Stomatologija. Baltic Dental and Maxillofacial Journal, 26: 68-76, 2024

Estimation of Human Islet Antigen Insulinoma-Associated-2 Autoantibody and Zinc Transporter 8 Autoantibody levels in saliva and serum in generalized periodontitis patients with and without Type 2 Diabetes mellitus – A cross-sectional study

Irene Ann Thomas[*], *Ashish S Nichani*[*], *BS Jagadish Pai*[*]

SUMMARY

Objective. To assess salivary and serum levels of Human Islet Antigen Insulinoma-Associated-2 Autoantibody (IA-2A) & Zinc Transporter 8 Autoantibody (ZnT8A) in generalized periodontitis patients with and without Type 2 Diabetes mellitus (T2DM) & healthy controls. Our hypothesis was that either/both these chronic, low-grade inflammatory conditions may influence each other and lead to altered salivary and/or serum IA-2A and ZnT8A levels.

Material and Methods. Periodontally & systemically healthy controls (G-I, n=20), generalized periodontitis without T2DM (G-II, n=20), T2DM without periodontitis (G-III, n=20) & generalized periodontitis with T2DM (G-IV, n=20) patients were enrolled. Periodontal, demographic, anthropometric & laboratory parameters were evaluated & analysed.

Results. Salivary and serum IA-2A & ZnT8A were significantly elevated in diabetic as compared to the non-diabetic patients ($p<0.001$). There was a strong & positive correlation between periodontal clinical parameters, fasting plasma glucose (FPG), glycosylated haemoglobin (HbA1c) & both salivary and serum IA-2A & ZnT8A. Regression analysis results determined both salivary and serum IA-2A and ZnT8A to be independent risk factors for periodontitis ($p<0.05$). Serum ZnT8A in T2DM patients and salivary IA-2A in non-T2DM patients were the most accurate markers to differentiate periodontitis from health.

Conclusion. Autoantibodies to IA-2 & ZnT8 may function as non-invasive markers to screen for periodontitis/or T2DM.

Keywords: Autoantibodies, Periodontitis, Insulinoma-Associated Protein 2, Type 2 Diabetes Mellitus, Zinc Transporter 8.

INTRODUCTION

Periodontal disease & Diabetes mellitus are chronic and highly prevalent. In Type 2 Diabetes Mellitus (T2DM) there is decreased insulin secretion/action or both (1). Periodontitis, a multifactorial inflammatory disease involves the tooth - supporting tissues. It is now recognized that periodontitis affects glycemic control and raises the risk of complications in T2DM. Being infection driven, periodontitis also increases cytokine levels which causes systemic inflammation and exacerbates insulin resistance (2). T2DM affects periodontitis by generating advanced glycation end products (AGEs), triggering a hyper-inflammatory response, and hindering bone healing (3).

In T2DM, an acute-phase disease of the innate immune system, the destruction of β-cells by upregulated cytokine levels especially interleukin (1L)-1β, is characteristic of low-grade, chronic inflammation. Autoimmune activation results from release of self-antigens from affected tissues caused by inflammation-induced tissue damage. Detection of islet autoantibodies ranges from 10-33% with the percentage being as high as 50% of T2DM patients & may indicate β-cell autoimmunity (4). Insulinoma-associated protein-2 antibody (IA-2A), & zinc

[*]*Department of Periodontology, The Oxford Dental College, Bangalore, India.*

Address correspondence to Ashish S Nichani, Department of Periodontology, The Oxford Dental College, Bangalore, India.
E-mail address: nichaniashish@hotmail.com

transporter-8 autoantibody (ZnT8A) are antigen-specific islet autoantibodies, associated with T2DM (5). Adults with T2DM, with one or more of these autoantibodies present, have lower body mass index (BMI), worsened glycemic control & an increased need for insulin therapy (6).

IA-2 (Insulinoma antigen-2), a transmembrane glycoprotein inherent to the protein tyrosine phosphatase (PTP) family of proteins & is expressed in the insulin granule membranes of β-cells (7). The presence of IA-2A elicits a humoral response secondary to low-grade systemic and islet inflammation & is related to progression of disease (8). Increased free-fatty acids and glucose in bloodstream triggers inflammatory signalling by stimulating NLRP3 (Nod-like receptor family pyrin domain-containing protein-3) inflammasome & activating toll-like receptors(TLRs), which increases IL-1β production. Hyperglycemia increases expression of IA-2, which makes β-cells more susceptible to IA-2A. NLRP3/ASC-caspase-1(apoptosis-associated speck-like protein that comprises caspase-recruitment domain) multiprotein complex influences innate immune response leading to endothelial dysfunction in both periodontitis and T2DM (9).

Zinc Transporter 8 (ZnT8) is a transmembrane protein and plays a role in sequestrating & transporting zinc into the secretory vesicles of β-cells (10). Changes in ZnT8 expression affect the progression of T2DM by influencing insulin synthesis & secretion. In T2DM & periodontal disease, metabolic & inflammatory stress disturbs zinc homeostasis intensifying oxidative stress and causing cell damage. Thus, detection of ZnT8A can aid in the diagnosis & prediction of both these diseases (11).

The inflammatory state in diabetes & periodontitis is responsible for increased cytokine secretion, elevated oxidative stress and upregulation of the receptor activator of nuclear factor kappa-B ligand/ Osteoprotegerin (RANKL/OPG) axis mediated by (Nuclear factor-kappa B) NF-κB. This in turn enhances bone resorption, periodontal connective tissue disintegration, and local tissue injury (12).

IA-2A and ZnT8A are important islet-autoantibodies & the degree of autoimmune reaction can differ based on age, despite the fact that both autoantigens are transmembrane proteins and their half-life is correlated. The age at which diabetes appears & its duration have a role to play not only on the autoantibody status but also on the severity of periodontal disease (13).

To the best of our understanding, there is dearth of data investigating the association between IA-2A and ZnT8A in periodontitis and T2DM. To address this gap, the primary goal of the present study was to determine whether IA-2A and ZnT8A were present in the saliva and serum of patients with generalized periodontitis and T2DM with age between 35-75 years and a BMI under 30 kg/m^2. The secondary aim was to compare & correlate the demographic, anthropometric, biochemical and periodontal clinical parameters between the study groups.

MATERIAL AND METHODS

Study design and clinical assessment

102 patients who presented to the department of periodontology between April–December 2023 were screened. 22 patients were excluded from this study due to their refusal to volunteer in the study or absence at first appointment. 80 patients aged between 35-75years were recruited once they gave written informed consent (Figure 1). The study was carried out after obtaining ethical clearance from the Institution's ethical committee (Ref.No: TODC/034/ECAL/2021-2022) issued on 20.05.2022 & in accordance with the Helsinki Declaration as amended in 2013 and adhered to the STROBE guidelines. Following data were collected: age, gender, number of teeth present, body mass index (BMI), family income & last dental check-up. Anthropometric measurements [height, weight, abdominal circumference (AC)], fasting plasma glucose (FPG) and glycosylated haemoglobin (HbA1c) were measured. Patients were grouped as: periodontally & systemically healthy controls (G-I, n=20), generalized periodontitis without Type 2 Diabetes Mellitus (T2DM) (G-II, n=20), T2DM without periodontitis (G-III, n=20) & generalized periodontitis with T2DM (G-IV, n=20).

Patients with <10% Bleeding on probing (BOP), Probing depth (PD)≤3 mm, no clinical attachment loss (CAL) & no loss of alveolar bone were included in the healthy group. Periodontitis was defined on framework for staging, grading, extent & distribution of periodontitis proposed in the 2017 World Workshop (14). Periodontitis cases had CAL≥4mm, PD≥5mm, BOP≥30% and radiographic bone loss extending from coronal 3rd of the root and beyond. Total periodontitis was defined by stages II, III and IV. Generalized periodontitis included those who had≥20 teeth (excluding 3rd molars) and those who didn't receive periodontal therapy in previous six months. T2DM was defined according to American Diabetes Association Classification (15). FPG and HbA1c values of previously diagnosed diabetic patients (FPG≥126mg/dl, HbA1c≥6.5%) validated their diabetic status. BMI (<30 kg/m^2) was calculated based on the weight/square of height. Exclusion criteria were: alcoholics, smokers, systemic diseases, previous history of T1DM,

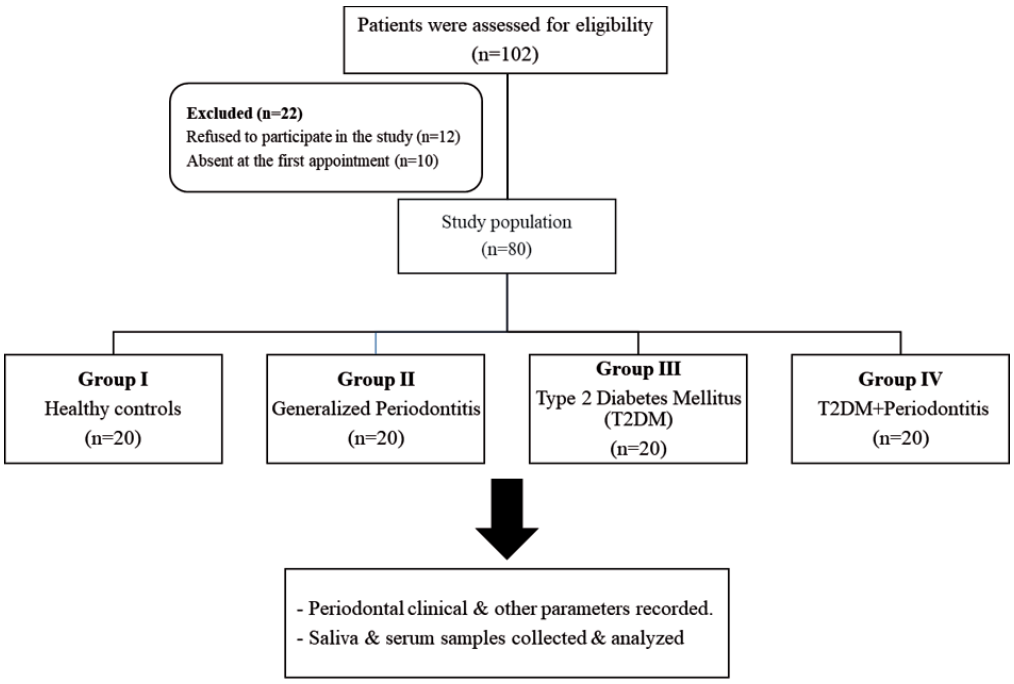

Fig. 1. Workflow of the study

post-menopausal, lactating, and pregnant women or those on oral contraceptives. Patients who consumed antibiotics, anti-epileptic, or anti-inflammatory drugs during the past six months were excluded.

Evaluation of periodontal clinical parameters

A single, calibrated examiner evaluated the periodontal status with a William's graduated periodontal probe (Hu-Friedy, Chicago, USA). Plaque index (PI), BOP (%), PD (mm), and CAL (mm) were measured at six sites per tooth. Orthopantomogram (OPG) was used for radiographic assessment of the alveolar bone level. Intra-examiner variability for PD and CAL, was 0.829 and 0.822, respectively. Intra-examiner agreement for BOP was>0.85, when analysed dichotomously and using the κ-light test.

Saliva and serum sample collection

Following an overnight fast, saliva was collected between 8-10 am into a 10 ml disposable, sterile container. No oral stimuli was allowed for 120mins prior to saliva collection. Antecubital vein was used to draw 10 ml of venous blood, allowed to stand for 30 mins and centrifuged. Samples were collected prior to assessment of periodontal clini-

Table 1. List of materials used and their characteristics

Patient characteristics/ biomarkers levels	Group I Healthy controls (n=20)	Group II Generalized periodontitis (n=20)	Group III T2DM (n=20)	Group IV Generalized periodontitis + T2DM (n=20)	GIII/ GI p-Value	GIII/ GII p-Value	GIV/GI p-Value	GIV/ GII p-Value
Age (years)	40.45±6.75 [b c d]	46.25±7.28 [a]	49.50±10.45 [a]	49.35±8.16 [a]	<0.002*	<0.606	<0.001*	<0.290
AC (inch)	38.35±6.00	34.70±3.69 [c d]	38.50±4.20 [b]	39.00±4.51 [b]	0.734	0.011*	0.989	0.003*
BMI (kg/m²)	21.76±1.56	22.35±1.94	22.30±2.70	23.35±3.29	NS	NS	NS	NS
FPG (mg/dl)	99.90±2.53 [c d]	99.05±1.43 [c d]	159.6±7.45 [a b d]	203.7±21.96 [a b c]	<0.001*	<0.001*	<0.001*	<0.001*
HbA1c (%)	5.33±0.20 [c d]	5.75±0.24 [c d]	6.78±0.19 [a b]	7.29±0.44 [a b]	<0.001*	<0.001*	<0.001*	<0.001*
PI	0.58±0.22 [b c d]	2.08±0.36 [a c]	1.55±0.16 [a b d]	2.28±0.30 [a c]	<0.001*	<0.001*	<0.001*	0.094
BOP (%)	1.80±1.04 [b d]	13.85±1.03 [a c]	2.59±1.84 [b d]	18.94±9.92 [a c]	0.276	<0.001*	<0.001*	0.807
PD (mm)	1.51±0.06 [b c d]	5.70±0.22 [a c d]	1.79±0.28 [a b d]	6.26±0.55 [a b c]	<0.001*	<0.001*	<0.001*	<0.001*
CAL (mm)	1.91±0.19 [b d]	6.51±0.37 [a c d]	2.09 ±0.26 [b d]	6.97±0.74 [a b c]	0.116	<0.001	<0.001*	<0.001*
Salivary IA-2A (pg/ml)	59.96±23.41 [b c d]	110.68±18.28 [a c d]	132.57±20.86 [a b d]	183.71±37.16 [a b c]	<0.001*	0.002*	<0.001*	<0.001*
Serum IA-2A (pg/ml)	22.18±9.47 [b c d]	41.24±4.72 [a c d]	58.56±6.45 [a b d]	128.97±72.19 [a b c]	<0.001*	<0.001*	<0.001*	<0.001*
Salivary ZnT8A (ng/ml)	1318.93±478.00 [b c d]	2294.15±257.92 [a c d]	2916.67±180.30 [a b d]	3270.38±513.39 [a b c]	<0.001*	<0.001*	<0.001*	<0.001*
Serum ZnT8A (ng/ml)	1024.00±162.07 [b c d]	1435.82±118.08 [a d]	1827.44±148.99 [a d]	3147.43±1045.85 [a b c]	<0.001*	<0.001*	<0.001*	<0.001*

*p<0.05 denotes statistical significance. ANOVA test with a Bonferroni correction: [a] significant difference in comparison with G-I; [b] significant difference from G-II; [c] significant difference from G-III; [d] significant difference from G-IV; BMI – body mass index; AC – abdominal circumference; FPG – fasting plasma glucose; HbA1c – glycated haemoglobin; PI – plaque index; BOP – bleeding on probing; PD – probing depth; CAL – clinical attachment loss.

cal parameters, stored at -80°C and analysed by a technician who was blinded to the study.

Assessment of biochemical parameters

Salivary and serum Human Islet Antigen Insulinoma-Associated-2 Autoantibody (IA-2A) and Zinc Transporter 8 Autoantibody (ZnT8A) concentrations were assessed using GENLISA™ ELISA kits (Krishgen Biosystems™, USA) as per instructions from the manufacturer. A microplate reader was used to measure colour change at 450nm. Respective assay standard curves were utilised to determine the concentrations. FPG levels were analysed using an automated enzymatic method. HbA1c values were measured by high performance liquid chromatography.

Statistical analyses

The primary outcome variables were IA-2A and ZnT8A concentrations in all study groups. The sample size was estimated using the GPower software v. 3.1.9.2. It was computed taking into consideration the effect size to be measured at 40, power at 80% and the α-error at 5%. A number of 76, which was rounded off to 80 was deemed fit. Each study group comprised of 20 patients [20 patients x 4 groups =80 patients]. All data were checked for errors, entered in Microsoft excel, and analysed using SPSS (version 20). Descriptive data were presented as mean ± SD and categorical estimations as (n) and percent (%). Kolmogorov-Smirnov test was used to check the normality of the variables. Non-parametric tests were used to analyse the data since all variables did not follow a normal distribution. The Kruskal-Wallis Chi square test was employed to evaluate the differences between all the numerical variables in the four analysed groups, and Mann Whitney test with Bonferroni correction was used to make post-hoc pairwise comparisons. Pearson Chi-square test was used to check the association of gender with the groups. Spearman's rank correlation was utilized to check the correlation between the independent variables. Receiver operating characteristic curve (ROC) and corresponding area under the curve (AUC) analyses for salivary & serum biomarkers were performed for differentiation of periodontitis from healthy patients. Logistic regression analysis was performed to examine the relationship between IA-2A and ZnT8A and various potential predictors using a stepwise method. A p-value less than 0.05 was considered *to be statistically significant.*

RESULTS

All 80 patients completed the study. Demographic, anthropometric, laboratory and periodontal clinical parameters are summarized in Table 1. Human Islet Antigen Insulinoma-Associated-2 Autoantibody (IA-2A) and Zinc Transporter 8 Autoantibody (ZnT8A) were observed in all salivary and serum samples. Abdominal circumference (AC), body mass index (BMI), periodontal parameters, Fasting plasma glucose (FPG), Glycosylated haemoglobin (HbA1c), salivary & serum concentrations of IA-2A and ZnT8A and were greatest in Group 4. The values were least in Group 1 when compared to other groups. Salivary & serum IA-2A & ZnT8A; FPG & Hba1c were significantly higher in diabetic as compared to the non-diabetic groups ($p<0.05$). There was no significant difference between gender, BMI & no. of teeth present between the study groups ($p>0.05$). Higher mean age was recorded in Group III followed by Groups IV, II and I respectively. The difference in mean age amongst the groups was found to be statistically significant ($p<0.05$).

Correlation analysis between all parameters in the groups is presented in Table 2. There was significant correlation between age & periodontal parameters (except for CAL, r=0.142; $p>0.05$), FPG & Hba1c in all groups ($p<0.05$). Salivary IA-2A & serum ZnT8A were found to have very strong positive relationship with probing depth (PD) (r=0.753, 0.705 respectively). All other periodontal parameters had a strong positive relationship with both IA-2A & ZnT8A ($p=<0.001$). Serum IA-2A, salivary ZnT8A, serum ZnT8A were found to have very strong positive correlation with HbA1c (r=0.773, 0.788, 0.769 respectively), Salivary IA-2A, serum IA-2A, salivary ZnT8A, serum ZnT8A were found to have very strong positive correlation with FPG (r=0.730, 0.833, 0.824, 0.826 respectively), serum IA-2A (r=0.867, 0.929, 0.931), salivary ZnT8A (r=0.879, 0.929, 0.923 respectively) and serum ZnT8A (r=0.888, 0.931, 0.923 respectively) which was statistically highly significant ($p=<0.001$).

Results of the stepwise logistic regression analysis presented in Table 3 indicate that both salivary & serum IA-2A and ZnT8A were statistically significant independent predictors of periodontitis ($p<0.05$). Screening efficacy of IA-2A and ZnT8A & their receiver-operating characteristics (ROC) curve analyses in periodontitis are presented in Table 4 & Figure 2. In DM, the most accurate biomarker for distinguishing patients with periodontitis from controls was serum ZnT8A (SN= 1.00, SP= 1.00, cut-off value = 2132.70 ng/mL; AUC = 1.000) whereas in non-DM patients, salivary IA-2A was the most accurate biomarker (SN= 1.00, SP= 1.00; cut-off value 88.62 pg/mL; AUC =1.000).

Table 2. Correlation of demographic, anthropometric, systemic health, periodontal clinical parameters and biochemical markers using Spearman's correlation

Correlations		Age	BMI	AC	PI	BOP	PD	CAL	HbA1C	FPG	Salivary IA-2A	Serum IA-2A	Salivary ZnT8A	Serum ZnT8A
Age (years)	r	1	0.009	-0.039	0.329	0.231	0.263	0.142	0.346	0.317	0.332	0.366	0.360	0.374
	p –value	---	0.939	0.728	0.003*	0.039*	0.018*	0.210	0.002*	0.004*	0.003*	0.001*	0.001*	0.001*
BMI (kg/m^2)	r	0.009	1	0.306	0.207	0.113	0.087	0.156	0.036	0.120	0.086	0.194	0.093	0.137
	p –value	0.939	---	0.006*	0.065	0.318	0.443	0.168	0.749	0.288	0.446	0.085	0.414	0.226
AC (inch)	r	-0.039	0.306	1	-0.068	-0.12	-0.008	-0.058	0.226	0.235	0.138	0.117	0.072	0.124
	p-value	0.728	0.006*	---	0.550	0.308	0.941	0.610	0.044*	0.036*	0.223	0.302	0.527	0.272
PI	r	0.329	0.207	-0.068	1	0.725	0.781	0.774	0.324	0.441	0.649	0.619	0.634	0.621
	p –value	0.003*	0.065	0.550	---	<0.001*	<0.001*	<0.001*	0.003*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
BOP (%)	r	0.231	0.113	-0.115	0.725	1	0.768	0.746	0.052	0.165	0.479	0.408	0.421	0.432
	p –value	0.039*	0.318	0.308	<0.001*	---	<0.001*	<0.001*	0.645	0.144	<0.001*	<0.001*	<0.001*	<0.001*
PD (mm)	r	0.263	0.087	-0.008	0.781	0.768	1	0.891	0.327	0.436	0.753	0.668	0.682	0.705
	p –value	0.018*	0.443	0.941	<0.001*	<0.001*	---	<0.001*	0.003*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
CAL (mm)	r	0.142	0.156	-0.058	0.774	0.746	0.891	1	0.216	0.348	0.572	0.565	0.562	0.581
	p –value	0.210	0.168	0.610	<0.001*	<0.001*	<0.001*	---	0.054	0.002*	<0.001*	<0.001*	<0.001*	<0.001*
HbA1C (%)	r	0.346	0.036	0.226	0.324	0.052	0.327	0.216	1	0.871	0.660	0.773	0.788	0.769
	p –value	0.002*	0.749	0.044*	0.003*	0.645	0.003*	0.054	---	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
FPG (mg/dL)	r	0.317	0.120	0.235	0.441	0.165	0.436	0.348	0.871	1	0.730	0.833	0.824	0.826
	p –value	0.004*	0.288	0.036*	<0.001*	0.144	<0.001*	0.002*	<0.001*	---	<0.001*	<0.001*	<0.001*	<0.001*
Salivary IA-2A (pg/ml)	r	0.332	0.086	0.138	0.649	0.479	0.753	0.572	0.660	0.730	1	0.867	0.879	0.888
	p –value	0.003*	0.446	0.223	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	---	<0.001*	<0.001*	<0.001*
Serum IA-2A (pg/ml)	r	0.366	0.194	0.117	0.619	0.408	0.668	0.565	0.773	0.833	0.867	1	0.929	0.931
	p –value	0.001*	0.085	0.302	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	---	<0.001*	<0.001*
Salivary ZnT8A (ng/ml)	r	0.360	0.093	0.072	0.634	0.421	0.682	0.562	0.788	0.824	0.879	0.929	1	0.923
	p –value	0.001*	0.414	0.527	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	---	<0.001*
Serum ZnT8A (ng/ml)	r	0.374	0.137	0.124	0.621	0.432	0.705	0.581	0.769	0.826	0.888	0.931	0.923	1
	p –value	0.001*	0.226	0.272	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	---

*p<0.05 denotes statistical significance. R – Correlation coefficient; PI – Plaque index; BOP – Bleeding on probing; PD – Probing depth; CAL – Clinical attachment loss; BMI – Body mass index; AC – Abdominal circumference; FPG – Fasting plasma glucose; HbA1c – Glycated hemoglobin; IA-2A – Human Islet Antigen Insulinoma Associated-2 Autoantibody; ZnT8A – Zinc transporter 8 autoantibody.

DISCUSSION

To the best of our knowledge the present study reports for the first time, the detection of Human Islet Antigen Insulinoma-Associated-2 Autoantibody (IA-2A) and Zinc Transporter-8 Autoantibody (ZnT8A) in periodontitis. Moreover, this study demonstrates the presence of autoimmunity in an infrequently documented cohort i.e. non-obese patients with Type 2 Diabetes Mellitus (T2DM). This was a cross sectional study with 80 individuals (28 females & 52 males) split equally into four groups. Salivary and serum levels of IA-2A & ZnT8A in individuals with periodontitis, with and without T2DM and healthy controls were assessed to analyse correlations between periodontal clinical & other parameters.

The present study demonstrated that patients with T2DM with generalised periodontitis & T2DM alone had higher concentrations of IA-2A and ZnT8A in saliva and serum compared with periodontitis patients & healthy controls. Results also suggested that in the samples analysed, diabetes might have contributed to the high levels of serum & salivary IA-2A and ZnT8A, lending credence to our hypothesis

that T2DM positively influenced IA-2A and ZnT8A levels. Results also show that Plaque index (PI) & Bleeding on probing (BOP), clinically associated with periodontal inflammation & Probing depth (PD) & Clinical attachment loss (CAL), associated with periodontal tissue destruction, were strongly & positively correlated with salivary and serum IA-2A & ZnT8A. The results derived from the stepwise logistic regression analysis indicated that both salivary and serum IA-2A & ZnT8A were statistically significant independent determinants of periodontitis. More specifically, receiver-operating characteristics (ROC) curve analysis indicated, serum ZnT8A in T2DM and salivary IA-2A in non-T2DM were the most accurate to distinguish between periodontitis & periodontal health. These unique results bring to the fore novel prospects for the non-invasive screening of generalised periodontitis regardless of the systemic health.

Diabetes and periodontitis affect each other. Pancreatic autoantibodies are present in approximately 36% of the phenotypic T2DM patients and intensive anti-hyperglycemic therapy has been associated with islet autoantibody positivity in these patients. The effects of islet autoimmunity are also seen in overweight & obese patients. IA-2 is a dominant autoantigen that appears later & participates in the autoimmune attack on the β cells in T2DM. IA-2A detection may indicate also rapidly progressing disease. β-cells express high levels of ZnT8 where it is responsible for zinc transport, insulin maturation and storage. Zinc deficiency accentuates oxidative stress which is also responsible for several complications seen in T2DM (16).

In the present study, salivary and serum IA-2A & ZnT8A levels were significantly elevated in diabetic as compared to the non-diabetic groups. Our findings support previous studies wherein the prevalence of IA-2A ranged from 2-12.5% & ZnT8A from 0.2–10.7% in T2DM cases & was significantly higher than found in non-diabetic individuals (10, 17). The Latent Autoimmune Diabetes in Adults (LADA) study reported that IA-2A was found in 26.1% of elderly LADA patients (18).

PI, BOP, PD & CAL were strongly and positively correlated with salivary and serum IA-2A and ZnT8A which were statistically significant. Both FPG & HbA1c were positively correlated with the periodontal parameters which was statistically significant. Our results are consistent with previous research. Mealey *et al.* (19) reported that periodontitis risk is elevated threefold in individuals with diabetes as compared with non-diabetics. An increased risk is dependent on glycemic control. The US National Health and Nutrition Examination Survey (NHANES) III, reported that after controlling for confounding factors, an HbA1c level of >9% resulted in a significant increase in periodontitis prevalence than in those adults without diabetes (20). Demmer and colleagues (21) reported increased HbA1c levels in patients with severe periodontal disease at baseline. It is well known that T2DM and periodontitis are both chronic, low-grade

Table 3. Stepwise logistic regression analysis

Parameter	Constant	B	SE (β)	p-value	OR	95% CI for OR	
						Lower Bound	Upper Bound
Age	1.639	0.034	0.026	0.186	1.035	0.984	1.089
BMI	-2.812	0.123	0.094	0.192	1.131	0.940	1.361
AC	1.940	-0.053	0.047	0.256	0.948	0.866	1.039
Diabetes	-0.100	0.100	0.447	0.823	1.105	0.460	2.657
IA-2A Saliva	-3.883	0.032	0.008	<0.001*	1.032	1.017	1.048
IA-2A Serum	-2.267	0.041	0.012	0.001*	1.042	1.017	1.067
ZnT8A Saliva	-3.341	0.001	0.000	<0.001*	1.001	1.001	1.002
ZnT8A Serum	-3.235	0.002	0.001	0.001*	1.002	1.001	1.003

*Values with p<0.05 have been determined as independent risk factors for peridontitis. CI – Confidence interval; OR – Odds ratio; SE – Standard error; BMI – Body mass index; AC – abdominal circumference; IA-2A – Human Islet Antigen Insulinoma Associated-2 Autoantibody; ZnT8A – Zinc transporter 8 autoantibody.

Table 4. Screening efficacy of IA-2A and ZnT8A markers in periodontitis

Type 2 Diabetes mellitus (+)								Type 2 Diabetes mellitus (-)						
Parameter	AUC	p-value	95% CI for AUC		Cut-off	SN	SP	AUC	p-value	95% CI for AUC		Cut-off	SN	SP
			Lower Bound	Upper Bound						Lower Bound	Upper Bound			
Salivary IA-2A	0.952	<0.001*	0.892	1.000	141.60	1.00	0.65	1.000	<0.001*	1.000	1.000	88.62	1.00	1.00
Serum IA-2A	0.989	<0.001*	0.964	1.000	63.96	1.00	0.75	0.956	<0.001*	0.890	1.000	34.38	1.00	0.90
Salivary ZnT8A	0.999	<0.001*	0.994	1.000	3196.05	1.00	0.95	0.972	<0.001*	0.918	1.000	1890.50	1.00	0.95
Serum ZnT8A	1.000	<0.001*	1.000	1.000	2132.70	1.00	1.00	0.955	<0.001*	0.883	1.000	1147.40	1.00	0.76

AUC – Area under curve; CI – Confidence interval; SN – Sensitivity; SP – Specificity; IA-2A – Human Islet Antigen Insulinoma Associated-2 autoantibody; ZnT8A – Zinc transporter 8 autoantibody.

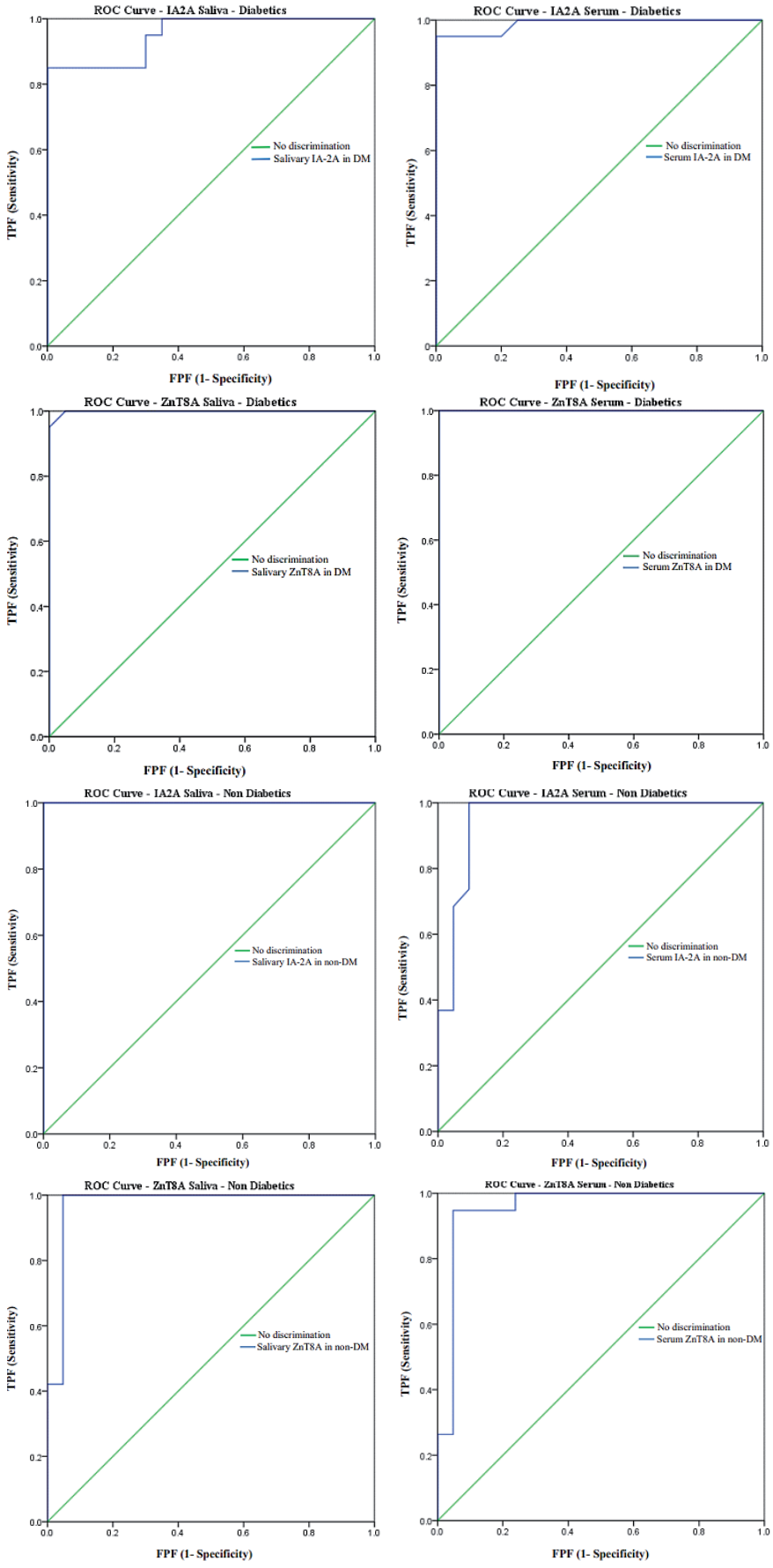

Fig. 2. Receiver-operating characteristic (ROC) curves of salivary and serum markers assessed for periodontitis screening. AUC- area under the curve; IA-2A- Human Islet Antigen Insulinoma Associated-2 Autoantibody; ZnT8A- Zinc Transporter 8 Autoantibody; TPF- True positive fraction; FPF- False positive fraction; DM- diabetes mellitus.

inflammatory diseases. Periodontitis is associated with an altered glucose metabolism & increased insulin resistance in T2DM, as well as increased risk of complications, including mortality (20).

There was a very strong & positive correlation between salivary & serum IA-2A & ZnT8A with HbA1c & FPG which was statistically significant. Our results are consistent with previous studies which reported that IA-2A & ZnT8A positive patients demonstrated higher FPG & HbA1c levels in T2DM (18, 22, 23). Results of this study report worsening glycaemic control associated with increased IA-2A & ZnT8A levels. Glycaemic control is the major objective in the treatment of T2DM. Islet-autoantibody positivity is related to lower β-cell function and insulin resistance. In T2DM, islet-autoantibodies are linked to variations in physiology and hyperglycaemia is driven more by a natural lack of insulin. However, Pilla *et al.* (6) reported HbA1c levels did not vary by IA-2A & ZnT8A status. Kibirige *et al.* (24) reported no significant differences in FPG & HbA1c between those with and without IA-2A & ZnT8A.

In the present study, age had a moderate to weak positive relationship with PI, BOP & PD which was statistically significant. There was a negligible relationship with CAL. There was a moderate positive relationship between age & IA-2A and ZnT8A which was statistically significant. Our results are in line with previous studies. Yohena *et al.* (22) reported a higher frequency of autoimmunity in older diabetic patients. Mehranpoor *et al.* (17) reported that mean age of T2DM patients was significantly higher in IA-2A positive patients. However, Trabucchi *et al.* (10) reported that clinical phenotype analysed according to presence of at least one marker showed association with

younger age. Other authors have reported IA-2A and ZnT8A showed a trend towards younger age (25, 26). Genovese *et al.* (23) reported no differences in age/duration of disease with respect to IA-2A. Borg *et al.* (27) reported IA-2A was positive in same frequency in adults and children. Similarly few authors have reported no significant differences between age and autoantibody-positivity to both IA-2A & ZnT8A (24, 28).

In the present study, the patients were of normal weight or slightly overweight. Body mass index (BMI) had a weak positive relationship with PI. Abdominal circumference (AC) and BMI had a no or negligible relationship with neither IA-2A & ZnT8A nor other periodontal parameters which was not statistically significant. These results are in line with previous studies that reported no significant differences in BMI and IA-2A & ZnT8A positivity (10, 16, 22, 24). However, Mehranpoor *et al.* (17) has stated that IA-2A positive patients had lower mean BMI & AC. Lampasona *et al.* (25) reported ZnT8A positive patients had lower BMI. Maddaloni *et al.* (29) reported IA-2A increased in frequency with BMI in T2DM.

There were no significant differences in mean values of periodontal parameters and IA-2A & ZnT8A levels between males and females. Our findings are consistent with earlier studies (26, 28). However, few authors have reported that among patients positive for autoantibodies, there were significantly more females (17, 22, 23) whereas few studies have reported men had higher prevalence of autoantibodies. This can be related to demographic characteristics and genetic diversity of populations (11, 27).

All diabetic patients in this study were on oral hypoglycemic drugs. In older T2DM patients, especially those on insulin, the appearance of islet autoantibodies is not uncommon & is 5–10% or higher. Previous studies have reported tendency for IA-2A & ZnT8A to disappear post-diabetes diagnosis (16, 30). In this study though diabetes duration ranged from 2-10 years, IA-2A & ZnT8A were detected in all samples. Levels of serum IA-2A & ZnT8A were increased and salivary IA-2A & ZnT8A were decreased in patients with long-standing diabetes.

The strength of the present study is in its capacity to look for possible correlations among a wide range of parameters. However it must be interpreted with some limitations. This study only represents current state of diabetes-associated antibodies due to its cross-sectional design. A bias in patient selection may have resulted from the study design's inability to perform randomization in patient selection process. Other study limitations include a relatively small sample size and a lack of information on potential confounders like the oral microbiome.

CONCLUSION

The present study for the first time reports on the role of Human Islet Antigen Insulinoma-Associated-2 Autoantibody (IA-2A) & Zinc Transporter 8 Autoantibody (ZnT8A) in periodontal disease. Disease-associated autoantigens when identified, play a dual role: their antibodies can act as predictive markers and the disease process might be halted/subdued by modulation of the immune response. Periodontists should contemplate the possibility of diabetes and impaired fasting glucose when examining patients even with normal body weight and no abdominal obesity. However, more multicentered, prospective, longitudinal and interventional studies involving a larger population should be carried out to validate the findings of the present study and to better understand the role of IA-2A & ZnT8A in the pathogenesis of both periodontal disease & T2DM.

Conflict of interest

The authors have made it clear they have no conflicts of interest.

Funding information: The authors received no financial support for the research.

REFERENCES

1. Bascones-Martinez A, Munoz-Corcuera M, Bascones-Ilundain J. Diabetes and periodontitis: a bidirectional relationship. Medicina Clinica 485;145:31-5.
2. Graziani F, Gennai S, Solini A, Petrini M. A systematic review and meta-analysis of epidemiologic observational evidence on the effect of periodontitis on diabetes an update of the EFP-AAP review. J Clin Periodontol 2018;45:167-87.
3. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. Lancet 2005;365:1333-46.
4. Brooks-Worrell B, Ismail H, Wotring M, Kimmie C, Felton J, Palmer JP. Autoimmune development in phenotypic type 2 diabetes patients. Clin Immunol 2007;123:66–67.
5. Brooks-Worrell B, Palmer JP. Immunology in the Clinic Review Series; focus on metabolic diseases: development of islet autoimmune disease in type 2 diabetes patients: potential sequelae of chronic inflammation. Clin Exp Immunol 2012;167:40-6.
6. Pilla SJ, Balasubramanyam A, Knowler WC, Lazo M, Nathan DM, Pi-Sunyer X, et al. Islet autoantibody positiv-

ity in overweight and obese adults with type 2 diabetes. Autoimmunity 2018;51:408-16.
7. Kawasaki E. Anti-Islet Autoantibodies in Type 1 Diabetes. Int J Mol Sci 2023;24:10012.
8. Buzzetti R, Spoletini M, Zampetti S, Campagna G, Marandola L, Panimolle F, et al. Tyrosine phosphatase-related islet antigen-2(256-760) autoantibodies, the only marker of islet autoimmunity that increases by increasing the degree of BMI in obese subjects with type 2 diabetes. Diabetes Care 2015;38:513-20.
9. Itariu BK, Stulnig TM. Autoimmune aspects of type 2 diabetes mellitus - a mini-review. Gerontology 2014; 60:189-96.
10. Trabucchi A, Faccinetti NI, Guerra LL, Puchulu FM, Frechtel GD, Poskus E, et al. Detection and characterization of ZnT8 autoantibodies could help to screen latent autoimmune diabetes in adult-onset patients with type 2 phenotype. Autoimmunity 2012;45:137-42.
11. Huang G, Xiang Y, Pan L, Li X, Luo S, Zhou Z. Zinc transporter 8 autoantibody (ZnT8A) could help differentiate latent autoimmune diabetes in adults (LADA) from phenotypic type 2 diabetes mellitus. Diabetes Metab Res Rev 2013;29:363-8.
12. Ateeq H, Zia A, Husain Q, Khan MS, Ahmad M. Effect of inflammation on bones in diabetic patients with periodontitis via RANKL/OPG system-A review. J Diabetes Metab Disord 2022;21:1003-9.
13. Kawasaki E, Oikawa Y, Okada A, Kanatsuna N, Kawamura T, Kikuchi T, et al. Different interaction of onset age and duration of type 1 diabetes on the dynamics of autoantibodies to insulinoma-associated antigen-2 and zinc transporter 8. J Diabetes Investig 2021;12:510-5.
14. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. J Periodontol 2018;89:159-72.
15. American Diabetes Association. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2019. Diabetes Care 2019;42:13-28.
16. Brooks-Worrell BM, Reichow JL, Goel A, Ismail H, Palmer JP. Identification of autoantibody-negative autoimmune type 2 diabetic patients. Diabetes Care 2011;34:168-73.
17. Mehranpoor M, Mohammadi M, Lagzian M. Prevalence and Metabolic Characteristics of Patients with Latent Autoimmune Diabetes in Adults: A Cross-Sectional Study. Clinical Diabetology 2023;12:362-9.
18. Niu X, Luo S, Li X, Xie Z, Xiang Y, Huang G, et al. Identification of a distinct phenotype of elderly latent autoimmune diabetes in adults: LADA China Study 8. Diabetes Metab Res Rev 2019;35:e3068.
19. Mealey BL, Rose LF. Diabetes mellitus and inflammatory periodontal diseases. Curr Opin Endocrinol Diabetes Obes 2008;15:135–41.
20. Preshaw PM, Bissett SM. Periodontitis. Oral Complication of Diabetes. Endocrinol Metab Clin North Am 2013;42:849–67.
21. Demmer RT, Desvarieux M, Holtfreter B, Jacobs Jr DR, Wallaschofski H, Nauck M, Völzke H, Kocher T. Periodontal status and A1C change: longitudinal results from the study of health in Pomerania (SHIP). Diabetes care 2010; 33:1037-43.
22. Yohena S, Penas-Steinhardt A, Muller C, Faccinetti NI, Cerrone GE, Lovecchio S, et al. Immunological and clinical characteristics of latent autoimmune diabetes in the elderly. Diabetes Metab Res Rev 2019;35:e3137.
23. Genovese S, Bazzigaluppi E, Gonçalves D, Ciucci A, Cavallo MG, et al.; Clinical phenotype and β-cell autoimmunity in Italian patients with adult-onset diabetes. Eur J Endocrinol 2006;154:441–7.
24. Kibirige D, Sekitoleko I, Balungi P, Kyosiimire-Lugemwa J, Lumu W, Jones AG, et al. Islet autoantibody positivity in an adult population with recently diagnosed diabetes in Uganda. PLoS One 2022;17:1-15.
25. Lampasona V, Petrone A, Tiberti C, Capizzi M, Spoletini M, di Pietro S, et al. Zinc transporter 8 antibodies complement GAD and IA-2 antibodies in the identification and characterization of adult-onset autoimmune diabetes: Non-Insulin Requiring Autoimmune Diabetes(NIRAD)4. Diabetes Care 2010;33:104-8.
26. Kawasaki E, Oikawa Y, Okada A, Kanatsuna N, Kawamura T, Kikuchi T, et al. Zinc transporter 8 autoantibodies complement glutamic acid decarboxylase and insulinoma-associated antigen-2 autoantibodies in the identification and characterization of Japanese type 1 diabetes. J Diabetes Investig 2020;11:1181-87.
27. Borg H, Gottsäter A, Fernlund P, Sundkvist G. A 12-year prospective study of the relationship between islet antibodies and beta-cell function at and after the diagnosis in patients with adult-onset diabetes. Diabetes 2002;51:1754-62.
28. Damanhouri LH, Dromey JA, Christie MR, Nasrat HA, Ardawi MS, Robins RA, et al. Autoantibodies to GAD and IA-2 in Saudi Arabian diabetic patients. Diabet Med 2005;22:448-52.
29. Maddaloni E, Lessan N, Al Tikriti A, Buzzetti R, Pozzilli P, Barakat MT. Latent Autoimmune Diabetes in Adults in the United Arab Emirates: Clinical Features and Factors Related to Insulin-Requirement. PLoS One 2015;10:e0131837.
30. Barinas-Mitchell E, Pietropaolo S, Zhang YJ, Henderson T, Trucco M, Kuller LH, et al. Islet cell autoimmunity in a triethnic adult population of the Third National Health and Nutrition Examination Survey. Diabetes 2004;53:1293–1302.

Received: 02 01 2024
Accepted for publishing: 23 09 2024