

# GEORGIAN MEDICAL NEWS

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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 ВИНТИ 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 ВИНТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

### WEBSITE

[www.geomednews.com](http://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](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. Корректур авторам не высылаются, 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 computer-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.nlm.nih.gov/bsd/uniform_requirements.html)  
[http://www.icmje.org/urm\\_full.pdf](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 საქურაღებოლ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დაიცვათ 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. რენტგენოგრაფიების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალებების შედეგების 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 ფხიხლებში 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.

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## CIRCULATING PEPTIDES OF THE TNF SUPERFAMILY AND TNF RECEPTOR SUPERFAMILY IN SUBJECTS WITH TYPE 1 DIABETES: RELATIONSHIPS WITH CLINICAL AND METABOLIC PARAMETERS

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### Abstract.

Tumor Necrosis Factor Superfamily (TNFSF) and Tumor Necrosis Factor Receptor Superfamily (TNFRSF) molecules play an essential role in the regulation of immune and inflammatory reactions and may be involved in the pathogenesis of type 1 diabetes (T1D). In this study, we aimed to assess serum levels of TNFSF and TNFRSF peptides in T1D subjects depending on their clinical and metabolic parameters.

**Material and methods:** Fifty-eight adults with T1D and 19 individuals with normal glucose tolerance (control) were included in the study. Concentrations of TNF- $\alpha$ , TNF- $\beta$ , TWEAK, APRIL, BAFF, LIGHT, sTNFR1, sTNFR2, and sCD30 were assessed by multiplex bead array assay. Time in range (TIR) and glucose variability (GV) were assessed by continuous glucose monitoring.

**Results:** Patients with T1D had increased levels of TNF- $\alpha$  and decreased levels of LIGHT compared to control ( $p=0.03$  and  $p=0.02$  respectively). Patients with TIR <70% when compared with those with TIR >70% demonstrated higher levels of TNF- $\alpha$  ( $p=0.008$ ), APRIL ( $p=0.01$ ) and lower levels of BAFF ( $<0.0001$ ). Serum APRIL and BAFF correlated differently with GV. Overweight or obese patients had higher levels of sTNFR1 and sTNFR2 than those with normal body weight (sTNFR1:  $p=0.01$ ; sTNFR2:  $p=0.02$ ). Patients with diabetic retinopathy compared to those without showed increased levels of APRIL ( $p=0.008$ ) and patients with declined renal function had higher concentrations of APRIL ( $p=0.03$ ), BAFF ( $p=0.03$ ), and sCD30 ( $p=0.04$ ). In multiple regression analysis, HbA1c was associated with TNF- $\alpha$ , eGFR was predictor for sCD30 and APRIL, BMI was associated with APRIL and sTNFR1, and TIR was associated with BAFF.

**Conclusions:** The results show the relationships between TNFSF and TNFRSF peptides, hyperglycemia, GV, obesity, and diabetic complications in T1D. Among the studied molecules, TNF- $\alpha$ , APRIL, BAFF, sTNFR1, and sCD30 can be considered as promising biomarkers for assessing metabolic and vascular risk in subjects with T1D.

**Key words.** Type 1 diabetes, hyperglycemia, time in range, glucose variability, inflammation, obesity, tumor necrosis factor, TNFSF, TNFRSF.

### Introduction.

Diabetes is recognized as one of the most serious problems for health systems worldwide due to its widespread and complications. According to the International Diabetes Federation, at least 537 million adults are living with diabetes; this number is predicted to rise to 783 million by 2045. In 2021, diabetes was responsible for 6.7 million deaths [1]. Identification of the pathogenic mechanisms and biomarkers of diabetes and its complications remains a challenge for medical science. In

pathogenesis of diabetic complications, role of chronic low-grade inflammation is widely discussed [2]. Hyperglycemia has traditionally been regarded as a trigger for inflammatory pathways in diabetes. Besides, a growing body of evidence points to the proinflammatory effect of enhanced glucose variability (GV) in both clinical and experimental diabetes [3,4]. The list of other contributing factors includes obesity, excessive lipid species, elevation of circulating lipopolysaccharide, and hypoxia [5].

The molecules of the Tumor Necrosis Factor Superfamily (TNFSF) play an essential role in the regulation of immunity and inflammation. These proteins are expressed predominantly by immune cells and regulate immune response, as well as cell proliferation, differentiation, and apoptosis. Tumor necrosis factor alpha (TNF- $\alpha$ ), the most known member of this family, was identified as a cytotoxic cytokine in 1968. Currently, TNFSF includes 19 proteins that can function as cytokines. The TNF receptor superfamily (TNFRSF) comprises 29 proteins [6]. Members of these two superfamilies are gaining more attention as important mediators, promising biomarkers, and target molecules for various processes, including inflammation, tissue remodeling, fibrosis, and cancer [6,7].

Up to date, little is known about the changes in the levels of most circulating TNFSF and TNFRSF molecules in people in diabetes, with the only exception of TNF- $\alpha$  and its receptors. Besides, the relationships between these molecules and glucose fluctuations have not been studied yet. Therefore, we aimed to assess serum levels of some TNFSF cytokines and TNFRSF molecules in subjects with type 1 diabetes (T1D) depending on clinical and metabolic parameters.

### Materials and Methods.

**Design:** We performed a cross-sectional one-center comparative study. Inclusion criteria for patients with T1D (observation group) were age from 18 to 70 years; diagnosis of T1D; and basal-bolus insulin therapy. Age from 18 to 70 years, normal (<5.6%) glycated hemoglobin A1c (HbA1c) level, and normal result of standard oral glucose tolerance test were inclusion criteria for non-diabetic subjects (control group).

A set of exclusion criteria included: current acute metabolic complications (diabetic ketoacidosis, hyperglycemic hyperosmolar state, and lactic acidosis); end-stage renal disease; congestive heart failure; COVID-19 and other infections within three months before the study; chronic inflammatory or autoimmune diseases or malignant neoplasm in medical history; pregnancy.

All subjects with diabetes underwent clinical examination at the clinic of RICEL – Branch of IC&G SB RAS, a tertiary referral hospital for diabetes care. The assessment of metabolic

status and in-depth screening/monitoring of complications were performed. All patients underwent continuous glucose monitoring (CGM) with assessment of Time in Ranges (TIRs) and GV parameters. The measurement of HbA1c and standard glucose tolerance test with 75 g of glucose load were performed in individuals without known diabetes.

The study protocol was approved by the institutional Ethical Committee (Protocol 158, June 1, 2020). All subjects provided their written informed consent prior to inclusion.

**Methods:** The levels of HbA1c, serum cholesterol, triglycerides, high-sensitivity C-reactive protein (hsCRP), urinary albumin and creatinine were measured with AU480 Chemical Analyzer (Beckman Coulter, USA) and commercially available cartridges. Estimated glomerular filtration rate (eGFR) was calculated by CKD-EPI formula (2009). Complete blood count was performed with the use of hematology analyzer (Analyticon Biotechnologies AG, Germany). Neutrophil-to-lymphocyte ratio was estimated as an inflammatory marker related to glycemic control in diabetes [8].

Serum was obtained from fasting blood samples. Concentrations of TNF- $\alpha$  (TNFSF2), tumor necrosis factor beta (TNF- $\beta$ /TNFSF1), TNF-related weak inducer of apoptosis (TWEAK/TNFSF12), A proliferation-inducing ligand (APRIL/TNFSF13), B-cell activating factor (BAFF/TNFSF13B), LIGHT<sup>1</sup> (TNFSF14), soluble TNF receptor 1 (sTNFR1/TNFRSF1A), soluble TNF receptor 2 (sTNFR2/TNFRSF1B), and soluble CD30 (sCD30/TNFRSF8) were assessed by multiplex bead array assay (Bio-Rad Laboratories, USA). The analysis was performed according to manufacturer's instructions. Fluorescence was measured on a two-beam laser automated analyzer Bio-Plex® 200 system. Data were acquired with Bio-Plex Manager Software 4.0. The values below the detection limit were set to zero.

Real-time CGM was performed with MMT-722 or MMT-754 monitoring systems and CareLink® Pro software (Medtronic, USA). Calibration was performed using a glucose meter One Touch Verio® (Lifescan, USA). Median CGM duration was 5.5 days (range: 2.7 – 10.5 days). Time In the target Range (TIR, 3.9 – 10.0 mmol/L), Time Above Range (TAR, >10.0 mmol/L), Time Below Range (TBR, <3.9 mmol/L) were estimated according to International Consensus on Use of Continuous Glucose Monitoring [9]. Coefficient of Variation (CV), Mean Amplitude of Glycemic Excursions (MAGE), and Mean Absolute Glucose change (MAG) were calculated for assessment of GV. Clinical significance of these parameters was reviewed recently [10,11]. The GV parameters were estimated with the use of EasyGV v. 9.0.R2 calculator [12]. The values of TIR >70% were considered as targeted and values of CV >36% as indicators of unstable glycemic control [9].

**Statistical analysis:** Statistics 12.0 software package (Dell, Round Rock, TX, USA) was used for analysis. Quantitative data are presented as medians (lower quartiles; upper quartiles); frequencies are presented as percentages (%). The Kolmogorov–Smirnov (KS) test was applied to test the normality of data distribution. As many parameters were not distributed normally, non-parametric Mann-Whitney U-test were used for the group comparisons. Spearman rank correlation analysis and stepwise multiple regression analysis with forward selection of variables

were applied to test the association between studied parameters. P values <0.05 were considered significant.

## Results.

**Clinical characteristics of the study participants:** Fifty-eight subjects with T1D, 28 women and 30 men, aged from 18 to 68 years (median 31 years), were included. Forty-two subjects had normal body mass index (BMI), fourteen were overweight (BMI 25-29.9 kg/m<sup>2</sup>), and two individuals had obesity (BMI >30 kg/m<sup>2</sup>). Twenty-eight patients received insulin in multiple daily injections and thirty were on continuous subcutaneous insulin infusion. The list of complications and associated diseases included diabetic retinopathy (n=31), chronic kidney disease (n=28), peripheral neuropathy (n=55), autonomic neuropathy (n=40), non-alcoholic fatty liver disease (n=8), dyslipidemia (n=44), arterial hypertension (n=14), and coronary artery disease (n=1). Clinical characteristics of patients are presented in Table 1.

The control group included 19 subjects with normal glucose tolerance (median HbA1c 5.1%, or 32 mmol/L), 8 men and 11 women, from 18 to 64 years of age (median 33 years).

Serum levels of TNFSF and TNFRSF molecules. Patients with T1D had markedly increased serum levels of TNF- $\alpha$  and decreased levels of LIGHT as compared to control (Table 2). The levels of sCD30 tended to be increased in subjects with diabetes. Other molecules demonstrated no statistical differences between the groups.

**Table 1.** Clinical characteristics of patients with T1D.

| Parameter                                    | Median (25; 75 percentile) |
|--|----------------------------|
| Age, years                                   | 31 (26; 39)                |
| BMI, kg/m <sup>2</sup>                       | 23 (21; 26)                |
| Waist circumference, cm                      | 78 (72; 87)                |
| Waist-to-hip ratio                           | 0.86 (0.82; 0.90)          |
| Diabetes duration, years                     | 15 (6; 19)                 |
| Daily insulin dose, IU/kg                    | 0.6 (0.56; 0.8)            |
| HbA1c, %                                     | 7.0 (6.2; 7.5)             |
| HbA1c, mmol/mol                              | 53 (44; 59)                |
| hsCRP, mg/mL                                 | 1.24 (0.75; 2.54)          |
| eGFR, mL/min/1.73 m <sup>2</sup>             | 96 (88; 105)               |
| Urinary albumin-to-creatinine ratio, mg/mmol | 0.4 (0.2; 0.7)             |
| Hemoglobin, g/L                              | 139 (129; 152)             |
| Neutrophil-to-lymphocyte ratio               | 1.72 (1.14; 2.49)          |

**Table 2.** Serum levels (pg/mL) of TNFSF cytokines and TNFRSF molecules in subjects with T1D and individuals with normal glucose tolerance.

| Molecule      | Groups            |                   | P    |
|---------------|-------------------|-------------------|------|
|               | Control (n = 19)  | T1D (n = 58)      |      |
| TNF- $\alpha$ | 2.1 (0.6; 8.6)    | 5.6 (1.8; 62)     | 0.03 |
| TNF- $\beta$  | 183 (9.8; 192)    | 204 (5.7; 234)    | 0.31 |
| TWEAK         | 222 (174; 275)    | 265 (183; 314)    | 0.22 |
| APRIL         | 4665 (2931; 5392) | 4148 (2734; 7836) | 0.69 |
| BAFF          | 1963 (1732; 2145) | 1702 (1345; 2179) | 0.34 |
| LIGHT         | 3.7 (0.6; 7.8)    | 0.6 (0; 4.5)      | 0.02 |
| sTNFR1        | 273 (180; 344)    | 243 (155; 412)    | 0.75 |
| sTNFR2        | 88 (73; 103)      | 93 (70; 121)      | 0.65 |
| sCD30         | 213 (171; 271)    | 299 (195; 386)    | 0.07 |

Associations of circulating TNFSF and TNFRSF molecules with clinical and laboratory parameters in individuals with T1D. In patients with diabetes, APRIL and sTNFR1 demonstrated weak positive correlations with age ( $r=0.28$ ,  $p=0.03$  and  $r=0.31$ ,  $p=0.03$ , respectively). Both sTNFR1 and sTNFR2 correlated positively with BMI ( $r=0.38$ ,  $p=0.003$  and  $r=0.28$ ,  $p=0.03$ , respectively), waist circumference ( $r=0.37$ ,  $p=0.004$  and  $r=0.30$ ,  $p=0.02$ ) and waist-to-hip ratio ( $r=0.34$ ,  $p=0.009$  and  $r=0.37$ ,  $p=0.004$ ). Overweight or obese patients had higher levels of sTNFR1 and sTNFR2 than those with normal body weight (sTNFR1: 396, 233 – 521 and 178, 137 – 318 pg/mL respectively,  $p=0.01$ ; sTNFR2: 118, 98 – 154 and 81, 65 – 112 pg/mL respectively,  $p=0.02$ ).

When studying associations with inflammatory markers, a positive correlation between BAFF and hsCRP was recorded ( $r=0.38$ ,  $p=0.003$ ) and sTNFR1 correlated weakly with neutrophil-to-lymphocyte ratio ( $r=0.27$ ,  $p=0.04$ ).

No associations were found between studied molecules and diabetes duration. Patients with diabetic retinopathy when compared to those without showed increased serum levels of APRIL (6585, 2932 – 12477 vs. 3649, 2280 – 4999 pg/mL,  $p=0.008$ ). Individuals with decreased eGFR ( $<60$  mL/min/1.73 m<sup>2</sup>) compared to the patients with normal renal function had significantly higher concentrations of APRIL (51370, 13250 – 89489 vs. 4079, 2694 – 6584 pg/mL,  $p=0.03$ ), BAFF (4051, 3293 – 4810 vs. 1643, 1345 – 2121 pg/mL,  $p=0.03$ ), sCD30 (639, 485 – 794 vs. 298, 186 – 383 pg/mL,  $p=0.04$ ). sTNFR1 demonstrated a negative correlation with eGFR ( $r=-0.28$ ,  $p=0.03$ ) and sCD30 correlated positively with serum creatinin ( $r=0.35$ ,  $p=0.004$ ). We were not able to detect any differences in the levels of studied molecules depending on other complications. The level of TNF- $\alpha$ , but not those of other molecules, correlated with HbA1c ( $r=0.28$ ,  $p=0.03$ ).

CGM-derived TIRs and GV parameters in subjects with T1D are summarized in Table 3.

Patients with TIR  $<70\%$  had significantly higher levels of TNF- $\alpha$  and TNF- $\beta$  and lower concentrations of BAFF and LIGHT compared to control (Table 4). In patients with TIR  $>70\%$  levels of all studied molecules, with exception of LIGHT, did not differ from control. There were significant differences between patients with TIR  $<70\%$  and TIR  $>70\%$  in the levels of TNF- $\alpha$ , APRIL, and BAFF.

Patients with high CV values ( $>36\%$ ) did not show any significant differences with those with lower CV in the concentrations of studied molecules (all  $p>0.05$ ). In correlation analysis, TNF- $\alpha$  demonstrated weak positive relationships with mean monitored glucose ( $r=0.27$ ,  $p=0.04$ ) and TAR ( $r=0.26$ ,  $p=0.048$ ). APRIL correlated positively with TAR ( $r=0.30$ ,  $p=0.02$ ). Besides, there was a negative correlation between APRIL and TIR ( $r=-0.33$ ,  $p=0.01$ ). BAFF showed negative correlations with mean monitored glucose ( $r=-0.29$ ,  $p=0.03$ ) and TAR ( $r=-0.34$ ,  $p=0.009$ ), this molecule also correlated positively with TIR ( $r=0.35$ ,  $p=0.007$ ). No significant correlations were found between the studied molecules and TBR.

When studying associations with GV parameters, APRIL showed a positive correlation with CV ( $r=0.32$ ,  $p=0.01$ ) and BAFF correlated negatively with CV and MAGE ( $r=-0.27$ ,

**Table 3.** CGM parameters in subjects with T1D.

| Parameter   | Median (25; 75 percentile) |
|---|----------------------------|
| Mean glucose, mmol/L  | 6.7 (6.2; 7.8)             |
| TIR, %  | 86 (72; 93)                |
| TAR, %  | 9.3 (3.3; 15.8)            |
| TBR, %  | 1.9 (0.7; 3.7)             |
| CV, %   | 32 (26; 36)                |
| MAGE, mmol/L  | 3.5 (2.6; 4.6)             |
| MAG, mmol $\times$ h <sup>-1</sup> $\times$ L <sup>-1</sup> | 1.87 (1.50; 2.47)          |

**Table 4.** Serum levels (pg/mL) of TNFSF and TNFRSF molecules in subjects with T1D depending on TIR.

| Molecule      | Groups             |                        | P         |
|---------------|--------------------|------------------------|-----------|
|               | TIR $>70\%$ (n=42) | TIR $\leq 70\%$ (n=16) |           |
| TNF- $\alpha$ | 3.2 (0; 6.8)       | 7.4 (2.8; 7.0)***      | 0.008     |
| TNF- $\beta$  | 180 (6.4; 225)     | 209 (4.7; 291)*        | 0.43      |
| TWEAK         | 262 (184; 295)     | 284 (181; 369)         | 0.35      |
| APRIL         | 3685 (2531; 5713)  | 7865 (3916; 12864)     | 0.01      |
| BAFF          | 1952 (1408; 2313)  | 1416 (1204; 1672)**    | $<0.0001$ |
| LIGHT         | 0.6 (0; 5.4)*      | 0 (0; 2.1)*            | 0.25      |
| sTNFR1        | 213 (139; 394)     | 309 (162; 477)         | 0.35      |
| sTNFR2        | 90 (66; 121)       | 95 (74; 125)           | 0.73      |
| sCD30         | 316 (196; 386)     | 276 (151; 446)         | 0.75      |

\*  $P < 0.05$  vs. control, \*\*  $P < 0.01$  vs. control, \*\*\*  $P < 0.001$  vs. control.

**Table 5.** Predictors of serum levels of TNFSF and TNFRSF molecules in subjects with T1D in multiple regression analysis.

| Molecule      | Significant predictors                        | Parameters of the model |       |
|---------------|---|-------------------------|-------|
|               |   | R <sup>2</sup>          | P     |
| TNF- $\alpha$ | HbA1c ( $\beta=0.235$ )                       | 0.09                    | 0.03  |
| APRIL         | eGFR ( $\beta=-0.51$ ), BMI ( $\beta=-0.38$ ) | 0.39                    | 0.002 |
| BAFF          | TIR ( $\beta=0.326$ )                         | 0.21                    | 0.007 |
| sTNFR1        | BMI ( $\beta=0.275$ )                         | 0.22                    | 0.02  |
| sCD30         | eGFR ( $\beta=-0.33$ )                        | 0.11                    | 0.01  |

$p=0.04$  and  $r=-0.29$ ,  $p=0.04$  respectively). We did not find any correlations between the studied molecules and MAG.

In the models of stepwise multiple regression analysis with inclusion of age, BMI, eGFR, HbA1c, TIR, TAR, CV, and MAGE as independent variables, HbA1c turned out to be predictor for TNF- $\alpha$ , eGFR predicted sCD30 and APRIL, BMI was predictor for APRIL and sTNFR1, and TIR was associated with BAFF (Table 5). However, we were unable to generate any valid models for TNF- $\beta$ , TWEAK, LIGHT, and sTNFR2.

## Discussion.

In this study, we assessed serum levels of TNFSF cytokines (TNF- $\alpha$ , TNF- $\beta$ , APRIL, BAFF, and LIGHT) and TNFRSF molecules (sTNFR1, sTNFR2, and sCD30) in patients with T1D depending on their clinical and CGM parameters. A particular area of our interest was GV and its relation to the studied regulators. When assessing GV, we took into account time-dependent characteristics (assessed by TIRs), the dispersion of glucose values around the mean (CV), the amplitude of glucose fluctuations (MAGE), and the rate of changes in glucose levels (MAG). One of the hypotheses tested was that GV, as a risk factor for vascular diabetic complications [11,13,14], could be

associated with changes in production of TNFSF and TNFRSF peptides.

The results indicate that the shifts in the levels of TNFSF and TNFRSF molecules in patients with T1D are differently associated with body weight, microvascular complications, inflammatory markers, hyperglycemia and GV. First of all, we found a significant increase in concentrations of TNF- $\alpha$  in subjects with diabetes and its association with hyperglycemia. These findings confirm earlier reports of an association between TNF- $\alpha$  levels and long-term indicators of glycemic control in patients with T1D [15-17]. In addition, we have shown the correlation of mean monitored glucose and TAR with serum TNF- $\alpha$ . Accordingly, patients with non-targeted TIR values had higher serum TNF- $\alpha$ . The findings are in agreement with previously reported data on the stimulating effect of high glucose on TNF- $\alpha$  production by human peripheral blood mononuclear cells [18].

TNF- $\alpha$  is usually considered as a pro-inflammatory cytokine. The effects of TNF- $\alpha$  are mediated by two main receptors: TNFR1 and TNFR2. It was found that TNFR1 is expressed in most cell types, whereas TNFR2 is restricted to particular cells, including endotheliocytes, fibroblasts, and immune cells. Effects mediated by the two receptors are quite different: TNFR1 signaling tends to be pro-inflammatory and apoptotic, whereas TNFR2 signaling is mostly anti-inflammatory and promotes cell proliferation [19]. In our study, we did not find any differences in the levels of receptors between subjects with normal glucose tolerance and T1D, or any relationships between the levels of receptors and glycemic control parameters. At the same time, in patients with diabetes, concentrations of both receptors correlated with body weight and waist-to-hip ratio. It is known that both TNF and its receptors are expressed in adipocytes; expression of TNF- $\alpha$  and TNFR2 has been reported to be increased in obese individuals, and TNFR1 expression is higher in visceral than in subcutaneous adipose tissue [20]. The raised level of TNF- $\alpha$  induces insulin resistance in adipocytes and other peripheral tissues by impairing the insulin signaling [21]. Moreover, anti-TNF- $\alpha$  treatment can reduce insulin resistance and improve glycemic status in patients with rheumatic diseases [22] and type 2 diabetes (T2D) [23]. Thus, increased TNF- $\alpha$  signaling induced by hyperglycemia and/or obesity may contribute to the development of insulin resistance. The latter, in turn, exacerbates hyperglycemia, closing a vicious circle. We found a negative correlation between sTNFR1 and eGFR. In the Diabetes Control and Complications Trial / Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) cohort, serum sTNFR1 was found to be an independent risk factor for macroalbuminuria and decreased renal function in patients with T1D [24,25]. Therefore, we could speculate that TNF- $\alpha$  signaling may be a link between hyperglycemia, obesity, insulin resistance and vascular complications in T1D.

Patients with TIR <70%, but not those with targeted TIR values, had significantly higher levels of TNF- $\beta$  compared to control. TGF- $\beta$  (TNFSF1) has been proved to inhibit the production and function of effector T cells and antigen-presenting dendritic cells, and can regulate natural killer cells, macrophages, dendritic cells, and granulocytes to inhibit inflammation.

Interestingly, TNF- $\alpha$  and TGF- $\beta$  can influence each other's production and signaling [26]. Therefore, it is possible that an imbalance between the production and signaling of TNF- $\alpha$  and TNF- $\beta$  in T1D may contribute to immune dysfunction and low-grade inflammation.

TWEAK (TNFSF12) is a cytokine that controls many cellular events including proliferation, migration, differentiation, apoptosis, angiogenesis, and inflammation. Membrane bound and soluble forms of TWEAK are produced and secreted by numerous cell types, mostly by monocytes [27]. The exact role of TWEAK in the pathophysiology of diabetes is unknown. Contrary to previously published data [28], we did not find a reduction in TWEAK levels in subjects with T1D. Serum TWEAK was not associated with TIRs and GV parameters.

In the panel of assessed molecules, the APRIL-BAFF system is of particular interest. Both molecules can be produced by dendritic cells, monocytes, macrophages, and T cells. APRIL and BAFF bind to two receptors, TACI (TNFRSF13B) and BCMA (TNFRSF17), having different affinity for them. These regulators are critical for the differentiation, survival, and function of B cells and the maintenance of humoral immunity. Recent studies identified overlapping signaling cascades between APRIL and BAFF and discovered their role in autoimmune diseases and cancer [6,29]. There are very few data on serum APRIL and BAFF levels in subjects with diabetes. A decrease in the levels of APRIL in T1D patients was reported [30], which does not correspond to our data. In pediatric patients with T1D, serum BAFF levels did not differ from healthy control, however, BAFFR-expressing B and T cell numbers were reduced, with the exception of patients with recent-onset disease demonstrating increase in these parameters [31]. It was shown that transient BAFF blockade inhibits T1D development in non-obese diabetic mice [32]. This suggests the pathogenic role of this molecule in T1D. In our patients, APRIL, similar to TNF- $\alpha$ , demonstrated associations with low TIR and hyperglycemia. Besides, it was associated with the presence of vascular complications (diabetic retinopathy and chronic kidney disease). We found diverse relationships between CV and serum APRIL and BAFF. Interestingly, patients with T1D with TIR <70% had lower BAFF concentrations than those with TIR >70%. Since the effect of glucose and its fluctuations on the production of the discussed regulators has not been studied, the nature of these findings requires further research.

LIGHT (TNFSF14) was the only factor in our study that showed a decrease in its serum level. LIGHT is primarily expressed in inflammatory effector cells, including dendritic cells, natural killer cells, macrophages, neutrophils, innate lymphoid cells, and CD4 and CD8 memory T cells. Dysregulation of the LIGHT network is considered to be a pathogenic mechanism in some autoimmune and inflammatory diseases, including COVID-19 pneumonia and inflammatory bowel diseases. It was also shown that LIGHT signaling affects adipocyte differentiation [33]. In subjects with different degrees of obesity and glucose intolerance serum LIGHT was positively associated with BMI, fat mass, and HbA1c, and patients with morbid obesity and T2D had increased LIGHT levels [34]. In this study, we did not find any relationships between LIGHT concentration, BMI, and

glycemic control parameters in subjects with T1D. Thus, the value of circulating LIGHT in diabetes needs further research.

We recorded a tendency to increase in the concentration of sCD30 in patients with T1D. The transmembrane receptor CD30 (TNFRSF8) and its ligand CD30L (CD153, TNFSF8) are expressed in subpopulations of activated T- and B-cells. Upregulation of CD30 expression was found in some hematological malignancies, autoimmune and inflammatory diseases [35]. CD30-mediated signaling is related to the nuclear factor-kappa B (NFkB) stimulation and mitogen-activated protein kinase/extracellular signal-regulated kinase pathways [36]. The expansion of the DR<sup>+</sup> CD30<sup>+</sup> subpopulation of activated T cells was reported in new-onset human T1D [37], which is consistent with experimental data on the involvement of the CD30 – CD30L system in the pathogenesis of autoimmune diabetes [38]. We found significantly increased concentration of sCD30 in T1D patients with reduced renal function. To our knowledge, changes in sCD30 levels in diabetic patients with chronic kidney disease have not been previously reported. At the same time, it was demonstrated that high serum sCD30 levels are associated with inflammatory disorders in end-stage renal disease [39] and poor outcome in renal transplantation [40].

Several scenarios can be considered to explain the changes in the cytokine production in T1D. First of all, the autoimmune nature of the disease must be taken into account. As noted above, some of the cytokines studied are involved in the pathogenesis of autoimmune beta cell damage. However, in our sample, most patients had a long diabetes duration and low residual beta cell function (data not shown). Therefore, it is hardly possible to assume the presence of an active autoimmune process at this stage of the disease. Damaged target organs may be a source of excess cytokines in the bloodstream in patients with diabetes. However, we did not include patients with advanced diabetic complications, such as end-stage renal disease or foot ulcers, in this study. Finally, cytokine imbalance may be a consequence of the stimulatory effect of hyperglycemia and GV on inflammatory pathways. Obesity, which develops over time in some patients with T1D, may exacerbate this effect. We received indirect confirmation of this hypothesis in our study.

Our study is not without limitations. Cross-sectional design does not prove causal relationships between parameters. A limited sample size could lead to a type II error in statistical hypotheses testing. The duration of CGM was quite short. At the same time, this is the first study to evaluate the changes in a panel of circulating TNFSF and TNFRSF molecules in T1D patients in relation to clinical data and CGM parameters. Future studies are needed to clarify the pathogenic and clinical significance of these regulators in diabetes.

### Conclusion.

Individuals with T1D demonstrate changes in the levels of circulating TNFSF and TNFRSF peptides associated with metabolic and clinical parameters. Serum levels of TNF- $\alpha$  are increased and LIGHT levels are decreased in subjects with T1D compared to non-diabetic control. TNF- $\alpha$  is associated with hyperglycemia (TAR and HbA1c), while BAFF is negatively related to TIR. APRIL and BAFF demonstrate opposite correlations with GV. The levels of sTNFR1 and sTNFR2 are

higher in obese T1D subjects, meantime, APRIL, BAFF and sCD30 are increased in those with diabetic nephropathy with declined kidney function. The results show the relationships between peptides of the TNFSF and TNFRSF, hyperglycemia, GV, obesity, and diabetic complications in T1D. Some of these peptides (TNF- $\alpha$ , APRIL, BAFF, sTNFR1, and sCD30) can be considered as promising biomarkers for assessing metabolic and vascular risk in subjects with T1D.

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