Impact of the Glycemic Level on the Salivary Proteome of Middle-Aged and Elderly People With Type 2 Diabetes Mellitus: An Observational Study

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Type 2 diabetes mellitus (T2DM) is an increasing global public health concern, but its impact on the salivary proteome is still unclear. To evaluate the effect of glycemic levels in middle-aged and elderly individuals with T2DM on salivary proteomics, we compared the differences by liquid chromatography tandem mass spectrometry (LC–MS/MS). Unstimulated whole saliva samples from 8 T2DM patients with good glycemic control (G group, HbA1c <6.5%) and 16 patients with poor control (P group, HbA1c ≥6.5%) were analyzed by LC–MS/MS in the data-independent acquisition mode (Clinical register number: ChiCTR1900023582.). After functional annotation, cluster analysis and receiver operating characteristic (ROC) curve analysis were carried out to screen and evaluate candidate proteins. A total of 5,721 proteins were quantified, while 40 proteins differed significantly. In the P group, proteins involved in oxidative stress-related processes were upregulated, whereas proteins related to salivary secretion were downregulated. The combination of thioredoxin domain-containing protein 17, zymogen granule protein 16B, and FAM3 metabolism regulating signaling molecule D yielded an area under the curve of 0.917 which showed a robust ability to distinguish the P and G groups. In conclusion, poorly controlled hyperglycemia may affect salivary proteins through various pathways, including oxidative stress and glandular secretion. Furthermore, the differentially expressed proteins, especially the three proteins with the best differentiation, might serve as an anchor point for the further study of hyperglycemia and oral diseases.

Keywords: Data-independent acquisition, mass spectrometry, type 2 diabetes mellitus, salivary proteome, salivary secretion, oxidative stress

1 INTRODUCTION

Diabetes mellitus (DM) is a common metabolic disease manifesting as defects in insulin secretion, insulin action or both, which results in a type of hyperglycemic status. Diabetes mellitus encompasses type 1, type 2, gestational diabetes and other types, and the estimate from the International Diabetes Federation (IDF) is projected to rise to over 640 million people with diabetes by 2040 (Cole and Florez, 2020). Type 2 diabetes mellitus (T2DM) accounts for over 90% of cases of DM worldwide and...
has become an increasing global public health concern. T2DM can lead to multiple complications, including diabetic neuropathy, diabetic retinopathy, diabetic nephropathy, and cardiovascular complications, which increase disabilities and mortalities in individuals (Zheng et al., 2018).

In addition to the complications of diabetes mentioned above, there are also many oral manifestations associated, including periodontitis, dental caries, oral candidiasis, and salivary dysfunction (Mauri-Obradors et al., 2017). In patients with diabetes, elevated glucose levels contribute to a decrease in the salivary flow rate, a reduction in pH concentration and changes in the oral environment, which easily cause changes in salivary traits (Velasco-Ortega et al., 2016). In addition, diabetic patients suffer from vascular endothelial damage due to specific vascular diseases and are vulnerable to invasion and injury, coupled with an increased systemic inflammatory response in hyperglycemia (Graves et al., 2020). All of these factors promote the occurrence and development of oral complications.

Saliva is a bodily fluid with a complex composition and specific roles. In recent years, salivary proteomics has made great progress, and more than 4,000 protein species have been identified (Siqueira and Dawes, 2011; Huang et al., 2021). Moreover, salivary proteome research has focused on the screening of novel biomarkers for oral and related diseases, including lactoferrin, interleukin-6, and matrix metalloproteinase-8 (Castagnola et al., 2017; Bermejo-Pareja et al., 2020; Kc et al., 2020; Boroumand et al., 2021). In addition, diabetes affects the composition of saliva, and alterations may impact the incidence or signs of oral lesions (Abd-Elraheem et al., 2017; Naseri et al., 2018). Overall, the impact of diabetes on salivary proteins has attracted the attention of many researchers. In particular, an article on comprehensive proteomic analysis of the human salivary protein in T2DM in 2004 (Gillet et al., 2012; Doerr, 2015; Ludwig et al., 2018). During the DIA process, it is independent of the composition of precursor ions for their fragmentation to implement the approach, and thus, this method improves the accuracy and precision of quantitation and has gained much popularity (Zhang et al., 2020a). This technique is widely used in various body fluids, including blood, cerebrospinal fluid, urine, and tears but is less applied in saliva (Li et al., 2020; van der Laan et al., 2020; Cheung et al., 2021; Jia et al., 2021).

In this study, we investigated the proteomic profile of whole saliva by LC-MS/MS with the DIA mode in T2DM patients with satisfactory glycemic control in comparison with the poorly-controlled individuals. The aim of the present work was to characterize the salivary proteome at different glucose control levels to identify differentially expressed proteins associated with regulated biological pathways and evaluate potential biomarkers for disease monitoring.

## 2 MATERIALS AND METHODS

### 2.1 Study Population

The observational research was approved by the Local Ethics Committee at the Peking University School and Hospital of Stomatology (PKUSSIRB-201944042). The clinical register number of this study is ChiCTR1900023582. From April 2019 to July 2019, we recruited 24 subjects, and they all signed written informed consent forms. After matching their periodontal condition and other indicators, they were divided into two groups according to their glycosylated hemoglobin A1c (HbA1c) levels: a well-controlled group (n = 8; one male and seven females; HbA1c <6.5%) and a poorly controlled group (n = 16; three males and 13 females; HbA1c ≥6.5%), with no statistical difference in age and sex distribution.

The inclusion criteria were as follows: 1) 50–75 years old; 2) a minimum of 15 teeth (excluding third molars); 3) a clinical diagnosis for at least 1 year with T2DM defined by the latest classification and diagnosis (American Diabetes Association, 2021); and 4) no change in diabetic treatment, including the dosage and formulation, during the last 3 months.

The exclusion criteria were as follows: 1) presence of systemic disease other than T2DM, for example, an acute cardiovascular event in the 12 months prior to the start of the study or stroke, renal failure, or liver dysfunction; 2) presence of the major complications of diabetes; and 3) presence of an active infection such as a periapical abscess.

### 2.2 Clinical Examination

The clinical physicians performed the periodontal examination for all participants using a periodontal probe (UNC15; Hu-Friedy, Chicago, IL, United States). The related parameters were recorded at six sites of each tooth. The measured parameters were as follows: probing pocket depth (PPD), clinical attachment loss (CAL) and bleeding index (BI). All clinical examination procedures were performed by two senior calibrated dentists.
2.3 Saliva Sampling and Storage
The participants were requested to refrain from eating, drinking, chewing and brushing for at least 2 h. Each person was asked to rinse their mouth with water for approximately 2 min and then wait 10 min. After that, the participants were taught to collect their whole unstimulated saliva according to a method described by Petros Papagerakis (Papagerakis et al., 2019). The saliva flowed in a sterile 5 ml Eppendorf tube and was transferred to the laboratory on ice. The saliva samples were centrifuged at 10,000 g/min for 10 min, and the supernatants were frozen at −80°C.

2.4 Laboratory Assays
2.4.1 Sample Preparation for Proteome Analysis
Samples were suspended in lysis buffer (1% sodium deoxycholate (SDS), 8M urea) which included appropriate 1 protease inhibitor cocktail and phosphatase inhibitor to inhibit protease activity. The mixture was allowed to vortex to mix well and then placed on ice for 30 min during which the sample were vortexed at every 10 min. After centrifugation at 12,000 g at 4°C for 20 min, the concentration of protein supernatant was determined by Bicinchoninic acid (BCA) method by BCA Protein Assay Kit. 50 μg proteins were suspended in 50 μl solution, reduced by adding 1μl 0.5M TCEP incubated at 37°C for 1 h, alkylated by adding 2 μl of 1M iodoacetamide in the dark at room temperature for 40 min. Then the sample was precipitated using 300 μl prechilled acetone at −20°C overnight. The precipitate was washed twice with cold acetone and then resuspended in 50 mM TEAB. Finally, the proteins were digested with trypsin (Promega, Madison, WI) at a substrate/enzyme ratio of 50:1 (w/w) at 37°C for 16 h.

2.4.2 High pH Reverse Phase Separation
The peptide mixture was redissolved and then fractionated by high pH separation using an Ultimate 3000 system (Thermo Fisher Scientific, MA, United States) connected to a reversed-phase column (XBridge C18 column, 4.6 mm × 250 mm, 5 μm, (Waters Corporation, MA, United States)). High pH separation was performed using a linear gradient, starting from 5% B to 45% B in 40 min (B: 20 mm ammonium formate in 80% ACN, pH = 10.0, adjusted with ammonium hydroxide).

2.4.3 Creation of the Spectral Library
To build the spectral library, the peptide solutions were analyzed by nano-HPLC–MS/MS. Specifically, the peptides were redissolved in 30 μl of solvent A (A: 0.1% formic acid in water) and analyzed by online nanospray LC–MS/MS on an Orbitrap Fusion Lumos coupled to an EASY-nLC 1200 system (Thermo Fisher Scientific, MA, United States). A 3 μl peptide sample was loaded onto an analytical column (Acclaim PepMap C18, 75 μm × 25 cm) and separated with a 120-min gradient from 5 to 35% B (B: 0.1% formic acid in ACN).

2.4.4 DIA Mode With Nano-HPLC–MS/MS Analysis and Data Acquisition
Each sample was analyzed using the nano-HPLC–MS/MS equipment and LC gradient described above for building the spectral library but using the DIA mode.

Raw DIA data were processed and analyzed by Spectronaut X (Biognosys AG, Switzerland) with default parameters. The retention time prediction type was set to dynamic iRT. Data extraction was determined by Spectronaut X based on extensive mass calibration. Spectronaut Pulsar X will dynamically determine the ideal extraction window depending on iRT calibration and gradient stability. A q-value (FDR) cutoff at the precursor and protein levels was applied at 1%. All selected precursors passing the filters were used for quantification. The average top three filtered peptides that passed the 1% q-value cutoff were used to calculate the major group quantities.

2.5 Data Analysis
All statistical analyses were performed using SPSS 24.0 software (SPSS; Chicago, IL, United States). Quantitative variables are described as the mean ± standard deviation (SD), and frequencies or rates were used for qualitative variables. Student’s t test was used to compare between-group differences, and a p-value <0.5 was defined as statistically significant. Additionally, for qualitative results such as sex, we used the chi-squared test to detect statistically significant differences. After Student’s t test, differentially expressed proteins were filtered if their q-value <0.05 and their absolute value of fold change (FC) ≥1.5. Proteins were annotated against the KEGG and GO databases to obtain their functions. Significant pathways and GO functions were examined within differentially expressed proteins with a p-value <0.05. In addition, we used the receiver operating characteristic (ROC) curve to screen and evaluate the performance of the model, and the area under the receiver operating characteristic curve (AUC) is a performance index ranging from 0 to 1. AUC is equal to 0.5 when the ROC curve corresponds to random chance and 1.0 for perfect accuracy, while an estimated AUC less than 0.5 represents a worse condition (Zou et al., 2007). A higher AUC value indicates superior discrimination performance.

3 RESULTS
3.1 Demographic and Clinical Data
In total, 24 individuals diagnosed with T2DM were recruited for the study, and according to their blood glucose level, they were divided into a well-controlled group and a poorly controlled group. The former included eight subjects (G group; mean age 65.25 ± 3.77 years, one male and seven females), and the latter...
included 16 subjects (P group; mean age 69.69 ± 5.51 years, three males and 13 females). There were no significant differences in age or sex between these two groups (p-value = 0.053 and 1.000, respectively). Regarding their clinical status, most indicators were not significantly different except for HBA1c (p-value = 0.002) and the fasting plasma glucose (p-value < 0.001). A total of 24 saliva samples were obtained. More details of these subjects are shown in Table 1.

### 3.2 Mass Spectrometry Data

As shown in Figure 1A, we used LC–MS/MS scanned by the DIA method to quantify and identify proteins in each sample. As a result, the spectral libraries of saliva contained 20,738 precursors, 16,185 peptides, 2,101 protein groups and 5,721 proteins. Following the generation of the library, identification and quantification were implemented. We identified 16,806 precursors, 12,985 peptides, 1,362 protein groups and 3,355 proteins.
proteins. The sum and percentage of proteins identified in the saliva of each group as well as the overlap between the samples were found in a Venn diagram (Figure 1B). In the P group, 3,327 proteins were quantified, while 3,172 proteins were quantified in the G group. There were 3,144 proteins identified in the two groups. In the PCA plot of sample relationships (Figure 1C), there was a relatively close distance within each group and a partial separation between groups. The composition of the samples within the group was similar, while the samples were distributed separately between groups, indicating a good agreement and a similarity between those samples in the reduced space.

3.3 Differentially Expressed Proteins in Saliva

In saliva samples from the P group, 40 proteins were expressed significantly differently compared to the G group under the screening conditions with a q-value <0.05 and a limit of fold change (FC) ± 1.5 (Figure 2A). Among these proteins, nine proteins were significantly upregulated in the P group compared to the G group. They included hemoglobin subunit beta, hemoglobin subunit alpha 1, and so on (Figure 2A; Table 2). In total, 31 proteins were significantly downregulated in the P group. These proteins included mucin 5B, lactoperoxidase, prolactin-induced protein, etc. (Table 3). The PCA plot of individuals in the G and P groups based on the differentially expressed proteins showed a partial separation of the groups (Figure 2B).

3.4 Functional Annotation of Differentially Expressed Proteins

To investigate the function of differentially expressed proteins, GO and KEGG pathway analyses of upregulated and downregulated proteins were performed separately.

For upregulated proteins, 288 significant enrichments were identified using GO analysis (p-value <0.05). GO is a universal resource for analysis and interpretation of high-throughput biological dataset (Kramarz and Lovering, 2019) and we performed GO analysis to obtain the functional characteristics of the differentially expressed proteins and to indicate the GO terms enriched in these proteins. There were three categories: molecular function (MF), biological process (BP) and cellular component (CC). In MF, the proteins were mainly associated with oxidative stress processes, including peroxidase activity, oxidoreductase activity, antioxidant activity and haptoglobin binding. The most significant terms for BP and CC were bicarbonate transport and cytoplasmic vesicle lumen, respectively (Figure 3B). KEGG analysis performed for differentially upregulated proteins showed that the upregulated proteins were significantly enriched in African trypanosomiasis and malaria (Figure 3A).

For downregulated proteins, the functional annotation showed that there were 91 significant GO terms, including ribonuclease T2 activity, low-density lipoprotein particle receptor binding, and endopeptidase inhibitor activity in MF. For BP and CC, the most significant terms were regulated exocytosis and extracellular space (Figure 3D).
KEGG pathway analysis revealed that the proteins were significantly enriched in the salivary secretion pathway (Figure 3C).

### 3.5 Analysis of the Discriminating Ability of Candidate Proteins

Among the differentially expressed proteins, three proteins with missing values in the samples were removed (Tables 2, 3). Among the remaining proteins, three differentially expressed proteins with high AUC values were selected for further analysis: thioredoxin domain-containing protein 17 (TXNDC17), zymogen granule protein 16B (ZG16B), and FAM3 metabolism regulating signaling molecule D (FAM3D). The expression levels of the three proteins in each sample were used in hierarchical cluster analysis (Figure 4A), which intuitively reflected expression differences between the P and G groups and showed that there was partly clear separation between the two groups. Samples G02, G04, G07, and G08 were far from the other samples in the G group. Generally, there are differences in the abundance of these three proteins between the two glycemic control states, and the sample clusters are relatively distinct.

Compared with the G group, the expression of TXNDC17 increased in saliva, and the other two decreased in the P group. There were significant differences in the expression of TXNDC17, ZG16B and FAM3D (p-value < 0.05) (Figure 4B).

ROC curve analysis of saliva was performed to verify the differentiation ability of the three proteins. The ability of these proteins to distinguish different HbA1c levels is shown in Figure 4C. The AUCs of each protein ranged from 0.802 to 0.823, whereas the AUC for their combined value was 0.917. ROC curve analysis indicated that the combination of candidate biomarkers in saliva to distinguish the G and P groups had the largest AUC area.

Figure 4D indicates the enriched biological processes of FAM3D, ZG16B, and TXNDC17. They were involved in a variety of functions. GO terms annotated by FAM3D included negative regulation of insulin and glucagon and signal transduction, whereas ZG16B was annotated in retinal homeostasis. TXNDC17 was enriched in biological processes related to the response to stimuli, such as peroxidase activity, cellular oxidant detoxification, and tumor necrosis factor-mediated processes. In addition, all of them were involved in biological regulation and response to stimulus, and thus, the two major functional processes were in the central position.

### TABLE 3 | Differentially down-regulated proteins in saliva.

| Symbol | Protein name | p-value   | q-value | AUC     |
|--------|--------------|-----------|---------|---------|
| FAM3D  | FAM3 metabolism regulating signaling molecule D | 0.0000    | 0.0034  | 0.823*  |
| ZG16B  | Zymogen granule protein 16B | 0.0000    | 0.0000  | 0.813*  |
| FCGBP  | Fc fragment of IgG binding protein | 0.0000    | 0.0000  | 0.792   |
| KT1    | Keratin 1    | 0.0006    | 0.0165  | 0.792   |
| OPE    | Carboxypeptidase E | 0.0000    | 0.0000  | 0.781   |
| PIP    | Prolactin induced protein | 0.0000    | 0.0000  | 0.771   |
| TCN1   | Transcobalamin 1 | 0.0000    | 0.00043 | 0.771   |
| CLTC   | Clathrin heavy chain | 0.0012    | 0.02292 | 0.771*  |
| TIMP1  | TIMP metallopeptidase inhibitor 1 | 0.0000    | 0.0136  | 0.75    |
| PSAP   | Prosaposin   | 0.0017    | 0.02967 | 0.75    |
| LPO    | Lactoperoxidase | 0.0000    | 0.00000 | 0.74    |
| WFD2   | WAP four-disulfide core domain 2 | 0.0003    | 0.00712 | 0.729   |
| CA6    | Carbonic anhydrase 6 | 0.0003    | 0.00795 | 0.729   |
| CLU    | Clusterin    | 0.0029    | 0.04507 | 0.729   |
| DG3    | Desmoglein 3 | 0.0001    | 0.00333 | 0.719   |
| LGALS3BP | Galectin 3 binding protein | 0.0000    | 0.00000 | 0.708   |
| BPIFB2 | BPI fold containing family B member 2 | 0.0000    | 0.00000 | 0.708   |
| NUCB1  | Nucleolin 1  | 0.0025    | 0.04052 | 0.708   |
| KLK11  | Kallikrein related peptidase 11 | 0.0010    | 0.02045 | 0.698   |
| CRNN   | Cornulin     | 0.0029    | 0.04507 | 0.698   |
| PRR27  | Proline rich 27 | 0.0000    | 0.00112 | 0.688   |
| NUCB2  | Nucleolin 2  | 0.0000    | 0.00000 | 0.677   |
| SMRSB  | Submaxillary gland androgen regulated protein 3B | 0.0000    | 0.00023 | 0.677   |
| NUCB2  | Nucleolin 2  | 0.0000    | 0.00000 | 0.677   |
| RNASET2| Ribonuclease T2 | 0.0033    | 0.04818 | 0.677   |
| MUC5B  | Mucin 5B, oligomeric mucus/gel-forming | 0.0000    | 0.00000 | 0.667   |
| CST3   | Cystatin C   | 0.0000    | 0.00000 | 0.656   |
| MvH9   | Myosin heavy chain 9 | 0.0006    | 0.01391 | 0.646   |
| TBS1   | Thrombospondin 1 | 0.0013    | 0.02292 | 0.646   |
| AC018630.2 | PRH1-PRR4 readthrough | 0.0030    | 0.04691 | 0.635   |
| PRH1   | Proline rich protein Haell subfamily 1 | 0.0030    | 0.04691 | 0.635   |

* AUC value >0.8.

The protein detected in samples with missing values were removed.
4 DISCUSSION

In this study, 24 patients with T2DM were recruited. After matching their periodontal and demographic status, the subjects were allocated to two groups according to their different control levels of blood glucose. The proteins in the saliva of individuals were detected by LC/LC–MS with DIA scanning. Two groups of differentially expressed proteins were obtained. Through ROC analysis of these proteins, TXNDC17, ZG16B, and FAM3D with high AUC values were finally selected.

In recent years, LC–MS/MS approaches that rely on the DIA method have gained more interest in proteomics because of their advantages in quantification reproducibility, specificity and accuracy, especially the quantification of low protein amounts (Barkovits et al., 2020). However, there are limited studies about DIA applied in oral science. Cecchettini A and Finamore F
explored the potential of salivary proteomics to identify primary Sjögren’s syndrome by applying LC-SWATH-MS technology (Cecchettini et al., 2019; Finamore et al., 2021). In our study, we used LC-MS/MS relying on the DIA method to test the subjects’ saliva to ensure the accuracy and coverage of protein quantification and identification. To our knowledge, this study is one of the limited studies collecting saliva to evaluate changes in the proteome of patients with different blood glucose levels by this technology. Undoubtedly, this is a challenge and supplement to apply DIA mass spectrometry technology in the dental field.

As a type of body fluid secreted by the salivary gland, saliva has vast potential for the diagnosis and prognosis of diseases for many reasons, such as its easy, safe, economical and noninvasive collection method (Castagnola et al., 2017). In our report, we found that 40 salivary proteins were expressed at significantly different levels in the P group compared to the G group. Our findings are consistent with those reported in several previous studies (Aitken et al., 2015; Abd-Elraheem et al., 2017; Shirzaiy and Dalirsani, 2019), which indicate that the composition of saliva changes under different blood glucose levels. However, a
and redox reactions, specifically involved in many biological processes, including oxidative stress.

A robust ability to distinguish the P and G groups. The AUC value was higher than any single one, which means that when combinations of biomarkers improved the predictive value compared with a single biomarker. When combinations of salivary TXNDC17/ZG16B/FAM3D levels were analyzed, the AUC value was higher than any single one, which means that the combined application of the three in saliva has the most robust ability to distinguish the P and G groups.

TXNDC17 is a novel and highly conserved disulfide reductase that can suppress NF-κB signaling that seems to be related to its inhibition of osteoclast differentiation and bone resorption. It is involved in many biological processes, including oxidative stress and redox reactions, specifically peroxidase activity and the reduction of H₂O₂. The redox balance within cells and tissues is maintained by antioxidant systems, which are vital to physiological homeostasis. When antioxidant systems are weakened or ROS production is excessive, a state called oxidative stress occurs that will cause direct damage to cells and tissues and active proinflammatory signal transduction. The pathological conditions of diabetes are associated with the generation of oxidative stress. Our results show that in the poorly controlled group, the expression level of TXNDC17 was significantly higher in saliva and that the ability of tissue to reduce ROS production was strengthened. It seems that these people encounter the breakdown of oxidative stress balance and are vulnerable to entering a stressful environment, which makes them more prone to inflammatory reactions and corresponding oral complications. Therefore, we can estimate that oxidative stress-related pathways could play a role in the regulation of salivary proteins by poorly controlled hyperglycemia, which is coincident with Masoomeh Shirzaiy, whose report proposed that hyperglycemia and some disturbance in the antioxidant system could cause damage to cell.

To date, ZG16B (zymogen granule protein 16B) is known as a secretory lectin protein that is proposed to play a regulatory role in lacrimal gland acinar cells to stimulate “flushing out” of the granule content during exocytosis. To the best of our knowledge, we observed for the first time that the expression of ZG16B was reduced in the saliva of patients with poor control of T2DM. ZG16B has been researched as a biomarker for the diagnosis and progression of many tumors, including pancreatic cancer and colon cancer, but its detailed biological function is also unknown. It is tempting to suggest that the secretory function of salivary glands under hyperglycemia will be affected, and thus, ZG16B, as a secreted protein, will inevitably be affected and its expression reduced. ZG16B showed little to no protein or transcript expression in other tissues or organs, including the mammary gland and pituitary gland. The decrease in ZG16B was observed in reflex tears detected in diabetes, and it is speculated that ZG16B may be a critical point in retinal homeostasis and ocular surface protection, which indicates that the decrease in ZG16B in saliva may indicate the condition of the retina and may even be related to the appearance of ocular lesions. However, the exact relationship of the differential expression of ZG16B in saliva and the occurrence of ocular complications remains to be further studied.

Sequence similarity three member D (FAM3D) is a member of a novel cytokine-like family, the FAM gene family, which is mainly derived from the gut. The expression level of FAM3D is affected by nutritional status, with postprandial elevation and a reduction after fasting, and the secretion timing of FAM3D is contrary to that of glucagon. Some studies found that FAM3D can induce Mac-1-mediated neutrophil recruitment and strongly chemotact human peripheral blood neutrophils and monocytes, which indicates that FAM3D may be regarded as a proinflammatory factor. The FAM3D protein in saliva may indicate the condition of the retina and may even be related to the appearance of ocular lesions. However, the exact relationship of the differential expression of FAM3D in saliva and the occurrence of ocular complications remains to be further studied.

In recent years, the changes in expression levels and expression patterns of salivary proteins under different disease states and physiological conditions have made them an essential breakthrough in studying the etiology, diagnosis and prognosis of these diseases. Changes in salivary protein in states of different blood glucose levels have become a hot spot in the study of the relationship between the oral cavity and diabetes. Alterations in the salivary proteins of T2DM patients have been associated with changes in their metabolism affected by chronic hyperglycemia. In recent years, a large number of studies have focused on the relationship between T2DM and oral conditions, but the specific mechanism remains unknown. It is possible that the salivary proteins, especially those that are downregulated in poorly controlled T2DM patients, play a role in the pathophysiological changes in the oral tissues, including the expression of FAM3D, which is consistent with previous studies.
number of studies have found many differentially expressed proteins in the state of hyperglycemia, including bone morphogenetic protein 7, lactoferrin, albumin, cathepsin D, etc. (Pappa et al., 2020; Fouani et al., 2021). In our research, we found 40 proteins differentially expressed between the two groups and finally screened out three proteins in saliva that distinguish the two groups well. These three proteins are involved in various functions. We speculate that in the hyperglycemic state, the body’s oxidative stress state, inflammatory state and hormone secretion balance are all disrupted, which causes a variety of macrovascular and microvascular complications, including retinopathy. This hypothesis can also reflect that salivary protein components can show vast aspects of the body affected by hyperglycemia. It will be of inestimable value and potential for further research on the impact of hyperglycemia on the body and oral cavity in the future.

However, there are still some limitations to consider in our study. The first limitation is the absence of subjects with normal glucose conditions. However, the conclusion still has a certain reference value as we have controlled the confounding factors. Second, due to the strict inclusion and exclusion criteria, the sample size is small while there are fewer males in the groups. Although there are no statistical differences in age and sex distribution between the two groups, the unbalanced male and female proportion may also have some impact on the findings. Third, as a cross-sectional study, the present study could not provide strong evidence for a cause-effect relationship. With these limitations, we have to make conclusions and extrapolate the findings with caution. In the future, we need more rigorously designed clinical trials with a larger sample size and healthy controls included for verification and complementation in further.

5 CONCLUSION

In conclusion, the results of this study showed that 40 proteins were differentially expressed in saliva between middle-aged and elderly people with good and poor glycemic control, indicating that, in a hyperglycemic state, oxidative stress state, gland secretion function, hormone regulation, inflammatory response and other processes are all affected. Moreover, the three proteins TXNDC17, ZG16B, and FAM3D showed the best ability to distinguish between the two different states of high and low blood glucose, and their combination could be used to further investigate the in-depth relationship between hyperglycemia and oral disease.

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DATA AVAILABILITY STATEMENT

The data presented in the study are deposited in the ProteomeXchange Consortium (http://proteomexchange.org) via the iProX partner repository (Ma et al., 2019) repository, accession number PXD029066.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Local Ethics Committee at the Peking University School and Hospital of Stomatology (PKUSSIRB-201944042). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SYJ: Writing—original draft, Experimentation, Data analysis, Data visualization. YLZ: Writing—original draft, Data analysis, Data visualization. XYS: Sample collection, Experimentation. CY: Conceptualization, Supervision, Data interpretation, Writing—review and editing, Data analysis, Data visualization. SGZ: Supervision, Data interpretation, Sample collection, Design of the study. All authors contributed to manuscript revision, read, and approved the submitted version.

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