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Salivary β1-Adrenergic Receptor-Related Immunoreactivity in Pediatric Type 1 Diabetes Mellitus

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ABSTRACT

Introduction: Type 1 diabetes mellitus is associated with several oral complications, including salivary gland dysfunction and alterations in salivary composition. Adrenergic pathways are involved in the regulation of salivary secretion; however, salivary β1-adrenergic receptor-related immunoreactivity has not been well investigated in children with type 1 diabetes mellitus. This exploratory case-control study aimed to compare salivary β1-adrenergic receptor immunoreactivity between children with type 1 diabetes mellitus and systemically healthy controls.

Materials and Methods: This observational case-control study included 66 children aged 7-13 years, comprising 33 children with type 1 diabetes mellitus and 33 systemically healthy controls within the same age range. Children with type 1 diabetes mellitus were clinically stable at enrollment and had acceptable metabolic control based on screening records and pediatric endocrinology evaluation. These glycemic criteria were used only for eligibility screening and were not included as analytic variables. Unstimulated whole saliva was collected using the spitting method after a minimum fasting period of 2 hours. Salivary β1-adrenergic receptor immunoreactivity was measured using a sandwich enzyme-linked immunosorbent assay. Data normality was assessed using the Shapiro-Wilk test, and the between-group comparison was performed using the Mann-Whitney U test.

Results: Salivary β1-adrenergic receptor immunoreactivity was significantly higher in children with type 1 diabetes mellitus than in healthy controls. The median salivary β1-adrenergic receptor immunoreactivity was 81.56 ng/L (IQR: 62.12-92.31) in the type 1 diabetes mellitus group and 36.43 ng/L (IQR: 27.56-45.92) in the control group. The between-group difference was statistically significant (Mann-Whitney U = 137.0, $p = 1.79 \times 10^{-7}$). The range of values was wider in the type 1 diabetes mellitus group (19.96-615.28 ng/L) compared with controls (4.67-188.29 ng/L).

Conclusion: Children with type 1 diabetes mellitus showed higher salivary β1-adrenergic receptor-related immunoreactivity than systemically healthy controls. This finding should be interpreted as preliminary evidence of altered salivary biomarker profiles rather than direct evidence of increased adrenergic receptor expression, receptor function, or sympathetic nervous system activity. Further studies with larger samples, community-based or matched controls, salivary flow rate measurement, oral health indices, and detailed diabetes-related variables are required to clarify the biological source and clinical relevance of this finding.

Keywords: Type 1 diabetes mellitus; Pediatric diabetes; Saliva; Salivary biomarkers; β1-adrenergic receptor; Immunoreactivity; ELISA.

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Introduction

Type 1 Diabetes Mellitus (T1DM) is a chronic autoimmune disorder characterized by autoimmune destruction of pancreatic β-cells, resulting in absolute insulin deficiency and persistent hyperglycemia, with onset commonly in childhood or adolescence [1,2]. The disease manifests a spectrum of microvascular and macrovascular complications and oral health alterations are increasingly recognized as relevant complications that may be influenced by metabolic, inflammatory, salivary, and behavioral factors [3-5]. These manifestations include periodontal disease, salivary gland dysfunction, xerostomia, increased susceptibility to oral infections, alterations in taste perception, and delayed healing of oral mucosal wounds. The oral cavity is particularly affected because of its rich vascular and neural supply and its continuous exposure to mechanical and microbial challenges [6,7].

Saliva has a fundamental role in maintaining oral health by providing lubrication, antimicrobial activity, buffering capacity, and support for tissue repair [8,9]. Several studies have reported alterations in salivary flow rate and salivary composition in patients with diabetes mellitus [10]. Reduced unstimulated salivary flow and increased salivary protein concentration are among the most frequently reported findings. These changes may contribute to the development of oral complications such as dental caries, mucosal lesions, and periodontal disease. Similar salivary alterations have been observed in both adult and pediatric patients with diabetes [8,11]. Salivary secretion is mainly regulated by the autonomic nervous system through the coordinated activity of parasympathetic and sympathetic pathways [12].

Parasympathetic stimulation typically results in high-volume saliva with low protein content, whereas sympathetic stimulation produces saliva with lower flow rate and higher protein concentration. Adrenergic receptor pathways are part of the physiological background of salivary secretion and may influence protein-rich salivary output; however, salivary biomarker measurements do not necessarily reflect receptor function or tissue-level signaling [13]. β-adrenergic receptors are G protein–coupled receptors that mediate the cellular effects of catecholamines, including epinephrine and norepinephrine. Among these receptors, the β1-adrenergic receptor subtype is involved in intracellular signaling pathways that regulate protein secretion through cyclic adenosine monophosphate–dependent mechanisms. Activation of β-adrenergic receptors in salivary gland cells promotes exocytosis of protein-rich secretory granules and influences the qualitative characteristics of saliva [14,15]. Although saliva-based biomarkers have been increasingly investigated in systemic and oral inflammatory conditions, evidence regarding salivary β1-adrenergic receptor immunoreactivity in pediatric type 1 diabetes mellitus remains limited. Therefore, the present case-control study aimed to compare salivary β1-adrenergic receptor immunoreactivity between children with type 1 diabetes mellitus and age-matched healthy controls using unstimulated whole saliva.

Materials and Methods

This study was designed as an exploratory observational case-control study. The study was conducted at the Children's Medical Center and the School of Dentistry, Tehran University of Medical Sciences. Participant recruitment, saliva collection, and laboratory procedures were performed after approval by the institutional ethics committee. The primary objective of the study was to compare salivary β1-adrenergic receptor immunoreactivity between children with type 1 diabetes mellitus and systemically healthy controls. The study was considered exploratory and hypothesis-generating because no previous disease-specific data were available regarding salivary β1-adrenergic receptor immunoreactivity in children with type 1 diabetes mellitus. A total of 66 children aged 7 to 13 years were included in the study. The case group consisted of 33 children with type 1 diabetes mellitus, and the control group consisted of 33 systemically healthy children within the same age range.

Children in the case group had a confirmed diagnosis of type 1 diabetes mellitus established by pediatric endocrinologists prior to enrollment. To reduce clinical heterogeneity within the diabetic group, only children who were clinically stable and had acceptable metabolic control during the preceding three months based on screening records and pediatric endocrinology evaluation were considered eligible. These glycemic criteria were used only at the participant selection stage and were not defined as study outcomes or analytic variables. Therefore, individual HbA1c and fasting blood sugar values were not included in the final statistical dataset. Control participants were recruited from systemically healthy children attending the School of Dentistry for routine dental examinations. Therefore, the control group should be considered a clinic-based convenience sample rather than a community-based sample. Controls were screened to exclude any known

systemic disease and were sampled using the same saliva collection protocol as the type 1 diabetes mellitus group. Both recruitment settings were university-affiliated public academic centers, which may have reduced major differences related to healthcare access context. However, formal individual matching for socioeconomic status, parental education, oral hygiene behavior, or dental attendance patterns was not performed. Children were eligible for the type 1 diabetes mellitus group if they had a confirmed diagnosis of type 1 diabetes mellitus established by a pediatric endocrinologist, were 7 to 13 years of age, were clinically stable at the time of saliva collection, and had acceptable metabolic control during the preceding three months based on screening records and pediatric endocrinology evaluation. Children were eligible for the control group if they were 7 to 13 years of age, had no known systemic disease, attended the School of Dentistry for routine dental examination, and were able to provide an unstimulated whole saliva sample.

Children in either group were excluded if they had any additional systemic disease, were unable to cooperate during saliva sampling, failed to comply with pre-sampling fasting instructions, or could not provide an adequate saliva sample for laboratory analysis. Written informed consent was obtained from the parents or legal guardians of all participants before enrollment. Because no previous study was available that directly reported salivary β1-adrenergic receptor immunoreactivity in children with type 1 diabetes mellitus, no disease-specific effect size could be used for an a priori sample size calculation. Therefore, the present study was designed as an exploratory and hypothesis-generating case-control investigation. A balanced sample of 33 children with type 1 diabetes mellitus and 33 systemically healthy controls was included based on the availability of eligible participants during the study period and the feasibility of saliva collection and ELISA-based laboratory assessment. To contextualize the statistical interpretability of the enrolled sample, a post-hoc sensitivity power analysis was performed. With 33 participants per group, a two-sided alpha level of 0.05, and equal group allocation, the study had approximately 80% power to detect a standardized between-group difference of d = 0.70 and approximately 90% power to detect a standardized difference of d = 0.81. Therefore, the sample size was considered adequate for detecting moderate-to-large between-group differences, but insufficient for reliably detecting small effects, subgroup differences, or adjusted multivariable associations. An earlier salivary biomarker study used an ELISA-based approach to evaluate salivary adrenergic receptor-related proteins in oral lichen planus [16]. However, that study involved a biologically distinct oral inflammatory condition and was not used as a disease-specific basis for sample size estimation in the revised manuscript. It is cited only to support the analytical feasibility of detecting adrenergic receptor-related immunoreactivity in saliva. The exposure variable was group status, defined as type 1 diabetes mellitus or systemically healthy control. The primary outcome variable was salivary β1-adrenergic receptor immunoreactivity in unstimulated whole saliva, reported in ng/L according to the ELISA output. The final analytic dataset included group status and salivary β1-adrenergic receptor immunoreactivity. Glycemic indices were used only for eligibility screening and were not included as analytic variables. Diabetes duration, insulin regimen, daily insulin dose, Tanner pubertal stage, unstimulated salivary flow rate, and standardized oral health indices such as DMFT/dmft, plaque index, and gingival index were not prospectively recorded in a standardized analyzable form. Accordingly, these variables were not included in descriptive tables, correlation analyses, or adjusted statistical models.

Unstimulated whole saliva samples were collected using the spitting method after a minimum fasting period of two hours. Participants were instructed to avoid eating and drinking during the pre-sampling fasting interval. The same saliva collection protocol was applied to both groups in order to reduce pre-analytical variability. After collection, saliva samples were centrifuged to remove cellular debris and particulate material. The supernatant was stored at −20°C until ELISA analysis. All samples were handled under the same pre-analytical conditions in both study groups. Although unstimulated whole saliva was collected under a standardized protocol, salivary flow rate was not measured using timed sialometry. Therefore, the measured values should be interpreted as salivary β1-adrenergic receptor immunoreactivity based on the ELISA-reported concentration, rather than as secretion rate or total salivary output. Salivary β1-adrenergic receptor-related immunoreactivity was quantified using a commercially available sandwich enzyme-linked immunosorbent assay kit designed to detect human β1-adrenergic receptor protein in biological fluids. Measurements were reported in ng/L according to the manufacturer's calibration curve. In the context of whole saliva, the ELISA-based signal should be interpreted as β1-adrenergic receptor-related immunoreactivity rather than direct measurement of intact functional membrane-bound

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receptors. The detected signal may reflect soluble receptor fragments, epithelial shedding products, salivary gland-derived components, or extracellular vesicle-associated proteins. Therefore, the assay provides an exploratory salivary biomarker measurement and does not directly assess receptor function, receptor density in salivary gland tissue, or sympathetic nervous system activity. Measurement bias was reduced by applying the same saliva collection protocol, storage conditions, and ELISA-based laboratory method to all participants. All samples were collected as unstimulated whole saliva after the same minimum fasting interval. Selection bias was addressed by applying predefined eligibility criteria to both groups. However, the control group was recruited from children attending a dental school for routine dental examinations, and therefore it represents a clinic-based convenience control group. Formal matching for socioeconomic status, parental education, dental attendance patterns, oral hygiene behavior, pubertal status, or oral health indices was not performed. These issues were considered in the interpretation of the findings and are acknowledged as limitations.

Statistical analysis was performed using SPSS version 20. All statistical tests were two-sided, and a p-value less than 0.05 was considered statistically significant. The distribution of salivary β1-adrenergic receptor-related immunoreactivity was evaluated using the Shapiro-Wilk test. Because the outcome variable showed a non-normal distribution, continuous values were summarized using median and interquartile range. Minimum and maximum values were also reported to describe the observed range of measurements. Between-group comparison of salivary β1-adrenergic receptor-related immunoreactivity was performed using the Mann-Whitney U test as the primary analysis. The Mann-Whitney U statistic and the two-sided numerical p-value were reported for the primary comparison. Because HbA1c and fasting blood sugar were used only for eligibility screening and were not retained as analytic variables in the final statistical dataset, no correlation analyses between salivary β1-adrenergic receptor-related immunoreactivity and glycemic indices were performed in the revised analysis. A supplementary log-transformed analysis was performed as a robustness assessment of the between-group difference. Natural log-transformed values were compared between groups using an independent-samples t test. Back-transformed geometric means, geometric mean ratio, 95% confidence interval, and p-value were reported. The primary analysis remained the non-parametric Mann-Whitney U test because the raw salivary β1-adrenergic receptor-related immunoreactivity values were skewed and included extreme observations. The study protocol was approved by the Ethics Committee of Tehran University of Medical Sciences, School of Dentistry, ethics code IR.TUMS.DENTISTRY.REC.1399.101. Written informed consent was obtained from the parents or legal guardians of all participants. Because this was a non-interventional observational case-control study and participants were not prospectively assigned to any health-related intervention, the study was not registered in a clinical trial registry.

Results

A total of 66 children were included in the final analysis, comprising 33 children with type 1 diabetes mellitus and 33 systemically healthy controls within the same age range. Group status and salivary β1-adrenergic receptor-related immunoreactivity were available for all 66 included participants, corresponding to a 100% completion rate for the final analytic variables. Glycemic indices were used only during eligibility screening and were not retained as analytic variables in the final statistical dataset. Therefore, HbA1c and fasting blood sugar values were not reported descriptively, and no correlation analyses between salivary β1-adrenergic receptor-related immunoreactivity and glycemic indices were performed. Diabetes duration, insulin regimen, daily insulin dose, pubertal stage, unstimulated salivary flow rate, and standardized oral health indices were not included in the final analytic dataset; therefore, completion rates could not be reported for these variables.

Salivary β1-adrenergic receptor-related immunoreactivity showed a non-normal distribution in both study groups based on the Shapiro-Wilk test. The Shapiro-Wilk test was statistically significant in healthy controls ($p = 1.31 \times 10^{-7}$) and in children with type 1 diabetes mellitus ($p = 2.51 \times 10^{-10}$). Therefore, values were summarized using median, interquartile range, minimum, and maximum, and the primary between-group comparison was performed using the Mann-Whitney U test. The median salivary β1-adrenergic receptor-related immunoreactivity was 36.43 ng/L (IQR: 27.56-45.92) in healthy controls and 81.56 ng/L (IQR: 62.12-92.31) in children with type 1 diabetes mellitus. Salivary β1-adrenergic receptor-related immunoreactivity was significantly higher in the type 1 diabetes mellitus group than in the healthy control group (Mann-Whitney U = 137.0, two-sided $p = 1.79 \times 10^{-7}$). The observed range was wider in children with

type 1 diabetes mellitus (19.96-615.28 ng/L) than in healthy controls (4.67-188.29 ng/L). A supplementary log-transformed analysis was performed as a robustness assessment and was not used as the primary analytical approach. In this analysis, the back-transformed geometric mean was 77.52 ng/L in the type 1 diabetes mellitus group and 34.26 ng/L in healthy controls. The geometric mean ratio was 2.26 (95% CI: 1.67-3.06; p = 1.13×10^{-6}). This supplementary analysis was consistent with the primary non-parametric comparison, while the Mann-Whitney U test remained the primary analysis because the raw values were skewed and included extreme observations.

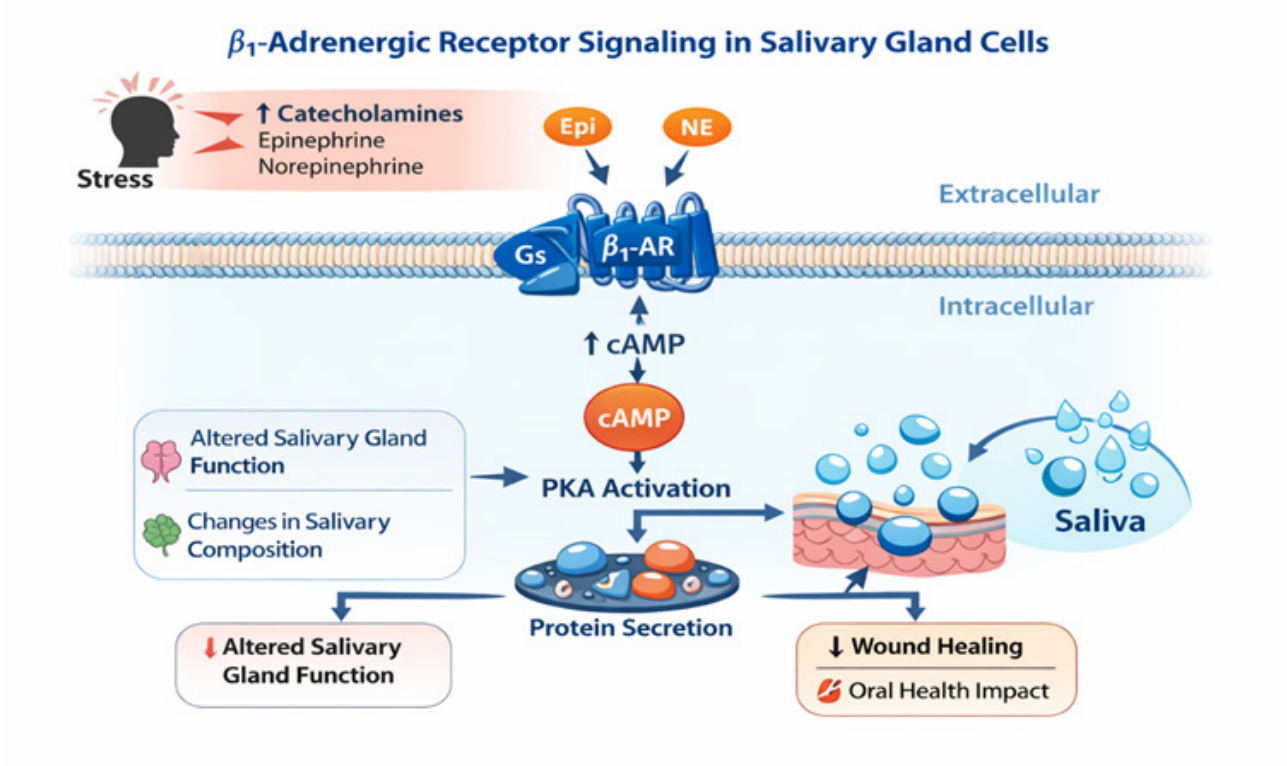

Figure 1. Schematic overview of the physiological background of β1-adrenergic receptor-related pathways in salivary gland function. The diagram is intended only to provide biological context and should not be interpreted as a mechanistic model tested in the present study. This study measured salivary β1-adrenergic receptor-related immunoreactivity in unstimulated whole saliva using ELISA and did not directly evaluate intact receptor expression, receptor activity, catecholamine release, autonomic function, or sympathetic nervous system activity.

Table 1. Salivary β1-adrenergic receptor-related immunoreactivity in children with type 1 diabetes mellitus and healthy controls.

Variable	*Group*	*n*	*Median (IQR) (ng/L)*	*Minimum (ng/L)*	*Maximum (ng/L)*	*Mann–Whitney U test*	*p-value*
Salivary β1-adrenergic receptor-related immunoreactivity, unstimulated whole saliva	*Healthy controls*	*33*	*36.43 (27.56–45.92)*	*4.67*	*188.29*	*137*	*1.79 × 10^-7*
	Type 1 diabetes mellitus	*33*	*81.56 (62.12–92.31)*	*19.96*	*615.28*		

Values are presented as median and interquartile range (IQR). Between-group comparison was performed using the Mann-Whitney U test.

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Discussion

The present exploratory case-control study evaluated salivary β1-adrenergic receptor-related immunoreactivity in children with type 1 diabetes mellitus and systemically healthy controls within the same age range. The main finding was that children with type 1 diabetes mellitus showed significantly higher salivary β1-adrenergic receptor immunoreactivity than controls. The median value was 81.56 ng/L in the type 1 diabetes mellitus group and 36.43 ng/L in the control group. This difference was statistically significant in the primary non-parametric analysis and remained in the same direction in the supplementary log-transformed assessment. These findings suggest that pediatric type 1 diabetes mellitus may be associated with an altered salivary β1-adrenergic receptor-related immunoreactive profile. However, this result should be interpreted carefully because the ELISA-detected signal in whole saliva does not directly demonstrate increased receptor expression, receptor function, receptor density in salivary gland tissue, or sympathetic nervous system activity.

This distinction is important for avoiding biological overinterpretation. β1-adrenergic receptors are membrane-associated G protein-coupled receptors, whereas the present study measured an immunoreactive signal in unstimulated whole saliva. Therefore, the measured outcome is more accurately described as salivary β1-adrenergic receptor-related immunoreactivity rather than receptor concentration in the strict biological sense. In whole saliva, such a signal may originate from several sources, including salivary gland-derived components, epithelial shedding products, soluble protein fragments, inflammatory exudates, serum-derived components, or extracellular vesicle-associated proteins. Accordingly, the present finding should be considered a preliminary salivary biomarker observation rather than evidence of increased functional adrenergic signaling. The oral environment in children with type 1 diabetes mellitus provides a relevant context for interpreting this finding. Type 1 diabetes may affect oral health through multiple mechanisms, including changes in salivary function, immune response, glycation-related inflammation, oral microbial ecology, and tissue repair. In a recent systematic review and meta-analysis, Triebl et al. reported that evidence regarding DMFT, periodontal indices, and salivary function in children with type 1 diabetes is heterogeneous, but salivary flow rate and glycemic control remain important variables in this population [17]. Liu et al. also reported that children and adolescents with type 1 diabetes may have higher caries experience and reduced salivary flow, although the certainty of evidence was not uniformly high [18]. These studies support the broader concept that pediatric type 1 diabetes may be associated with measurable changes in salivary and oral health parameters. They do not, however, provide direct evidence about β1-adrenergic receptor-related immunoreactivity. Recent clinical evidence further suggests that oral inflammatory status may influence salivary biomarker profiles in young individuals with type 1 diabetes. Catamo et al. evaluated children and young adults with type 1 diabetes and healthy controls and found higher frequencies of caries and gingivitis in the diabetes group. They also reported that salivary cytokine profiles differed according to oral condition, with specific changes in inflammatory and anti-inflammatory mediators among participants with caries, gingivitis, or both conditions [19]. This is highly relevant to the present study because standardized oral health indices, including DMFT/dmft, plaque index, gingival index, bleeding on probing, and periodontal status, were not included in the final analytic dataset. Therefore, unmeasured oral inflammatory or dental conditions may have contributed to the observed difference in salivary β1-adrenergic receptor-related immunoreactivity.

The absence of salivary flow rate measurement is another important consideration. Whole saliva biomarker values may be influenced by dilutional effects, total protein content, and secretion rate. Reduced salivary flow can increase the apparent value of some salivary proteins even when total secretion is not increased. Previous studies in pediatric type 1 diabetes have reported changes in salivary flow and salivary composition, although findings have varied across studies [17,18]. In the present study, unstimulated whole saliva collection was standardized across both groups, but timed sialometry was not performed. Therefore, the results cannot distinguish between a higher salivary immunoreactive signal and possible differences related to salivary volume, dilution, or flow. The relationship between the present finding and glycemic status also cannot be determined. In this study, glycemic information was used during eligibility screening to reduce clinical heterogeneity and to include clinically stable children with acceptable metabolic control. However, individual HbA1c and fasting blood sugar values were not retained as analytic variables in the final dataset. As a result, no descriptive glycemic table or correlation analysis with glycemic indices could be provided. This limits interpretation and prevents assessment of whether salivary β1-adrenergic receptor-related immu-

noreactivity differs according to metabolic control, glycemic variability, disease duration, insulin regimen, or insulin dose. Therefore, the present findings should not be interpreted as independent of glycemic status. They should instead be considered preliminary evidence of a between-group difference in a clinically selected subgroup of children with type 1 diabetes mellitus. Only limited evidence is available regarding salivary adrenergic receptor-related measurements. Gholizadeh et al. evaluated salivary α1- and β1-adrenergic receptor levels in patients with oral lichen planus using an ELISA-based method and reported higher salivary adrenergic receptor levels in oral lichen planus compared with healthy controls [16]. That study is useful because it demonstrates the analytical feasibility of detecting adrenergic receptor-related immunoreactivity in saliva. However, oral lichen planus is biologically and clinically different from pediatric type 1 diabetes mellitus. The two conditions differ in age distribution, disease mechanisms, inflammatory context, and oral tissue involvement. Therefore, the study by Gholizadeh et al. should not be used as a direct biological comparator for the present findings and should not be considered a disease-specific basis for sample size estimation. Its relevance to the present study is limited to showing that ELISA-based assessment of salivary adrenergic receptor-related proteins is technically feasible.

The biological source of the ELISA-detected signal remains uncertain. Saliva is a complex biofluid containing secretions from major and minor salivary glands, gingival crevicular fluid, immune cells, desquamated epithelial cells, microbial products, serum-derived components, and extracellular vesicles. Recent reviews have emphasized that salivary extracellular vesicles can carry proteins, nucleic acids, and other biomolecules that may reflect both local oral and systemic biological processes [20]. Salivary extracellular vesicles may also improve biomarker detection by protecting molecular cargo from degradation and reducing some forms of sample complexity. At the same time, experimental evidence indicates that extracellular vesicle isolation methods can influence particle yield, size distribution, and protein content, and that some biomarkers may be detectable in extracellular vesicle fractions but not in whole saliva [21]. Since the present study did not isolate or characterize extracellular vesicles, it is not possible to determine whether the observed β1-adrenergic receptor-related immunoreactivity was associated with soluble fragments, extracellular vesicle-associated proteins, epithelial shedding, or other salivary components.

Pre-analytical factors may also influence salivary biomarker studies. Mortazavi et al. reviewed saliva collection, transportation, preparation, and storage methods and showed that human saliva studies vary substantially in collection technique, handling procedures, centrifugation, storage temperature, and timing of analysis [22]. In the present study, the same saliva collection and storage protocol was applied to both groups, which reduces differential measurement bias. However, the lack of universally standardized protocols for salivary biomarker research remains a general methodological challenge. This issue is particularly relevant for exploratory biomarkers, where differences in sample processing may affect protein stability, measured values, or downstream assay performance. Several limitations should be considered when interpreting the present findings. First, the case-control design does not allow causal inference. The study demonstrates a between-group difference in salivary β1-adrenergic receptor-related immunoreactivity, but it cannot determine whether this difference is caused by type 1 diabetes mellitus or by other metabolic, oral, inflammatory, developmental, or methodological factors.

Second, the study was exploratory and included 33 participants per group. Because no previous disease-specific data were available for salivary β1-adrenergic receptor-related immunoreactivity in pediatric type 1 diabetes mellitus, the study was not based on a directly relevant a priori effect size. Although the enrolled sample was adequate for detecting a moderate-to-large between-group difference, it was not sufficient for subgroup analyses, multivariable adjustment, or reliable evaluation of small effects. Third, glycemic indices were used only during eligibility screening and were not retained as analytic variables in the final dataset. Therefore, individual HbA1c and fasting blood sugar values could not be reported descriptively, and the relationship between salivary β1-adrenergic receptor-related immunoreactivity and metabolic control could not be assessed. In addition, diabetes duration, insulin regimen, daily insulin dose, and glycemic variability were not available in a standardized analyzable form. These omissions limit interpretation of whether the observed salivary difference is related to diabetes severity, treatment characteristics, or metabolic status. Fourth, pubertal status was not recorded. This is relevant because the included age range, 7 to 13 years, covers a developmental period in which pubertal maturation may influence hormonal status, immune function, salivary composition, and metabolic physiology. Fifth, unstimulated salivary flow rate was not measured

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using timed sialometry. Therefore, the ELISA-reported values could not be normalized to salivary flow or interpreted as secretion rates. Because whole saliva biomarker values may be influenced by dilutional effects and total salivary output, the absence of salivary flow measurement limits biological interpretation of the measured immunoreactivity. Sixth, standardized oral health indices were not included in the final analytic dataset. DMFT/dmft, plaque index, gingival index, bleeding on probing, and periodontal status were not recorded in a standardized form suitable for analysis. This is an important limitation because caries, plaque accumulation, gingival inflammation, and periodontal status may influence salivary protein composition and inflammatory biomarker profiles. Seventh, psychological stress and autonomic-related variables were not assessed. Validated stress questionnaires, salivary catecholamines, salivary α-amylase, and autonomic function tests were not included. Therefore, the present findings should not be interpreted as evidence of stress-related mechanisms, autonomic dysregulation, or sympathetic nervous system activity.

Eighth, the biological origin of the ELISA-detected β1-adrenergic receptor-related signal remains uncertain. In whole saliva, this signal may reflect soluble receptor fragments, epithelial shedding products, salivary gland-derived components, inflammatory exudates, or extracellular vesicle-associated proteins rather than intact functional membrane-bound receptors. Because extracellular vesicles were not isolated or characterized, the present study cannot determine the source or functional significance of the measured immunoreactivity. Finally, the control group was recruited from systemically healthy children attending a dental school for routine dental examinations. Therefore, the controls represent a clinic-based convenience sample rather than a community-based sample. Although both groups were recruited from university-affiliated public academic settings, formal matching for socioeconomic status, parental education, oral hygiene behavior, or dental attendance patterns was not performed. This may limit external validity and should be addressed in future studies using community-based or formally matched controls. Despite these limitations, the study has several strengths. It addresses a scarcely investigated salivary biomarker in a pediatric type 1 diabetes population and uses a non-invasive sampling method that is suitable for children. The same unstimulated whole saliva protocol and ELISA-based laboratory approach were applied to all participants. The revised interpretation also distinguishes the ELISA-detected salivary signal from functional adrenergic receptor activity and avoids unsupported conclusions regarding glycemic correlations or sympathetic nervous system activity. Future studies should use prospective designs with larger samples and community-based or formally matched controls. They should collect detailed glycemic variables, including HbA1c, fasting blood sugar, continuous glucose monitoring indices, diabetes duration, insulin regimen, and insulin dose. They should also include pubertal staging, timed unstimulated salivary flow rate, total salivary protein, oral health indices, gingival and plaque assessment, and validated measures of psychological stress. Mechanistic studies should combine ELISA-based saliva measurements with extracellular vesicle characterization, catecholamine assessment, autonomic function testing, and, where feasible, tissue-level or cellular analyses. Such studies are needed to determine whether salivary β1-adrenergic receptor-related immunoreactivity has clinical relevance in pediatric type 1 diabetes mellitus or mainly reflects broader changes in salivary protein composition, oral inflammatory status, or extracellular vesicle-associated biomarker profiles.

Conclusion

In conclusion, children with type 1 diabetes mellitus showed significantly higher salivary β1-adrenergic receptor-related immunoreactivity than systemically healthy controls within the same age range. This finding should be interpreted as a preliminary salivary biomarker observation, not as direct evidence of increased β1-adrenergic receptor expression, receptor function, tissue-level adrenergic signaling, or sympathetic nervous system activity. Because glycemic indices, diabetes duration, insulin-related variables, pubertal status, salivary flow rate, oral health indices, stress measures, and extracellular vesicle characterization were not included in the final analytic dataset, the biological source and clinical meaning of this difference remain uncertain. Larger prospective studies with detailed metabolic, oral, salivary, and mechanistic assessments are required to confirm these findings and clarify the potential role of salivary β1-adrenergic receptor-related immunoreactivity in pediatric type 1 diabetes mellitus.

Conflict of Interest

There is no conflict of interest to declare.

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