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ASSESSMENT OF PROGRANULIN AND FAM19A5 PROTEIN BLOOD LEVELS IN PATIENTS WITH METABOLIC SYNDROME

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Progranulin and family with sequence similarity 19, member A5 (FAM19A5) protein are adipokines with growing importance in the context of metabolic diseases. The study aimed to determine the serum concentration of progranulin and FAM19A5 in people with metabolic syndrome (MS) compared to those without MS. The concentration of progranulin and FAM19A5 was determined in 45 people with MS (group A) and in 35 healthy people without MS (group B). Body composition analysis, blood pressure, blood oxygen saturation and anthropometric measurements were performed. There were no differences in the blood levels of progranulin and FAM19A5 between the groups. In group A, the level of progranulin was 29.25±36.92 pg/ml and in group B it was 46.00±60.12pg/ml (p=0.2693). The level of FAM19A5 was 163.16±55.11 pg/ml and 197.57±112.89 pg/ml (p=0.1341) in subjects with and without metabolic syndrome, respectively. In group A, there was a correlation between FAM19A5 and diastolic blood pressure (DBP) (R= –0.40) and high-density lipoprotein (HDL) level (R= –0.37). In group B, correlations were found between progranulin and waist circumference (R= –0.43) and progranulin and triglyceride (TG) levels (R= –0.42). Both groups together showed correlations between progranulin level and body mass index (R= –0.24), HDL (R=0.25) and TG levels (R= –0.25) and between FAM19A5 level and DBP (R= –0.34). In conclusion, patients with and without MS do not differ in the range of progranulin and FAM19A5 serum levels. In patients with MS, elevated FAM19A5 serum levels may be an indicator of dyslipidaemia development. FAM19A5 appears to be a better predictor of MS than progranulin.

Key words: *family with sequence similarity 19, member A5, progranulin, diabetes, dyslipidemia, metabolic syndrome, obesity, blood pressure, triglycerides, oxygen saturation, adipokines*

INTRODUCTION

In the last few years, there have been numerous reports on the newly discovered progranulin cytokine and the family with sequence similarity 19, member A5 (FAM19A5) protein. Progranulin is a multifunctional regulatory protein synthesised in the process of spermatogenesis and secreted by cells such as adipocytes, macrophages and chondrocytes. The clinical significance of progranulin became particularly evident in 2006 when it was found that heterozygous mutations in the *GRN* gene encoding progranulin on chromosome 17 lead to haploinsufficiency and are one of the main causes of temporo-frontal dementia (1-4). Today, it is known that full-length progranulin, through the reduction in the concentration of pro-inflammatory cytokines and the increase in the number of anti-inflammatory cytokines, has a growth-promoting, neuroprotective and anti-inflammatory effect. On the other hand, granulin peptides seem to have pro-inflammatory properties (1, 5, 6). Through its growth-stimulating function, progranulin participates in processes such as wound healing, bone growth and reconstruction and angiogenesis (1, 7).

The recently described neurotrophic and neuroimmuno-modulatory properties of progranulin are still not well understood, but the inclusion of a progranulin substitute in therapeutic management seems promising; the lentiviral vector delivery of progranulin to brain regions associated with the disease has been found to significantly reduce the severity of Parkinson's disease and Alzheimer's disease phenotypes in mice (1, 8, 9). The newly discovered adipokine is also not indifferent in oncology. It has been proven that many tumours express progranulin at levels that are much higher than in normal tissue, which may be of value in cancer diagnosis and prognosis; this also implies the important role of progranulin in cancer progression. There are scientific studies that prove the importance of progranulin in the neoplastic process and indicate the possibility of using it in innovative therapies (1, 10).

It has not yet been proven that progranulin plays an important role in the link between obesity, insulin resistance and metabolic syndrome in childhood (11). Therefore, investigating the relationship between progranulin and metabolic syndrome (MS) markers, in particular with the addition of adipose tissue and diet, is important and may form the basis for further research on the use of progranulin in the treatment of diet-related diseases.

In turn, FAM19A5 is an adipokine discovered in 2015 (12). In 2018, it was noticed that the level of FAM19A5 was decreased in obese mice, which is associated with the activation of smooth muscle cells of the blood vessels and the formation of neointima in damaged vessels. Studies in mice have also shown that FAM19A5 has a protective function by activating the receptor 2-G12/13-RhoA sphingosine-1-phosphate. It was suggested that lowering the level of FAM19A5 in obesity may promote the occurrence of cardiometabolic complications. The novel adipokine is associated with various metabolic and cardiovascular risk factors in humans, such as fasting blood glucose, glycated haemoglobin and mean heart rate (HR), suggesting its potential as a biomarker of cardiovascular diseases (13-17).

The expression of the progranulin gene in visceral adipose tissue is increased in obesity and often results in insulin resistance. Obesity increases the risk of developing type 2 diabetes, hypertension, cardiovascular disease, myocardial infarction and some types of cancer (6, 18, 19). Research indicates that the TAFA-1 and TAFA-2 genes encoding the FAM19A5 protein may also be involved in the development of cardiovascular complications in obesity (20).

The aim of the study was to assess the concentration of progranulin and FAM19A5 in the serum of adults with metabolic syndrome. We hypothesised that the serum concentration of progranulin in adults with MS is higher than in adults without MS and that the concentration of FAM19A5 in the serum of adults with MS is lower than in adults without MS. We chose these two proteins to look for new adipocytokines as markers of cardiovascular risk in obese patients.

In this project, we determined the concentration of progranulin and FAM19A5 in the blood serum of Polish adults with metabolic syndrome, which makes the project innovative and unique due to the investigated population.

MATERIALS AND METHODS

Study design

The study was designed as a case-control study. STROBE (strengthening the reporting of observational studies in epidemiology) guidelines were implemented. The study protocol was approved by the Ethics Committee of Poznan University of Medical Sciences in Resolution No. 353/20 and registered at ClinicalTrials.gov (identifier: NCT04451616). The study protocol can be accessed at: https://clinicaltrials.gov/ct2/show/NCT04451616. The study was conducted in accordance with the guidelines included in the Declaration of Helsinki (1975 revision with amendments).

Patients who fulfilled the inclusion criteria and did not meet the exclusion criteria were enrolled into the study. Patients were divided into two groups: group A (n=45) with MS or control group B (n=35) without MS. In all enrolled patients, anthropometric and body composition analyses were performed, blood pressure (BP), pulse and blood oxygen saturation (SpO_2) were measured and blood samples were collected.

The study procedures were conducted in the Department of Treatment of Obesity, Metabolic Disorders and Clinical Dietetics, Poznan University of Medical Sciences, Poznan, Poland. The study was conducted between July 2020 and June 2021.

Participants

Written informed consent was obtained from each patient. The inclusion criteria were as follows: age 18–70 years; MS diagnosed in accordance with the International Diabetes Federation (IDF) and American Heart Association/National Heart, Lung and Blood Institute (AHA/NHLBI) 2009 guidelines (21) (group A); or the lack of MS (group B).

The exclusion criteria were the following: secondary form of obesity; substitution of progranulin and/or FAM19A5 protein; body mass reduction >5% of the initial body mass in the last 3 months prior to recruitment; liposuction and/or other treatments that reduce adipose tissue; pacemaker implantation; cardioverter/cardiac defibrillator implantation; condition after stroke; Alzheimer's disease; frontotemporal dementia or other neurodegenerative diseases; clinically significant neurological disorders; inflammatory autoimmune diseases; lysosomal storage diseases; clinically significant abnormal liver, kidney or thyroid gland function; acute or clinically significant inflammatory process in the respiratory, digestive or urogenital systems; connective tissue diseases or arthritis; active neoplastic disease; occurrence of a cardiovascular event in the 6 months prior to the study, cancer in the 5 years prior to the study, change in the treatment of components of the metabolic syndrome or change of drugs or change of drug doses during the 3 months prior to the study, alcohol or drug addiction; pregnancy or childbirth during recruitment or 3 months before recruitment; current lactation or lactation in the 3 months prior to recruitment; and/or any other condition that the investigators believe would make participation not in the best interest of the patient or could reduce the credibility of the study. Patients' sex and age were self-reported.

Anthropometric and body composition analysis

Anthropometric measurements and body composition analyses were performed in the morning, after a night-long rest and when fasting. During measurements, the patients were wearing light clothes and no shoes. Neck circumference (NC), waist circumference (WC) and hip circumference (HC) measurements were taken to the nearest 0.5 cm with the use of non-stretchable tape. Height measurement was performed to the nearest 0.5 cm using a manual stadiometer (WPT 100/200 OW; Radwag, Radom, Poland). Body mass was measured with the use of electric scales (InBody 370 Body Composition Analyzer, InBodyBldg, Seoul, Korea) to the nearest 0.01 kg. Body mass index (BMI) was calculated using mass and height measurement results. The following formula was used to calculate BMI (22):

$$\text{BMI} = \text{body weight [kg]} / (\text{height [m]} \times \text{height [m]})$$

Overweight was diagnosed in the case of BMI in the range of 25.0–29.99 kg/m^2, obesity in the case of BMI equal or above 30 kg/m^2. The BMI range, which proved the correct body weight, was in the range of 18.50–24.99 kg/m^2 (22).

Body composition was analysed by the method of electrical bioimpedance with the use of the InBody 370 device (InBodyBldg, Seoul, Korea). This measurement determined parameters such as body mass, body fat content, skeletal muscle mass content and total water content.

Blood pressure, pulse and blood oxygen saturation

Blood pressure was measured with a digital electronic tensiometer (model 705IT TM, Omron Corporation, Kyoto, Japan). During the measurements, patients were sitting for >5 minutes in a chair, with their back supported, feet on the floor and arm supported on a desk, with an empty bladder, after relaxation. Caffeine consumption and exercise were not allowed for at least thirty minutes before the measurements. Three measurements were made and the mean was calculated. HR was determined by stethoscopic auscultation of the heart in the same conditions.

Blood SpO_2 measurements were taken on the patient's finger using the non-invasive method of pulsoximetry using a pulse oximeter (23).

Blood sample collection and biochemical analysis

A fasting blood sample was collected into serum separated tubes from the forearm vein, in the morning, after a whole night of sleep and after half an hour in the supine position. After collection and preparation, the serum samples were frozen immediately and stored at –80°C until biochemical analysis.

In the serum samples, the concentrations of progranulin and FAM19A5 protein were determined using enzyme-linked immunosorbent assay (ELISA) and commercial kits (Human PGRN [progranulin] ELISA Kit, ELK8906, ELK Biotechnology Co., Ltd; Human FAM19A5 [Protein FAM19A5] ELISA Kit; ELK8907; ELK Biotechnology Co., Ltd., Wuhan, China). Serum concentration of total cholesterol (TC), high-density lipoprotein (HDL), triglycerides (TG), glucose, creatinine, alanine transaminase (ALAT), aspartate transaminase (AspAT) and gamma-glutamyltransferase (GGT) were determined in the commercial laboratory. The LDL serum concentration was calculated using the Friedewald formula (24). The estimated glomerular filtration rate (eGFR) value was calculated with the use of Modification of Diet in Renal Disease (MDRD) method (25).

Criteria for the diagnosis of metabolic syndrome

Metabolic syndrome was diagnosed according to the common position of the IDF, NHLBI, AHA, World Heart Federation (WHF), International Atherosclerosis Society (IAS) and International Association for the Study of Obesity (IASO). To diagnose metabolic syndrome, any 3 of the 5 following criteria should be met:

- increased waist circumference: ≥80 cm in women and ≥94 cm in men;
- serum triglycerides >150 mg/dl (1.7 mmol/l) or treatment of hypertriglyceridaemia;
- serum HDL <50 mg/dl (1.3 mmol/l) in women and <40 mg/dl (1.0 mmol/l) in men or treatment for this lipid disorder;
- systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg, or treatment of previously diagnosed arterial hypertension;
- fasting plasma glucose ≥100 mg/dl (5.6 mmol/l) or pharmacological treatment of type 2 diabetes (26).

Statistical methods and data anonymization

Data are presented as means ± standard deviation (SD) and medians, first quartile and third quartile for each parameter. The Shapiro-Wilks test was used to check the normality of data distribution. The comparison between study groups was performed with the use of t-test for independent samples (for data with normal distribution) or Mann-Whitney-U-test (for data without normal distribution). Spearman's rank correlation test was performed to identify correlations between data. The results obtained were considered significant when $p<0.05$.

The primary outcome for the study was serum concentrations of progranulin and FAM19A5 protein in subjects with metabolic syndrome (group A) compared to subjects without metabolic syndrome (group B). Secondary outcomes were comparison between group A and group B results of measurement of height, body mass BMI, WC, HC, NC, body fat content, diastolic blood pressure (DBP), systolic blood pressure (SBP), pulse, SpO_2, serum concentrations of TC, HDL, LDL, TG, glucose, C-reactive protein (CRP), creatinine, estimated glomerular filtration rate (eGFR), ALAT, AspAT, GGT.

All patients enrolled in the study were assigned a unique code as an identifier by one study team member. All study documentation and biological samples were marked with only the subject's unique code.

The serum concentration of FAM19A5 was selected as a primary outcome and basis for the calculation of the number of volunteers to be recruited for a suitably powered study. The minimum detectable difference (Study group versus Control group) in the primary outcome has been established. It was calculated that a sample size of at least 46 subjects in the study population would yield at least 80% power in detecting the significant difference at the 0.05 α level. Missing data was not included in the statistical analyses.

RESULTS

In this study, we enrolled 45 and 35 subjects with and without MS, respectively. Group A included 45 patients who met the inclusion criteria and did not meet the exclusion criteria from Outpatient Clinic of Metabolic Disorders and Hypertension, Poznan University Hospital of Lord's Transfiguration. During the screening 38 patients were not included in the study as they did not meet the inclusion criteria or presented exclusion criteria, or because they did not agree to participate in the study.

The mean age was 52.2 years in group A and 43.0 years in group B. All participants from both groups declared that they live in Poland. The study population characteristics are presented in *Table 1*.

Patients' WC, HC, NC, body mass and BMI were higher in group A than group B. The detailed results of anthropometric and body composition analyses are shown in *Table 2*.

There was a statistically significant difference in the levels of HDL, TG and glucose between groups A and B. Detailed results of biochemical analyses are shown in *Table 3*.

Patients' DBP was higher in group A compared to group B, but SpO_2 was higher in group B than in group A. The detailed results are shown in *Table 4*.

Table 1. Study population characteristics.

Parameter	**Group A**	**Group B**	**p-value**
Participants	45	35	–
Female	23.5%	28.8%	–
Age [years] (mean ±SD)	52.2±15.64	43.0±13.20	**0.001**$^{\#}$
Body mass [kg] (mean ±SD)	110.3±3.0	66.6±12.2	**0.001**$^{\#}$
BMI [kg/m^2] (mean ±SD)	37.9±10.8	24.4±5.1	**0.001**$^{\#}$
TC [mmol/l] (mean ±SD)	4.9±1.5	5.3±0.7	0.241*

BMI, body mass index; SD, standard deviation; TC, total cholesterol; *Student's t-test for independent variables, $^{\#}$Mann-Whitney U test.

Table 2. Anthropometric and body composition analysis results.

Parameter	**Group A** (n=45)		**Group B** (n=35)		**p-value**
	mean±SD	**median [Q1; Q3]**	**mean±SD**	**median [Q1; Q3]**	
Height [m]	1.70±0.11	1.72 [1.60; 1.78]	1.68±0.07	1.68 [1.64; 1.72]	0.47622*
Body mass [kg]	110.26±3.02	104.10 [85.00; 134.90]	66.62±12.17	62.55 [59.20; 71.80]	**0.001**#
Body mass index [kg/m^2]	37.92±10.83	35.83 [29.50; 45.40]	24.37±5.12	23.41 [21.84; 24.33]	**0.001**#
Body fat content [kg]	45.72±20.40	38.80 [28.05; 60.02]	18.75±8.06	17.10 [13.80; 20.40]	**0.001**#
Skeletal muscle mass content [kg]	35.44±8.16	35.90 [28.05; 42.65]	25.85±3.53	25.40 [23.00; 27.90]	**0.001**#
Total water content [l]	46.58±10.08	48.10 [37.15; 55.50]	34.58±4.38	34.10 [31.20; 37.50]	**0.001**#
Waist circumference [cm]	114.92±16.18	118.00 [106.00; 121.00]	77.05±12.19	78.00 [70.50; 85.50]	**0.001**#
Hip circumference [cm]	124.33±22.37	126.50 [106.00; 137.00]	99.71±10.99	99.00 [95.50; 103.50]	**0.001**#
Neck circumference [cm]	45.50±5.69	40.50 [39.00; 46.00]	35.87±10.65	34.00 [32.00; 36.50]	**0.001**#

Q1, first quartile; Q3, third quartile; SD, standard deviation; *Student's t-test for independent variables; #Mann-Whitney U test

Table 3. Serum concentration of TC, HDL, LDL, TG, glucose, serum creatinine, ALAT, AspAT, GGT and eGFR.

Parameter	**Group A** (n=45)		**Group B** (n=35)		**p-Value**
	mean±SD	**median [Q1; Q3]**	**mean±SD**	**median [Q1; Q3]**	
Total cholesterol [mmol/l]	4.87±1.52	4.94 [3.90; 5.61]	5.25±0.68	5.38 [4.85; 5.65]	0.2415*
High-density lipoprotein [mmol/l]	1.11±0.30	1.08 [0.83; 1.38]	2.70±0.28	1.72 [1.42; 1.85]	**0.001**#
Low-density lipoprotein [mmol/l]	3.12±1.26	3.37 [2.02; 4.01]	3.15±0.86	3.15 [2.73; 3.00]	0.8989*
Triglycerides [mmol/l]	3.21±5.19	1.85 [1.27; 2.73]	1.07±0.45	1.04 [0.74; 1.35]	**0.001**#
Glucose [mmol/l]	7.12±2.99	6.10 [5.30; 7.23]	5.01±0.50	5.90 [4.72; 5.20]	**0.001**#
Serum creatinine [µmol/l]	86.84±59.12	77.00 [61.00; 84.00]	70.07±11.72	68.50 [63.74; 75.58]	0.3734#
Alanine transaminase [U/l]	32.16±22.02	26.50 [19.00; 39.00]	24.76±14.56	20.00 [16.00; 27.00]	0.8980#
Aspartate transaminase [U/l]	27.09±16.95	22.00 [16.00; 31.00]	22.60±5.35	22.00 [18.50; 26.50]	0.9184#
Gamma-glutamyltransferase [U/l]	53.91±84.35	32.00 [18.75; 54.00]	26.00±13.06	20.00 [14.50; 34.00]	0.1747#
Estimated glomerulalar filtration rate [ml/min]	74.39±22.35	85.00 [66.00; 90.00]	77.42±21.01	90.00 [68.50 90.00]	0.6564#

Q1, first quartile; Q3, third quartile; SD, standard deviation; *Student's t-test for independent variables; #Mann-Whitney U test

There are no significant differences in the levels of progranulin and FAM19A5 between the two groups. Details are shown in *Table 5*.

Data analysis revealed a range of significant correlations between serum levels of progranulin and FAM19A5 with other investigated parameters. In group A, the concentration of FAM19A5 was negatively correlated with DBP (–0.40) and HDL (–0.37). In group B, the level of progranulin was negatively correlated with WC (–0.43) and TG (–0.42). The detailed results of correlation analysis are presented in *Table 6*.

DISCUSSION

In our study, we have investigated serum concentrations of FAM19A5 in subjects with metabolic syndrome for the first time

Table 4. Comparison of systolic blood pressure, diastolic blood pressure, heart rate and oxygen saturation measurement results between group A and group B.

Parameter	**Group A** (n=45)		**Group B** (n=35)		**p-Value**
	mean±SD	**median [Q1; Q3]**	**mean±SD**	**median [Q1; Q3]**	
Systolic blood pressure [mmHg]	130.80±24.26	127.50 [122.00; 143.00]	117.29±16.23	116.00 [103.25; 130.50]	**0.0008**#
Diastolic blood pressure [mmHg]	82.13±12.4	79.00 [75.00; 91.00]	76.04±8.67	74.00 [68.75; 79.25]	**0.0277***
Pulse [x/min]	77.27±12.63	64.50 [70.00; 85.00]	68.97±11.05	71.00 [62.75; 73.75]	**0.0029**#
Oxygen saturation [%]	93.16±9.38	89.00 [95.00; 98.00]	97.48±2.10	98.00 [97.50; 99.00]	**0.0003**#

Q1, first quartile; Q3, third quartile; SD, standard deviation; *Student's t-test for independent variables; #Mann-Whitney U test.

Table 5. Serum level of progranulin and FAM19A5.

Parameter	**Group A** (n=45)		**Group B** (n=35)		**p-Value**
	mean ± SD	**median [Q1; Q3]**	**mean ± SD**	**median [Q1; Q3]**	
Progranulin [pg/ml]	29.25 ± 36.92	14.68 [12.71; 21.12]	46.00 ± 60.12	16.18 [12.89; 48.78]	0.2693#
FAM19A5 [pg/ml]	163.16 ± 55.11	154.62 [124.09; 180.93]	197.57 ± 112.89	169.92 [133.38; 204.64]	0.1341#

Q1, first quartile; Q3, third quartile; SD, standard deviation; #Mann-Whitney U test.

Table 6. Significant correlations of progranulin and FAM19A5 serum levels with other investigated parameters.

Correlations in group A		
Parameters	**Spearman's R value**	**p-Value**
FAM19A5 & Diastolic blood pressure	–0.40	**0.001**
FAM19A5 & High density lipoprotein	–0.37	**0.001**
Correlations in group B		
Parameters	**Spearman's R value**	**p-Value**
Progranulin & Waist circumference	–0.43	**0.001**
Progranulin & Triglycerides	–0.42	**0.001**
Correlations in the whole study population (group A and B together)		
Parameters	**Spearman's R value**	**p-Value**
Progranulin & Body mass	–0.23	**0.001**
Progranulin & Body mass index	–0.24	**0.001**
Progranulin & High density lipoprotein	0.25	**0.001**
Progranulin & Triglycerides	–0.25	**0.001**
FAM19A5 & Diastolic blood pressure	–0.34	**0.001**

Data are presented as Spearman's R value.

to our knowledge in humans. We found no significant differences between group A and group B in the range of FAM19A5 and progranulin serum levels, thus we did not confirm our preliminary hypothesis. However, we found correlations between serum levels of FAM19A5 and DPB and FAM19A5 and HDL in patients with MS. Furthermore, we found some interesting correlations between serum concentrations of progranulin with WC and TG levels in the control group and correlations between serum levels of progranulin and body mass, BMI, HDL and TG serum levels in the entire study population. We also found correlations between serum levels of FAM19A5 and DBP in the entire study population. As new markers of cardiovascular risk are still urgently sought to determine a patient's cardiovascular state in an increasingly precise manner, in light of our results, serum FAM19A5 and progranulin levels may be considered as new useful tools in this scientific area; in this context, our research appears innovative.

Several publications present an association between progranulin and components of MS. Based on previous studies,

it can be stated that progranulin is involved in the inflammation process and glucose and insulin homeostasis, as well as impacting on metabolic parameters. Studies on progranulin gene expression in adipose tissue show that this parameter, next to progranulin blood concentrations, is positively correlated with components of MS such as visceral obesity, insulin resistance, type 2 diabetes and dyslipidaemia (27-31). Moreover, progranulin levels in the blood are positively correlated with WC, DBP, SBP, CRP, glucose, TG and total cholesterol levels, BMI, body fat mass, visceral fat and pro-inflammatory interleukins such as interleukin-6 (IL-6) or tumor necrosis factor-alpha (TNF-α) levels (27-31). Youn *et al*. observed that progranulin serum concentrations were significantly higher in individuals with type 2 diabetes and obese 209 subjects with predominant visceral fat accumulation in comparison to 60 normal glucose tolerance subjects (27), while Xu *et al*. (28) showed that concentrations of progranulin and other inflammatory markers are elevated in the sera of 84 type 2 diabetes patients with microangiopathy. Furthermore, in the second-mentioned study, serum progranulin levels had remarkable positive correlations with the levels of inflammatory markers such as TNF-α, IL-6 and white blood cells and were associated with obesity, lipid metabolism disorders and hypertension (27, 28). On the other hand, Niklowitz *et al*. did not observe any differences between progranulin levels in 88 obese and 23 normal-weight children. The authors stated that progranulin was not associated with homeostatic model assessment-insulin resistance (HOMA-IR) or transaminases (11). Similarly, Shafaei *et al*. demonstrated that there were no significant differences in serum progranulin levels in 60 type 2 diabetes patients with and without MS and 30 healthy controls. Furthermore, in this study, serum progranulin levels were not correlated with any components of MS (31). In the Waluga-Kozlowska *et al*. study, serum levels of progranulin and chemerin in obese subjects with type 2 diabetes were higher compared to healthy subjects (32). These peptides and their relationship to each other may be involved in the pathomechanism of type 2 diabetes and insulin resistance (32).

One of the key findings of our study is the negative correlation between progranulin serum levels and WC in non-MS patients. While WC is one of the diagnostic criteria of MS, our study shows that possibly low progranulin serum concentrations could be a prognostic marker of developing MS in non-MS patients. This could allow the implementation of prevention intervention in non-MS subjects characterized by low progranulin serum levels. Furthermore, elevated WC is a significant marker of cardiovascular risk (34). Moreover, in non-MS and in the entire study population, lower progranulin blood levels correlated with higher TG levels, which is a marker of atherosclerosis development (34). In non-MS patients, this correlation was stronger than in the whole study population. Furthermore, progranulin and HDL levels were positively correlated in the entire population. Since low HDL levels and high TG levels are markers of increased cardiovascular risk, it is possible that a lower progranulin level could be a marker for elevated risk of cardiovascular diseases. Furthermore, in the whole population, we observed that cardiovascular risk markers - body mass and BMI were negatively correlated with progranulin serum concentrations. Moreover, especially in group A, progranulin may serve as an early marker of atherosclerosis. Taking all of these findings together, we can presume that low progranulin serum levels should be considered a cardiovascular risk marker in non-MS rather than MS subjects. Dyslipidemia, and consequently atherosclerosis, as a component of the MS is one of the factors increasing the risk of acute cardiovascular diseases and stroke. The Lasek-Bal *et al*. study showed a positive correlation between the concentration of progranulin on the first day of stroke and the severity of the functional state on the 30th day of the disease in 138 patients. The authors suggest that progranulin may play a role in this disorder as a pro-inflammatory cytokine (35).

Increased DBP and decreased HDL blood concentrations are among the components of the diagnosis of MS (21). In our study, the serum concentration of FAM19A5 was negatively correlated with DBP and HDL levels in the group of patients with MS. There is no such relationship in the group of patients without MS. There were also no correlations between the concentration of progranulin and the components of MS in the group of MS patients.

Few studies show a correlation of FAM19A5 with the occurrence of metabolic syndrome or with the components of MS (20, 36, 37). In 2019, Lee *et al*. demonstrated that serum concentrations of FAM19A5 were increased in 178 patients with type 2 diabetes compared with 45 subjects without. The serum concentration of FAM19A5 was positively correlated with WC, waist-hip ratio, ALAT, fasting plasma glucose and glycated haemoglobin (20). Our study found no such correlations in people with MS. In the study conducted by Xie *et al.* which included 55 children with obesity and 48 healthy controls, serum level of FAM19A5 was decreased in obese children. The authors suggested that FAM19A5 plays important role in glucose metabolism in obese children (40).

As decreased levels of blood HDL is a marker of lipid disorders, it is possible to hypothesise that high serum levels of FAM19A5 may be a marker of dyslipidemia in MS patients. Dyslipidaemia can lead to atherosclerosis and cardiovascular disease (38), so high blood level of FAM19A5 may be considered as a cardiovascular risk marker in MS patients. Yari *et al*. showed a significant negative correlation between plasma FAM19A5 levels and BMI, visceral fat, ALAT and AspAT in the serum of 37 patients with non-alcoholic fatty liver disease (36). It is worth highlighting the results of research conducted by Wang *et al*. who showed that the FAM19A5 protein was abundantly expressed in adipose tissue in mice with normal mass but was reduced in adipose tissue in obese mice (37).

A low serum concentration of FAM19A5 in patients with MS and the general population is a marker of the risk of increasing DBP and, thus, the development of diastolic hypertension. Diastolic hypertension is a form of hypertension resulting from an increase in arterial stiffness. Such an increase is most common in people with diabetes and dyslipidaemia (MS components) and in the elderly (39). Thus, we can hypothesise that decreased serum levels of FAM may be a risk marker for the development of diastolic arterial hypertension in the general population and especially in MS patients. To summarise, based on our results we can presume that FAM19A5 serum levels may be a better predictor of MS components than progranulin.

However, it should be emphasized that the treatment of the metabolic syndrome should be carried out with the use of pharmacological and non-pharmacological methods. Recent data show the importance of total body electromyostimulation (WB-EMS) training in women with MS. In the Reljic *et al*. study, this training improved overall cardiometabolic risk score, body composition, and muscle strength in 29 women with metabolic syndrome (41). In addition to physical activity, appropriate nutritional strategies should be part of the treatment of metabolic syndrome. The latest data show that the Mediterranean diet is the most effective method of nutrition in obese patients with complications, including cardio-metabolic conditions. However, there is a lack of data on the influence of nutrition on the concentrations of progranulin and FAM19A5 protein in people with the metabolic syndrome (42).

So far, the relationship between the concentrations of progranulin and FAM19A5 in people suffering from COVID-19 has not been established. The data so far show higher levels of IL-6 and C-reactive protein in patients with more severe forms

of COVID-19. Taking into account the fact that patients with obesity and the metabolic syndrome have a higher risk of death, it seems valuable for the future to determine the concentration of these adipokines in the serum of COVID-19 patients (43).

As the findings of many studies suggest a link between FAM19A5, obesity and diabetes, more research is needed to fully clarify the role of FAM19A5 in the development of metabolic syndrome. Subsequent studies should take into account the level of physical activity in patients.

In our work, we investigated for the first time to our knowledge the serum levels of FAM19A5 in humans with MS and determined its associations with parameters of MS. We also demonstrated a range of correlations between serum progranulin levels and some indices of MS. Our study allows progranulin and FAM19A5 serum levels to be considered as new markers for the development of MS components.

The main limitation of our study was the lack of assessment of the influence of individual drugs (*e.g.*, antihypertensive drugs) on the results in people with MS. This issue requires further scientific investigation in the future.

The findings of this study demonstrate that patients with metabolic syndrome do not differ significantly from patients without metabolic syndrome in the range of progranulin and FAM19A5 serum concentrations. However, exclusively in patients with metabolic syndrome, elevated FAM19A5 serum levels may be an indicator of the development of dyslipidaemia. FAM19A5 serum level seems to be a more adequate indicator of metabolic disturbances associated with metabolic syndrome than progranulin blood concentration. The issue of serum levels of FAM19A5 and progranulin in obese patients and their associations with metabolic syndrome development requires further investigation.

Abbreviations: CRP, C-reactive protein; AHA, American Heart Association; ALAT, alanine transaminase; AspAT, aspartate transaminase; BMI, body mass index; BP, blood pressure; BUN, blood urea nitrogen; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; ELISA, enzyme-linked immunosorbent assay; GGT, gamma-glutamyltransferase; HC, hip circumference; HDL, high-densitylipoprotein; HOMA-IR, homeostatic model assessment-insulin resistance; HR, heart rate; IAS, International Atherosclerosis Society; IASO, International Association for the Study of Obesity; IDF, International Diabetes Federation; LDL, low-densitylipoprotein; MDRD, modification of diet in renal disease; NC, neck circumference; NHLBI, National Heart, Lung and Blood Institute; SBP, systolic blood pressure; SD, standard deviation; SpO_2, oxygen saturation; STROBE, Strengthening the reporting of observational studies in epidemiology; TC, total cholesterol; TG, triglycerides; UAER, urinary albumin excretion rate; WBC, white blood cells; WC, waist circumference; WHF, World Heart Federation.

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REFERENCES

1. Bateman A, Cheung ST, Bennett HP. A brief overview of progranulin in health and disease. *Methods Mol Biol* 2018; 1806: 3-15.
2. Jian J, Li G, Hettinghouse A, Liu C. Progranulin: a key player in autoimmune diseases. *Cytokine* 2018; 101: 48-55.
3. Batzu L, Westman E, Pereira JB; Alzheimer's Disease Neuroimaging Initiative. Cerebrospinal fluid progranulin is associated with increased cortical thickness in early stages of Alzheime's disease. *Neurobiol Aging* 2019; 88: 61-70.
4. Paushter DH, Du H, Feng T, Hu F. The lysosomal function of progranulin,a guardian against neurodegeneration. *Acta Neuropathol* 2018; 136: 1-17. doi: 10.1007/s00401-018-1861-8
5. Wei J, Hettinghouse A, Liu C. The role of progranulin in arthritis. *Ann NY Acad Sci* 2016; 1383: 5-20.
6. Gorken U, Inal ZO, Inal HA, Bostanci MO. Serum progranulin levels are elevated in infertile women with obesity. *Endokrynol Polska* 2018; 69: 661-666.
7. Wei J, Zhang L, Ding Y, *et al*. Progranulin promotes diabetic fracture healing in mice with type 1 diabetes. *Ann NY Acad Sci* 2020; 1460: 43-56.
8. Minami SS, Min SW, Krabbe G, *et al*. Progranulin protects against amyloid β deposition and toxicity in Alzheimer's disease mouse models. *Nat Med* 2014; 20: 1157-1164.
9. Van Kampen JM, Baranowski D, Kay DG. Progranulin gene delivery protects dopaminergic neurons in a mouse model of Parkinson's disease. *PLoS One* 2014; 9: e97032. doi: 10.1371/journal.pone.0097032
10. Zhang Y, Bateman A, *et al*. The glycoprotein growth factor progranulin promotes carcinogenesis and has potential value in anti-cancer therapy. *J Carcinogene Mutagene* 2012; S2: doi: 10.4172/2157-2518.S2-001
11. Niklowitz P, Rothermel J, Lass N, Barth A, Reinehr T. Is there a link between progranulin, obesity, and parameters of the metabolic syndrome in children? Findings from a longitudinal intervention study. *Pediatr Diabetes* 2019; 20: 1047-1055.
12. Janvilisri T, Leelawat K, Roytrakul S, Paemanee A, Tohtong R. Novel serum biomarkers to differentiate cholangiocarcinoma from benign biliary tract diseases using a proteomic approach. *Dis Markers* 2015; 2015: 105358. doi: 10.1155/2015/105358
13. Lee YB, Hwang HJ, Kim JA, *et al*. Association of serum. FAM19A5 with metabolic and vascular risk factors in human subjects with or without type 2 diabetes. *DiabVasc Dis Res* 2019; 16: 530-538.
14. Wang Y. Chen D, Zhang Y, *et al*. Novel adipokine, FAM19A5, inhibits neointima formation after injury through sphingosine-1-phosphate receptor 2. *Circulation* 2018; 138: 48-63.
15. Kang D, Kim HR, Kim KK, *et al*. Brain-specific chemokine FAM19A5 induces hypothalamic inflammation. *Biochem Biophys Res Commun* 2020; 19; 523: 829-834.
16. Park M. Kim HS, Lee M, *et al*. FAM19A5, a brain-specific chemokine, inhibits RANKL-induced osteoclast formation through formyl peptide receptor 2. *Sci Rep* 2017; 7: 15575. doi: 10.1038/s41598-017-15586-0

17. Lee HL, Seok HY, Ryu HW, *et al*. Serum FAM19A5 in neuromyelitisoptica spectrum disorders: Can it be a new biomarker representing clinical status? *Mult Scler* 2019; 26: 1700-1707.
18. Yoo HJ, Hwang SY, Hong HC, *et al*. Implication of progranulin and C1q/TNF-related protein-3 (CTRP3) on inflammation and atherosclerosis in subjects with or without metabolic syndrome. *PLoS One* 2013; 8: e55744. doi: 10.1371/journal.pone.0055744
19. Kloting N, Fasshauer M, Dietrich A, *et al*. Insulin-sensitive obesity. *Am J Physiol Endocrinol Metab* 2010; 299: E506-E515.
20. Lee Y-B, Hwang HJ, Kim JA, *et al*. Association of serum FAM19A5 with metabolic and vascular risk factors in human subjects with or without type 2 diabetes. *Diab Vasc Dis Res* 2019; 16: 530-538.
21. Alberti KG, Eckel RH, Grundy SM, *et al*. Harmonizing the metabolic syndrome. *Circulation* 2009; 120: 1640-1645.
22. Body mass index - BMI. [Online] World Health Organization, 5 January 2022. https://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi.
23. Bosch D, *et al*. Pulsoxymetrie. Lungenfunktionsprufung. Berlin, Springer, 2013.
24. Friedewald W. Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; 18: 499-502.
25. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002; 39 (2 Suppl 1): S1-S266.
26. Cabral LS, Cherubini PA, de Oliveira MA, Bianchini L, Torres CM, Bianchin MM. Diagnostic yield and accuracy of different metabolic syndrome criteria in adult patients with epilepsy. *Front Neurol* 2017; 8: 460. doi: 10.3389/fneur.2017.00460
27. Youn BS, Bang SI, Kloting N, *et al*. Serum progranulin concentrations may be associated with macrophage infiltration into omental adipose tissue. *Diabetes* 2009; 58: 627-636.
28. Xu L, Zhou B, Li H, *et al*. Serum levels of progranulin are closely associated with microvascular complication in type 2 diabetes. *Dis Markers* 2015; 2015: 357279.doi: 10.1155/2015/357279
29. Yilmaz Y, Eren F, Yonal O, *et al*. Serum progranulin as an independent marker of liver fibrosis in patients with biopsy-proven nonalcoholic fatty liver disease. *Dis Markers* 2011; 31: 205-210.
30. Qu H, Deng H, Hu Z. Plasma progranulin concentrations are increased in patients with type 2 diabetes and obesity and correlated with insulin resistance. *Mediat Inflamm* 2013; 2013: 360190. doi: 10.1155/2013/360190
31. Shafaei A, Marjani A, Khoshnia M. Serum progranulin levels in type 2 diabetic patients with metabolic syndrome. *Rom J Intern Med* 2016; 54: 211-216.
32. Waluga-Kozlowska E, Kuznik-Trocha K, Komosinska-Vassev K, *et al*. Progranulin and chemerin plasma level in obese patients with type 2 diabetes treated with a long-acting insulin analogue and premixed insulin analogue. *J Physiol Pharmacol* 2021; 72: 895-903.
33. Lo K, Huang YQ, Shen G, *et al*. Effects of waist to height ratio, waist circumference, body mass index on the risk of chronic diseases, all-cause, cardiovascular and cancer mortality. *Postgrad Med J* 2021; 97: 306-311.
34. Nordestgaard B. Triglyceride-rich lipoproteins and atherosclerotic cardiovascular disease: new insights from epidemiology, genetics, and biology. *Circ Res* 2016; 118: 547-563.
35. Lasek-Bal A, Jedrzejowska-Szypulka H, Student S, *et al*. The importance of selected markers of inflammation and blood-brain barrier damage for short-term ischemic stroke prognosis. *J Physiol Pharmacol* 2019; 70: 209-217.
36. Yari FA, Shabani P, Karami S, Sarmadi N, Poustchi H, Bandegi AR. Circulating levels of FAM19A5 are inversely associated with subclinical atherosclerosis in non-alcoholic fatty liver disease. *BMC Endocr Disord* 2021; 21: 153. doi: 10.1186/s12902-021-00820-8
37. Wang Y, Chen D, Zhang Y, *et al*. Novel adipokine, FAM19A5, inhibits neointima formation after injury through sphingosine-1-phosphate receptor 2. *Circulation* 2018; 138: 48-63.
38. Kostis JB. The importance of managing hypertension and dyslipidemia to decrease cardiovascular disease. *Cardiovasc Drugs Ther* 2007; 21: 297-309.
39. Smulyan H, Lieber A, Safar ME. Hypertension, diabetes type II, and their association: role of arterial stiffness. *Am J Hypertens* 2016; 29: 5-13.
40. Xie K, Liu L, Yin C, *et al*. Follistatin-like 1 and family with sequence similarity to 19 member A5 levels are decreased in obese children and associated with glucose metabolism. *Ann Nutr Metab* 2022; Apr 20: doi: 10.1159/000524624.
41. Reljic D, Konturek PC, Herrmann HJ, Neurath MF, Zopf Y. Effects of whole-body electromyostimulation exercise and caloric restriction on cardiometabolic risk profile and muscle strength in obese women with the metabolic syndrome: a pilot study. *J Physiol Pharmacol* 2020; 71: 89-98.
42. Jamka M, Wasiewicz-Gajdzis M, Walkowiak J. Effectiveness of different dietary strategies in the management of obesity and obesity-related comorbidities. *J Med Sci* (*JMS*) 2021; 90: e253. doi:10.20883/medical.e523
43. Kapala P, Versace M, Tuchendler E, Marciniak A, Guzik P. Pulmonary embolism in patients with the Coronavirus Disease 2019. *J Med Sci* (*JMS*) 2022; 91: e602. doi:10.20883/medical.e602

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