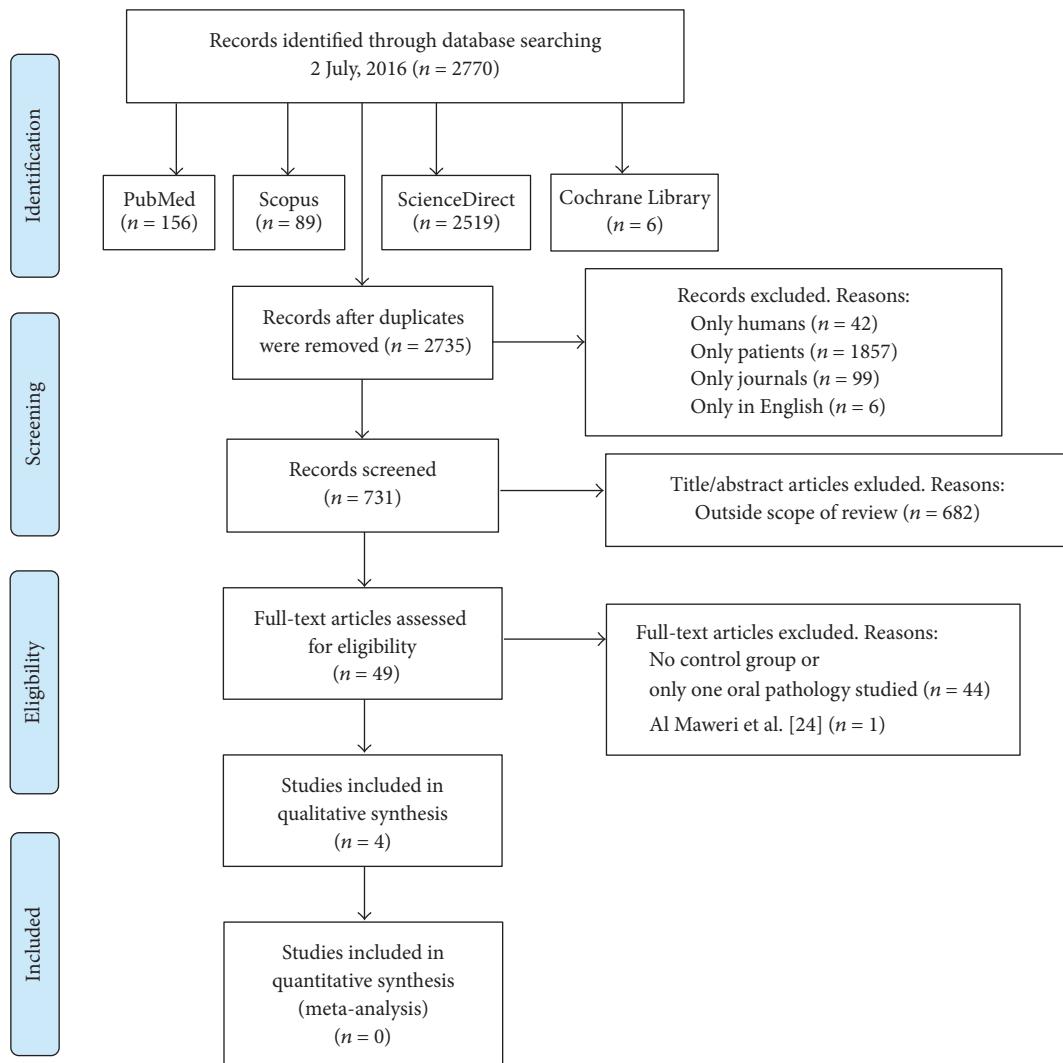


FIGURE 1: Flow diagram of the literature search, according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). PubMed/MEDLINE, Scopus, and ScienceDirect: (diabetes OR “diabetes mellitus”) AND (“oral mucosal lesions” OR “oral diseases” OR “oral pathology”) AND (prevalence OR diagnosis); Cochrane Library: (diabetes OR (diabetes mellitus)) AND ((oral mucosal lesions) OR (oral diseases) OR (oral pathology)) AND (prevalence OR diagnosis).

2. Materials and Methods

We prepared this systematic review by following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P) 2015 statement [17, 18].

2.1. Focused Question. Based on the PRISMA guidelines, a focused question was constructed. The addressed focused PICO question (population, intervention, comparison, and outcome) was the following: do diabetes patients have higher prevalence of oral mucosal disorders compared with a control group?

2.2. Search Strategy. A comprehensive search of the literature was conducted without date restriction until 2 July 2016 in the following databases: MEDLINE, Scopus, ScienceDirect, and the Cochrane Library. The search strategy used was

a combination of Medical Subject Headings (MeSH) terms: (diabetes OR diabetes mellitus) AND (oral mucosal lesions OR oral diseases OR oral pathology) AND (prevalence OR diagnosis) according to each database (Figure 1). Moreover, to ensure completeness of the systematic literature review, an additional hand search to find potential eligible studies was performed and all the references in the articles deemed eligible for inclusion in the study were searched.

2.3. Study Selection

2.3.1. Inclusion Criteria. Full-text articles were included regardless of time period of study and year of publication.

Types of Studies. The studies had to be (a) original articles published in scientific journals and (b) only cross-sectional studies written in English idiom.

Types of Population. Individuals with DM could have T1DM or T2DM. We also considered other diabetes classifications, namely, insulin-dependent DM (IDDM) and non-insulin-dependent DM (NIDDM). A healthy non-DM population as control group must exist.

Outcomes. We considered both oral alterations and oral mucosal lesions as disorders. The studies must evaluate the prevalence of oral mucosal lesions or alterations in DM patients. The results must be compared with a healthy control group. The results must specify oral mucosal lesions or alterations in both the DM group and the non-DM group.

2.3.2. Exclusion Criteria. Studies excluded were (a) those published in languages other than English, (b) those studies that compared only one oral pathology (e.g., Lichen planus) to a healthy control group, (c) those studies which were not carried out on humans, and (d) review articles, experimental studies, longitudinal studies, case reports, commentaries, letters to the Editor, and unpublished articles.

2.4. Data Collection and Extraction. Two independent researchers (José González-Serrano and Julia Serrano) compared search results to ensure completeness and then duplicates were removed. Those articles not meeting study eligibility criteria using limits such as “only humans,” “only patients,” “only in English,” and “only scientific journals” were also removed. Then the reviewers screened full title and abstracts of the remaining papers individually. Differences in eligible studies were resolved by discussion with a third reviewer (Víctor Manuel Paredes). They went on to obtain the full papers for all potentially eligible studies, which were then checked for eligibility using the standard abstraction forms characteristics, first authors, type of study, country in which study was conducted, recruitment of patients, title of the paper, journal, sample characteristics (population, age, and gender), type of DM, period of time suffering DM, treatment for DM, oral mucosal disorders diagnosis criteria, clinical examination method, clinical observer, and experience (Table 1), and confounding factors such as tobacco, other drugs taken, prosthesis users, DM diagnosis, glycosylated hemoglobin, and diabetic complications (Table 2). The eligible papers were then included in the systematic review. The reported statistical significance was extracted if it was available.

2.5. Quality Assessment. The methodological quality in the final selection of eligible studies was evaluated following the Joanna Briggs Institute Prevalence Critical Appraisal Tool [19] (Table 3), which incorporates 10 domains:

- (1) Was the sample representative of the target population?
- (2) Were study participants recruited in an appropriate way?
- (3) Was the sample size adequate?
- (4) Were the study subjects and the setting described in detail?

- (5) Was the data analysis conducted with sufficient coverage of the identified sample?
- (6) Were objective, standard criteria used for the measurement of the condition?
- (7) Was the condition measured reliably?
- (8) Was there appropriate statistical analysis?
- (9) Are all the important confounding factors/subgroups/differences identified and accounted for?
- (10) Were subpopulations identified using objective criteria?

A study was considered to have a low quality assessment if 0–5 criteria were met and high quality assessment if studies met 5–10 criteria. Two reviewers (Gonzalo Hernández and Rosa María López-Pintor) conducted a critical appraisal independently of each other. The reviewers met to discuss the results of their critical appraisal; if the two reviewers disagreed on the final critical appraisal, a third reviewer (Elisabeth Casañas) was required.

2.6. Statistical Methods. The prevalence of oral mucosal disorders from the included studies was presented as a percentage. The results of each oral mucosal disorder were shown along with the number of DM patients and controls, their respective percentages, and their statistical significance when available (Table 4). A meta-analysis was not possible due to the differences between the selected papers: different types of DM, different types of oral disorders, and heterogeneous demographic characteristics (age and ethnic origin).

3. Results

3.1. Study Selection. The response to the search strategy yielded 2770 results, of which 2735 remained after removing those that were duplicated. We restricted the search to those articles published in English, in humans and patients, and excluded all results that were not published in journals, leaving a total of 731 references. Then, 2 independent researchers (José González-Serrano and Julia Serrano) reviewed all the titles and abstracts, obtaining 49 potential references. Finally, 45 were discarded due to the absence of a control group or because only one selected oral pathology was studied. Only 4 papers were included in our systematic review [20–23] (Figure 1).

Due to similarity between study populations in the papers realized by the groups of Saini et al. [21] and Al Maweri et al. [24], authors were asked if patients of one study were included in another one. The answer was affirmative, proposing us to select only the paper written by the group of Saini et al. [21], since it was more complete.

3.2. Study Characteristics. The selected articles were published between 2000 and 2014. A total of 2570 patients were studied, of which 1366 were cases (434 T1DM and 932 T2DM cases) and 1204 were controls. The mean age of the subjects ranged from 33 to 53 years in DM group and from 31 to 51

TABLE 1: General characteristics of selected studies.

Type of study	Guggenheim et al., 2000 [20]	Saini et al., 2010 [21]	Bastos et al., 2011 [22]	Mohsin et al., 2014 [23]
Country	Cross-sectional USA	Cross-sectional Malaysia	Cross-sectional Brazil	Cross-sectional Pakistan
Patients recruited at	Department of Oral Medicine, University of Pittsburgh	Endocrinology Clinic of Medical Hospital and Department of Dental School	Clinic of Periodontics, Estadual Paulista University	Badai Institute of Diabetology and Endocrinology
Sample	Cases 405	Controls 268	Cases 420	Cases 395
Age (years)	32.5 ± 0.3 33 ± 0.4	32.5 ± 0.3 31.8 ± 0.49	52.96 ± 10.52 51.80 ± 11.58	53.10 ± 7.9 51.4 ± 10.3
Gender	Cases Male, 204 Female, 201	Controls Male, 108 Female, 160	Cases Male, 185 Female, 235	Cases Male, 109 Female, 148
Type of DM	T1DM	T1DM, 29 T2DM, 391	T1DM, 29 T2DM, 391	T2DM
Period of time with DM	U	8.36 ± 6.08 years: <5 years: 170 (40.5%) 6–10 years: 138 (32.9%) >10 years: 112 (26.7%)	<10 years: 36 (24.7%) ≥10 years: 110 (75.3%)	U
Treatment for DM	Insulin 405	Oral hypoglycemics, 274 Insulin, 49 Both, 97	Oral hypoglycemics, 98 (67.1%) Insulin, 29 (19.8%) Both, 19 (13.1%)	U
Oral mucosal disorders diagnosis criteria	Based on onset, duration, oral habits, clinical appearance, history of trauma, and previous episodes	Based on WHO guide to epidemiology and diagnosis of oral mucosal diseases	U	U
Biopsy when needed	U	Yes	Yes	Yes
Clinical examination method	Examination light Dental mirror Gauze square	Electrical overhead light Mouth mirror Tweezers Gauze	Artificial light Dental mirror Gauze square	Visible light Dental mirror Cotton gauze
Clinician and experience	2 oral medicine specialists with 10 years of experience	Single examiner assessed by an oral medicine specialist with more than 7 years of experience	Stomatologist	U

TABLE 2: Confounding factors of selected studies.

	Guggenheim et al., 2000 [20]			Saini et al., 2010 [21]			Bastos et al., 2011 [22]			Mohsin et al., 2014 [23]			
	Cases	Controls	U	Cases	Controls	Excluded	Cases	Controls	Excluded	Cases	Controls	U	
Tobacco	Now, 19.4% Ever, 37.5%												
Other drugs taken	Cases Cardiovascular agents, 19.8%, $p < 0.01$ Immunosuppressants, 2.7%, $p < 0.05$ Anticonvulsants, 2.7%, $p < 0.05$ Thyroid supplements, 8.4%, $p < 0.001$ Antimicrobials, 10.4% Unknown, 5.2%	Controls Cardiovascular agents, 6% Immunosuppressants, 0.4% Anticonvulsants, 0.4% Thyroid supplements, 1.1% Antimicrobials, 8.6% Unknown, 7.1%	U	Cases Cardiovascular agents, 22.4% Antibiotics, 2.4% NSAID, 3.3% Others, 2.4%	Controls Cardiovascular agents, 10% Antibiotics, 1% NSAID, 1.4% Antihistaminic drugs, 1.4% Others, 1.7%		Cases Cardiovascular agents, 39.2% taking a daily medication, of which 73.3% were antihypertensives and 56% were antidepressants	Controls Cardiovascular agents, 30 (27%)				U	
Dentures users	Cases Complete or partial dentures, 12.3%, $p < 0.01$	Controls Complete or partial dentures, 3%		Cases	Controls	U	Cases 8.49 ± 2.25	Controls: excluded by fasting blood glucose level	U	Cases 8.49 ± 2.25	Controls: excluded by fasting blood glucose level	U	
DM diagnosis		U					Good (<75), 172 (41%) Moderate (76-89), 92 (21.9%) Poor (>9), 156 (37.1%)	Adequate (<7): 38 (26%) Inadequate (≥7): 108 (74%)	U	Good (<75), 172 (41%) Moderate (76-89), 92 (21.9%) Poor (>9), 156 (37.1%)	Adequate (<7): 38 (26%) Inadequate (≥7): 108 (74%)	U	U
Glycosylated hemoglobin (HbA1c)	11 ± 0.1			Nephropathy, 23.2% Neuropathy, 26.9% Retinopathy, 44.4% Peripheral vascular disease, 10.6%			65 (44.5%) Nephropathy, 20.3% Neuropathy, 16.5% Retinopathy, 63.2%			14.5% Diabetic complications		Excluded	

U: unspecified.

TABLE 3: JBI Critical Appraisal Checklist for studies reporting prevalence data.

	Guggenheimer et al., 2000 [20]	Saini et al., 2010 [21]	Bastos et al., 2011 [22]	Mohsin et al., 2014 [23]
(1) Was the sample representative of the target population?	Y	Y	Y	U
(2) Were study participants recruited in an appropriate way?	U	U	U	U
(3) Was the sample size adequate?	U	Y	U	Y
(4) Were the study subjects and setting described in detail?	U	U	U	U
(5) Is the data analysis conducted with sufficient coverage of the identified sample?	U	U	U	U
(6) Were objective, standard criteria used for measurement of the condition?	U	U	N	N
(7) Was the condition measured reliably?	U	U	U	U
(8) Was there appropriate statistical analysis?	Y	Y	Y	Y
(9) Are all the important confounding factors/subgroups/differences identified and accounted for?	N	N	N	N
(10) Were subpopulation identified using objective criteria?	U	Y	Y	U
Total number of "Y"	2	4	3	2
Quality assessment	low	low	low	low

Y: yes; N: no; U: unclear; N/A: not applicable.

years in controls. Regarding gender, we studied 1315 women and 1255 men, 673 women and 657 men for DM cases and 606 women and 598 men for the controls (Table 1).

3.3. Main Findings. The prevalence of having one or more oral mucosal disorders in T2DM patients was significantly greater than that in the control group according to Saini et al. (45% × 38.3%) [21], Bastos et al. (88% × 45%) [22], and Mohsin et al. (60.8% × 39.2%) [23]. In T1DM patients, the prevalence of having one or more oral disorders was significantly higher than that in the control group (44.7% × 25%) according to Guggenheimer et al. [20].

The types of oral disorders that were found to be statistically significant in more than one of the studies included in DM patients compared with the control group were coated tongue [22, 23], fissured tongue [20, 22, 23], migratory glossitis [21, 22], and denture stomatitis [20, 21]. Every oral disorder found in DM patients and control groups of the selected papers is recorded in Table 4.

3.4. Risk of Bias in Individual Studies. Using the predetermined 10 domains for the methodological quality assessment according to the Joanna Briggs Institute Prevalence Critical Appraisal Tool [17], we determined all the selected papers [20–23] to have a low quality assessment (0–5 domains) and none of them to have a high quality assessment (5–10 domains). Table 3 shows a more detailed description of the articles included.

4. Discussion

We identified 4 studies reporting prevalence of oral mucosal disorders in DM population compared to non-DM population. Comparisons between studies were limited due to different types of DM, different types of oral disorders, and heterogeneous demographic characteristics (age and ethnic origin) of the studied population. In addition, the quality assessment of studies was low. Hence, no meta-analysis was performed. Nevertheless, there are some patterns that can be described.

In the present systematic review, higher prevalence of oral mucosal disorders was found in patients with DM compared to non-DM patients. This prevalence ranged from 45–88% in T2DM patients to 38.3–45% in non-DM groups and from 44.7% in T1DM patients to 25% in non-DM population. This increased prevalence of oral disorders in DM groups may be due to an inadequate metabolic control of DM or a slow healing process [25]. According to some authors, its cause might be oxidative stress, a decreased antioxidant capacity, or higher levels of inflammatory cytokines, as they are considered as major alternative pathways contributing to the pathogenesis of diabetic complications [26, 27].

Changes of the tongue are more frequent in DM patients than in controls, such as fissured tongue [20, 22, 23], migratory glossitis [21, 22], or coated tongue [22, 23]. There is a strong association between migratory glossitis and fissured tongue [28]. The pathogenesis of fissured tongue is considered to be a genetically determined variant of development,

TABLE 4: Distribution of oral mucosal disorders in DM patients and controls.

	Guggenheim et al., 2000 [20]		Saini et al., 2010 [21]		Bastos et al., 2011 [22]		Mohsin et al., 2014 [23]	
	Cases n (%)	Controls n (%)	Cases n (%)	Controls n (%)	Cases n (%)	Controls n (%)	Cases n (%)	Controls n (%)
Subjects with one or more oral disorders	180 (44.4) <i>p</i> < 0.0001	67 (25)	189 (45) <i>p</i> < 0.05	161 (38.3) <i>p</i> < 0.05	129 (88) <i>p</i> < 0.001	50 (45)	225 (60.8) <i>p</i> < 0.0001	145 (39.2)
Angular cheilitis	13 (3.2)	3 (1.1)	10 (2.4)	3 (0.7)	22 (15)	10 (9)		
Aphthous stomatitis	6 (1.5)	8 (3.0)	5 (1.2)	3 (0.7)				
Atrophy of tongue papillae	36 (8.9)	6 (2.2)						
Pseudomembranous candidiasis	<i>p</i> < 0.001							
Denture stomatitis	2 (0.5)	1 (0.4)	45 (10.7) <i>p</i> < 0.05	26 (6.2)				
Epulis fissuratum	19 (4.7) <i>p</i> < 0.05	4 (1.5)	0 (0.0)					
Fissured tongue	3 (0.7)	22 (5.4) <i>p</i> < 0.0001	1 (0.4)	114 (27.1)	112 (26.7) <i>p</i> < 0.001	26 (17.8) <i>p</i> < 0.001	4 (3.6)	63 (15.9) <i>p</i> < 0.05
Fistulous tract	4 (1.0)	1 (0.4)						
Gingival hyperplasia	7 (1.7)	4 (1.15)						
Herpes labialis	1 (0.2)	2 (0.7)						
Inflammatory papillary hyperplasia	3 (0.7)	0 (0.0)						
Fibroma	10 (2.5) <i>p</i> < 0.05	1 (0.4)	5 (1.2)	5 (1.2)				
Lichen planus	2 (0.5)	2 (0.7)	2 (0.5)	0 (0)	9 (6.1) <i>p</i> < 0.01	0 (0)	7 (1.8)	4 (1)
Median rhomboid glossitis	29 (7.2) <i>p</i> < 0.0001	1 (0.4)	4 (1)	5 (1.2)				
Geographic tongue	22 (5.4)	9 (3.4)	17 (4) <i>p</i> < 0.05	4 (1)	8 (5.4) <i>p</i> < 0.01	1 (0.9)	5 (1.3)	4 (1)
Papilloma	1 (0.2)	1 (0.4)						
Traumatic ulcer	14 (3.5)	3 (1.1)	8 (1.9)	2 (0.5)				
Frictional keratosis	<i>p</i> < 0.05							
Coated tongue		10 (2.4)	14 (3.3)		42 (28.7) <i>p</i> < 0.0001	9 (8.1)	106 (26.8) <i>p</i> < 0.0001	32 (7.9)
Varices					30 (20.5) <i>p</i> < 0.001	6 (5.4)		
Melanin pigmentation					12 (8.2) <i>p</i> < 0.01	2 (1.8)	60 (15.2)	45 (11.1)
Leukoedema					8 (5.4) <i>p</i> < 0.05	2 (1.8)		

TABLE 4: Continued.

	Guggenheim et al., 2000 [20]			Saini et al., 2010 [21]			Bastos et al., 2011 [22]			Mohsin et al., 2014 [23]		
	Cases n (%)	Controls n (%)		Cases n (%)	Controls n (%)		Cases n (%)	Controls n (%)		Cases n (%)	Controls n (%)	
Actinic cheilitis							37 (25.3) <i>p < 0.0001</i>	6 (5.4)		14 (3.5)	12 (3)	
Leukoplakia				6 (2.7)	1 (1.8)							
Nicotinic stomatitis				3 (2)	2 (1.8)							
Oral submucous fibrosis									8 (2)	12 (3)		
Linea alba									31 (7.1)	12 (3)		
Fordyce granules									<i>P < 0.05</i>			
									9 (2.3)	0 (0)		

the result of aging, or changes in the oral environment. Migratory glossitis is thought to have hereditary and environmental components [28]. Coated tongue can be associated with a decreased salivary flow present in DM population [9]. These tongue alterations uncommonly require treatment.

DM patients are more susceptible to suffering from fungal infections by *Candida albicans*, especially if they wear prostheses [29]. Guggenheimer et al. [20] and Saini et al. [21] showed that DM patients suffered significantly more denture stomatitis compared to the control groups. Guggenheimer et al. found that the use of dentures was a factor significantly associated with the presence of *Candida pseudohyphae* in T1DM subjects [15]. Thus, diabetes patients using prostheses should have dental check-ups more frequently to prevent this infection. Dental professionals should also provide hygiene measures in order to prevent fungal infections.

Regarding potentially malignant disorders, Bastos et al. found significantly higher prevalence of actinic cheilitis and oral lichen planus in DM patients with regard to the control group [22], while Saini et al. and Mohsin et al. did not find higher prevalence [21, 23]. These findings do not clarify whether there is a need for regular clinical examinations to ensure early diagnosis and treatment of potentially malignant disorders of the oral mucosa in DM patients.

Ujpál et al. saw that smoking diabetes patients are more susceptible to developing leukoplakia [30]. However, tobacco as a confounding factor has not been identified in all studies (Table 2). Guggenheimer et al. only specified tobacco consumption in T1DM patients group [20], Saini et al. excluded tobacco in both groups [21], and Mohsin et al. did not specify this variable [23]. The only authors that included tobacco in both T2DM patients and the control group were Bastos et al., obtaining statistically significant differences in the appearance of nicotine stomatitis in T2DM; nevertheless these authors did not find statistically significant differences of leukoplakia between two groups [22]. Future studies about this topic should take into account this risk factor to establish a possible correlation with the presence of different oral disorders.

A biopsy was performed in three of the four studies included in order to diagnose oral mucosal disorders when required [21–23], but none of them specified how the process was done (fresh tissue for direct immunofluorescence technique or in formaldehyde for a traditional anatomical pathology analysis). It is worth mentioning that none of the selected studies include patients diagnosed with vesiculobullous lesions such as pemphigus vulgaris or benign mucous membrane pemphigoid. However, we do have experience of patients with T2DM and pemphigus vulgaris [31]. Moreover, Heelan et al. in a study of 295 patients diagnosed with different types of pemphigus found that 18% of them were diabetic [32]. The absence of vesiculobullous lesions in the included studies may be due to the absence of direct immunofluorescence diagnostic tests.

Oral hypoglycemics can generate oral and/or skin lichenoid reactions, as seen with tolazamide, tolbutamide, chlorpropamide, glimepiride, or glyburide [33, 34]. It seems strange that none of the studies collected this type of lesions, as they might have classified them as lichen planus. These

lesions appear temporarily while taking the drug. Other main drugs taken were collected in three of the four studies [20–22]. In the study of Guggenheimer et al., 2.7% ($p < 0.05$) of T1DM patients were taking immunosuppressive drugs. However, they did not specify how their consumption may influence the occurrence of oral lesions. López-Pintor et al. saw in renal transplant patients under immunosuppressive therapy that the appearance of oral lesions was of 54.7% compared to 19.4% in a healthy control group [35]. For these reasons, it is important to register all drugs taken by patients in order to study a possible connection with oral disorders.

Due to the fact that only articles published in the English language were reviewed, bias due to the language publication could not be ruled out. Although we searched four databases, we cannot guarantee that some related papers might not have been identified. However, we checked the reference lists of reviewed articles to identify relevant studies. The studies reviewed, as we observed previously, presented different types of DM (T1DM and T2DM) which could cause detection bias.

Firstly, none of the included studies specified the blood glucose values that have been used for the diagnosis of DM [20–23]. Only studies by Saini et al. and Mohsin et al. evaluated blood glucose in the control group [21, 23]. Therefore, DM patients could have been present in the control groups of the rest of studies [20, 22]. Secondly, most of the studies did not take into account whether cases of DM are consecutive or not and the observation period. With respect to oral disorders, the type of biopsy taken was unspecified and differing criteria for diagnosing oral mucosal disorders were used, which could also cause bias. Guggenheimer et al. based their diagnosis on onset, duration, oral habits, clinical appearance, history of trauma, and previous episodes [20], Saini et al. based their diagnosis on WHO guide to epidemiology and diagnosis of oral mucosal diseases [21], and the two others did not specify what they based their diagnosis on [22, 23]. Finally, most of studies did not correctly match smoking habit, the use of drugs, and the presence of dentures with oral disorders. These risk factors are very important in some oral disorders etiology.

Prevalence of DM increases with age and T2DM is much more common than T1DM (the latter only accounts for about 10% of DM patients) [36]. Therefore, T2DM population presents greater probability to have oral mucosal disorders. Fungal infections, especially in adult dentures users, will be also easier to find in a daily clinical practice. Thus, periodical oral check-ups should be made in DM population.

5. Conclusion

The review conducted demonstrated that the prevalence of oral mucosal disorders in DM patients is statistically higher than that in non-DM individuals. Fungal infections related to dentures (denture stomatitis) and tongue alterations such as coated tongue and fissured tongue or migratory glossitis were the most frequent disorders in the oral cavity. Owing to the high degree of heterogeneity regarding the types of DM, diagnosis of DM, and differing diagnosis criteria of oral disorders, it was difficult to compare the studies. In addition,

the quality assessment showed the low quality of the existing studies. Therefore, the results of this systematic review were inconsistent.

We recommend that new studies analyzing the prevalence of oral mucosal disorders in DM population should use more precise and current definitions concerning the determination and diagnosis of DM patients and oral mucosal disorders. New studies should also specify the relationship between the presence of oral disorders and risk factors such as smoking, dentures, and drugs taken by DM patients.

Competing Interests

The authors declare that they have no competing interests.

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Research Article

Oxidative Damage to the Salivary Glands of Rats with Streptozotocin-Induced Diabetes-Temporal Study: Oxidative Stress and Diabetic Salivary Glands

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Objective. This study evaluated oxidative damage caused to the salivary glands in streptozotocin-induced diabetes (DM). **Materials and Methods.** Rats were divided into 4 groups: groups 1 and 2, control rats, and groups 3 and 4, DM rats. 8-Hydroxy-2'-deoxyguanosine (8-OHdG), protein carbonyl (PC), 4-hydroxynonenal protein adduct (4-HNE), oxidized and/or MDA-modified LDL-cholesterol (oxy-LDL/MDA), 8-isoprostanes (8-isoP), and oxidative stress index (OSI) were measured at 7 (groups 1 and 3) and 14 (groups 2 and 4) days of experiment. **Results.** The unstimulated salivary flow in DM rats was reduced in the 2nd week, while the stimulated flow was decreased throughout the duration of the experiment versus control. OSI was elevated in both diabetic glands in the 1st and 2nd week, whereas 8-isoP and 8-OHdG were higher only in the parotid gland in the second week. PC and 4-HNE were increased in the 1st and 2nd week, whereas oxy-LDL/MDA was increased in the 2nd week in the diabetic parotid glands. **Conclusions.** Diabetes induces oxidative damage of the salivary glands, which seems to be caused by processes taking place in the salivary glands, independently of general oxidative stress. The parotid glands are more vulnerable to oxidative damage in these conditions.

1. Introduction

Long-term metabolic disorders in the course of type 1 diabetes mellitus cause irreversible damage to many organs [1, 2]. The leading cause of diabetic complications is chronic hyperglycaemia, which induces a series of mechanisms that generate excessive production of reactive oxygen species (ROS). Diabetes is also associated with the reduction in the total antioxidant capacity, which results from disruption of

endogenous antioxidant enzyme activities as well as increased formation of ROS. Events lead to the development of chronic oxidative stress in aerobic organisms [3].

The oxidative stress is defined as "a situation where the steady-state of ROS is transiently or chronically enhanced, disturbing cellular metabolism and its regulation and damaging cellular macromolecules" [4]. The target of ROS damage includes all groups of biomolecules which in the case of weakening of antioxidant systems may result in permanent

changes in the redox state of DNA, RNA, proteins, lipids, and carbohydrates and leads to the loss of the biological function of the cells. It has been shown that the oxidation of amino acid residues results in a change of their native structure and biological activity of cellular proteins [5]. It has also been demonstrated that lipid peroxidation inhibits activity of enzymes, transporters, and receptors present in cell membranes [6]. The impact of ROS on DNA leads to damage of single nucleotide bases modifications, DNA double-strand breaks (DSBs), and formation of DNA adducts. The numerous oxidation markers are used to assess oxidative changes. The most widely used and applied approaches are generation of isoprostanes (8-isoP), oxidized and/or MDA-modified LDL-cholesterol (oxy-LDL/MDA), and reactive aldehydes, such as malondialdehyde (MDA) and 4-hydroxynonenal protein adduct (4-HNE protein adduct) [4], for protein-information of protein carbonyls (PC) [7] for DNA 8-hydroxy-D-guanosine (8-OHdG) [8].

The concentration of reactive aldehydes such as malondialdehyde (MDA) is determined via their direct reaction with thiobarbituric acid (TBA). Direct TBA method is a nonspecific for MDA analysis. The pretreatment of biological samples for avoiding the possible interferences derived other reactive aldehydes which could be performed according to the recent sample preparation method [9]. It was shown that long-lasting streptozotocin-induced diabetes increased the MDA level only in the submandibular glands of rats in comparison to the control [10]. Deconte et al. [11] showed an increase in the MDA content in parotid glands of rats 30 days after STZ injection. Zalewska et al. [12] claimed that changes in MDA depend on the duration of streptozotocin-induced diabetes and the type of the salivary glands of rats.

The aforementioned oxidation biomarkers are among the most commonly used biomarkers, albeit not the only ones for the quantification of oxidative stress. Moreover, in certain cases, the levels of ROS modified molecules may also be decreased or unchanged as compared to the control due to their elimination by specific biological systems [4], which may falsely suggest a normal redox homeostasis. The oxidative stress index (OSI) is also used to fully illustrate the activity of oxidative stress. The OSI helps to assess the relationship between total oxidants (TOS) and total antioxidants (TAS) and may be regarded as a validated standard when assessing the oxidative stress [13].

The aim of our study was to assess the level and progression of oxidative damage measured by PC, 8-isoP, 4-HNE protein adduct, oxy-LDL/MDA, 8-OHdG, and OSI in the salivary glands of rats in two time periods of streptozotocin-induced diabetes.

The association between various oxidative stress markers such as PC, 8-isoP, 4-HNE protein adduct, oxy-LDL/MDA, 8-OHdG, and OSI and the secretory function of the salivary glands in the course of diabetes has not been studied so far. We aim to increase the understanding of the connection between oxidative stress and the dysfunction of salivary glands in two time periods of streptozotocin-induced diabetes.

2. Materials and Methods

2.1. Experimental Animals. Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations. The protocol of the present study was approved by the Local Committee on the Ethical Use of Animals of the Medical University in Białystok, Poland (resolution number 96/2015; date of approval 09.06.2015).

The experiment was carried out on male Wistar rats, caged separately, kept in standard animal holding conditions (at 21–22°C, in a cycle 12 h light/12 h dark, at constant humidity). Rats had unrestricted access to water and food (Agropol Motycz, Poland; consisting of 10.3% fat, 24.2% protein, and 65.5% carbohydrate). One week after arrival, the 8-week-old rats were divided randomly into control (C, n = 16) and diabetic (DM, n = 16), followed by a subdivision into two subgroups (8 rats in each subgroup) based on the number of days counted from the date of induction of diabetes in the DM group to the date of the end of the experiment (7 and 14 days).

2.2. Study Description. After overnight fasting, rats assigned to the DM groups were injected with a single, intraperitoneal dose of streptozotocin (freshly prepared in ice-cold citrate buffer) (STZ, Sigma Chemical Co., St. Louis, MO, USA; 50 mg/kg of body weight, sufficient diabetogenic effect, 100% the survival of rats) by the same experienced person [2, 12]. The control animals only received a citrate buffer (intraperitoneal injection). We chose the intraperitoneal method for simplicity of handling; moreover it was shown that intravenous injection of STZ caused mortality around 25% of the rats within the 1st week [14]. 48 hours after the STZ injection, the development of diabetes was confirmed by tail blood glucose analysis. Blood glucose levels, not urine glucose, were determined due to simplicity of handling. All 16 rats in both DM subgroups reached blood glucose level > 250 mg/dL, so they were considered diabetic. Seven and fourteen days after the STZ injection diabetic and control rats were anaesthetized by intraperitoneal injection with pentobarbital (80 mg/kg body weight), always in the morning (8.00–11.00) [12]. The rats were placed on a heated couch (37°C) at an angle 30°. Under anaesthesia, nonstimulated saliva secretion rate was measured for 15 min, using preweighted cotton balls inserted into the oral cavity [15]. Afterwards, salivation was stimulated with an intraperitoneal injection of pilocarpine nitrate (5 mg/kg BW, Sigma Chemical Co., St. Louis, MO, USA). Stimulated saliva secretion was measured in a way analogous to the unstimulated secretion, 5 minutes after the injection of pilocarpine, for 5 minutes [16]. The salivary flow rate was calculated by subtracting the initial weight from the final weight of the cotton balls. We assumed that 1 mg is equal to 1 μL [17].

Next, still under anaesthesia, tail blood glucose analysis was performed (Accu-Check, Roche) followed by blood sampled from the abdominal aorta and the parotid and submandibular glands excised for further analysis. The salivary glands were weighted and immediately freeze-clamped with aluminium tongs, precooled in liquid nitrogen, and stored

at -80°C . Blood was collected into glass tubes containing heparin and centrifuged (5 min, 4°C , 3000 g, MPW 351, MPW Med. Instruments, Warsaw, Poland). The obtained plasma was precooled in liquid nitrogen and stored at -80°C . No haemolysis was observed in any of the resulting plasma. The salivary glands and plasma were defrosted (4°C), washed with cold PBS, and reweighed. The salivary glands were cut into small pieces, following 10x dilution in ice-cold PBS (portion of the salivary gland intended for the determination of carbonyl groups were 10x diluted in 50 mM phosphate buffer), and homogenized with the addition of the protease inhibitor (1 tablet/10 mL of the buffer) (Complete Mini Roche, France) on ice with a glass homogenizer (Omni TH, Omni International, Kennesaw, GA, USA). Next, homogenates were sonificated with an ultrasonic cell disrupter (1800 J per sample, 20 s \times 3, on ice) (UP400S, Hielscher, Teltow, Germany). The homogenates were spun (10 min, 4°C , 5000 g, MPW 351, MPW Med. Instruments, Warsaw, Poland). The resulting supernatants were analysed on the same day.

2.3. Assays. The plasma insulin and free fatty acids (FFA), plasma and salivary glands PC, 4-HNE protein adduct, oxy-LDL/MDA, 8-isoP, and 8-OHdG, total protein, TAS, TOS, and OSI were determined. Data were shown as the total amount (ratio of the examined parameter to total protein). Normalization to total proteins made it possible to assess differences in the ratio of biochemical analytes present in the biological fluids and tissue homogenates. All determinations were performed in duplicate. The final result is the arithmetic mean of the two measurements.

Plasma FFA concentrations were determined using the method described by Bligh and Dyer [18]. The plasma pH was measured using a pH meter SevenMulti, Mettler Toledo.

The insulin, 4-HNE protein adduct, oxy-LDL/MDA, 8-isoP, and 8-OHdG were determined by ELISA using commercially available kits (Shibayagi Co., Gunma, Japan; Cell Biolabs, Inc. San Diego, CA, USA; Immundiagnostik, Bensheim, Germany; Cayman Chemicals, Ann Arbor, MI, USA; and USCN Life Science, Wuhan, China, resp.) following the attached instructions. The supernatants, plasma, controls, and standards were incubated in microplate wells coated with monoclonal antibody to insulin, 4-HNE protein adduct, oxy-LDL/MDA, 8-isoP, and 8-OHdG. Next, avidin conjugated to horseradish peroxidase (HRP) was added and incubated, followed by incubations with the TMB substrate. Only plasma or supernatants that contained complex insulin, 4-HNE protein adduct, oxy-LDL/MDA, 8-isoP or 8-OHdG, biotin-conjugated antibody, and enzyme-conjugated avidin changed their colour. The reaction was terminated by the addition of sulphuric acid and the colour change was determined spectrophotometrically (microplate reader, Mindray MR-96, China).

The PC was determined according to the method introduced by Reznick and Packer [19]. The supernatant and plasma were incubated with 10 mM DNPH (2,4-dinitrophenylhydrazine; POCH S.A. (Polskie Odczynniki Chemiczne Spółka Akcyjna, Gliwice, Poland)) in 2.5 M HCl. The PC was calculated from the peak (355–390 nm)

absorbance using the molar absorption coefficient $\varepsilon = 22,000 \text{ M}^{-1} \text{ cm}^{-1}$. Guanidine hydrochloride (Sigma Chemical Co., St. Louis, MO, USA) was used as a blank.

The TAS was assessed with a kit supplied by Randox (Crumlin, UK). ABTS (2,2'-azino-di-[3-ethylbenzthiazoline sulphonate]) was incubated with peroxidase (metmyoglobin) and hydrogen peroxide to yield the cation ABTS⁺. ABTS⁺ had a blue-green colour, which was assessed spectrophotometrically at the wavelength 600 nm (microplate reader, Mindray MR-96, China). Antioxidants present in the sample reduce this colour formation proportional to their content.

The TOS determination was performed by commercial kit (PerOx, TOS/TOC) supplied by Immune Diagnostic (Bensheim, Germany). In this assay, peroxidase reacts with peroxides in the sample followed by the conversion of TMB to a coloured product. Addition of the stop solution stopped the reaction and caused change in colour. Absorbance of the sample was measured at 450 nm in a microtiter plate reader (Microplate Reader, Mindray MR-96, China). The quantification was performed by the delivered calibrator.

The oxidative stress index was calculated from the formula TOS/TAS $\times 100$ [20].

The protein concentration was assessed by the bicinchoninic acid method (BCA), with bovine serum albumin as a standard (Thermo Scientific PIERCE BCA Protein Assay Kit, Rockford, IL, USA).

2.4. Statistical Analysis. Statistical analysis was performed using Statistica version 10.0 (Statsoft, Cracow, Poland). To show the significant differences between groups, the Kruskal-Wallis ANOVA test was performed. The Spearman Correlation Coefficient was used to study the associations between the variables. Results were presented as a median and minimum and maximum. The statistical significance was defined as $p \leq 0.05$.

3. Results

3.1. Effect of Streptozotocin-Induced Diabetes on Body Weight, Plasma Insulin and pH, Fatty Acids, Glucose Concentration, Salivary Glands Weight, and Food Intake. Streptozotocin-induced diabetes caused reduction in the body weight in the DM groups as compared to the control groups in the first (12%, $p = 0.03$) and second (19.5%, $p = 0.042$) week of the experiment. In the control group, the body weight of rats was significantly increased between the first and the second week of the study (7%, $p = 0.026$), whereas the diabetic rats weight remained the same during the study (Table 1). Furthermore, in both periods of streptozotocin-induced diabetes a dramatic reduction in fasting plasma insulin concentration was observed (below the lower limit of detection), followed by 2.5 ($p = 0.001$) and 5.6 times ($p = 0.0001$), respectively, elevation in glucose concentration in DM groups as compared to the control rats. In the control group the glucose concentration remained the same during the study, whereas the diabetic rats showed significantly increased blood glucose concentrations between the first and the second week of the study (131%, $p = 0.003$) (Table 1). The

TABLE 1: The body weight, salivary unstimulated and stimulated flow rate, and the concentrations of plasma: insulin, glucose, free fatty acids, and pH of the control and streptozotocin-diabetic rats.

	Body weight (mg) M (min-max)	Salivary flow rate ($\mu\text{L}/\text{min}$)		Insulin (ng/mL) M (min-max)	Glucose (mg/dL) M (min-max)	FFA (nmol/mL) M (min-max)	pH M (min-max)
		UWS	SWS				
C1	262.5 (223–279)	0.35 (0.2–0.56)	88.6 (75.9–130.2)	0.854 (0.799–1.456)	95.25 (76.53–99.74)	97.77 (74.39–103.87)	7.71 (7.7–7.9)
C2	282.5 (264–297)	0.36 (0.22–0.61)	86.4 (70.2–109.8)	1.066 (0.991–1.682)	97.86 (80.26–102.47)	91.56 (88.77–96.72)	7.71 (7.6–7.94)
DM1	230.5 (201–260)	0.32 (0.06–0.52)	63.79 (59.94–78.7)	0.0	237 (129–255)	156.43 (133.65–198.66)	7.71 (7.49–7.9)
DM2	227.5 (209–250)	0.27 (0.067–0.91)	52.7 (48.56–69.24)	0.0	548.02 (425.38–594.33)	162.98 (143.52–186.52)	7.71 (7.68–7.93)
<i>P</i> (C1:C2)	<0.05 (↑)	ns	ns	ns	ns	ns	ns
<i>P</i> (DM1:DM2)	ns	ns	ns	ns	<0.005 (↑)	ns	ns
<i>P</i> (C1:DM1)	<0.05 (↓)	ns	<0.05 (↓)	<0.000005 (↓)	<0.005 (↑)	<0.005 (↑)	ns
<i>P</i> (C2:DM2)	<0.05 (↓)	<0.05 (↓)	<0.05 (↓)	<0.000005 (↓)	<0.00005 (↑)	<0.005 (↑)	ns

C1: control group in the first week of the experiment, C2: control group in the second week of the experiment, DM1: diabetic group in the first week of the experiment, DM2: diabetic group in the first second of the experiment, SWS: stimulated whole saliva, UWS: unstimulated whole saliva, insulin: plasma insulin concentration, glucose: plasma glucose concentration, FFA: plasma free fatty acids concentration, M: median, min: minimum, max: maximum, ns: not significant, *p*: statistical significance < 0.05, (↓): decrease, and (↑): increase.

TABLE 2: Comparison of the examined parameters between parotid and submandibular glands of the control and streptozotocin-diabetic rats.

Group N = 8	PG	SMG	<i>p</i> PG : SMG
Salivary glands weight (g) M (min–max)			
C1	0.09 (0.08–0.12)	0.19 (0.18–0.22)	ns
C2	0.09 (0.79–0.13)	0.21 (0.17–0.24)	ns
DM1	0.09 (0.07–0.09)	0.21 (0.14–0.24)	ns
DM2	0.07 (0.06–0.09)	0.18 (0.11–0.19)	ns
TAS ($\mu\text{mol}/\text{mg}$ of protein) M (min–max)			
C1	2.84 (2.01–3.81)	1.94 (1.75–2.06)	<0.005 (↑)
C2	2.69 (2.15–2.99)	1.89 (1.84–2.10)	<0.005 (↑)
DM1	2.36 (2.30–2.89)	2.16 (2.10–2.30)	<0.05 (↑)
DM2	1.40 (0.42–1.74)	1.76 (1.17–1.82)	ns
TOS ($\mu\text{mol}/\text{mg}$ of protein) M (min–max)			
C1	0.04 (0.02–0.21)	0.014 (0.01–0.11)	<0.0005 (↑)
C2	0.042 (0.02–0.26)	0.02 (0.01–0.30)	<0.005 (↑)
DM1	0.057 (0.05–0.08)	0.02 (0.018–0.021)	<0.0005 (↑)
DM2	0.067 (0.01–0.09)	0.03 (0.008–0.04)	<0.005 (↑)
OSI M (min–max)			
C1	14.08 (6.32–17.52)	7.22 (7.17–12.17)	ns
C2	15.61 (5.21–22.5)	10.58 (4.97–19.35)	ns
DM1	24.15 (19.97–39.81)	9.26 (6.67–12.54)	<0.0005 (↑)
DM2	47.86 (36.52–59.86)	17.05 (6.02–25.33)	<0.0005 (↑)
PC (nmol/mg of protein) M (min–max)			
C1	0.051 (0.025–0.10)	0.051 (0.019–0.065)	ns
C2	0.055 (0.019–0.100)	0.049 (0.016–0.070)	ns
DM1	0.061 (0.058–0.084)	0.056 (0.036–0.064)	<0.05 (↑)
DM2	0.068 (0.039–0.127)	0.061 (0.040–0.136)	<0.05 (↑)
4-HNE protein adduct ($\mu\text{g}/\text{mg}$ of protein) M (min–max)			
C1	0.32 (0.24–0.38)	0.30 (0.28–0.34)	ns
C2	0.34 (0.30–0.39)	0.36 (0.33–0.37)	ns
DM1	0.46 (0.43–0.57)	0.39 (0.26–0.41)	<0.05 (↑)
DM2	0.65 (0.57–0.74)	0.45 (0.39–0.54)	<0.005 (↑)
oxy-LDL/MDA (ng/mg of protein) M (min–max)			
C1	0.31 (0.26–0.33)	0.28 (0.25–0.29)	ns
C2	0.31 (0.22–0.32)	0.29 (0.27–0.32)	ns
DM1	0.37 (0.35–0.49)	0.32 (0.28–0.4)	<0.05 (↑)
DM2	0.38 (0.35–0.53)	0.33 (0.32–0.38)	<0.05 (↑)
8-isoP (pg/mg of protein) M (min–max)			
C1	0.022 (0.019–0.029)	0.022 (0.019–0.033)	ns
C2	0.020 (0.015–0.021)	0.019 (0.014–0.025)	ns
DM1	0.023 (0.021–0.057)	0.019 (0.011–0.021)	<0.05 (↑)
DM2	0.028 (0.021–0.044)	0.023 (0.019–0.028)	<0.05 (↑)
8-OHdG (pg/mg of protein) M (min–max)			
C1	0.64 (0.30–1.40)	0.57 (0.51–0.61)	ns
C2	0.70 (0.41–2.00)	0.54 (0.49–0.68)	ns
DM1	0.53 (0.31–0.63)	0.52 (0.32–0.63)	ns
DM2	0.91 (0.74–1.65)	0.51 (0.41–0.97)	<0.005 (↑)

C1: control group in the first week of the experiment, C2: control group in the second week of the experiment, DM1: diabetic group in the first week of the experiment, DM2: diabetic group in the first second of the experiment, PG: parotid gland, SMG: submandibular gland, TAS: total antioxidant status, TOS: total oxidant status, OSI: oxidative status index, PC: protein carbonyls, 4-HNE protein adduct: 4-hydroxynonenal protein adduct, oxy-LDL/MDA: oxidized and/or MDA-modified LDL-cholesterol, 8-isoP: 8-isoprostanes, 8-OHdG: 8-hydroxy-D-guanosine, ns: not significant, *p*: statistical significance < 0.05, M: median, min: minimum, max: maximum, and (↑): increase.

plasma FFA concentration increased by 60%, *p* = 0.001 (1st week), and 78%, *p* = 0.001 (2nd week), in DM in comparison to the control group. In the control and DM groups, the FFA blood concentration remained the same during the study (Table 1). The plasma pH was similar in all groups during

the experiment (Table 1). The weight salivary glands were similar in both DM and control groups in the first and the second week of the experiment. In the control and the DM groups, the salivary glands weight remained the same during the study (Table 2).

3.2. Effect of Streptozotocin-Induced Diabetes on Salivary Unstimulated and Stimulated Flow Rate. The median of the unstimulated flow rate was significantly 15% ($p = 0.034$) reduced in the DM rats as compared to the control rats only in the second week of the study. The median of the stimulated flow rate was significantly reduced in the DM rats as compared to the control rats throughout the duration of streptozotocin-induced diabetes (28%, $p = 0.03$, and 39%, $p = 0.011$, resp.) (Table 1).

3.3. Effect of Streptozotocin-Induced Diabetes on Plasma TAS, TOS, OSI, PC, 4-HNE Protein Adduct, oxy-LDL/MDA, 8-isoP, and 8-OHdG. One- and two-week lasting streptozotocin-induced diabetes resulted in a significantly higher median of the total amount of TOS ($p = 0.0001$ and $p = 0.0002$, resp.), OSI ($p = 0.003$ and $p = 0.001$, resp.), PC ($p = 0.046$ and $p = 0.049$, resp.), 4-HNE protein adduct ($p = 0.00001$ and $p = 0.00001$, resp.), oxy-LDL/MDA ($p = 0.009$ and $p = 0.0006$, resp.), and 8-isoP ($p = 0.01$ and $p = 0.007$, resp.) in plasma of the DM rats as compared to the control rats. The median of the total amount of plasma TAS of DM rats was significantly lower ($p = 0.003$) as compared to the median of the total amount of plasma TAS of the control rats, whereas the median of the total amount of 8-OHdG ($p = 0.009$) in plasma of DM rats was significantly elevated in the second week of the study as compared to the control rats (Table 3).

3.4. Effect of Streptozotocin-Induced Diabetes on Parotid Glands TAS, TOS, OSI, PC, 4-HNE Protein Adduct, oxy-LDL/MDA, 8-isoP, and 8-OHdG. The medians of the total amount of TAS (17%, $p = 0.002$, and 48%, $p = 0.0001$, resp.) were significantly reduced, whereas the medians of the total TOS (43%, $p = 0.006$, and 60%, $p = 0.0001$, resp.), OSI (72%, $p = 0.0001$, and 207%, $p = 0.00001$, resp.), PC (20%, $p = 0.03$, and 25%, $p = 0.01$, resp.) (Figure 1(a)), and 4-HNE protein adduct (43%, $p = 0.001$, and 92%, $p = 0.0001$, resp.) (Figure 1(b)) were significantly elevated in the parotid glands of the DM rats as compared to the control rats at each step of the experiment. The medians of the total oxy-LDL/MDA (24%, $p = 0.03$), 8-isoP (40%, $p = 0.004$), and 8-OHdG (30%, $p = 0.001$) were significantly higher in the parotid glands of the DM rats as compared to the control rats only in the second week of the streptozotocin-induced diabetes (Figure 1(b)).

3.5. Effect of Streptozotocin-Induced Diabetes on Submandibular Glands TAS, TOS, OSI, PC, 4-HNE Protein Adduct, oxy-LDL/MDA, 8-isoP, and 8-OHdG. The medians of TAS were significantly higher (11%, $p = 0.04$) in diabetic submandibular glands as compared to the healthy control after one week of streptozotocin-induced diabetes, whereas the medians of the total amount of TOS (43%, $p = 0.03$, and 50%, $p = 0.02$, resp.) and OSI (28%, $p = 0.03$, and 61%, $p = 0.002$, resp.) were significantly upregulated in the submandibular glands of DM rats as compared to the control rats throughout the duration of streptozotocin-induced diabetes (Figure 2(a)). The median of the total TAS was significantly reduced (7%, $p = 0.04$) (Figure 2(a)), whereas the medians of the total amount of oxy-LDL/MDA (14%, $p = 0.02$) and 4-HNE protein adduct (25%,

$p = 0.002$) were significantly elevated in the submandibular glands of the DM rats as compared to the control rats only in the second week of the streptozotocin-induced diabetes (Figure 2(b)).

3.5.1. Parotid versus Submandibular Glands

Diabetes. Parotid glands of the diabetic rats showed significantly higher medians of the total amount of TAS (10%, $p = 0.039$), TOS (185%, $p = 0.0002$), OSI (161%, $p = 0.0001$), PC (10%, $p = 0.02$), 4-HNE protein adduct (15%, $p = 0.032$), oxy-LDL/MDA (15%, $p = 0.03$), and 8-isoP (21%, $p = 0.04$) in the first week of the streptozotocin-induced diabetes as compared to the submandibular glands of DM rats.

However, in the second week of the study parotid glands of DM rats showed no significant differences only for TAS; all other parameters, TOS (123%, $p = 0.002$), OSI (181%, $p = 0.0001$), PC (11%, $p = 0.04$), 4-HNE protein adduct (44%, $p = 0.001$), oxy-LDL/MDA (14%, $p = 0.03$), 8-isoP (21%, $p = 0.039$) and 8-OHdG (78%, $p = 0.001$), were significantly higher as compared to the submandibular gland of diabetic rats (Table 2).

Control Rats. Parotid glands of the control rats showed higher medians of total amounts of TAS (46%, $p = 0.001$; 42%, $p = 0.001$, resp.) and TOS (185%, $p = 0.0001$; 110%, $p = 0.001$) as compared to the submandibular glands of the control rats, both the first and the second week of the experiment (Table 2).

Correlation

One Week

Submandibular Glands. Correlation was observed between the total amount of TAS and 4-HNE protein adduct and 8-isoP ($p = 0.035$, $r = -0.45$; $p = 0.043$, $r = -0.37$, resp.)

2 Week

Submandibular Glands. Correlation was observed between glucose concentrations and the total amount of 4-HNE protein adduct and oxy-LDL/MDA ($p = 0.033$, $r = 0.37$; $p = 0.011$, $r = 0.57$, resp.)

Parotid Glands. Correlation between the total amount of 4-HNE protein adduct and SWS ($p = 0.017$, $r = -0.61$) and correlation between glucose concentrations and a total amount of 4-HNE protein adduct, oxy-LDL/MDA, PC, and 8-isoP ($p = 0.023$, $r = 0.57$; $p = 0.009$, $r = 0.71$; $p = 0.034$, $r = 0.54$; $p = 0.01$, $r = 0.64$, resp.) were observed.

4. Discussion

In this paper, we assessed the level and progression of the oxidative damage to the diabetic salivary glands using various biomarkers of oxidative stress and oxidative injury. We investigated the relationship between oxidative stress and secretory function of the salivary glands in the course of streptozotocin-induced diabetes.

TABLE 3: Plasma TAS, TOS, OSI, protein carbonyls (PC), 4-HNE protein adduct, oxy-LDL/MDA, 8-isoprostanes (8-isoP), and 8-OHdG of the control and streptozotoxin-diabetic rats.

	TAS ($\mu\text{mol}/\text{mg of protein}$) M (min–max)	TOS ($\mu\text{mol}/\text{mg of protein}$) M (min–max)	OSI M (min–max)	PC (pmol/mg of protein) M (min–max)	4-HNE protein adduct (ng/mg of protein) M (min–max)	oxy-LDL/MDA (pg/mg of protein) M (min–max)	8-isoP (pg/mg of protein) M (min–max)	8-OHdG (pg/mg of protein) M (min–max)
C1	0.1 (0.06–0.15)	1.03 (0.32–2.44)	1.03 (0.095–1.17)	1.41 (1.07–1.48)	0.004 (0.001–0.005)	1.57 (0.99–2.64)	0.0019 (0.0018–0.0022)	0.20 (0.17–0.24)
C2	0.1 (0.05–0.16)	1.05 (0.88–1.28)	0.7 (0.47–1.13)	1.48 (1.31–2.01)	0.005 (0.001–0.007)	1.62 (1.47–2.24)	0.0018 (0.0011–0.0023)	0.21 (0.19–0.22)
DM1	0.16 (0.09–0.20)	2.84 (2.54–3.27)	1.78 (1.57–2.51)	1.57 (1.51–2.11)	6.8 (6.7–7.5)	2.29 (1.21–2.95)	0.0023 (0.0019–0.0026)	0.26 (0.24–0.30)
DM2	0.077 (0.05–0.15)	2.67 (2.48–2.74)	1.31 (1.21–1.83)	1.59 (1.09–2.19)	6.5 (6.0–7.7)	3.65 (3.08–4.01)	0.0022 (0.0017–0.0026)	0.26 (0.25–0.29)
<i>p</i> (C1:C2)	ns	ns	ns	ns	ns	ns	ns	ns
<i>p</i> (DM1:DM2)	ns	ns	ns	ns	<0.00005 (\uparrow)	<0.005 (\uparrow)	<0.005 (\uparrow)	ns
<i>p</i> (C1:DM1)	ns	<0.00005 (\uparrow)	<0.005 (\uparrow)	<0.005 (\uparrow)	<0.00005 (\uparrow)	<0.05 (\uparrow)	<0.05 (\uparrow)	ns
<i>p</i> (C2:DM2)	<0.05 (\downarrow)	<0.00005 (\uparrow)	<0.005 (\uparrow)	<0.005 (\uparrow)	<0.00005 (\uparrow)	<0.005 (\uparrow)	<0.05 (\uparrow)	<0.05 (\uparrow)

C1: control group in the first week of the experiment, C2: control group in the second week of the experiment, DM1: diabetic group in the first week of the experiment, DM2: diabetic group in the first second of the experiment, TAS: total antioxidant status, TOS: total oxidant status, OSI: oxidative status index, PC: protein carbonyls, 4-HNE: protein adduct: 4-hydroxyneonal protein adduct, oxy-LDL/MDA: oxidized and/or MDA-modified LDL-cholesterol, 8-isop: 8-isoprostanes, 8-OHdG: 8-hydroxy-D-guanosine, ns: not significant, *p*: statistical significance < 0.05, M: median, min: minimum, max: maximum, (\downarrow): decrease, and (\uparrow): increase.

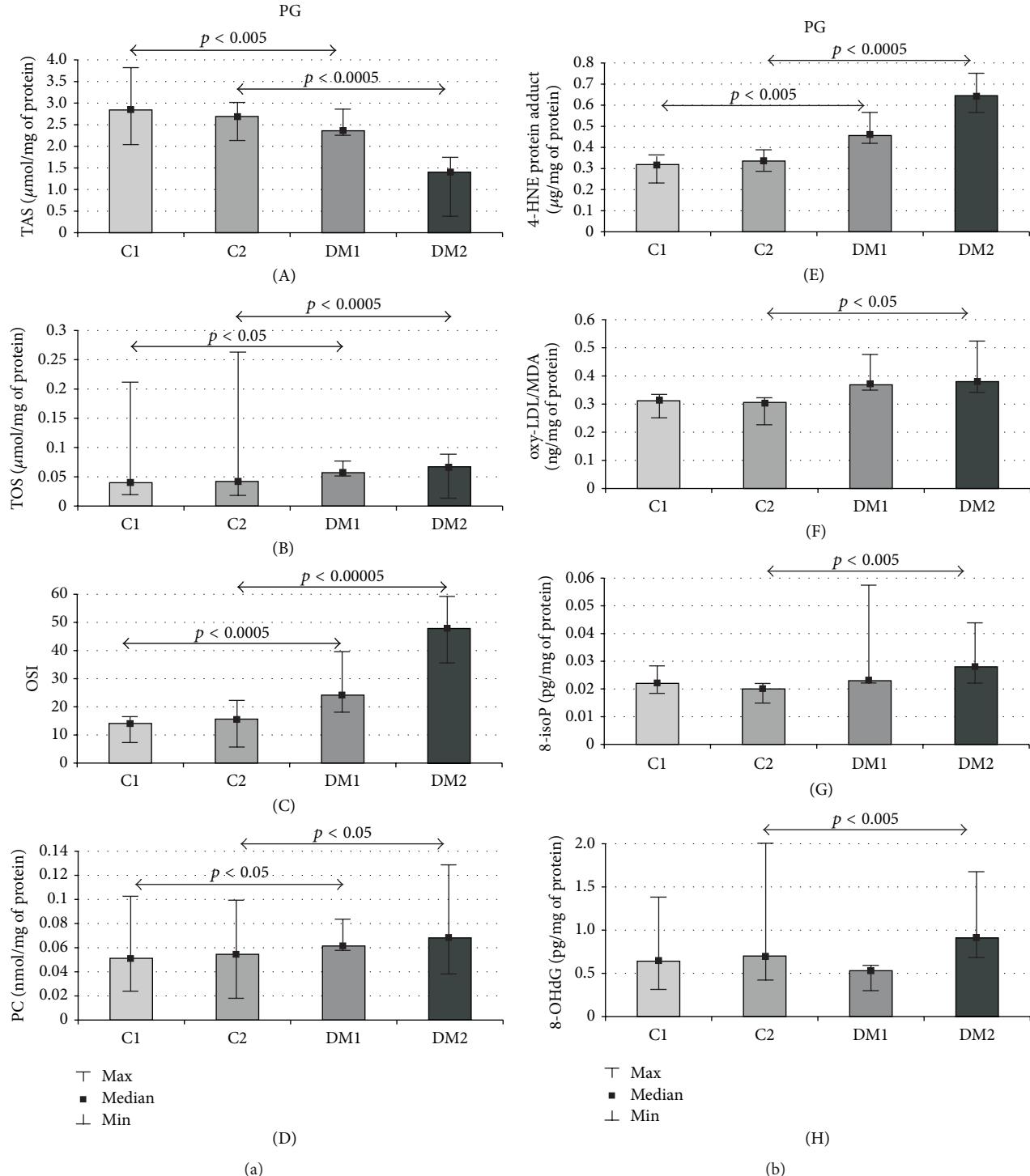


FIGURE 1: (a) Parotid gland TAS, TOS, OSI, and PC of the control and streptozotocin-diabetic rats. PG: parotid gland, C1: control group in the first week of the experiment, C2: control group in the second week of the experiment, DM1: diabetic group in the first week of the experiment, DM2: diabetic group in the second of the experiment, TAS: total antioxidant status, TOS: total oxidant status, OSI: oxidative status index, PC: protein carbonyls, p : statistical significance < 0.05 , min: minimum, and max: maximum. (b) Parotid gland 4-HNE protein adduct, oxy-LDL/MDA, 8-isoP, and 8-OHdG of the control and streptozotocin-diabetic rats. PG: parotid gland, C1: control group in the first week of the experiment, C2: control group in the second week of the experiment, DM1: diabetic group in the first week of the experiment, DM2: diabetic group in the second of the experiment, 4-HNE protein adduct: 4-hydroxyneononal protein adduct, oxy-LDL/MDA: oxidized and/or MDA-modified LDL-cholesterol, 8-isoP: 8-isoprostanes, 8-OHdG: 8-hydroxy-D-guanosine, p : statistical significance < 0.05 , M: median, min: minimum, and max: maximum.

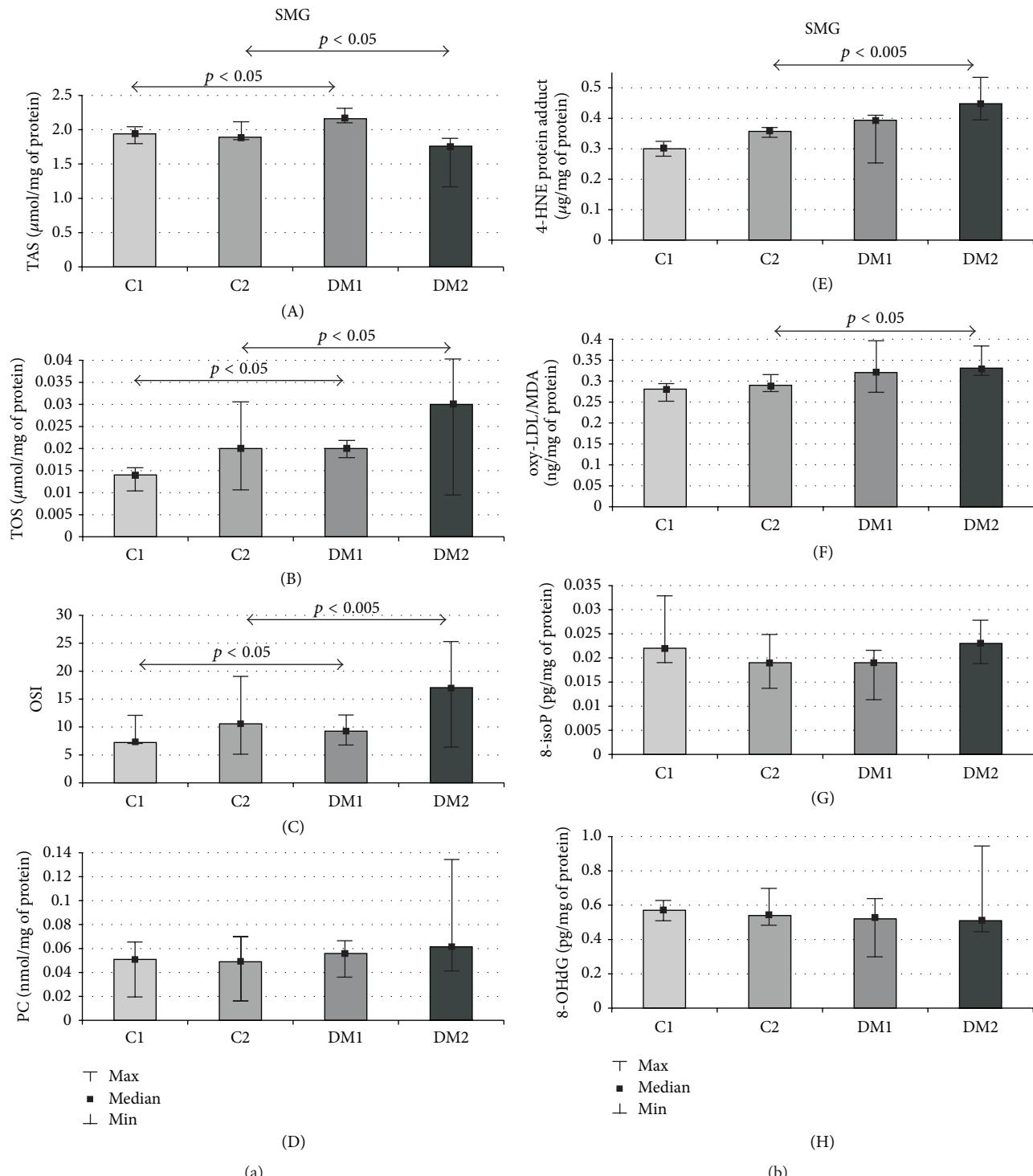


FIGURE 2: (a) Submandibular gland TAS, TOS, OSI, and PC of the control and streptozotocin-diabetic rats. SMG: submandibular gland, C1: control group in the first week of the experiment, C2: control group in the second week of the experiment, DM1: diabetic group in the first week of the experiment, DM2: diabetic group in the second of the experiment, TAS: total antioxidant status, TOS: total oxidant status, OSI: oxidative status index, PC: protein carbonyls, p : statistical significance < 0.05 , min: minimum, and max: maximum. (b) Submandibular gland 4-HNE protein adduct, oxy-LDL/MDA, 8-isoP, and 8-OHdG of the control and streptozotocin-diabetic rats. SMG: submandibular gland, C1: control group in the first week of the experiment, C2: control group in the second week of the experiment, DM1: diabetic group in the first week of the experiment, DM2: diabetic group in the second of the experiment, 4-HNE protein adduct: 4-hydroxynonenal protein adduct, oxy-LDL/MDA: oxidized and/or MDA-modified LDL-cholesterol, 8-isoP: 8-isoprostanates, 8-OHdG: 8-hydroxy-D-guanosine, p : statistical significance < 0.05 , M: median, min: minimum, and max maximum.

The present model of type 1 diabetes was based on streptozotocin- (STZ-) induced diabetes. The dose of STZ is a determining factor for the extent of its diabetogenic action. Experimental diabetes type 1 is induced in animals using STZ in doses from 45 to 100 mg/dL [21]; doses of STZ < 40 mg/dL may not induce diabetes and doses higher than 80 mg/dL may lead to excess rats mortality [21]. Based on the literature [22, 23] and our own experience, we chose a dose of 50 mg/dL, because this amount determines sufficient diabetogenic effect at 100% survival of rats over two time periods [12]. STZ model uses the fact that STZ is selectively toxic to beta (β) cells of the pancreas [24] and is recommended as a clinically relevant animal model and it is most commonly used to explore human diabetes type 1 [21]. In line with our expectations, we received severe hyperglycaemia with simultaneous reduction of fasting plasma insulin levels (below the limit of detection) as early as 1 week after STZ injection. The obtained results demonstrated the destruction of pancreatic β cells and, consequently, endocrine failure of this organ. We also experienced progression of the impaired endocrine function of pancreatic β cells; between the first and the second week of the study, diabetic rats showed significantly increased blood glucose concentrations. Furthermore, despite the fact that diabetic rats consumed similar amounts of food as the control rats, their body weight was significantly lower as compared to the control group.

Under physiological conditions, there is a balance between the generation and elimination of ROS in living organisms, and therefore oxidative damage cannot be observed. However, when this redox balance is disrupted, an elevated production of ROS is observed, followed by an increase in ROS modified molecules, which are widely used biomarkers of oxidative damage. It is considered that the assessment of oxidative injury using only single oxidative modification marker is not sufficient in organisms due to "the different sensitivity, dynamics, nature, and results of ROS-involving process" [25].

Several products derived from biomolecule oxidation were detected in various diabetic organs and plasma [26–30]; however, the only examined marker of oxidative stress in diabetic salivary glands is MDA. Lushchack [25] claims that the reaction of MDA with thiobarbituric acid is not specific and many compounds (amino acids, carbohydrates, and aldehydes) may react with TBA and may interfere with the assay, so these methods as well as interpretation of the obtained results should be evaluated with caution. On one hand, Cebe et al. [9] recently proposed that the pretreatment of biological samples for avoiding the possible interferences derived other reactive aldehydes which could be eliminated according to their recent sample preparation method. In the present experiment, we decided to evaluate the recommended and most commonly used oxidative injury biomarkers: PC, 8-isoP, oxy-LDL/MDA, 4-HNE protein adduct, and 8-OHdG as well as OSI which is an objective assessment of the relationship between total antioxidant mechanisms and the oxidants level [20].

Oxidative damage in the form of a significant increase in 4-HNE protein adduct and PC in the first week after the STZ administration was noted only in the salivary parotid

glands. However, a significant increase in the total amount of OSI in the diabetic submandibular glands as compared to the control demonstrates the redox imbalance also in this gland. The submandibular glands, in contrast to the diabetic parotid glands, seem to prevent their destruction in the process of OS through antioxidant defence system activation (TAS). The negative correlation between the total amounts of TAS, 4-HNE protein adduct, and 8-isoP shows that the increase in the antioxidant capacity elevates cell resistance to oxidative damage and it is sufficient to maintain ROS modified biomolecules within the basal state [31]. It is difficult to explain these differences based solely on the results of the present experiment. Increased TAS levels in the submandibular glands may suggest, on the one side, the adaptive response to the excess production of ROS in this gland; however, on the other hand, it may be an attempt to compensate dysfunctional parotid glands in terms of antioxidant features. These changes may also suggest that, at least in the early stages of type 1 diabetes, the oral cavity will be sufficiently protected against free radicals, even if parotid glands, the physiological source of oral antioxidants, are deficient.

ROS formation in diabetes is directly associated with hyperglycaemia. Hyperglycaemia promotes the generation of free radicals by modulation of the mitochondrial respiratory chain, nonenzymatic glucose autoxidation, and protein glycation [32] and a reduction in the antioxidant capacity [33, 34]. The present results showed that oxidative injury in both glands is directly influenced by hyperglycaemia only in the second week of the experiment, which was observed by a positive correlation between plasma glucose levels and a total amount of 4-HNE protein adduct and oxy-LDL/MDA, for both glands, and 8-isoP and PC for the parotid gland. It is not surprising that the progression of endocrine insufficiency of the pancreas was accompanied by an extensive oxidation of major cellular components in both salivary glands, in addition to a significant reduction in TAS. Greater intensification of oxidative damage was observed in the diabetic parotid salivary glands. However, in the diabetic parotid gland, in addition to the 4-HNE protein adduct and PC and also oxy-LDL/MDA, 8-isoP and 8-OHdG were significantly higher as compared to the control glands. Selectively, from all evaluated oxidation products, a significant increase in the lipid peroxidation products such as 4-HNE protein adduct and oxy-LDL/MDA in the diabetic submandibular glands versus control could be a proof that these diabetic glands undergo an early stage of oxidative damage. Lipid peroxidation is thought to be the earliest marker of oxidative damage occurring via OS, which results from the fact that cell membrane is first exposed to free radicals before the other cellular components undergo oxidative modification [35]. It should be noted that in the present study we investigated only selected damage products. Determination of the other markers of oxidative damage (e.g., advanced oxidation protein product, disulphide groups, and others) may partially contradict this conclusion. Obviously, we cannot exclude that hyperglycaemia, increased endothelial permeability, allows passage of oxidation products from vessels to salivary glands nor that evaluated oxidation products arise directly or only in

the salivary glands, especially when we observed an increase in overall plasma oxidative modification and also cellular redox imbalance already in the first week after the STZ administration. However, the lack of any correlation between plasma and salivary oxidative stress parameters suggests that observed results may be caused by processes taking place in the salivary glands, independently from plasma/general oxidative stress.

Interestingly, regardless of the oxidative stress and oxidative damage intensity, we observed that the capacity of the parotid glands in the response to external stimuli (SWS) is depressed throughout the whole experiment. Basal activity (UWS) of the submandibular glands versus control is depressed only in the second week of the experiment; in the first week of the study, despite the shifting of the antioxidants/oxidants balance towards the oxidative status (\uparrow OSI), the mechanisms of saliva secretion in the diabetic submandibular glands are unaffected, which is reflected as no significant differences in the UWS as compared to healthy control. It should be noted that, in the absence of stimulation, the submandibular gland provides about 60% of total salivary secretion and it is the main source of unstimulated saliva. After saliva stimulation, only the parotid glands increase their secretion by about 10–15%, and thus parotid glands may be considered to be the main source of stimulated saliva [36].

The observed negative correlation between 4-HNE protein adduct and a stimulated salivary flow in the second week seems interesting due to the fact that the 4-HNE protein adduct is able to enhance the expression of proinflammatory cytokines and metalloproteinase production in the course of mitochondrial ROS-mediated stimulation of Akt/NF-kappaB signalling pathways [37]. It has been shown that inflammatory mediators and matrix metalloproteinase may decrease the response of residual acinar cells to acetylcholine and/or block their receptors leading to a reduction in saliva production and secretion [38].

Our results showed greater extent and diversity of oxidative injury in the parotid diabetic glands than in the submandibular diabetic glands, which we also observed in morbid obesity [39]. Perhaps the observed differences may result from differing intensity of morphological changes in both diabetic salivary glands [11, 40, 41]. Histological analysis of dietary salivary glands revealed, amongst other things, a massive accumulation of adipocytes in the parenchyma of vesicular and mucosal cells, which can be seen particularly in parotid glands and slightly marked in submandibular glands. By releasing monocyte chemoattractant protein-1 (MCP-1) the adipocytes cause an influx of monocytes and promote their transformation into macrophages [42]. Moreover, synthesis of MCP-1 is induced by the 4-HNE protein adduct [43]. Macrophages release cytokines (TNF, IL6, and IL1 β) and promote the development of inflammation which leads to the stimulation of respiratory processes in the phagocytic cells, activation of NADPH oxidase, and formation of large amounts of ROS. Perhaps, these additional sources of free radicals exceed the antioxidant/repair capabilities of the parotid glands and their oxidative damage is also observed throughout the whole experiment.

Analyzing the results of our experiment, attention should be paid to its limitations. The present research has been conducted on an animal experimental model; although such model provides invaluable assistance, it is not entitled to its direct impact on humans. The dose of STZ, duration of STZ diabetes, and determination of the other markers of oxidative damage could partially contradict our results.

5. Conclusion

- (1) Both parotid and submandibular glands undergo oxidative stress in the course of streptozotocin diabetes, irrespective of the duration of the disease.
- (2) The parotid glands seem more exposed and vulnerable than submandibular glands to an oxidant attack generated in the course of streptozotocin diabetes, regardless of the duration of the disease.
- (3) Oxidative damage in the course of streptozotocin diabetes caused the dysfunction of the salivary glands, wherein only the reduction of the stimulated saliva secretion is observed in the first week of the experiment. In the advanced stages of the disease, simulated saliva secretion is significantly more reduced than the secretion of nonstimulated saliva.
- (4) Oxidative damage to the salivary glands in the course of STZ diabetes seems to be caused by processes taking place in the salivary glands, independently from plasma/general oxidative stress.

Competing Interests

The authors declare no conflict of interests.

Authors' Contributions

M. Maciejczyk and I. Daniszewska had equal participation in the study.

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Review Article

Xerostomia, Hyposalivation, and Salivary Flow in Diabetes Patients

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The presence of xerostomia and hyposalivation is frequent among diabetes mellitus (DM) patients. It is not clear if the presence of xerostomia and hyposalivation is greater in DM than non-DM patients. The aims of this systematic review are (1) to compare the prevalence rates of xerostomia, (2) to evaluate the salivary flow rate, and (3) to compare the prevalence rates of hyposalivation in DM versus non-DM population. This systematic review was conducted according to the PRISMA group guidelines by performing systematic literature searches in biomedical databases from 1970 until January 18th, 2016. All studies showed higher prevalence of xerostomia in DM patients in relation to non-DM population, 12.5%–53.5% versus 0–30%. Studies that analyzed the quantity of saliva in DM population in relation to non-DM patients reported higher flow rates in non-DM than in DM patients. The variation flow rate among different studies in each group (DM/CG) is very large. Only one existing study showed higher hyposalivation prevalence in DM than non-DM patients (45% versus 2.5%). In addition, quality assessment showed the low quality of the existing studies. We recommend new studies that use more precise and current definitions concerning the determination and diagnosis of DM patients and salivary flow collection.

1. Introduction

Diabetes mellitus (DM) is an endocrine disease characterized by a deficit in the production of insulin with consequent alteration of the process of assimilation, metabolism, and balance of blood glucose concentration. DM has become a worldwide public health problem. In recent years, the global prevalence of DM has increased substantially, reaching 8.3% in 2014, which corresponds to 387 million patients [1]. Essentially, there are two types of DM: type 1 DM (T1DM) and type 2 DM (T2DM). T1DM accounts for approximately 5% of diagnosed diabetes cases [2].

Xerostomia is a subjective complaint of dry mouth, whereas hyposalivation is an objective decreased of salivary flow. The clinical method most often employed for the diagnosis of salivary dysfunction is a sialometry test. Hyposalivation is considered to appear when salivary flow rates are under 0.1 mL/min at rest (UWS) or 0.7 mL/min under stimulation (SWS). Xerostomia is often associated with

hyposalivation, but not always. And many cases of xerostomia have been described in patients with a normal salivary flow rate [3–6].

Several factors are capable of inducing salivary disorders in DM patients such as ageing, head and neck radiotherapy, systemic disorders, and several drugs [5]. Systemic diseases associated with xerostomia include rheumatologic chronic inflammatory disorders (Sjögren syndrome, rheumatoid arthritis, and systemic lupus erythematosus), endocrine disorders (DM, hyperthyroidism, and hypothyroidism), neurologic disorders (depression and Parkinson's disease), genetic disorders, metabolic disorders (dehydration, bulimia, anaemia, and alcohol abuse), infectious disorders (HIV/AIDS, HCV infection), and others (fibromyalgia, graft-versus-host-disease, sarcoidosis, and chronic pancreatitis). Many cases of xerostomia are also related to psychological conditions like depression and anxiety [5, 6].

Both types of DM, T1DM and T2DM, have been associated previously with xerostomia [7–12]. There are also studies

that have showed a decreased salivary flow in DM patients in relation to non-DM patients [7, 8, 12–21]. The reason for these problems could be due to damage to the gland parenchyma, alterations in the microcirculation to the salivary glands, dehydration, and disturbances in glycemic control [5].

Considerable debate exists surrounding the issue, if the presence of xerostomia and hyposalivation is greater in DM than non-DM patients. No systematic review has been performed up to now. Given the lack of systematic knowledge, we have conducted the first systematic review concerning the prevalence of xerostomia and hyposalivation in DM (compared to non-DM) patients. We also have analyzed the differences in the rate of salivary flow between DM and non-DM patients.

The main objectives of this review were (1) to compare the prevalence rates of xerostomia in the DM and non-DM population, (2) to evaluate the salivary flow rate in the DM and non-DM population, and (3) to compare the prevalence rates of hyposalivation in the DM and non-DM population.

2. Materials and Methods

The systematic review was performed according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [23].

2.1. Focused Question. Based on the PRISMA guidelines, 3 focused questions were constructed. The addressed focused questions (PICO) were as follows: (1) Do DM patients have higher xerostomia prevalence than non-DM patients? (2) Is the salivary flow rate lower in DM patients compared to non-DM patients? (3) Do DM patients have higher hyposalivation prevalence than non-DM patients?

2.2. Search Strategy. A comprehensive literature search was conducted by searching the international biomedical literature databases. PubMed/MEDLINE (National Library of Medicine, Bethesda, Maryland), Scopus, and Cochrane database were searched from 1970 until January 18th, 2016, using different combinations of the following keywords: diabetes; xerostomia; dry mouth; hyposalivation; and salivary flow. Moreover, we performed an additional handsearch to find potential eligible studies as reference lists of review articles and relevant studies.

2.3. Study Selection

2.3.1. Inclusion Criteria. Full-text articles were included if they met the inclusion criteria with respect to types of studies, types of population, and the main outcome/s regardless of the time period of study and the year of publication.

Types of Studies. The studies had to be (1) original studies, (2) cross-sectional studies, (3) comparative studies (DM group and healthy control group (CG)), and (4) only in humans. As we evaluated prevalence rates review articles, experimental studies, longitudinal studies, case-reports, commentaries,

and Letters to the Editor were excluded. We did not include unpublished articles.

Types of Population. Individuals with diabetes could have T1DM or T2DM. We also considered other diabetes classifications, namely, insulin-dependent (IDDM) and non-insulin-dependent DM (NIDDM). The total population with DM did not have to suffer specific diseases apart from DM (e.g., end-stage renal disease and hypertension). Individuals without DM were also considered with the aim of comparing prevalence and flow rates between the DM and non-DM population. Individuals without DM did not have to have specific diseases.

Outcomes. The definitions of xerostomia, quantity of salivary flow rate, and hyposalivation are detailed below. Different questions to assess xerostomia were considered: Does your mouth feel dry frequently? Does your mouth usually feel dry, especially during meals? Does your mouth feel dry when you are eating a meal? Do you have difficulties swallowing foods if you eat without additional fluids? Positive response to one of these questions and the consideration of patient's subjective feeling of dry mouth were considered to be xerostomia. Different types of salivary flow rate were considered: UWS (non-stimulated salivary flow), SWS (stimulated salivary flow), USP (nonstimulated parotid flow), SSP (stimulated parotid flow), and SSS (stimulated submandibular/sublingual flow). Furthermore, hyposalivation was considered when UWS < 0.1 mL/min or SWS < 0.7 mL/min, but we included studies that considered hyposalivation when UWS < 0.3 mL/min and SWS < 0.5 mL/min. The main outcomes were the prevalence of xerostomia and/or hyposalivation in percentage and/or the quantity of salivary flow rate in mL/min.

2.3.2. Exclusion Criteria. Studies were excluded if they were published in a language other than English. They were also excluded if they solely reported prevalence of xerostomia/hyposalivation and salivary flow rates among persons with DM in relation to the total population (DM and non-DM) and not exclusively to the diabetic (possibly compared to the non-DM) population.

2.4. Data Collection and Extraction. Two authors (Rosa María López-Pintor and Elisabeth Casañas) independently screened all the retrieved titles and abstracts identified through the search strategies to identify potentially eligible articles. Full texts of relevant studies judged by title and abstract were read and independently assessed with reference to the eligibility criteria by two authors (Rosa María López-Pintor and José González-Serrano). Disagreements were resolved by discussion with a third reviewer (Julia Serrano). Data extraction was performed including information about first author, publication year, country, study population, mean age, type of DM, DM diagnosis (if available), definition of xerostomia, definition of hyposalivation (if available), type of flow rate, and data sources of the study. With regard to the results, xerostomia prevalence (%) and salivary flow rate (mL/min), as well as hyposalivation prevalence (%) of DM

TABLE 1: JBI critical appraisal checklist for studies reporting prevalence data.

Assessment items	Yes	No	Unclear	Not applicable
(1) Was the sample representative of the target population?				
(2) Were study participants recruited in an appropriate way?				
(3) Was the sample size adequate?				
(4) Were the study subjects and the setting described in detail?				
(5) Was the data analysis conducted with sufficient coverage of the identified sample?				
(6) Were objective, standard criteria used for the measurement of the condition?				
(7) Was the condition measured reliably?				
(8) Was there appropriate statistical analysis?				
(9) Are all important confounding factors/subgroups/differences identified and accounted for?				
(10) Were subpopulations identified using objective criteria				

and non-DM groups, were extracted. The reported statistical signification was extracted if it was available.

2.5. Quality Assessment. In the final selection of eligible studies, we assessed features that could potentially bias the estimates of xerostomia/flow rate/hyposalivation using the Joanna Briggs Institute Prevalence Critical Appraisal Tool (Table 1) [24]. Using this tool we defined criteria based on clinical and epidemiological expertise and ranked potential sources of bias into low or high risk of bias. Scores of 0–5 were evaluated as “low quality” while those of 5–10 were considered to indicate “high quality.”

Critical appraisal was conducted by two reviewers (Gonzalo Hernández and Lucía Ramírez) independently of each other. The reviewers met to discuss the results of their critical appraisal; if the two reviewers disagreed on the final critical appraisal and could not be resolved through discussion, a third reviewer (Julia Serrano) was required.

2.6. Categorization of Studies. Due to the high heterogeneity of the studies, we analyzed the outcomes of interest in accordance with the prevalence of xerostomia or salivary quantity flow rate/hyposalivation (if available), type of DM, and age (adults ≥ 19 years old/children and adolescents). There were studies that reported xerostomia prevalence and flow rate; therefore, there could be two groups. The following categories were the result: (1) xerostomia studies in adults T2DM, (2) xerostomia studies in adults NIDDM, (3) xerostomia studies in children and adolescents T1DM, (4) salivary flow rate studies in adults T1DM, (5) salivary flow rate studies in adults IDDM, (6) salivary flow rate/hyposalivation prevalence studies in adults T2DM, (7) salivary flow rate/hyposalivation prevalence studies in children and adolescents T1DM, and (8) salivary flow rate/hyposalivation prevalence studies in children and adolescents IDDM.

2.7. Statistic Methods. The results of xerostomia prevalence from the included studies were presented as a percentage. The results of quantity salivary flow rate were presented as mean \pm standard deviation (if available). Hyposalivation prevalence results were shown as a percentage. The age of different populations was presented as mean \pm standard deviation, but

there were studies that categorized the age or presented only the mean. We showed the possible statistical signification if it was available.

Due to heterogeneity of results, we did not perform a meta-analysis.

3. Results

3.1. Searching and Inclusion. The initial search yielded 53 studies. Thirty-eight studies, which did not fulfill the eligibility criteria, were excluded (the Appendix). A total of 15 articles were included and processed for data extraction. The selection procedure is presented in Figure 1.

3.2. Study Design and Quality Assessment. With regard to the main outcome, 7 papers considered xerostomia prevalence (Table 2), and 12 articles considered quantity of salivary flow rate in DM patients (Table 3), while 4 papers considered both. Only one paper about salivary flow rate in DM population considered hyposalivation prevalence as outcome (Table 3). The results are presented in two parts, xerostomia studies and salivary flow rate/hyposalivation studies.

3.2.1. Xerostomia Studies. We found 7 studies about xerostomia prevalence that met our inclusion criteria. Two of them, written by Sandberg et al. [9, 10], presented the same study population. Therefore, we considered these two studies as one study in Table 2. The majority of studies that reported prevalence of xerostomia in DM patients were performed in adults ($n = 6$), 5 studies in T2DM patients and one in NIDDM. Only one study was performed in children and adolescents T1DM. One study carried out in adults T2DM [18] did not show xerostomia prevalence rates, but it was included due to presence in the results of explanation of no significant correlation in xerostomia in DM/CG patients.

With respect to the recruitment of patients, three studies had selected their DM patients from an endocrinology service or a diabetic care unit of a specialized medical care or hospital, two from a geriatric center and one (the two studies realized by Sanberg et al. [9, 10] with the same population) had sourced the DM patients from a register of primary health care. Control patients were selected from oral health centers ($n = 4$) and geriatric centers ($n = 2$).

TABLE 2: Xerostomia prevalence studies.

Author, publication year, country	Study population (DM/CG)	Mean age (years) DM/CG	Type of diabetes	DM diagnosis	Definition of xerostomia	Xerostomia DM/CG%	Significant association	Matched variables (DM/CG)	JBI scoring
(1) Studies in adults T2DM									
Vasconcelos et al. 2010, Brazil [7]	40/40 (i) DM: endocrinology service of center for specialized medical care (ii) CG: Stomatology Clinic of School of Dentistry (iii) Smokers, drinkers, pregnant, edentulous, receptors of salivary gland surgery, radiotherapy of the head and neck region, Sjögren syndrome, rheumatoid arthritis, or lupus erythematosus excluded	57.7 ± 8.9/50.2 ± 12.3	T2DM	NS	Does your mouth feel dry frequently?	12.5%/2.5%	No	Gender Age	3
(1) Studies in adults T2DM									
Bernardi et al. 2007, Brazil [8]	82/18 (i) DM: diabetic care unit of a local hospital (ii) CG: oral health center (same city) (iii) Those using total prostheses and mouth breathers were excluded. (iv) WCDM: HbA1c ≤ 8% (23%) (v) PCDM: HbA1c > 8% (77%)	PC 54.3 ± 10.1; WC 63.6 ± 12.3; CG 57.7 ± 15.6	T2DM	WHO criteria 2006 Fasting blood glucose levels ≥ 126 mg/dL	Does your mouth usually feel dry?	52.43%/0% WCDM = 47% PCDM = 54%	Yes $p = 0.0001$	Age	4
Sandberg et al. 2001, Sweden [10], and Sandberg et al. 2000, Sweden [9]	102/102 (i) DM: diabetes register in Primary Health Care (ii) CG: Public dental service clinics as the diabetic patients visited for the clinical examination	64.8 ± 8.4/64.9 ± 8.5	T2DM	NS	Patient's subjective feeling of dry mouth	53.5%/28.4%	Yes $p = 0.0003$	Age Gender	5

TABLE 2: Continued.

TABLE 2: Continued.

Author, publication year, country	Study population (DM/CG)	Mean age (years)	Type of diabetes DM diagnosis	Definition of xerostomia DM/CG%	Significant association	Matched variables (DM/CG)	JBI scoring
(3) <i>Studies in children and adolescents T1DM</i>							
48/40							
(i) DM: diabetic care unit of a local hospital							
(ii) CG: oral health centre							
Javed et al. 2009, Pakistan [12]	(iii) Smokers, hepatitis B or C, AIDS, HIV, and narcotic drug used are excluded	15 (10–19)/14.6 (10–19)	T1DM	NS	Does your mouth usually feel dry, especially during meals?	WCDM = 80% PCDM = 100% CG = 0%	Socioeconomic status 3
	(iv) WCDM: HbA _{1c} levels < 6.5 (<i>n</i> = 12)						
	(v) PCDM: HbA _{1c} levels ≥ 6.5 (<i>n</i> = 36)						

DM, diabetes mellitus; WCDM, well controlled diabetes mellitus; PCDM, poorly controlled diabetes mellitus; CG, control group; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; JBI, Joanna Briggs Institute Prevalence Critical Appraisal Tool.

TABLE 3: Salivary flow rate/hyposalivation studies.

Author, publication year, Study population (DM/CG) country	Mean age (years) DM/CG	Type of diabetes	DM diagnosis	Type and QFR mL/min	Definition of hyposalivation	Hyposalivation in DM/CG%	Significant association	Matched variables (DM/CG)	JBI scoring
(1) Studies in adults T1DM									
Edblad et al. 2001, Sweden [13]	(i) DM: Department of Paediatrics, Medical Centre Hospital, T1DM since childhood (ii) CG: randomly chosen from the Swedish register (iii) WCDM: HbA _{1c} ≤ 8% (n = 26) (iv) PCDM: HbA _{1c} > 8% (n = 15)	21 (1.6)/21 (1.6)	T1DM	NS	SWs (paraffin, spitting method) (i) DM: 1.30 (ii) PCDM: 1.31 (iii) WCDM: 1.24 (iv) CG: 1.54	—	—	Nonsignificant (NS)	Age Gender Living in the same county
(2) Studies in adults IDDM									
Ben-Aryeh et al. 1988, Israel [22]	(i) DM: Consecutive patients from diabetes service and research unit (ii) CG: healthy volunteers from the hospital staff who were taking no drugs including oral contraceptives	31.2 ± 7.4/29 ± 6.2	IDDM	NS	UWS (spitting method) 0.35 ± 0.24/0.48 ± 0.23	—	—	Yes (p = 0.036)	Age Gender 2
(3) Studies in adults T2DM									
Lasisi and Fasammade 2012, Nigeria [15]	(i) DM: endocrine unit of the medical outpatients department, University College (ii) CG: members of the university community	58.4 ± 10.6/50.2 ± 9.2	T2DM	NS	UWS (spitting method) 0.5/0.75	—	—	Yes (p = 0.04)	Gender 3
Vasconcelos et al. 2010, Brazil [7]	(i) DM: endocrinology service of center for specialized medical care (ii) CG: Stomatology Clinic of School of Dentistry (iii) Smokers, drinkers, pregnant, edentulous, receptors of salivary gland surgery, radiotherapy of the head and neck region, Sjögren syndrome, rheumatoid arthritis, or lupus erythematosus excluded	57.7 ± 8.9/50.2 ± 12.3	T2DM	NS	UWS and SWS (spitting method) (i) UWS: 0.21 ± 0.16/0.33 ± 0.20 (ii) SWS: 0.63 ± 0.43/1.20 ± 0.70	UWS < 0.1 mL/min SWS < 0.5 mL/min	45%/2.5%	Yes (i) UWS (p = 0.002) (ii) SWS (p = 0.0001) (iii) Hyposalivation (p = 0.001)	Age Gender 3
de Lima et al. 2008, Brazil [16]	(i) DM/CG: University Dental School (ii) Wearing complete maxillary or maxillary and mandibular dentures.	60 (9)/63 (12)	T2DM	Fasting blood glucose DM ≥ 126 mg/dL	SWs 0.95 (0.6)/1.14 (0.87)	SWs < 0.7 mL/min	NS	Nonsignificant (p = 0.331)	Gender Age Race 3

TABLE 3: Continued.

Author, publication year, Study population (DM/CG country)	Mean age (years) DM/CG	Type of diabetes	DM diagnosis	Type and QFR ml/min	Definition of hyposalivation	Hyposalivation in DM/CG%	Significant association	Matched variables (DM/CG)	JBI scoring
82/18 Bernardi et al. 2007, Brazil [8]	(i) DM: diabetic care unit of a local hospital (ii) CG: oral health center (same city) (iii) Those using total prostheses and mouth breathers were excluded. (iv) WCDM: HbA1 _c ≤ 8% (23%) (v) PCDM: HbA1 _c > 8% (77%)	PC 54.3 ± 10.1; WC 63.6 ± 12.3; CG 57.7 ± 15.6	T2DM	WHO criteria Fasting blood glucose DM ≥ 126 mg/dL CG < 110 mg/dL	SWS (spitting method), (i) PCDM: 0.65 ± 0.62 (ii) WCDM: 0.81 ± 0.47 (iii) CG: 1.95 ± 0.73	— — —	Yes SWS ($p = 0.001$)	Age	4
243/240 Dodds et al. 2000, USA [17]	(i) DM/CG: Participants in the Oral Health San Antonio Longitudinal Study of Aging (ii) CG: those subjects who reported no major health problems and were not taking any medications, other than vitamins or occasional analgesics.	Age is specified by sex per group (i) Female: 61.2 (37–78)/55.3 (ii) Male: 63.9 (39–78)/55.9 (36–79)	T2DM	Modified WHO criteria Fasting blood glucose ≥ 126 mg/dL or currently taking diabetic medications	UWS 0.36/0.44 SSP 0.28/0.36 USS 0.08/0.12 SS 0.31/0.41	— — —	UWS and USP: nonsignificant; USS and SS significantly reduced in DM	NS	5
29/23 Chavez et al. 2000, USA [18]	(i) DM: community-living and geriatric center (ii) CG: geriatric center (iii) Only dentate adults (iv) WCDM: HbA1 _c ≤ 9% ($n = 11$) (v) PCDM: HbA1 _c ≥ 9% ($n = 18$)	(i) Mean age NS (ii) Divided into ≤ 71 years (14/9) and > 71 years (15/14)	T2DM	Blood glucose levels ≥ 140 g/dL at 2 hours after oral glucose tolerance test	DM/CG/WCDM/PCDM UWS (spitting method) 0.26 ± 0.29/0.16 ± 0.21/0.14 ± 0.13/0.17 ± 0.25	— — —	Nonsignificant (DM/CG) Nonsignificant (CG/WCDM/PCDM)	Age Gender Race	2

TABLE 3: Continued.

Author, publication year, Study population (DM/CG country)	Mean age (years) DM/CG	Type of diabetes	DM diagnosis	Type and QFR mL/min	Definition of hyposalivation	Hyposalivation in DM/CG%	Significant association	Matched variables (DM/CG)	JBI scoring
(4) Studies in children and adolescents T1DM									
51/51									
(i) DM: paediatric endocrinology service of hospital									
Alves et al. 2012, Brazil [19]	11.3 ± 3.4/11.9 ± 3.4	T1DM	American Diabetes Association criteria (2010)	UWS (spitting method) ± 0.14/0.41 ± 0.28	UWS < 0.3 mL/min	NS	Yes UWS ($p = 0.02$)	Socioeconomic status Lived in the same area	2
(ii) CG: NS glycemic control was established by the determination of glycated haemoglobin concentration									
48/40									
(i) DM: diabetic care unit of a local hospital									
Javed et al. 2009, Pakistan [12]	15 (10–19)/14.6 (10–19)	T1DM	NS	UWS (spitting method) (i) DM: 0.2 (0.1–0.4) mL/min (ii) WCDM: 0.2 (0.1–0.4) mL/min (iii) PCDM: 0.1 (0.1–0.3) mL/min (iv) GC: 0.5 (0.3–0.7) mL/min	—	—	DM/CG, yes (UWS $p = 0.01$) WCDM/PCDM, nonsignificant	Socioeconomic status Tanner pubertal state between I and III	3
(ii) CG: oral health centre									
(iii) Smokers, hepatitis B or C, AIDS, HIV, and narcotic drug used are excluded									
(iv) WCDM: HbA1c levels < 6.5 ($n = 12$)									
(v) PCDM: HbA1c levels ≥ 6.5 ($n = 36$)									
20/21									
(5) Studies in children and adolescents IDDM									
López et al. 2003, Argentina [20]	9.4 ± 3.9/8.3 ± 1.8	IDDM	NS	UWS = saliva 5 min production collected with sterile syringe No stimulation or spitting $0.15 \pm 0.11/0.25 \pm 0.13$	—	—	Yes (NS)	Gender Socioeconomic status Tanner pubertal state between I and III	1
(i) DM: hospital endocrinology service									
(ii) CG: NS									
(iii) CG: absence of active disease, no history of drug treatment or therapy within the previous months, and no history of diabetes									
10/10									
(i) DM: newly diagnosed diabetic children, Diabetic Department of Paediatric Clinic (5–17)									
Belazi et al. 1998, Greece [21]	6.8 (4–15)/10.5	IDDM	NS	UWS (spitting method), $0.79 \pm 0.46/1.06 \pm 0.37$	NS	—	Nonsignificant ($p = 0.17$)	NS	1
(ii) CG: NS									
(iii) DM/CG: free from any other acute or systemic disease									

DM, diabetes mellitus; CG, control group; QFR, quantity of flow rate; NS, nonspecific; WC, well controlled; UWS, nonstimulated salivary flow; SWS, stimulated salivary flow; USP, nonstimulated parotid flow; SSP, stimulated parotid flow; USS, nonstimulated submandibular/sublingual flow; JBI, Joanna Briggs Institute Prevalence Critical Appraisal Tool.

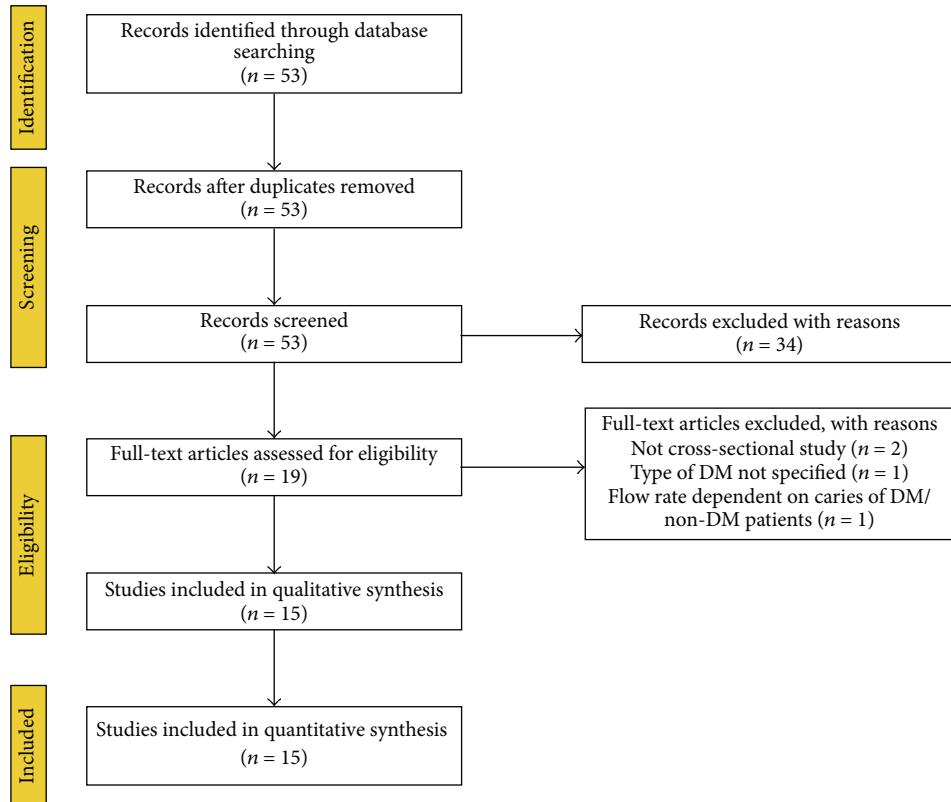


FIGURE 1: Flowchart of the systematic review process.

The studies included a minimum of 29 and a maximum of 102 DM patients and 18–102 control patients. Only two studies specified the DM diagnosis, one WHO criteria 2006 (fasting blood glucose greater ≥ 126 mg/dL) and another one blood glucose levels ≥ 140 mg/dL at 2 hours after oral glucose tolerance test. No one study reported duration of DM and three studies [8, 12, 18] reported the HbA_{1c} levels and classified the patients in well controlled DM (WCDM) and poorly controlled DM (PCDM).

DM and CG participants were matched by gender in 4 studies, by age in 5 studies, by race distribution in one, by diuretics and antidepressants treatment in one, and by socioeconomic status in another one. With regard to statistical significance, three studies [8–10, 12] found that DM patients had more significant xerostomia prevalence than non-DM patients. Only one study [18] did not realize the appropriate statistical methods.

Regarding quality assessment all studies obtained scores ≤ 5 ; therefore the studies were evaluated as “low quality” (Table 2). Due to the poor quality of the included studies no meta-analysis was performed.

3.2.2. Salivary Flow Rate/Hyposalivation Studies. We found 12 studies about quantity of salivary flow rate that met our inclusion criteria; one of them considered hyposalivation prevalence as outcome (Table 3). The majority of studies were carried out in adults ($n = 8$), 6 studies in T2DM patients, one

in T1DM patients, and another one in IDDM. Four studies were carried out in children and adolescents, 2 in T1DM patients and 2 in IDDM.

Three studies recruited their DM patients from a diabetes care unit of a hospital, 3 from an endocrine unit, 3 from a pediatric endocrinology service, one from a university dental school, one from an oral health study, and another one from community-living/geriatric centers. Non-DM patients came from varied origins: oral health centers ($n = 3$), Swedish register ($n = 1$), healthy volunteers from a hospital staff ($n = 1$), members of a university community ($n = 1$), patients of a university dental school ($n = 2$), and participants in an oral health study of aging ($n = 1$), and 3 studies did not specify the origin. The studies included a minimum of 10 and a maximum of 243 DM patients and a minimum of 10 and a maximum of 240 non-DM patients.

Five studies specified the DM diagnosis, two WHO criteria 2006 (fasting blood glucose ≥ 126 mg/dL), one modified WHO criteria 2006 (fasting blood glucose ≥ 126 mg/dL) or currently taking diabetic medications, one blood glucose levels ≥ 140 mg/dL at 2 hours after oral glucose tolerance test, and the last one American Diabetes Association criteria 2010 (HbA_{1c} levels $\geq 6.5\%$ or fasting blood glucose ≥ 126 mg/dL). One study [13] reported that DM patients suffered T1DM since childhood, and there was another study [21] that only included newly diagnosed diabetic children. With respect to dental condition, one study [7] did not include edentulous patients, one study [16] recruited only patients wearing

complete maxillary or maxillary and mandibular dentures, and another one [8] excluded patients using total prostheses and mouth breathers. Four studies [8, 12, 13, 18] reported the HbA_{1c} levels and classified the patients in WCDM and PCDM.

DM and non-DM participants were matched by gender in 7 studies, by age in 6 studies, by race distribution in 2, by socioeconomic status in 3, by living in the same area in two, and by Tanner puberty states in another one. With regard to the type of flow rate 9 studies collected UWS, 4 SWS, 2 USP, one SSS, one USS, and one collected SSP.

Three studies did not explain the hour of collection of saliva and 4 studies did not specify the saliva collection duration. Two studies collected salivary flow during 10 minutes and 6 studies during 5 minutes. Five studies [13, 17, 18, 20, 21] did not show or clarify correctly the statistical methods. Regarding quality assessment, only one study [13] obtained JBI scores ≥ 5 (Table 3). Therefore, due to the poor quality of the majority of the included studies no meta-analysis was performed.

Only one study reflected prevalence of hyposalivation as outcome [7]. The definition of hyposalivation was UWS < 0.1 mL/min and SWS < 0.5 mL/min (actually <0.7 mL/min is considered). The study showed that DM patients had significantly greater hyposalivation prevalence than CG.

3.3. Main Findings

3.3.1. Prevalence of Xerostomia in the DM/CG Population. The prevalence of xerostomia was analyzed in 7 studies (Table 2). In adults T2DM xerostomia prevalence varied between 12.5% and 53.5%, compared to 0–28.4% in the CG [7–10]. Only three studies [8–10] (two with the same study population [9, 10]) showed that DM patients suffered significantly more xerostomia than non-DM patients. One study realized by Bernardi et al. [8] showed that PCDM patients suffered more xerostomia prevalence than WCDM patients, 54% and 47%, respectively.

There was only one study about xerostomia in adults NIDDM [11]. This study showed that prevalence of xerostomia in NIDDM patients is greater than in CG population, 50% versus 30%, but this result was not significant.

Only one work was realized in children and adolescents T1DM between 10 and 19 years old. This study showed that prevalence of xerostomia was greater in T1DM patients than non-T1DM patients (0%), and the prevalence was greater in PCDM patients (100%) than WCDM patients (80%).

3.3.2. Quantity of Salivary Flow Rate in the DM/CG Population. The quantity of salivary flow rate was analyzed in 12 studies (Table 3). There was only one study in adults T1DM [13]; this study showed that SWS flow rate was lower in DM versus non-DM patients, 1.30 versus 1.54 mL/min, and obtained higher salivary flow rate in PCDM than WCDM (1.31 versus 1.34 mL/min). The study did not show significant statistical results. In adults IDDM it was another study [22] that found significantly lower UWS flow rate in DM patients than non-DM patients, 0.35 ± 0.24 versus 0.48 ± 0.23 mL/min.

A considerable part of studies were realized in adults T2DM [7, 8, 15–18]. Four of them evaluated UWS [7, 15, 17, 18]; the UWS flow rate in T2DM and non-T2DM patients varied between 0.16–0.5 mL/min and 0.26–0.75 mL/min, respectively. Two of these studies [7, 15] obtained greater significant UWS flow rate in T2DM than in CG patients. In addition, Chavez et al. [18] assessed the UWS flow rate in WCDM and PCDM adults T2DM; they found higher rates in PCDM than WCDM.

Three studies assessed SWS flow rate in T2DM [7, 8, 16]. The rates of SWS in T2DM and non-T2DM patients varied between 0.63–0.95 mL/min and 1.14–1.95 mL/min, respectively. Two of them [7, 8] showed significant statistical results. The study of Bernardi et al. [8] showed that WCDM had greater SWS rates than PCDM.

USP flow rates were analyzed in two studies [17, 25]; only in one of them [17] did T2DM patients show lower rates than non-DM patients; none obtained significant results.

There were four studies [12, 19–21] that reported salivary flow rates in children and adolescents T1DM and IDDM between 4 and 19 years old. All studies evaluated UWS; the rates in DM population varied between 0.15 and 0.79 mL/min and in non-DM patients 0.25 and 1.06 mL/min. Three studies [12, 19, 20] obtained significant lower rates in T1DM and IDDM patients. Javed et al. [12] showed that WCDM had greater UWS rates than PCDM, but this result was nonsignificant.

3.3.3. Prevalence of Hyposalivation in the DM/CG Population. Only one study evaluated this outcome and showed that hyposalivation prevalence was significantly greater in T2DM versus CG patients, 45% versus 2.5%.

4. Discussion

Multiple epidemiologic studies have suggested that xerostomia is frequent among DM patients. In addition, there are studies that have shown that DM patients presented lower salivary flow rates than non-DM population [26]. These salivary disorders could be associated with a poor quality of life and could increase the susceptibility to caries and oral infections in DM patients, particularly when there has been dehydration and inadequate blood glucose control [18]. DM is probably the most frequent metabolic disease with salivary implications, due to its high frequency. This systematic review was performed to analyze the prevalence of xerostomia and hyposalivation and the rates of salivary flow in DM patients in relation to non-DM patients. We specified explicit eligibility criteria, conducted comprehensive searches, and assessed risk of bias using criteria specific to this review.

4.1. Risk of Bias within Studies. Selection bias regarding the study population was minimized through the restriction to population-based studies. At the same time, we detected some sources of information bias. Firstly, the majority of studies [7, 9–13, 15, 16, 20–22] do not specify the DM diagnosis. Secondly, most of the studies [7, 8, 11, 12, 16, 18, 20–22]

did not show the observation period and the type of recruitment of DM cases. With respect to the salivary flow rate, not all the studies reported the same type of salivary flow and the same technique, and these could also cause bias. Finally, DM and non-DM are not correctly matched; there are studies that did not even match age and gender [8, 12, 15, 19, 20] and there is no study that matched correctly the use of drugs and illness (apart DM), so important in xerostomia/hyposalivation etiology. As we can see in Tables 2 and 3, the sample size in the majority of studies was small (especially in adults T2DM), considering that DM is a very frequent disease. With respect to the statistical analysis, not all the studies reported continuous variables in mean \pm standard deviation.

4.2. Risk of Bias across Studies. Due to the fact that only articles published in the English language were reviewed, publication (language) bias could not be ruled out. Although we searched three databases, we cannot guarantee that some related papers might not have been identified. However, we did check the reference lists of reviewed articles to identify relevant studies. The studies reviewed presented different types of DM and DM and non-DM patients of different age (see Section 2) that could cause detection bias. We minimized it by grouping together studies with similar age and the same DM type in every outcome.

4.3. Main Findings. We identified 15 studies reporting prevalence of xerostomia/hyposalivation and rates of salivary flow in DM population. Comparisons between studies were limited due to different types of DM, different types of salivary flow, and heterogeneous demographic characteristics (age, ethnic origin) of the studied individual. In addition, the quality assessment of studies was low. Hence, no quantitative data synthesis was performed. Nevertheless, there are some patterns that can be described.

4.3.1. Xerostomia Prevalence. All studies about this outcome showed higher prevalence of xerostomia in DM patients in relation to non-DM population, 12.5%–53.5% compared to 0–30% [7–12, 18]. Nevertheless, only four studies [8–10, 12] (two with the same study population [9, 10]) have shown significant statistical results. Two studies [8, 12] showed that WCDM patients have lower xerostomia prevalence than PCDM.

4.3.2. Salivary Flow Rates. All studies [7, 12, 15, 17–22] that analyzed the quantity of UWS in DM population in relation to non-DM patients reported higher UWS rates in non-DM than in DM patients. The variation flow rate among the different studies in each group (DM/CG) is very large. Six [7, 12, 15, 19, 20, 22] of these studies showed significant statistical results. The large variation flow rate among the studies could be due to the different criteria used to measure UWS. The time of measurement strongly influences the flow rate, so the saliva test (not only UWS) has to be performed at a fixed time-point of a limited time interval early morning due to the circadian rhythm of salivary flow [4, 27]. In addition,

the duration of salivary collection is also important [4], and not all studies reflected the same duration. In the studies, where the time of flow rate collection is present, this time varied between 5 and 10 minutes. In addition, it is not clear if WCDM patients have higher UWS rates than PCDM; of two studies [12, 18] discussing this topic only one [12] showed nonsignificant higher rates for WCDM patients.

The comparison of the SWS rates between DM and non-DM patients showed that rates were higher in non-DM patients [7, 8, 13, 16], but only half of the studies showed significant statistical results [7, 8]. The SWS flow rate varies very much among the different studies, in the manner of UWS; the possible reason was specified previously.

4.3.3. Hyposalivation Prevalence. Only one study [7] was about hyposalivation; this study showed significant statistical higher hyposalivation prevalence in DM than non-DM patients (45% versus 2.5%). The hyposalivation SWS level in this study is not actually accepted (<0.7 mL/min) if not <0.5 mL/min; therefore, the results could be biased.

4.4. Strengths and Limitations. The selection of studies for this systematic review was based on a systematic search approach with clearly determined search strategies. We included only those studies reporting xerostomia prevalence/salivary flow rate/hyposalivation within the DM population in relation to a non-DM control group. Moreover, we analyzed these outcomes in separate groups according to age and type of DM. This approach allows limited comparison of the studies despite a high degree of heterogeneity. Our review also has some limitations. Although three databases were searched, we cannot rule out having missed relevant studies, also due to publication bias. The studies published in languages other than English were not included. Most studies reporting our outcomes were conducted in economically developed areas such as USA and Sweden and thus do not represent a worldwide perspective.

In addition, there are studies previous to the year 2000. The change in the diagnostic criteria for DM from 140 mg/dL (7.8 mmol/L) to 126 mg/dL (7.0 mmol/L) in the fasting plasma glucose level in 1997 [28] led to an increase of the diabetic population due to the inclusion of less severe stages of the disease, and this must be taken into consideration when interpreting the results. Criteria for the diagnosis of prediabetes and DM could change periodically [2]; therefore, it is very important to realize the studies according to the current criteria.

5. Conclusions

The review conducted demonstrated the considerable variation in prevalence of xerostomia and salivary flow rates among DM population in relation to non-DM patients. Most studies found a higher prevalence of xerostomia and lower salivary flow rates in DM with respect to CG. We found only a study about hyposalivation that showed higher prevalence in DM than non-DM patients. A few studies showed that WCDM patients have lower xerostomia prevalence and

higher salivary flow rates than PCDM patients. Owing to the high degree of heterogeneity regarding the types of DM, diagnosis of DM, age of patients, and types and techniques of salivary flow collection, it was difficult to compare the studies. In addition, the quality assessment showed the low quality of the existing studies. Therefore, the results of this systematic review were inconsistent.

We recommend that new studies analyzing the xerostomia and salivary flow rate in the DM population should use more precise and current definitions concerning the determination and diagnosis of DM patients and salivary flow rate collection. New studies should match correctly DM and non-DM patients, keeping in mind xerostomia associated drugs and illness (other than DM). New studies are required that consider hyposalivation in DM patients because a reduction in salivary flow is not always pathological.

Appendix

List of Excluded Studies and Reason of Exclusion

- [1] F. Javed, HB. Ahmed, A. Mehmood, A. Saeed, K. Al-Hezaimi, and LP. Samaranayake, "Association between glycemic status and oral Candida carriage in patients with prediabetes", *Oral surgery, oral medicine, oral pathology and oral radiology*, vol. 117, no. 1, pp. 53201358, 2014. (The outcomes were not present.)
- [2] E. de la Rosa-Garcia, M. Miramontes-Zapata, LO. Sanchez-Vargas, and A. Mondragon-Padilla, "Oral colonisation and infection by *Candida* sp. in diabetic and non-diabetic patients with chronic kidney disease on dialysis", *Nefrologia*, vol. 33, no. 6, pp. 764–770, 2013. (Study about oral candidiasis in DM and non-DM patients.)
- [3] DH. Han, MS. Kim, HS. Shin, KP. Park, and HD. Kim, "Association between periodontitis and salivary nitric oxide metabolites among community elderly Koreans", *Journal of Periodontology*, vol. 84, no. 6, pp. 776–784, 2013. (Study not performed in DM patients. Study about periodontitis.)
- [4] G. Teratani, S. Awano, I. Soh, A. Yoshida, N. Kinoshita, T. Hamasaki et al., "Oral health in patients on haemodialysis for diabetic nephropathy and chronic glomerulonephritis", *Clinical oral investigations*, vol. 17, no. 2, pp. 483–489, 2013. (Study about oral health in haemodialysis patients.)
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Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contributions

Rosa María López-Pintor conceived and designed the experiments. Rosa María López-Pintor, Elisabeth Casañas, José González-Serrano, Julia Serrano, and Lucía Ramírez performed the experiments. Rosa María López-Pintor, Elisabeth Casañas, José González-Serrano, Julia Serrano, Lucía Ramírez, and Gonzalo Hernández analyzed the data. Lorenzo de Arriba contributed reagents/materials/analysis tools. Rosa María López-Pintor wrote the paper. Gonzalo Hernández contributed to the concept, design, and drafting of the protocol. Rosa María López-Pintor, Elisabeth Casañas participated in the development of the systematic search strategies. Gonzalo Hernández, Lorenzo de Arriba made major contributions to the write-up and editing of systematic review. Gonzalo Hernández, Lorenzo de Arriba, and Elisabeth Casañas critically revised the paper for important intellectual content and approved the final version.

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Research Article

Insulin Resistance and Obesity Affect Lipid Profile in the Salivary Glands

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In today's world wrong nutritional habits together with a low level of physical activity have given rise to the development of obesity and its comorbidity, insulin resistance. More specifically, many researches indicate that lipids are vitally involved in the onset of a peripheral tissue (e.g., skeletal muscle, heart, and liver) insulin resistance. Moreover, it seems that diabetes can also induce changes in respect of lipid composition of both the salivary glands and saliva. However, judging by the number of research articles, the salivary glands lipid profile still has not been sufficiently explored. In the current study we aim to assess the changes in the main lipid fractions, namely, triacylglycerols, phospholipids, free fatty acids, and diacylglycerols, in the parotid and the submandibular salivary glands of rats exposed to a 5-week high fat diet regimen. We observed that the high caloric fat diet caused a significant change in the salivary glands lipid composition, especially with respect to PH and TG, but not DAG or FFAs, classes. The observed reduction in PH concentration is an interesting phenomenon frequently signifying the atrophy and malfunctions in the saliva secreting organs. On the other hand, the increased accumulation of TG in the glands may be an important clinical manifestation of metabolic syndrome and type 2 diabetes mellitus.

1. Introduction

Carbohydrates and lipids are the two most important classes of molecules in respect of the body energy provisions. In today's world, however, wrong nutritional habits together with a low level of physical activity have given rise to the development of obesity and its comorbidity, insulin resistance (IR). More specifically, a lot of research indicates that lipids are vitally involved in the onset of a peripheral tissue (e.g., skeletal muscle, heart, liver, and adipose tissue) insulin resistance [1–3]. The latter seems to occur in relation to an imbalance between superfluous cellular free fatty acids

(FFAs) supply and their inadequate utilization in the process of mitochondrial β -oxidation [4]. Moreover, many of the previously conducted studies have confirmed the perturbing effects of FAs or their cellular intermediates, on the insulin signaling pathway [5], whereas palmitic acid is commonly applied to evoke IR in both animals [6] and cell lines [7]. Furthermore, the existence of a positive correlation between intramyocellular lipids content and the degree of insulin resistance has been strongly established in the scientific literature. In addition, triacylglycerol (TG) and diacylglycerol (DAG) are probably the two most commonly discussed, with respect to lipid-induced deterioration of insulin sensitivity,

lipids fractions [4, 8]. Interestingly, some research has indicated that the accumulation of a various size cytoplasmic lipid droplets occurs also in the diabetic rat's salivary glands [9, 10].

The main function of the salivary glands is, unsurprisingly, the synthesis of saliva, a watery fluid containing electrolytes, mucus, and enzymes. Alongside its role in digestion saliva serves also some protective functions in respect of the oral mucosa and gingiva. Moreover, research of Tomita et al. indicates that the secretions of the parotid and the submandibular salivary glands contain also lipid component (in an amount of about 5–10 mg per 100 mL of the secretion) [11]. Interestingly, not only do the salivary glands play an important role in respect of oral hygiene maintenance, but also they are associated with hyperglycemia and other metabolic disturbances occurring after the onset of diabetes [12, 13]. Moreover, Sandberg et al. showed that oral health problems are the health complications affecting approximately half the people with hyperglycemia [14]. Other common diabetes codiseases/coailments include periodontitis, gingivitis, xerostomia (dry mouth syndrome), tooth decay (also known as dental caries) and loss, and lesions of the tongue and the oral mucosa [15, 16]. Furthermore, diabetes also seems to be associated with both the parotid and the submandibular salivary glands atrophy, as confirmed by the glands weight and size reductions accompanied by a degeneration of the acinar cells and a decrement in the secretory granules diameter [17–19].

Previously conducted research indicates that diabetes frequently leads to the imbalance in the salivary glands lipid metabolism which subsequently results in the cytoplasm lipid droplets accumulation. However, judging by the limited number of the research articles, the salivary glands lipid profile still has not been sufficiently explored. In the current study we aim to assess, in detail, changes in the main lipid fractions, namely, triacylglycerols, phospholipids, free fatty acids, and diacylglycerols, concentration in the parotid (PSG) and the submandibular (SMSG) salivary glands of rats exposed to a 5-week high fat diet regimen.

2. Materials and Methods

2.1. Experimental Model. The research was conducted on male Wistar rats randomly assigned to one of the experimental groups (8 specimens in each group). Prior to any experiments all procedures concerning animal treatment and maintenance were approved by the Local Ethical Committee for Animal Experiments of the Medical University of Białystok. The rats were maintained in the appropriate conditions. Throughout the experiment a stable temperature (21–22°C), humidity, twenty-four-hour rhythm (12 h/12 h light-dark cycle), and free access to food and water were preserved.

At the beginning of the experiment the animals were randomly allocated into one of the two groups: control (C): with unrestricted access to a standard rodent diet; and high fat diet fed group (HFD): with unrestricted access to a high caloric research diet (60% of energy derived from

fats, as described before [3]). All tissue collection procedures were performed at the beginning of the 6th week; prior to it the animals were fasted overnight and subsequently intraperitoneally injected with pentobarbital (80 mg/kg of body weight). The parotid and the submandibular salivary glands samples were cut out, afterwards immediately frozen, and stored in liquid nitrogen for the time of further analyses.

2.2. Blood Parameters. In addition to the abovementioned procedures also blood from the abdominal aorta was collected. The blood was destined for the analysis of the fasting glucose (Accu-check glucometer, Byer, Germany), insulin (chemiluminescence, Abbot, USA), and free fatty acids levels (as described by Bligh and Dyer [20]). Based on these assessments we have estimated the animals' insulin resistance using widely accepted HOMA-IR formula; that is, HOMA = glucose * insulin/405.

2.3. Salivary Glands Lipids Composition. According to the protocol [20], four previously selected lipid fractions, that is, triacylglycerols, phospholipids, free fatty acids, and diacylglycerols, concentrations were assessed. For this reason the excised salivary glands samples were pulverized by grinding them in a liquid nitrogen precooled mortar and pestle. The obtained tissue powder was then moved to glass tubes for the subsequent lipids extraction according to the protocol developed by Bligh and Dyer [20]. The TG, DAG, and PH fractions separations were performed on the basis of thin-layer chromatography [3]. For the identification and quantification of individual fatty acid methyl esters measurements of the gas-liquid chromatography standards retention times were applied. The abovementioned were run on a Hewlett-Packard 5890 Series II gas chromatograph, with a Varian CP-SIL capillary column (50 m × 0.25 mm internal diameter) and flame-ionization detector (FID) (Agilent Technologies, USA). In the abovementioned measurements the following standards were used: (C 17:0) heptadecanoic acid, as a standard for FFAs quantification; (C 17:0) 1,2-diheptadecanoin, as a standard for DAG quantification; (C 17:0) triheptadecanoin, as a standard for TG quantification; (C 17:0) 1,2-diheptadecanoyl-sn-glycero-3-phosphocholine, as a standard for PH quantification. Total lipid content in each class (TG, PH, FFAs, and DAG) is expressed in nanomoles per gram of the tissue and was calculated from the sum of the particular fatty acid species in a given class.

2.4. Statistical Analysis. Between groups differences were detected using unpaired Student's *t*-test (α set at 0.05). In the case of data distribution other than normal and/or variance heteroscedasticity the *U* test (Mann-Whitney) was applied. All of the obtained results are presented in tables and in the form of bar-plots with the mean as a bar height and whiskers as the standard deviation (1 SD) value. Sample size was set based on a previously conducted pilot study; the power of the test was set at 0.8.

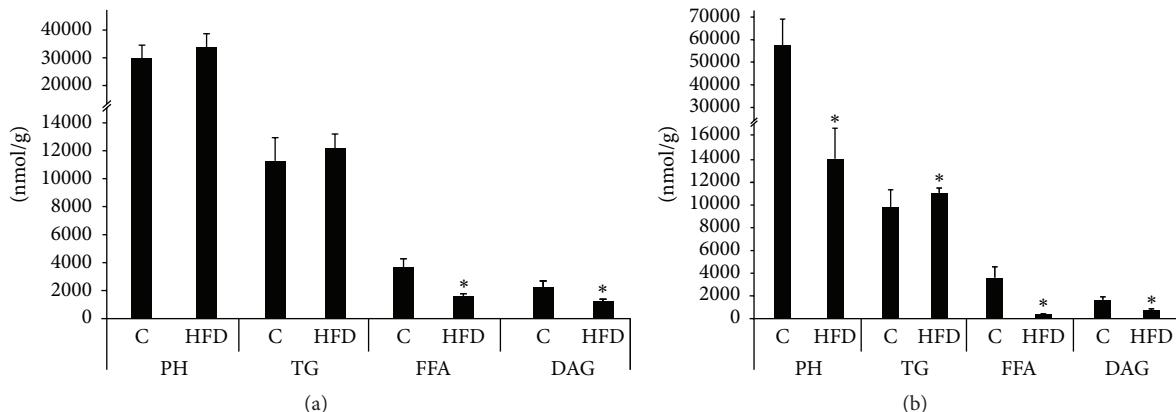


FIGURE 1: Effects of high fat diet feeding on the salivary glands lipid profile ((a) parotid salivary glands; (b) submandibular salivary glands). Ctrl: control group ($n = 8$); DG: diacylglycerols; FFAs: free fatty acids; HFD: high fat diet fed group ($n = 8$); PH: phospholipids; PSG: parotid salivary glands; SMSG: submandibular salivary glands; TG: triacylglycerols; *: difference versus Ctrl ($p < 0.05$).

TABLE 1: Effect of high fat diet feeding on body weight, fasting serum glucose level, fasting serum insulin level, the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index, and serum FFA level (measured at the beginning of the 6th week).

	C	HFD
Body weight (g)	315.6 ± 17.0	$375.4 \pm 18.1^*$
Glucose level (mg/dL)	101.3 ± 6.4	$164.5 \pm 12.4^*$
Insulin level ($\mu\text{U/mL}$)	4.6 ± 0.6	$55.7 \pm 5.7^*$
HOMA-IR	1.6 ± 1.1	$20.0 \pm 2.5^*$
FFA level ($\mu\text{mol/L}$)	88.6 ± 10.4	$152.4 \pm 10.1^*$

C: control group. HFD: group fed with high fat diet.

Results are based on 8 independent preparations for each experimental treatment (means \pm SD).

* $p < 0.05$ compared with C group.

3. Results

3.1. Effects of High Fat Diet Feeding on the Body Weight, Glucose Homeostasis, and Plasma Free Fatty Acids Level (Table 1). The average daily food intake was similar in both studied groups. The high fat diet fed rats were characterized by a significantly increased body mass, as compared with the control group ($p < 0.05$). Moreover, the high fat diet feeding also affected glucose homeostasis. We noticed an increase in the fasting glucose level as well as the level of insulin in the HFD group in comparison with the control animals ($p < 0.05$ and $p < 0.05$, resp.). Furthermore, we observed that the high fat diet feeding led to the development of insulin resistance, as assessed based on the elevated HOMA-IR index, in the HFD group in comparison with the control group ($p < 0.05$). Finally, in comparison with the control group the rats fed with the high fat diet were characterized by an increased plasma free fatty acids concentration ($p < 0.05$).

3.2. Effects of High Fat Diet Feeding on PH Content in the Salivary Glands (Figure 1, Tables 2 and 3). In the parotid salivary glands we did not observe any significant differences in total PH concentrations between the C and HFD group.

TABLE 2: Phospholipids composition in the parotid salivary glands (nmol/g).

Fatty acid	Control	High fat diet
Myristic (14:0)	10677.79 ± 245.404	1064.25 ± 183.734
Palmitic (16:0)	18308.42 ± 3549.057	20006.66 ± 3724.717
Palmitoleic (16:1)	2262.11 ± 752.999	1716.34 ± 255.614
Stearic (18:0)	1666.38 ± 290.83	1936.75 ± 250.423
Oleic (18:1)	3515.13 ± 567.385	$4726.36 \pm 910.837^*$
Linoleic (18:2)	1920.61 ± 575.49	$2656.63 \pm 432.943^*$
Arachidic (20:0)	101.46 ± 21.18	118.36 ± 36.992
α -linoleic (18:3)	257.25 ± 90.915	212.67 ± 30.417
Behenic (22:0)	167.36 ± 28.941	131.77 ± 32.863
Arachidonic (20:4)	737.81 ± 101.194	731.71 ± 123.755
Lignoceric (24:0)	98.77 ± 18.389	120.46 ± 37.501
Eicosapentaenoic (20:5)	30.46 ± 8.774	$55.63 \pm 8.098^*$
Nervonic (24:1)	88.62 ± 13.577	80.72 ± 19.464
Docosahexaenoic (22:6)	451.08 ± 69.753	$614.43 \pm 175.756^*$
UFA	9263.07 ± 971.175	10794.47 ± 1410.346
SFA	21410.18 ± 3888.879	23378.25 ± 3956.379
Total	30673.25 ± 4728.9	34172.72 ± 4828.508

Results are based on 8 independent preparations for each experimental treatment (means \pm SD). * $p < 0.05$ compared with control group.

However, we noticed an increment of oleic (18:1), linoleic (18:2), eicosapentaenoic (20:5), and docosahexaenoic (22:6) acids concentration in the HFD group in comparison with the C group ($p < 0.05$).

On the contrary, the submandibular salivary glands total PH content was significantly decreased ($p < 0.05$) in the HFD group compared with the C group. Furthermore, we found that in the HFD group in comparison with the C group both saturated and unsaturated fatty acids contents were decreased ($p < 0.05$). Among the unsaturated fatty acids in the HFD group compared with the C group the following fractions were decreased: palmitoleic (16:1), oleic (18:1), linoleic (18:2), α -linoleic (18:3), arachidonic (20:4), nervonic (24:1), eicosapentaenoic (20:5), and docosahexaenoic (22:6) ($p < 0.05$).

TABLE 3: Phospholipids composition in the submandibular salivary glands (nmol/g).

Fatty acid	Control	High fat diet
Myristic (14:0)	1812.03 ± 410.021	220.06 ± 50.003*
Palmitic (16:0)	20700.35 ± 5080.428	4084.86 ± 826.012*
Palmitoleic (16:1)	4888.46 ± 1330.273	347.52 ± 90.284*
Stearic (18:0)	2551.09 ± 568.847	1235.59 ± 228.771*
Oleic (18:1)	15946.36 ± 3926.205	4915.29 ± 1193.102*
Linoleic (18:2)	10439.62 ± 3124.438	2841.45 ± 698.514*
Arachidic (20:0)	46.67 ± 14.163	31.41 ± 3.803*
α-linoleic (18:3)	488.17 ± 261.042	157.67 ± 37.696*
Behenic (22:0)	51.8 ± 7.359	32.97 ± 2.694*
Arachidonic (20:4)	391.03 ± 70.611	196.61 ± 18.14*
Lignoceric (24:0)	14.08 ± 3.693	12.93 ± 2.181
Eicosapentaenoic (20:5)	15.41 ± 3.307	18.88 ± 1.696*
Nervonic (24:1)	13.09 ± 2.719	5.57 ± 0.947*
Docosahexaenoic (22:6)	92.91 ± 26.462	38.75 ± 5.619*
UFA	32275.05 ± 6588.666	8521.74 ± 1983.94*
SFA	25176.03 ± 5704.184	5617.82 ± 1005.162*
Total	57451.08 ± 11482.449	14139.56 ± 2787.592*

Results are based on 8 independent preparations for each experimental treatment (means ± SD). * $p < 0.05$ compared with control group.

Moreover, among the saturated fatty acids myristic (14:0), palmitic (16:0), stearic (18:0), arachidic (20:0), and behenic (22:0) acid were significantly decreased in the HFD group in comparison with the C group ($p < 0.05$).

3.3. Effects of High Fat Diet Feeding on FFAs Content in the Salivary Glands (Figure 1, Tables 4 and 5). In the parotid salivary glands total FFAs content was significantly decreased ($p < 0.05$) in the HFD group, as compared with the C group. Moreover, we found that in the HFD group in comparison with the C group both saturated and unsaturated fatty acids contents were decreased ($p < 0.05$). Among the unsaturated fatty acids in the HFD group compared with the C group the following fatty acid species were decreased: palmitoleic (16:1), oleic (18:1), linoleic (18:2), α-linoleic (18:3), arachidonic (20:4), eicosapentaenoic (20:5), nervonic (24:1), and docosahexaenoic (22:6) ($p < 0.05$). Moreover, among the saturated fatty acids myristic (14:0), palmitic (16:0), stearic (18:0), behenic (22:0), and lignoceric (24:0) acids were significantly decreased in the HFD group in comparison with the C group ($p < 0.05$).

Also in the submandibular salivary glands total FFAs content was significantly decreased ($p < 0.05$) in the HFD group, as compared with the C group. Furthermore, we found that in the HFD group in comparison with the C group both saturated and unsaturated fatty acids contents were decreased ($p < 0.05$). Among the unsaturated fatty acids in the HFD group compared with the C group the following fatty acid species were decreased: palmitoleic (16:1), oleic (18:1), linoleic (18:2), α-linoleic (18:3), arachidonic (20:4), eicosapentaenoic (20:5), nervonic (24:1), and docosahexaenoic (22:6) ($p < 0.05$). Moreover, among the saturated fatty acids myristic (14:0), palmitic (16:0), stearic (18:0), arachidic (20:0), behenic (22:0), and arachidonic acid (24:0) were significantly decreased in the HFD group in comparison with the C group ($p < 0.05$).

TABLE 4: Free fatty acids composition in the parotid salivary glands (nmol/g).

Fatty acid	Control	High fat diet
Myristic (14:0)	73.74 ± 11.539	38.11 ± 4.69*
Palmitic (16:0)	1225.19 ± 312.786	721.51 ± 90.679*
Palmitoleic (16:1)	475.43 ± 90.237	87.46 ± 21.989*
Stearic (18:0)	430.62 ± 120.932	230.14 ± 43.29*
Oleic (18:1)	369.43 ± 50.221	156.9 ± 39.858*
Linoleic (18:2)	337.67 ± 50.205	151.54 ± 35.83*
Arachidic (20:0)	6.67 ± 1.861	6.25 ± 0.806
α-linoleic (18:3)	31.86 ± 4.276	11.21 ± 3.25*
Behenic (22:0)	18.35 ± 4.609	4.2 ± 0.822*
Arachidonic (20:4)	558.74 ± 242.799	136.16 ± 41.227*
Lignoceric (24:0)	5.3 ± 1.381	3.97 ± 0.72*
Eicosapentaenoic (20:5)	28.94 ± 17.181	9.99 ± 3.425*
Nervonic (24:1)	3.88 ± 0.962	2.15 ± 0.29*
Docosahexaenoic (22:6)	70.78 ± 7.997	5.88 ± 1.923*
UFA	1876.72 ± 267.732	561.29 ± 134.462*
SFA	1759.88 ± 383.428	1004.18 ± 134.206*
Total	3636.6 ± 622.343	1565.47 ± 188.869*

Results are based on 8 independent preparations for each experimental treatment (means ± SD). * $p < 0.05$ compared with control group.

TABLE 5: Free fatty acids composition in the submandibular salivary glands (nmol/g).

Fatty acid	Control	High fat diet
Myristic (14:0)	50.36 ± 7.533	13.05 ± 2.304*
Palmitic (16:0)	1044.21 ± 293.655	136.95 ± 22.83*
Palmitoleic (16:1)	136.64 ± 33.283	3.2 ± 0.647*
Stearic (18:0)	673.43 ± 215.344	147.14 ± 21.13*
Oleic (18:1)	374.01 ± 68.926	21.52 ± 4.774*
Linoleic (18:2)	347.78 ± 77.772	11.45 ± 2.765*
Arachidic (20:0)	10.32 ± 2.892	5.01 ± 0.88*
α-linoleic (18:3)	31.6 ± 11.089	1.97 ± 0.495*
Behenic (22:0)	11.71 ± 0.945	2.39 ± 0.336*
Arachidonic (20:4)	828.63 ± 332.348	5.27 ± 1.259*
Lignoceric (24:0)	3.67 ± 0.97	2.53 ± 0.444*
Eicosapentaenoic (20:5)	24.19 ± 6.655	1.55 ± 0.656*
Nervonic (24:1)	3.78 ± 0.859	1.32 ± 0.417*
Docosahexaenoic (22:6)	46.98 ± 7.058	1.1 ± 0.145*
UFA	1793.6 ± 488.952	47.38 ± 7.813*
SFA	1793.71 ± 515.048	307.07 ± 44.118*
Total	3587.31 ± 959.758	354.44 ± 46.163*

Results are based on 8 independent preparations for each experimental treatment (means ± SD). * $p < 0.05$ compared with control group.

behenic (22:0), and arachidonic acid (24:0) were significantly decreased in the HFD group in comparison with the C group ($p < 0.05$).

3.4. Effects of High Fat Diet Feeding on DAG Content in the Salivary Glands (Figure 1, Tables 6 and 7). In the parotid salivary glands total DAG content was significantly decreased

TABLE 6: Diacylglycerols composition in the parotid salivary glands (nmol/g).

Fatty acid	Control	High fat diet
Myristic (14:0)	87.79 ± 22.188	56.03 ± 7.414*
Palmitic (16:0)	984.94 ± 210.904	575.82 ± 94.842*
Palmitoleic (16:1)	184.17 ± 24.947	49.8 ± 9.994*
Stearic (18:0)	431.31 ± 101.976	200.57 ± 29.904*
Oleic (18:1)	134.86 ± 21.235	93.98 ± 12.105*
Linoleic (18:2)	171.37 ± 35.577	130.41 ± 16.157*
Arachidic (20:0)	5.98 ± 1.84	5.05 ± 0.855
α-linoleic (18:3)	7.31 ± 1.464	5.26 ± 0.914*
Behenic (22:0)	12.08 ± 3.819	3.79 ± 0.715*
Arachidonic (20:4)	184.27 ± 76.864	94.84 ± 19.375*
Lignoceric (24:0)	3.77 ± 1.47	3.24 ± 0.514
Eicosapentaenoic (20:5)	7.5 ± 3.514	5.38 ± 0.913
Nervonic (24:1)	1.26 ± 0.382	1.31 ± 0.221
Docosahexaenoic (22:6)	28.71 ± 5.259	5.89 ± 0.927*
UFA	719.47 ± 127.861	386.87 ± 51.931*
SFA	1525.87 ± 303.224	844.5 ± 99.547*
Total	2245.33 ± 426.18	1231.37 ± 128.942*

Results are based on 8 independent preparations for each experimental treatment (means ± SD). * $p < 0.05$ compared with control group.

TABLE 7: Diacylglycerols composition in the submandibular salivary glands (nmol/g).

Fatty acid	Control	High fat diet
Myristic (14:0)	48.38 ± 11.363	38.27 ± 7.62*
Palmitic (16:0)	599.62 ± 94.795	281.06 ± 47.48*
Palmitoleic (16:1)	58.54 ± 19.612	4.72 ± 0.704*
Stearic (18:0)	364.07 ± 54.254	261.05 ± 48.843*
Oleic (18:1)	142.47 ± 40.036	44.75 ± 5.25*
Linoleic (18:2)	186.84 ± 50.092	43.5 ± 5.515*
Arachidic (20:0)	9.04 ± 1.714	6.11 ± 0.856*
α-linoleic (18:3)	8.62 ± 1.633	3.13 ± 0.448*
Behenic (22:0)	7.08 ± 0.724	2.94 ± 0.475*
Arachidonic (20:4)	184.5 ± 48.12	35.4 ± 3.616*
Lignoceric (24:0)	2.3 ± 0.579	2.14 ± 0.393
Eicosapentaenoic (20:5)	4.65 ± 1.736	2.32 ± 0.334*
Nervonic (24:1)	0.75 ± 0.188	1.04 ± 0.211*
Docosahexaenoic (22:6)	13.61 ± 2.375	2.06 ± 0.324*
UFA	600 ± 134.423	136.92 ± 10.521*
SFA	1030.49 ± 155.56	591.57 ± 101.192*
Total	1630.49 ± 266.998	728.49 ± 107.393*

Results are based on 8 independent preparations for each experimental treatment (means ± SD). * $p < 0.05$ compared with control group.

($p < 0.05$) in the HFD group, as compared with the C group. Moreover, we found that in the HFD group in comparison with the C group both saturated and unsaturated fatty acids contents were decreased ($p < 0.05$). Among the unsaturated fatty acids in the HFD group compared with the C group the following fatty acid species were decreased: palmitoleic (16:1), oleic (18:1), linoleic (18:2), α-linoleic (18:3), arachidonic (20:4), and docosahexaenoic acid (22:6) ($p < 0.05$). Among the saturated fatty acids in the HFD group compared with the C group the following fatty acid species were decreased: myristic (14:0), palmitic (16:0), stearic (18:0), arachidic (20:0), and behenic acid (22:0) ($p < 0.05$).

TABLE 8: Triacylglycerols composition in the parotid salivary glands (nmol/g).

Fatty acid	Control	High fat diet
Myristic (14:0)	95.58 ± 16.557	93.13 ± 10.494
Palmitic (16:0)	3753.26 ± 577.212	4106.75 ± 355.387
Palmitoleic (16:1)	725.82 ± 311.454	457.56 ± 60.399
Stearic (18:0)	1545.87 ± 203.585	1739.3 ± 179.592
Oleic (18:1)	740.19 ± 141.33	758.58 ± 60.541
Linoleic (18:2)	1745.84 ± 508.939	2123 ± 239.624*
Arachidic (20:0)	6.46 ± 0.973	6.98 ± 1.074
α-linoleic (18:3)	15.69 ± 5.458	14.51 ± 3.86
Behenic (22:0)	57.18 ± 12.029	25.77 ± 5.108*
Arachidonic (20:4)	2336.43 ± 247.191	2462.9 ± 323.202
Lignoceric (24:0)	6.2 ± 1.302	7.04 ± 1.189
Eicosapentaenoic (20:5)	63.45 ± 20.07	114.81 ± 19.906*
Nervonic (24:1)	6 ± 3.685	4.11 ± 2.077
Docosahexaenoic (22:6)	175.64 ± 37.506	250.05 ± 49.041*
UFA	5809.07 ± 895.885	6185.53 ± 508.847
SFA	5464.55 ± 774.298	5978.97 ± 530.721
Total	11273.62 ± 1662.308	12164.5 ± 1031.873

Results are based on 8 independent preparations for each experimental treatment (means ± SD). * $p < 0.05$ compared with control group.

($p < 0.05$). Moreover, among the saturated fatty acids myristic (14:0), palmitic (16:0), stearic (18:0), and behenic acid (22:0) were significantly decreased in the HFD group in comparison with the C group ($p < 0.05$).

Also in the submandibular salivary glands total DAG content was significantly decreased ($p < 0.05$) in the HFD group, as compared with the C group. Furthermore, we found that in the HFD group in comparison with the C group both saturated and unsaturated fatty acids contents were decreased ($p < 0.05$). Among the unsaturated fatty acids in the HFD group compared with the C group the following fatty acid species were decreased: palmitoleic (16:1), oleic (18:1), linoleic (18:2), α-linoleic (18:3), arachidonic (20:4), eicosapentaenoic (20:5), nervonic (24:1), and docosahexaenoic acid (22:6) ($p < 0.05$). Moreover, among the saturated fatty acids myristic (14:0), palmitic (16:0), stearic (18:0), arachidic (20:0), and behenic acid (22:0) were significantly decreased in the HFD group in comparison with the C group ($p < 0.05$).

3.5. Effects of High Fat Diet Feeding on TG Content in the Salivary Glands (Figure 1, Tables 8 and 9). In the parotid salivary glands we did not observe any significant differences in total TG concentrations between the C and HFD groups. However, we noticed an increment of linoleic (18:2), eicosapentaenoic (20:5), and docosahexaenoic acid (22:6) concentration coexisting with a decrement of behenic acid content (22:0) in the HFD group in comparison with the C group ($p < 0.05$).

On the contrary, the submandibular salivary glands total TG content was significantly increased ($p < 0.05$) in the HFD group compared with the C group. Among the unsaturated fatty acids in the HFD group compared with the C group the fatty acid species, oleic (18:1), eicosapentaenoic (20:5),

TABLE 9: Triacylglycerols composition in the submandibular salivary glands (nmol/g).

Fatty acid	Control	High fat diet
Myristic (14:0)	72.45 ± 10.702	47.77 ± 3.466*
Palmitic (16:0)	2978.17 ± 381.425	2979.54 ± 157.422
Palmitoleic (16:1)	183.65 ± 28.609	67.47 ± 4.61*
Stearic (18:0)	1575.82 ± 255.862	2004.72 ± 79.831*
Oleic (18:1)	732.15 ± 62.753	953.72 ± 35.941*
Linoleic (18:2)	1416.05 ± 347.755	2068.17 ± 272.292
Arachidic (20:0)	14.95 ± 4.395	19.21 ± 3.536
α-linoleic (18:3)	15.17 ± 5.569	14.62 ± 1.299
Behenic (22:0)	31.32 ± 9.904	28.75 ± 5.13
Arachidonic (20:4)	2542.74 ± 561.505	2542.69 ± 176.752
Lignoceric (24:0)	15.01 ± 1.78	12.17 ± 1.746*
Eicosapentaenoic (20:5)	40.37 ± 13.144	95.49 ± 20.396*
Nervonic (24:1)	9.62 ± 4.199	6.56 ± 1.041*
Docosahexaenoic (22:6)	171.85 ± 52.848	239.27 ± 29.357*
UFA	5111.61 ± 933.047	5988 ± 227.362
SFA	4687.72 ± 623.025	5092.15 ± 206.762
Total	9799.33 ± 1548.921	11080.15 ± 422.14*

Results are based on 8 independent preparations for each experimental treatment (means ± SD). * $p < 0.05$ compared with control group.

and docosahexaenoic acid (22:6), contents were increased ($p < 0.05$), whereas palmitoleic (16:1) and nervonic acid (24:1) contents were decreased ($p < 0.05$). Moreover, among the saturated fatty acids myristic (14:0) and arachidonic acid (24:0) contents were significantly increased, whereas the quantity of stearic acid (18:0) was decreased in the HFD group in comparison with the C group ($p < 0.05$).

4. Discussion

Obesity is currently a predominant medical condition because for several dozens of years an alarming increase in its prevalence has been observed. Unfortunately, raised body mass index (BMI) is an important risk factor for the development of several diseases, including insulin resistance and type 2 diabetes. Tissues which are responsible for the onset and development of the aforementioned conditions are mainly skeletal muscles, liver, and white adipose tissue. However, glucose homeostasis imbalance may affect any other tissue, including the salivary glands. Therefore, in the present study we examined the effect(s) of a diet induced insulin resistance and obesity on lipid profile in both the parotid and the submandibular salivary glands.

Some of the previously published reports indicate that chronic high fat diet feeding causes hyperglycemia which can contribute to the accumulation of lipid droplets in the cytoplasm of many nonadipose tissues, including skeletal muscles, liver, and even salivary glands [3, 9, 10]. Therefore, not surprisingly, our 5-week high fat diet feeding has led to a significantly greater animals' body mass which was accompanied by increased levels of blood glucose, insulin, and plasma FFAs together with the build-up in the salivary glands lipid concentration. All of the abovementioned

changes were in accordance with previously conducted research [3]. Moreover, based on the HOMA-IR values we can claim that the prolonged high fat diet feeding decreased the whole body insulin sensitivity. Finally, although not tested here, it is widely acknowledged that FFAs exert a negative influence in respect of the regulation of cellular glucose uptake and glycogen synthesis, thus leading to the development of hyperglycemia (as observed in our HFD group) [2]. It is important to emphasize that chronic high fat diet feeding may cause a reduction in unstimulated and stimulated salivary flow rate. In the group of obese patients disturbances in the oxidant/antioxidant homeostasis were previously observed [21]. On the other hand, the parotid and the submandibular glands of rats react differently to insulin resistance conditions, with more pronounced changes observed in the submandibular glands [22]. The observed different responsiveness of the glands to high fat diet feeding is not easy to explain; it was, however, observed in some other studies [23]. Ibuki et al. [23], for instance, showed that there was a significant decline in the submandibular salivary glands mass in the time-course of diabetes. Moreover, the authors reported an increased level of MCD (malondialdehyde which is a marker of increased lipid peroxidation) with the changes more pronounced in the case of the SMSG. The observed different responsiveness could be, perhaps, a reflection of a different tissue metabolism (PSG, oxidative, SMSG, glycolytic) [23]. If so, then the response pattern of the salivary glands (to high fat diet feeding) differs from the one observed in skeletal muscles where more pronounced changes in lipid metabolism are observed in the case of the more oxidative ones (e.g., soleus versus white gastrocnemius) [3].

Scientific investigation has led to the discovery of more than 30 different biological components present in the salivary glands, of which lipids seem to be a particularly important representative [24, 25]. Phospholipids, for instance, are the most abundant component in a plasmalemma and are vitally involved in the transport processes taking place across this biological membrane. Interestingly, according to some previous research several pathological conditions, that is, diabetes, aging, or weaning, may contribute to the decreased salivary glands PH content. Tomita et al., for example, indicated a probable negative correlation between the salivary glands PH concentration and the age of animals [11]. This phenomenon could be of particular importance to the discussed topic given commonly observed age-progressing decrement of peripheral tissues insulin sensitivity and the reduction of PH content observed in this study. Moreover, also data from Kamata et al. seem to indirectly confirm this assumption [26]. In the aforementioned investigation the authors reported that type 1 diabetes resulted in a degeneration of the salivary glands acinar cells together with a concomitant decline in their secretory granules number. The researchers postulated that the observed results could be, at least partially, evoked by a significant reduction in PH concentration, which in turn could negatively impact cell membranes stability [26]. Therefore, it seems conceivable that the changes in the salivary glands PH content, as observed by us and other authors, might lead to the detrimental

changes in the salivary glands structure, which in turn could be reflected in their functions, for example, by causing a decreased production of saliva, that is, hyposalivation.

On opposition to the above-discussed phospholipid fraction free fatty acids are believed to be heavily implicated in the processes of intracellular signal transmission. Previously published observations performed on insulin resistant diabetic animals clearly demonstrated increased FFAs concentrations in many tissues, including skeletal muscles and liver [2, 3]. Increased intracellular FFAs content strongly affects insulin signaling pathway and contributes to insulin resistance condition. Surprisingly, in the present study we observed, despite the increased blood FFAs supply, that the insulin resistant diabetic animals were characterized by a decreased FFAs content in both the parotid and the submandibular salivary glands. Furthermore, we found that the feeding with high fat diet led to a decrement in both saturated and unsaturated fatty acids contents. This phenomenon could be explained by either a decreased FFAs uptake from the blood or their transformation into other lipid fractions (perhaps TG, since their concentration increased significantly in the submandibular glands of the rats fed with high fat diet). Furthermore, it is well established that the fatty acid synthesis is a multistep process, with one of the steps, desaturation, catalyzed by desaturases. Interestingly, it has been shown that the activity of desaturases, including those originating from the salivary glands, decreases in the absence of insulin or during insulin resistance. As an end result of the decreased desaturase activity a decline in the number and amount of some unsaturated FFAs species occurs, since desaturase introduces "unsaturated" double carbon bond into a fatty acid chain. Similar findings were also reported by Mahay et al. [27]; however, not all research groups confirm them [28].

Interestingly, in the present study we have noticed a decreased level of DAG in the parotid and the submandibular salivary glands of the diabetic rats. We demonstrated, likewise in the case of FFAs, that this decrement applies to both the saturated and the unsaturated fatty acid species. At first glance, this novel and intriguing finding seems to be quite unexpected. Many authors point on the causative relationship between DAG over accumulation and insulin resistance in peripheral tissues (e.g., skeletal muscles, adipose tissue, and liver) [2, 4]. However, we should be aware that the salivary glands are not as vitally involved in the development of this metabolic condition as the aforementioned tissues (due to their relatively large mass and high metabolic activity) are. Moreover, closer literature data review indicates that in the salivary glands DAG intracellular signaling may contribute to the muscarinic-induced saliva secretion; thus any reduction in its (DAG) concentration would result in a hyposalivation, a condition observed in both human and animal diabetic individuals [29].

Another plausible explanation for the observed reductions in the salivary glands lipid content is a possible tissue atrophy frequently observed in some of the previous studies [19, 23]. Although the aforementioned studies tested the glands metabolism in the case of rats with streptozotocin induced diabetes they do, however, share significant

similarities with the model applied by us. With respect to the discussed topic it is important to note that both type 1 and type 2 diabetes are characterized by elevated levels of serum glucose and free fatty acids concentrations [3, 30]; thus the cellular external environment is somewhat alike. Ibuki et al. [23], for instance, showed that there was a significant decline in the submandibular, but not the parotid, salivary glands mass between the 28th and 45th day of the experiment (our experiment (HFD) lasted 5 weeks, that is, 35 days). Moreover, the authors reported increased level of MCD (malondialdehyde, which is a marker of increased lipid peroxidation) in both of the examined glands. This could indicate an increased cellular atrophy in the tissues and therefore increased lipids degeneration. The possibility of such cellular atrophy was not directly tested in our study; however, if it were the case one would expect it to be visible in all of the examined lipid fractions. According to our data (Tables 2–9, Figure 1), though, it seems that it did not happen in the case of the two most abundant lipid classes: PH (a decrease only in the submandibular salivary glands) and TG fractions (no difference in the case of PSG and an increase in the case of SMSG). Therefore, it is possible that the observed changes are not the result of the cellular atrophy itself but may reflect the diabetic condition in a broader context.

Some of the previously published studies indicate that the salivary glands intraorgan lipid accumulation occurs along with the time-course of insulin resistance and diabetes; moreover, its magnitude usually accompanies the changes in the serum glucose concentration [28, 31]. Furthermore, immunohistochemical scrutiny revealed that these lipid droplets are composed mostly of triacylglycerol that had been, probably, accumulated as a consequence of its increased generation and/or decreased utilization for secretory purposes [31]. It has been proven that the intracellular TG accumulation is associated with insulin resistance, although nowadays this phenomenon is recognized more as a marker of accumulated lipids rather than a direct cause of type 2 diabetes. Surprisingly, Morris and coworkers discovered that even two weeks of streptozotocin induced diabetes may lead to the pronounced salivary glands lipids accumulation as confirmed by histological evaluation [28]. Interestingly, the aforementioned changes were easily reversible after barely 1 week of insulin administration. Thus, it appears that the salivary glands lipid accumulation occurs quite early in the development of diabetes and, at least at that time point, is fairly easily reversible [28]. In the present study we have demonstrated that an increment in the salivary glands TG accumulation (greater TG content in the submandibular salivary glands) may also occur in the time-course of insulin resistance and type 2 diabetes. Therefore, it has been postulated that the pronounced build-up in the salivary glands lipid content (mostly in TG fraction) not only is associated with high BMI, fatty liver disease, or coronary artery disease risk factors, but also may be a relevant clinical symptom of metabolic syndrome and/or type 2 diabetes [32].

In conclusion, due to the scarcity of the literature data the precise effects of insulin resistance and type 2 diabetes with respect to the salivary glands lipid profile were not, so far, extensively elucidated. In the present study we aimed

to fill this gap. Firstly, we observed that the high fat diet regimen had caused significant changes in the salivary glands lipid composition, especially in regard to PH and TG, but not DAG or FFAs, classes. The observed reduction in PH concentration is an interesting phenomenon frequently signifying the atrophy and malfunctions in the saliva secreting organs. On the other hand, the increased accumulation of TG in the glands may be an important clinical manifestation of metabolic syndrome and type 2 diabetes mellitus.

Competing Interests

The authors declare that there are no competing interests regarding this paper publication.

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