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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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Содержание:

SALIVARY AND SERUM PROTEIN Z, AND B-ARRESTIN-1 AS A NOVEL DIAGNOSTIC MARKER OF PATIENTS WITH DIABETES MELLITUS TYPE 2

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Abstract.

Aim: To investigate serum and salivary protein Z and β-arrestin-1 levels as predictors of T2DM as compared to the healthy controls.

Methods: The assay comprised 98 subjects, enrolled of both genders in the age range of 30–58 years old. Volunteers were categorized into two groups: 49 healthy individuals and 49 DM patients. The blood and salivary samples were collected and sent for biochemical analysis to measure the protein Z and β-arrestin-1 levels.

Results: The DM group exhibited higher salivary and serum β-arrestin-1 compared to the control group. The findings revealed noticeably elevated serum and salivary protein levels. Z, HbA1c and glucose levels in the DM group. Protein Z showed significant negative correlations with HbA1c, supporting the observation of decreased protein Z in poorer glycemic control. Further analysis showed that serum and salivary protein Z are (87.8 and 46.9)% sensitive and (89.8 and 100.0)% specific with a cut-off value (≤1.500, and ≤141.6), respectively. The ROC Curve cut-off value of serum and salivary alpha β-arrestin-1observed was (≥14.80, and ≥2.290 pg/ml), respectively. The sensitivity was (95.9, 63.3)% and the specificity was (91.8 and 93.9)%, respectively.

Conclusion: Our findings demonstrated that higher salivary levels of β-arrestin-1 and lower levels of protein Z were associated with diabetes mellitus and could serve as an adjuvant diagnostic tool for diabetes.

Key words. Diabetes, pancreas, saliva, insulin, protein Z, β-arrestin-1.

Introduction.

The pancreas is an essential endocrine-exocrine organ that generates a number of hormones and enzymes [1]. The majority of islet cells in all vertebrates are β–cells, which release insulin when blood glucose levels rise [2]. Type-1 and Type-2 diabetes mellitus, which together afflict millions of people globally, are caused by impaired insulin secretion [3]. Diabetes mellitus is a prevalent metabolic disease marked by hyperglycemia that is becoming more widespread in all age groups and socioeconomic strata [4].

In addition to being a part of the metabolic syndrome and a known risk factor for periodontal disease, type 2 diabetes (T2D) is characterized by insulin resistance (IR) [5]. IR and β-cell dysfunction play major roles in the pathophysiology of type 2 diabetes, while the exact cause of the disease is unknown [6]. Hyperglycemia and salivary malproduction are linked to metabolic disturbances [7].

The salivary glands in the mouth produce and exude saliva, an extracellular fluid that contains a variety of indicators useful for identifying both local and systemic illnesses [8,9]. Saliva testing has shown great promise in the long-term monitoring of diabetes in recent years and is a non-invasive technique with benefits such as easy collection, low cost, and good patient compliance [10,11]. Compared to blood tests and other diagnostic procedures, saliva offers several advantages as a diagnostic tool, including ease of collection, non-invasiveness, accessibility, safety, and precision [12].

The liver is primarily responsible for producing the vitamin K-dependent anticoagulant plasma glycoprotein protein Z, and it functions as a serpin activator [13]. Protein Z-dependent protease inhibitor (ZPI) is more abundant than protein Z in human plasma, and protein Z and PZI are found together in the blood [14]. The vast variation in normal plasma protein Z concentrations among individuals may be partially attributed to heredity. protein Z acts as a cofactor for the inhibition of factor (F) Xa via a ZPI by forming a calcium ion-dependent complex with factor Xa on phospholipid surfaces [15].

The scaffold protein β-arrestin 2 (ARRB2) is an intracellular signalling molecule that can block G protein-coupled receptor (GPCR) signalling [16]. Additionally, these proteins have the ability to control numerous critical metabolic processes, such as β-cell activity, in a way that is independent of G proteins. β-arrestin-1 modulates insulin secretion and regulates β-cell survival and functions [17,18]. The purpose of this study was to assess β-arrestin-1 and serum and salivary protein Z levels as predictors of type 2 diabetes in comparison to healthy controls.

Materials and Methods.

In this investigation, diagnosed diabetic patients were recruited from Tikrit Teaching Hospital (Tikrit City, Iraq) between February and December 2025. The age range of participants was between 30 and 58 years old. The participants were divided into two groups: 49 were patients with DM, and 49 healthy individuals who served as a group of controls from the same areas as the patients and were randomly selected.

Diabetes was diagnosed based on HbA1c 6.5%, fasting serum glucose 126 mg/dL, and 2-hour plasma glucose with classic hyperglycemic symptoms.

Exclusion criteria comprised individuals with type 1 diabetes, acute infections, chronic inflammatory diseases, liver dysfunction, renal impairment, malignancy, or those on antioxidant supplements.

The collection took place between 8:00 a.m. and 11:00 a.m. 5 ml of whole unstimulated saliva from each patient was collected

Table 1. *Demographic parameters of the studied groups.*

Variable	Control (n=49)	DM (n=49)	p value
Age, years	42±6	45±12	0.12
Sex, M/F	22/27	29/20	0.01
BMI, kg/m²	24.9±9.2	27.8±5.4	0.06
Duration of DM, years	----	6.1±2.3	----

Chi square used for sex parameter and two sample unpaired t-test used for other parameters, p values considered significant at values less than 0.05

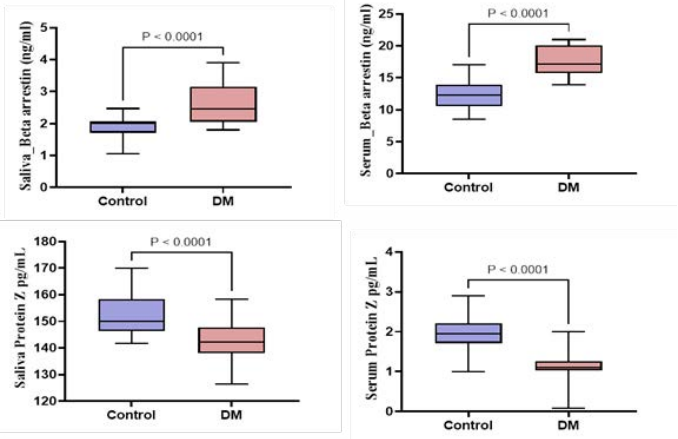

Figure 1. *Boxplots show the distribution of protein Z and β-arrestin in saliva and serum of the studied groups. Blue boxes represent the Control group; red boxes represent the DM group.*

in the morning after fasting for at least 12 hours. Serum samples were kept at -80°C while blood was centrifuged at 3,000 RPM for 10 minutes at room temperature. After centrifuging the saliva samples at 2,800 rpm for 10 minutes at 4°C, the supernatants were moved to storage tubes and stored at -80°C. Sandwich Enzyme-linked immunosorbent assay (ELISA) kits are used to evaluate protein Z and β-arrestin-1 levels (Creative Diagnostics, USA). Based on kits instructions supplied by Biolabo (France), glucose levels and HbA1c were measured based on colourimetric and immunofluorescence assay, respectively.

Statistical analyses: Data analyzed using GraphPad Prism (V10, USA). To assess the normal distribution of data, Shapiro-Wilk test was used. Independent samples two-tailed t-tests were employed for normally distributed data and Mann-Whitney U test was used for non-normally distributed data. the differences were considered significance at $p<0.05$. Correlations were assessed using Spearman's rank correlation coefficient.

Ethical approval was obtained from Tikrit University (Approval Letter Code MIDTUH3 on 14 January 2025).

Results.

The demographic parameters indicated well matched age and BMI indicating non-significant differences at p value less than 0.05. There is variation in sex distribution between groups with more female in control compared to DM group (p=0.01) (Table 1).

The DM group demonstrated substantially elevated levels of glycemic markers, including HbA1c (6.87% vs 4.72%), serum glucose (227.58 vs 97.17), and saliva glucose (5.78 vs 1.06). β-arrestin-1 levels (pg/ml) showed pronounced elevation in DM patients (saliva: 0.868 vs 0.150; serum: 2.503 vs 1.521), suggesting activation of metabolic stress pathways. Notably, protein Z levels (ng/ml) demonstrated an inverse pattern, with lower concentrations in the DM group (saliva: 142.45 vs 151.98; serum: 1.18 vs 1.94), potentially indicating consumption or reduced synthesis in the diabetic state. Normality was assessed using the Shapiro-Wilk test. Due to significant deviation from normality in most variables (particularly in the DM group), for group comparisons, non-parametric Mann-Whitney U tests were used (Table 2 and Figure 1).

All biomarkers demonstrated statistically significant differences between groups ($p<0.001$). Importantly, all effect sizes were large ($|d|\geq0.8$), indicating clinically meaningful differences. The largest effect sizes were observed for:

- Saliva glucose (d=12.13): Reflecting the dramatic elevation of glucose in diabetic saliva.
- Serum glucose (d=7.80): The hallmark feature of diabetes mellitus.

The negative effect sizes for protein Z (saliva d=-1.21, serum d=-2.10) confirm significantly lower levels in the DM group, potentially reflecting altered hemostatic function in diabetes.

Correlation Analysis:

All biomarkers demonstrated significant correlations with HbA1c ($p<0.001$), confirming their relationship with glycemic control. The strongest positive correlations were observed for glucose (Serum r=0.751, saliva r=0.736), β-arrestin-1 (Serum r=0.748, saliva r=0.750), whereas protein Z, which showed significant negative correlations with HbA1c (Saliva: r=-0.521; Serum: r=-0.595), supports the observation of decreased protein Z in poorer glycemic control (Table 3).

Correlation between serum and salivary markers:

The scatter plot of glucose demonstrated a positive correlation between saliva and serum glucose levels with differential

Table 2. *Comparison of biomarker levels between the control and DM groups.*

Variable	Control	DM	p value	Cohen's d	Sig.
Saliva β-arrestin-1, pg/ml	1.863±0.334	2.637±0.603	<0.001	1.586	***
Serum β-arrestin-1, pg/ml	12.35±1.94	17.75±2.16	<0.001	2.630	***
Saliva protein Z, ng/ml	151.98±7.54	142.45±8.20	<0.001	-1.210	***
Serum protein Z, ng/ml	1.940±0.413	1.180±0.303	<0.001	-2.100	***
HbA1c, %	4.720±0.601	6.872±0.473	<0.001	3.979	***
Saliva glucose, mmol/L	1.060±0.024	5.776±0.549	<0.001	12.134	***
Serum glucose, mmol/L	97.17±3.67	227.58±23.36	<0.001	7.800	***

Data expressed as Mean± SD, Significance: *** p<0.001. Cohen's d interpretation: |d|<0.2 negligible, 0.2-0.5 small, 0.5-0.8 medium, ≥0.8 large effect.

clustering trends for control versus DM patients, with no overlap between the two groups. The scatter plot of β-arrestin-1 demonstrated positive correlation between saliva and serum β-arrestin-1 levels with considerable variability trends for control versus DM patients, with no strong linear correlation. The scatter plot of protein Z demonstrated a positive correlation between saliva and serum protein Z levels, with considerable variability trends for control versus DM patients, with a weak correlation (Figure 2).

ROC analysis: Saliva glucose, serum glucose, and HbA1c demonstrated complete sensitivity, specificity, and accuracy at cutoffs of ≥5.000, ≥201.0, and ≥6.320, respectively. Serum β-arrestin-1 (cutoff ≥14.80) 95.9% sensitivity, 91.8% specificity, and 93.9% accuracy. Serum protein Z (cutoff of ≤1.500) also revealed 87.8% sensitivity, 89.8% specificity, and 88.8% accuracy. Saliva β-arrestin-1(cutoff ≥2.290) demonstrated 63.3% sensitivity, 93.9% specificity, and 78.6% accuracy. Saliva protein Z (cutoff ≤141.6) demonstrated 46.9% sensitivity, 100% specificity, and 73.5% accuracy (Table 4).

Classification Performance: Based on the cutoff values provided in Table 5, glucose levels (saliva or serum) demonstrated précised differentiation for all 98 participants with no false positives or negatives. HbA1c demonstrated only 2 false negatives and no false positives. Serum β-arrestin-1 (cutoff ≥14.80) demonstrated 4 false positives and 2 false negatives. Serum protein Z demonstrated 5 false positives and 6 false negatives. Saliva β-arrestin-1 correctly defined 77 participants, with 3 false positives and 18 false negatives, while Saliva protein Z correctly defined 72 participants, with 26 false negatives, with no false positives.

Likelihood Ratios and Youden's Index:

The results of saliva glucose, serum glucose, and HbA1c demonstrated a positive likelihood ratio (LR+), reflecting that the test outcomes are positively linked with the disease, with no false positives and thereby resulting in a perfect Youden's J score of 1.000, further confirmed by HbA1c LR- of 0.04 and a Youden's J of 0.959.

Serum β-arrestin-1 shows a strong positive association with disease (LR+ = 11.71) and a very low LR- (0.04), resulting in a high Youden's J of 0.878. Serum protein Z revealed good diagnostic power (LR+ = 8.59, Youden's J = 0.776). Saliva protein Z demonstrated only "Fair" acceptability for diagnosis, reflected by a Youden's J of 0.469, with a modest LR of 0.53, suggesting a greater rate of false negatives (Table 6). Therefore,

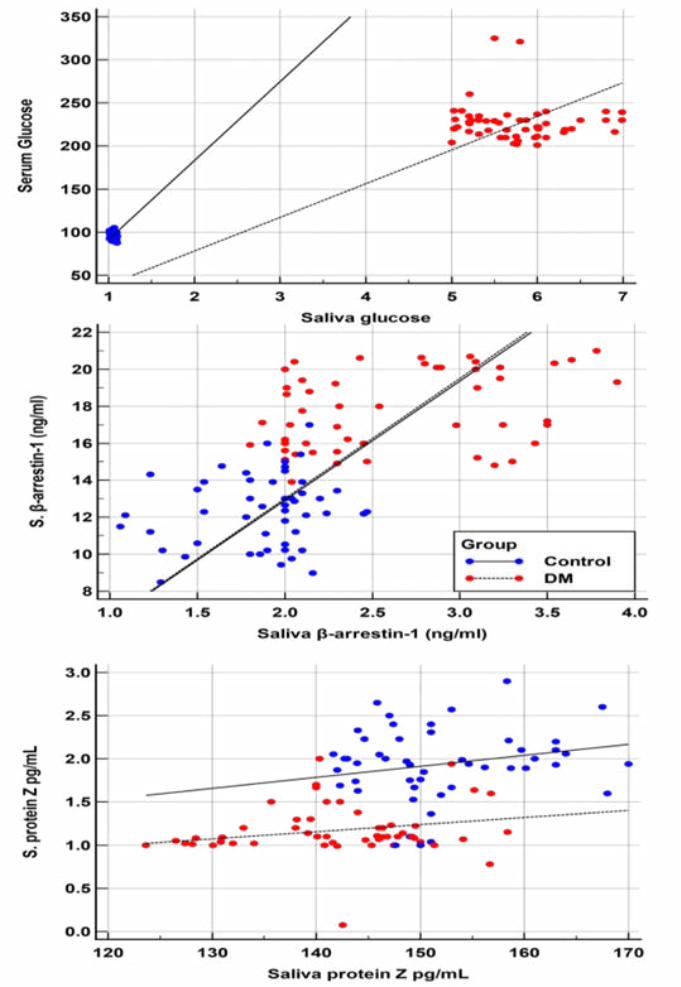

Figure 2. *Scatter plots depicting the relationship between saliva and serum concentrations for each biomarker. Blue points represent Control subjects; red points represent DM patients.*

Table 3. *Spearman correlations of biomarkers with HbA1c.*

Variable	r	p-value	Significance
Saliva protein Z, ng/ml	-0.521	<0.001	***
Serum protein Z, ng/ml	-0.595	<0.001	***
Saliva glucose, mmol/L	0.736	<0.001	***
Serum glucose, mmol/L	0.751	<0.001	***
Saliva β-arrestin-1, pg/ml	0.750	<0.001	***
Serum β-arrestin-1, pg/ml	0.748	<0.001	***
*** p<0.001; r: Spearman correlation coefficient			

Table 4. *ROC Analysis: Diagnostic Performance of Biomarkers (Ranked by AUC).*

Biomarker	AUC	95% CI	Cutoff	Sens%	Spec%	PPV%	NPV%	Acc%
Saliva glucose, mmol/L	1.000	1.000-1.000	≥5.000	100.0	100.0	100.0	100.0	100.0
Serum glucose, mmol/L	1.000	1.000-1.000	≥201.0	100.0	100.0	100.0	100.0	100.0
HbA1c, %	0.989	0.968-1.000	≥6.320	95.9	100.0	100.0	96.1	98.0
Serum β-arrestin-1, pg/ml	0.975	0.943-1.000	≥14.80	95.9	91.8	92.2	95.7	93.9
Saliva β-arrestin-1, pg/ml	0.871	0.799-0.943	≥2.290	63.3	93.9	91.2	71.9	78.6
Serum protein Z, ng/ml	0.910	0.849-0.970	≤1.500	87.8	89.8	89.6	88.0	88.8
Saliva protein Z, ng/ml	0.802	0.714-0.890	≤141.6	46.9	100.0	100.0	65.3	73.5
AUC: Area Under the Curve; CI: Confidence Interval; Sens: Sensitivity; Spec: Specificity; PPV: Positive Predictive Value; NPV: Negative Predictive Value; Acc: Accuracy. Cutoffs derived using Youden's Index.								

Table 5. *Classification Performance at Optimal Cutoff Points.*

Biomarker	Cutoff	TP	TN	FP	FN	Correct	Misclass.
Saliva glucose, mmol/L	≥5.000	49	49	0	0	98	0
Serum glucose, mmol/L	≥201.0	49	49	0	0	98	0
HbA1c, %	≥6.320	47	49	0	2	96	2
Serum β-arrestin-1, pg/ml	≥14.80	47	45	4	2	92	6
Serum Protein Z, ng/ml	≤1.500	43	44	5	6	87	11
Saliva β-arrestin-1, pg/ml	≥2.290	31	46	3	18	77	21
Saliva Protein Z, ng/ml	≤141.6	23	49	0	26	72	26

TP: True Positives; TN: True Negatives; FP: False Positives; FN: False Negatives; Correct: Total correctly classified; Misclass.: Total misclassified. Total sample n=98 (49 Control, 49 DM).

Table 6. *Likelihood Ratios and Youden's Index.*

Biomarker	LR+	LR-	Youden's J	Interpretation
Saliva glucose, mmol/L	∞	0.00	1.000	Excellent
Serum glucose, mmol/L	∞	0.00	1.000	Excellent
HbA1c, %	∞	0.04	0.959	Excellent
Serum β-arrestin-1, pg/ml	11.71	0.04	0.878	Good
Serum protein Z, ng/ml	8.59	0.14	0.776	Good
Saliva protein Z, ng/ml	∞	0.53	0.469	Fair

LR+: Positive Likelihood Ratio; LR-: Negative Likelihood Ratio; Youden's J = Sensitivity + Specificity - 1. LR+ >10 and LR- <0.1 indicate excellent diagnostic utility. ∞ indicates perfect positive or negative predictive ability.

serum β-arrestin-1 and serum protein Z might be used as good biomarkers, preferably serum measurements.

Discussion.

The present study has explored salivary protein Z and β-arrestin-1 in patients with DM. In diabetic patients, β-arrestin increased in serum and saliva; conversely, protein Z decreased in serum and saliva compared to the control group. Moreover, the results demonstrated that the levels of protein Z and β-arrestin-1 have reciprocally harmonised and correlated with the salivary concentration of glucose and HbA1c, reflecting a potential link of these proteins with the hyperglycemic status of individual patients. Therefore, serum β-arrestin-1 and serum protein Z might be used as good biomarkers, preferably serum measurements.

Isoforms of β-arrestins (β-arrestin-1 and β-arrestin-2) are a group of intracellular proteins that participate in metabolic functions and play a role in the pathophysiology of diabetes [19]. In an experimental study conducted in mice, Luan et al. (2009) demonstrated that reduced levels of β-arrestin-2 in the liver and skeletal muscle resulted in insulin hyposensitivity, and that restoring the β-arrestins expression can resolve glucose sensitivity, reflecting its critical role in insulin pathways [20]. Despite sharing pathophysiological properties of gestational diabetes regarding involvement of β-arrestin [21], the gestational diabetes was associated with reduced levels of β-arrestin [22], which contradicts our findings of elevated β-arrestin, taking into consideration that the population involved in the present study is Type 2 diabetes. In an experimental animal study, it has been reported that β-arrestin-2 encourages glucose consumption by increasing insulin sensitivity and glucose utilisation in diabetic mice [23].

The present study also confirmed a positive correlation between glycemic parameters and β-arrestin-2. Perhaps this connection is related to the depicted role of β-arrestin in pancreatic β-cell function under normal and pathological conditions, hence its reduction weakens insulin secretion [24]. Moreover, it has been reported that β-arrestin-2 reduces hepatic glucose synthesis via blocking glucagon receptors [25]. In an alternative study, it has been found that blocking β-arrestin-1 leads to cellular insulin hyposensitivity [26], and continuous insulin therapy is associated with degradation of β-arrestin-1, resulting in desensitisation of G protein-coupled receptors [27] and controlling GLP-1-mediated insulin production [28]. Perhaps finding that the response to sulfonylurea increased with β-arrestin-1 signalling in the pancreas [29].

In the present study, the levels of protein Z were associated with a reduction in DM compared to the control group. In a proteomic cytokine array conducted to test 310 cytokines, 41 of which were 1.5-fold elevated, except for protein Z, which was 2.2-fold downregulated in prediabetic and 2.5-fold in T2DM compared to the healthy group [30]. In bioinformatics genetic analysis, findings revealed that protein Z is specifically associated with diabetes rather than other diseases [31-35]. Moreover, protein Z has also been involved in vascular complications potentially associated with diabetic complications [36-38]. Moreover, hyperlipidemia coincides with diabetes, which is further associated with modulated protein Z levels [39].

Protein Z showed negative correlations with HbA1c. In line with the present study, Bae et al. (2021) have also reported a negative association of protein Z with glycemic parameters in diabetic patients, reflecting that reduced protein Z concentration is associated with worsening of glycemic status [30].

The analysis of ROC results revealed that salivary and serum β-arrestin-1 are highly specific and sensitive as a diagnostic tool, resulting in high accuracy of more than 90%, close to glucose and HbA1c accuracy. However, protein Z has shown weaker sensitivity and specificity, with accuracy even lower than 80%. moreover, likelihood ratios and Youden's index reflected that β-arrestin-1and protein Z provided good interpretation for

association with glycemic parameters for diagnosis, Hence, β-arrestin-1 is more reflective of diabetic status and comparable to glycemic parameters and could be used as adjuvant diagnostic tool, taking into consideration that salivary measured parameters were reflective mirror for that of serum, providing a chance for non-invasive strategy for diabetes diagnosis.

Limitation.

The limitation of this study includes a small sample size, hindering the prediction of the exact relationship between diabetes and β-arrestin and protein Z. More proteins are required to be analysed, which could be better reflective of salivary concentration and hence more mimicking the serum levels of that marker to be used as an adjuvant for glycemic markers. A longitudinal follow-up study is required to assess protein Z as an adjuvant biomarker for the diagnosis of prediabetes and newly diagnosed T2DM, providing a platform for teasing out the pathophysiology of glucose dysmetabolism.

Conclusion.

It is likely that higher concentrations of β-arrestin-1, while lower concentrations of protein Z, can be detected in the saliva and serum of DM patients compared to the control group. In order to fully investigate the potential of salivary biomarkers as early markers of metabolic dysregulation linked to DM and evaluate their therapeutic value, future research should focus on filling in these gaps.

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