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OBESITY IN PATIENTS WITH CHRONIC INFLAMMATORY BOWEL DISEASES

ABSTRACT

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The defense materials are available in the Scientific Department of Medical University - Varna and are published on the website of Medical University - Varna.

Note: In the abstract, the numbers of tables and figures noncorrespond to the numbers in the thesis.

CONTENTS

ABREVIATIONS USED

CD	Crohn's disease
WAT	White adipose tissue
VAT	Visceral adipose tissue
GIT	Gastrointestinal tract
DNA	Deoxyribonucleic acid
T2DM	Diabetes mellitus type 2
mRNA	Information ribonucleic acid
BMI	Body mass index
CLS	Crown-like structures
MS	Metabolic syndrome
NAFLD	Non-alcoholic fatty liver disease
SAT	Subcutaneous adipose tissue
rRNA	Ribosomal ribonucleic acid
CVD	Cardiovascular diseases
tTRA	Transport ribonucleic acid
FCP	Fecal calprotectin
IBD	Chronic inflammatory bowel diseases
UC	Ulcerative colitis
MRI	Magnetic Resonance Imaging
AJ	Adhesive bonds
AJC	Apical junctional complex
APC	Antigen-presenting cells
AZA	Azathioprine
BAT	Brown adipose tissue
CDEIS	Endoscopic Crohn's Disease Severity Index
CRP	C-reactive protein
CT	Computed tomography
DC	Dendritic cells
HDL	High-density lipoprotein
HR	Hazard ratio

IDF	International Diabetes Federation
IFN-g	Gamma-interferon
IL	Interleukin
miRNAs	Microribonucleic acids
MP	6-mercaptopurine
ncRNA	Non-coding ribonucleic acid
NGAL	Neutrophil gelatinase
NOD2	Nucleotide-binding oligomerization domain
OR	Odds ratio
pre-RNA	Precursor miRNA
pri-miRNA	Primary miRNA
PRR	Pattern recognition receptors
RIPK2	Receptor-interacting serine/threonine kinase 2
RISC	RNA-induced silencing complex
RNAi	RNA interference
scRNA	Small cytoplasmic ribonucleic acids
siRNAs	Small interfering ribonucleic acids
snoRNA	Small nucleic ribonucleic acids
snuRNA	Small nuclear ribonucleic acids
STAT	Signal transducer and activator of transcription
TJ	Close ties
UTR	3'-untranslated regions
human Lipocalin-2/ NGAL	Lipocalin 2
TNF-α	Tumor necrosis foctor - apha

I. INTRODUCTION

The nature of the etiology and pathogenesis of inflammatory bowel disease (IBD) are not yet fully understood. Individuals with certain genetic mutations in the presence of an unknown trigger mechanism develop dysbiosis, disruption of the gastrointestinal tract epithelial barrier, and bacterial translocation that irritate the immune system and induce avalanche-like secretion of proinflammatory cytokines with reduced and/or absent release of anti-inflammatory factors [148, 241, 277]. The control, monitoring, and which factors influence immune-mediated inflammation still present a major challenge to gastroenterologists [137].

Traditionally, many clinicians associate IBD with low or subnormal body weight. In recent years, a clear trend has emerged: an increase in the incidence of IBD and a parallel increase in overweight and obesity among these patients. Evidence has emerged in the literature that the increased prevalence of obesity in the general population increases the risk of developing Crohn's disease (CD). On the other hand, about 15-40% of patients with IBD are obese. Metainflammation is a possible contributor to disease progression. Different clinical studies in this field present conflicting data regarding the effect of obesity on the natural history and course of IBD. Adipocyte hypertrophy of intra-abdominal adipose tissue results in metainflammation that induces multiple proinflammatory mediators, including tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and leptin, among others [241]. The increase in these inflammatory mediators can be reflected in elevated levels of C-reactive protein (CRP) and fecal calprotectin (FCP) [65].

The impact of obesity on the efficacy of IBD-related therapies remains a poorly understood area, although data from other autoimmune diseases suggest that obesity

leads to suboptimal response to therapy, rapid clearance of biologic agents, and consequently low plasma concentrations. Therefore, the need has arisen to provide contemporary characterizations of obesity in IBD patients by comparing classical assessment methods and novel biomarkers, and to determine disease activity, the response to convectional therapy, and novel biologic agents in patients with IBD who are overweight and obese.

II. AIM, OBJECTIVES AND HYPOTHESIS

2.1. Aim

The aim of this study was to assess obesity in IBD patients (Crohn's disease and ulcerative colitis).

2.2. Objectives

1. To investigate some anthropometric characteristics in obese patients with Crohn's disease and ulcerative colitis.
2. To investigate the expression of a panel of miRNAs in patients with Crohn's disease and ulcerative colitis according to BMI.
3. To investigate the expression of human Lipocalin-2/NGAL in patients with Crohn's disease and ulcerative colitis.
4. To assess inflammation in IBD patients with obesity by comparing some anthropometric parameters, levels of a panel of circulating miRNAs and serum expression of human Lipocalin-2/NGAL.
5. To assess the circulating levels of a panel of investigational miRNAs and the serum expression of human Lipocalin-2/NGAL according to the activity of CD and UC and the conventional or biologic treatment administered.

2.3. Hypothesis

The expression of a panel of miRNAs and human Lipocalin-2/NGAL may serve as a reliable noninvasive marker to assess the activity and the treatment in overweight and obese CD and UC patients.

III. MATERIAL AND METHODS

3.1. Subject of the study

A panel of miRNAs (hsa-miR-17-5p, hsa-miR-29a-5p, hsa-miR-146a-5p, hsa-miR-142-3p, hsa-miR-155-5p, cel-miR-39-3p) and human Lipocalin-2/NGAL in serum were investigated in patients with proven CD and UC at the stage of disease activity and at the stage of remission achieved with medication.

3.2. Object of study

Seventy-eight IBD patients, divided into 2 groups, 40 with CD (20 with active CD and 20 in remission) and 38 with UC (16 with active UC and 22 in remission), respectively, who went through the Clinic of Gastroenterology at the University Multiprofessional Hospital for Active Treatment "Sv. Marina" - Varna from 02.2021 to 11.2022.

3.3. Specific studies

Serum expression level of miRNAs: hsa-miR-17-5p, hsa-miR-29a-5p, hsa-miR-146a-5p, hsa-miR-142-3p, hsa-miR-155-5p and control cel-miR-39-3p and human Lipocalin-2/NGAL were examined in all participants.

The material used for microRNAs testing was blood serum. Blood serum was obtained by venipuncture with a closed BD Vacutainer™ SST™ II Advance 5 ml system (Becton Dickinson, USA), after sampling the blood was allowed to clot at room temperature for 30 minutes then centrifuged at 1500×g for 15 minutes at room temperature and the serum was separated and divided into 500 µl portions and stored at -80 °C until analysis.

Isolation of miRNAs was performed from 200 µl of serum using a ready-to-use commercial miRNeasy Serum/Plasma Kit (50), catalogue № 217184 (QIAGEN, Germany) according to the manufacturer's protocol, as a normalization control was added to each serum sample 3.5 µl (1.6×10^8 copies per µl) control miRNA C. *elegans* miR-39: miRNeasy Serum/Plasma Spike-In Control, catalogue № 219610 (QIAGEN, Germany),

and the samples were eluted in 25 µl of RNAase-free water.

Each sample was then subjected to reverse transcription using a commercially prepared miRCURY LNA RT Kit, catalog № 339340 (QIAGEN, Germany) according to the manufacturer's protocol of 1.0 µl of eluted miRNA in a final volume of 10 µl and incubation at 42 °C for 60 min and enzyme inactivation at 95 °C for 5 min.

Each of the samples was then subjected to quantitative real-time PCR using a commercially prepared miRCURY LNA SYBR Green PCR Kit (200) and (600), catalog numbers № 339345 (200) and № 339346 (600) (QIAGEN, Germany) and prepared miRCURY LNA miRNA PCR Assay primers, catalog number № 339306 (QIAGEN, Germany) according to the manufacturer's protocol: 0.1 µl complementary DNA (cDNA) in 10 µl reactions in duplicate for 6 target microRNAs in 96-well plates.The primers used are miRCURY LNA miRNA PCR Assay, catalog № 339306 (QIAGEN, Germany) as follows (QIAGEN GeneGlobe reference number is indicated in parentheses): cel-miR-39-3p (YP00203952), hsa-miR-142-3p (YP00204291), hsa-miR-29a-5p (YP00204430), hsa-miR-146a-5p (YP00204688), hsa-miR-17-5p (YP02119304), hsa-miR-155-5p (YP02119311). The temperature parameters used were as follows: holding for 2 minutes at 95 °C to activate the enzyme; 40 cycles of 10 seconds at 95 °C; 60 seconds at 56 °C with fluorescence readout; melting curve analysis to demonstrate amplification specificity: initial denaturation for 15 seconds at 95 °C and cooling to 60 °C for 60 seconds and increasing to 95 °C at a rate of +0.05 °C per second and fluorescence readout. Analysis was performed on a QuantStudio Dx instrument from Applied Biosystems (USA) and a threshold cycle (Ct) was reported for each sample.

The primers used are miRCURY LNA miRNA PCR Assay (100), catalog № 218300 (QIAGEN, Germany) as follows (name and reference number in parentheses):

- hsa-miR-17-5p (MIMAT0000070:5'CAAAGUGCUUACAGUGCAGGUAG; GeneGlobe ID - YP02119304),
- hsa-miR-29a-5p (MIMAT0004503:5'ACUGAUUUCUUUUGGUGUUCAG; GeneGlobe ID - YP00204430),
- hsa-miR-146a-5p (MIMAT0000449:5'UGAGAACUGAAUUCCAUGGGUU; GeneGlobe ID - YP00204688),
- hsa-miR-142-3p (MIMAT0000434:5'UGUAGUGUUUCCUACUUUAUGGA; GeneGlobe ID - YP00204291),
- hsa-miR-155-5p (MIMAT0000646:5'UUAAUGCUAAUCGUGAUAGGGGUU; GeneGlobe ID - YP02119311),
- cel-miR-39-3p (MIMAT0000010: 5'UCACCGGGUGUAAAUCAGCUUG; GeneGlobe ID - YP00203952).

The temperature parameters used were as follows: holding for 15 minutes at 95°C to activate the enzyme; 40 cycles of 15 seconds at 94°C; 30 seconds at 70°C with fluorescence readout; melting curve analysis to demonstrate amplification specificity: initial denaturation for 15 seconds at 95°C and cooling to 55°C for 60 seconds and increasing to 95°C at a rate of +0.05°C per second and fluorescence readout. Analysis was performed on a QuantStudio Dx instrument from Applied Biosystems (USA) and a threshold cycle (Ct) was reported for each sample.

Samples from all participating IBD patients and healthy volunteers were given an identification number that contained no sensitive patient data.

In order to achieve the research goal and to solve the previously formulated tasks, the data of patients with IBD were studied and analyzed, who underwent examinations according to the standard clinical approach: history of the disease and comorbidities, physical examination, abdominal ultrasonography, CT enterography or MR-enterography (for CD patients and at the onset of IBD), ileocolonoscopy to assess endoscopic activity and morphological examination. On the basis of these findings, a diagnosis of CD or UC was made and these patients received therapy with mesalazine, corticosteroids, azathioprine or biologic therapy.

Fifty IBD patients were prospectively tested in the active or remission period for serum expression levels of miRNAs: hsa-miR-17-5p, hsa-miR-29a-5p, hsa-miR-146a-5p, hsa-miR-142-3p, hsa-miR-155-5p and control: cel-miR-39-3p.

All 78 IBD patients were examined prospectively during the period of activity or remission for serum human Lipocalin - 2/NGAL expression levels.

Lipocalin-2/NGAL levels were determined in blood serum using a ready-to-use Human Lipocalin-2/NGAL ELISA Kit, catalogue number RD191102200R, from BioVendor, Czech Republic, with a sensitivity (detection threshold) of 0.02 ng/ml and a linear range of 0.3-10 ng/ml. The test material was serum collected with a closed gel separation system Vacutainer SST II Advance from Beckton Dickinson. After venipuncture, the blood was left for 30 minutes at room temperature to allow clotting to take place and the serum was then separated by centrifugation for 15 minutes at 1 500 × g and stored at -80 °C until analysis. Human Lipocalin-2/NGAL analysis was performed according to the manufacturer's protocol, following the recommendation for 30-fold dilution of serums. Human Lipocalin-2/NGAL concentration in ng/ml was calculated based on the respective standards by 5-parameter logistic nonlinear regression using GraphPad Prism software version 9.5.1 by GraphPad Software.

Each group of the studied patients was detailed according to sex, age, disease activity and treatment administered, presence of intestinal complications (IC - subileus, ileus, fistulisation, abscessation, stenosis) and extra-intestinal manifestations (EIM -arthropathy, steatosis, hepatitis, cholelithiasis, iron deficiency anaemia, B12 deficiency, malabsorption, erythema nodosum, pyoderma gangrenosum, aphthous stomatitis, ocular manifestations). In all patients, the disease was proven by clinical, endoscopic, imaging (transversal-abdominal echoscopy and/or CT enterography, MR-enterography) and morphological criteria. The distribution according to localization (L/E) was performed using the Montreal classification.

Activity assessment in CD patients was performed by the Crohn's Disease Activity Index (CDAI), in UC patients by the Montreal severity classification (severity, S) and Partial Mayo score (Endoscopic Mayo score).

3.4. Methods applied to realize the aim and objectives of the study

3.4.1. Clinico-laboratory examinations

Serum expression level of miRNAs: hsa-miR-17-5p, hsa-miR-29a-5p, hsa-miR-146a-5p, hsa-miR-142-3p, hsa-miR-155-5p and control: cel-miR-39-3p and human Lipocalin - 2/NGAL were quantified in IBD patients with Crohn's disease in activity and remission and in ulcerative colitis in activity and remission.

Lipid profile study: triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol.

3.4.2. Anthropometric measurements

- BMI
- Waist circumference
- Waist/height ratio (WHtR)
- Skinfold measurement with caliperometer

BMI. Methodology: BMI is the ratio of body weight (kg) to height (m2). BMI= body mass(kg)/height(m2). Its calculation requires measurement of body weight to the nearest 0.1 kg and the patient's height without shoes and with light clothing [3].

Waist circumference. Methodology: According to WHO protocol, waist circumference is measured with a non-stretchable, plastic tape measure placed at the level of the horizontal plane midway between the superior border of the iliac bone and the inferior border of the 10th rib at the end of normal resting expiration. The waist-to-height ratio (wHtR) in adults is recommended to be less than -0.5 [322].

Skinfold measurement with caliperometer. Methodology: the measurement of the abdominal skinfold is made along a line through the umbilicus by lifting horizontally 3 cm laterally and 1 cm lower than the umbilicus. It is embedded in the first recommendation of the

Joint Clinical Guideline on Nutrition and Metabolism in Gastrointestinal and Liver Diseases issued in September 2022 with a total of 19 recommendations for IBD patients [28, 343].

3.4.3. Statistical methods

- Dispersion analysis (ANOVA)
- Variance analysis
- Correlation analysis
- Regression analysis
- Risk assessment analysis
- ROC curve analysis
- Comparative analysis (evaluation of hypotheses)
- Graphical and tabular method of displaying the obtained results

A significance level of $p<0.05$ at 95% confidence interval was assumed for all analyses performed.

The relative concentration of the target miRNAs studied was calculated by the ΔΔCt method [190] relative to a reference miRNA - normalization control C. elegans miR-39 relative to a reference sample - represented by the arithmetic mean of the Ct of all individuals in the control group was calculated using Microsoft Office Excel 2016 and is presented as a ratio relative to a reference sample.

$$\Delta CT = CT - CT0 \qquad \Delta\Delta CT = \Delta CT - \Delta CTcontrol \qquad FC = 2^{-\Delta\Delta CT}$$

Where: CT - Cycle Threshold

FC - Fold Change

Data were processed with SPSS v. 20.0 for Windows.

The clinical study was conducted after obtaining permission from the Research Ethics Committee at MU-Varna - protocol/decision №100, meeting on 25.02.2021. All study participants signed informed consent.

The study was funded-conducted after the permition of the Fund „Science of Medical University - Varna, project №20002 "Metabolic syndrome in patients with inflammatory bowel diseases".

IV. RESULTS

4.1.Characteristics of the studied groups and determination of the threshold values of miRNAs and human Lipocalin-2/NGAL

Between February 2021 and November 2022, studies with five miRNAs and one control miRNA and human Lipocalin-2/NGAL were performed in 108 individuals who were divided into five groups (Fig. 1).

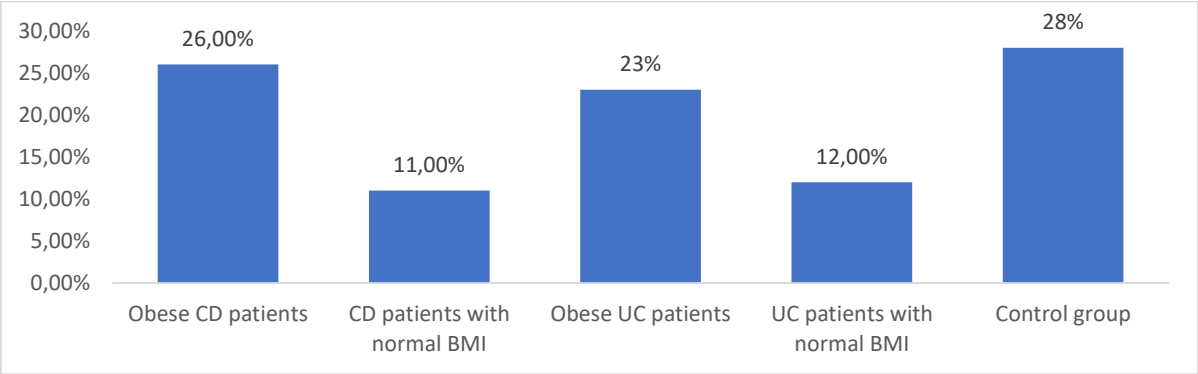

Fig. 1. Distribution of groups participating in the study

Individuals in the control group were selected according to well-defined criteria for healthy individuals - normal weight, no comorbidities, non-smokers and non-medicated. The mean age of the healthy controls was 26.2 ± 6.2 years (18-42 years), with an even gender distribution (50:50 male:female).

Table 1 presents the general characteristics of the clinical group. There was no significant difference according to the investigated parameters between UC and CD patients, making the results of subsequent analyses comparable.

Since validated threshold values of miRNAs and serum human Lipocalin-2/NGAL in normal-weight subjects are lacking, these were calculated for the present study.

Table 1. General characteristics of the examined persons

Indicator		Ulcerative colitis (n=38)	Crohn's disease (n=40)	P value
Age, years (mean±SD, range)	Momentary	42.3±15.0 (18-75)	42.8±14.8 (20-69)	>0.05
	Beginning of complaints	32.2±10.8 (12-55)	34.7±13.6 (10-66)	>0.05
	Making a diagnosis	34.4±12.3 (12-63)	36.4±12.3 (16-66)	>0.05
Gender	Male	16/42.1%	22/55.0%	>0.05
	Female	22/57.9%	18/45.0%	
Prevalence of IBD, months	(mean±SD, range)	128.5±105.0 (5-504)	113.7±78.1 (4-342)	> 0.05
CRP	(mean±SD, range)	10.9±16.9 (0.58-71.5)	10.3±14.9 (0.2-79.7)	>0.05
	< 5 mg/l	20/54.1%	18/47.4%	>0.05
	> 5 mg/l	17/45.9%	20/52.6%	
FCP	(mean±SD, range)	406.25±504.28 (1-2000)	282.52±434.73 (2-1745,50	>0.05
	< 50µg/g	11/37.9%	9/33.3%	>0.05
	> 50µg/g	18/62.1%	18/66.7%	
Fasting blood sugar	(mean±SD, range)	5.27±0.75 (3.50-7.20)	5.41±0.97 (4-7.96)	>0.05
Triglycerides	(mean±SD, range)	1.93±1.85 (0.63-9.19)	1.77±0.96 (0.45-4.85)	>0.05
Total cholesterol	(mean±SD, range)	5.06±1.34 (2.21-9.19)	4.67±1.53 (2.11-7.84)	>0.05
HDL-cholesterol	(mean±SD, range)	1.30±0.29 (0.74-1.84)	1.29±0.41 (0.58-2.81)	>0.05
LDL-cholesterol	(mean±SD, range)	3.05±1.08 (1.35-6.50)	2.66±1.01 (0.86-4.45)	>0.05
Fibrinogen	(mean±SD, range)	3.61±0.92 (2.10-5.66)	3.78±1.18 (1.89-6.63)	>0.05
BMI	(mean±SD, range)	28.0±6.5 (16.7-50.5)	27.7±6.7 (14.7-44.7)	>0.05
	< 25kg/m^2	13/34.2%	12/30.0%	>0.05
	> 25 kg/m^2	25/65.8%	28/70.0%	
Activity/Remission	Activity	16/42.1%	20/50.0%	>0.05
	Remission	22/57.9%	20/50.0%	>0.05

Weight, kg. (mean±SD, range)	Momentarily	81.55±23.84 (47-188)	79.83±21.07 (40.4-118.75)	>0.05
	Making the diagnosis	75.2±22.8 (45-136)	73.6±21.4 (37-120)	>0.05
	Difference	7.28±15.3 (-27.15-58)	8.25±12.9 (-14.6-44.55)	>0.05
Waist measurement, cm (mean±SD, range)		95.14±16.3 (67-155)	95.87±15.8 (65-123)	>0.05
Abdominal fold size, mm (mean±SD, range)		28.16±10.9 (5-60)	27.67±11.6 (3-58)	>0.05
Biological therapy	None	14/36.8%	12/30.0%	>0.05
	Anti-TNF	19/50.0%	25/62.5%	
	Anti-integrin	5/13.2%	3/7.5%	

The threshold values (Cut-off) of the expression of the studied miRNAs and human Lipocalin-2/NGAL to distinguish IBD patients with obesity from those with normal body weight are presented in table 2. This table also shows the sensitivity and specificity of the respective miRNAs and serum human Lipocalin-2/NGAL.

Table 2. Threshold value of circulating miRNAs and serum human Lipocalin-2/NGAL in subjects of normal body weight

miRNAs	**Cut-off**	**AUC 95% CI**	**P value**	**Sensitivity/ Specificity**
hsa-miR-17-5p	0.99	0.603 (0.400-0.805)	< 0.05	60.0%/60.0%
hsa-miR-29a-5p	1.54	0.580 (0.366-0.794)	< 0.05	60.0%/62.5%
hsa-miR-142-3p	1.15	0.605 (0.382-0.828)	< 0.05	60.0%/60.0%
hsa-miR-146a-5p	1.06	0.518 (0.319-0.716)	< 0.05	50.0%/50.0%
hsa-miR-155-5p	1.32	0.513 (0.314-0.711)	< 0.05	52.5%/60.0%
human Lipocalin - 2/NGAL	63.15	0.626 (0.486-0.766)	< 0.05	60.0%/60.4%

ROC curves of the investigated circulating miRNAs and serum human Lipocalin - 2/NGAL are presented in the following figures.

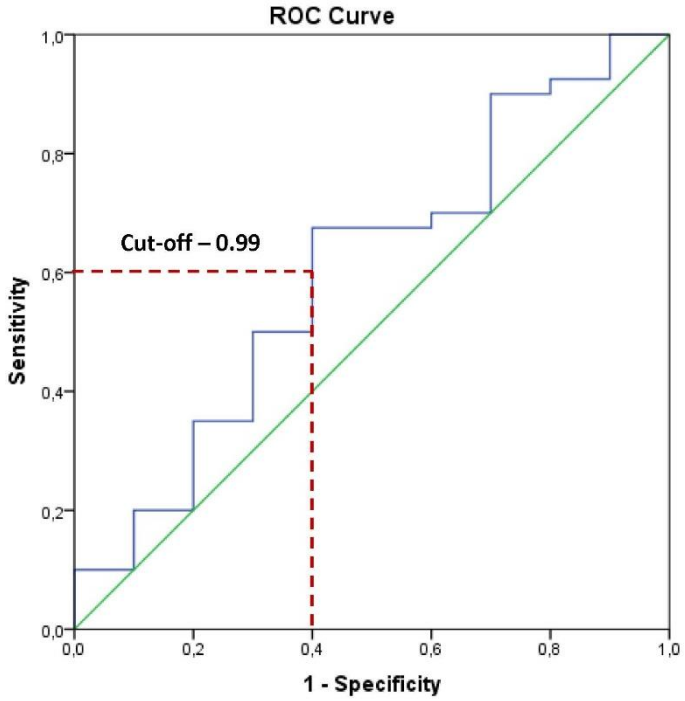

Fig. 2. ROC curve analysis to determine the threshold value of hsa-miR-17-5p expression to distinguish IBD patients with obesity

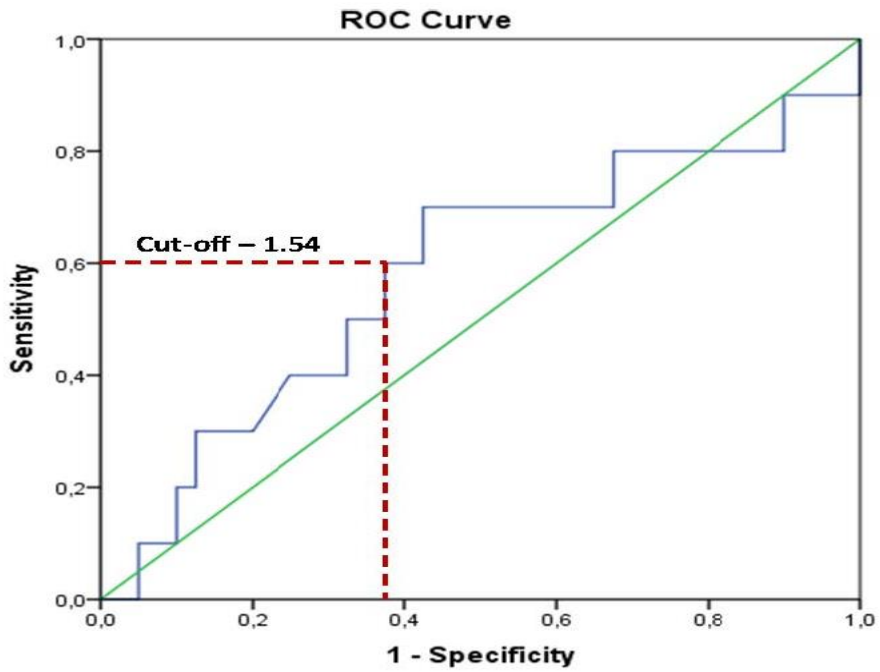

Fig. 3. ROC curve analysis to determine the threshold value of hsa-miR-29a-5p expression to distinguish IBD patients with obesity

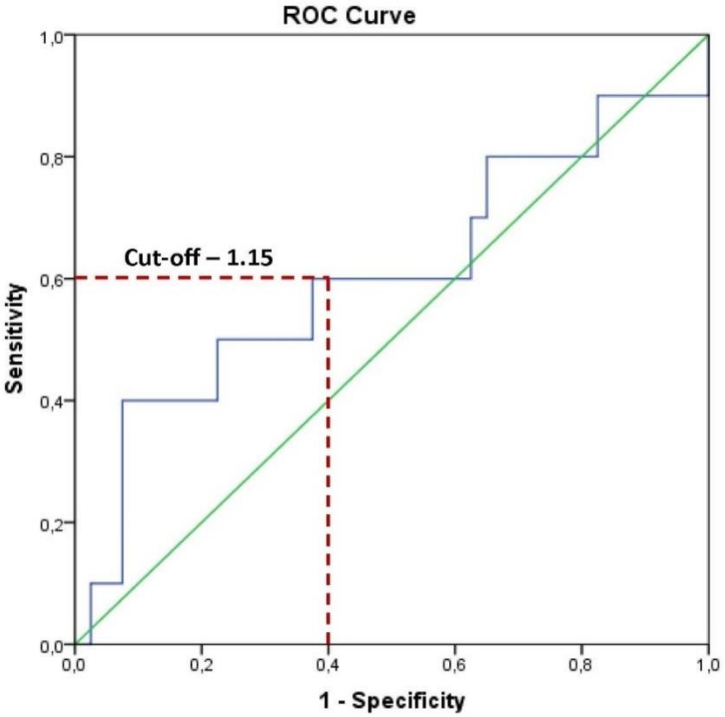

Fig. 4. ROC curve analysis to determine the threshold value of hsa-miR-142-3p expression to distinguish IBD patients with obesity

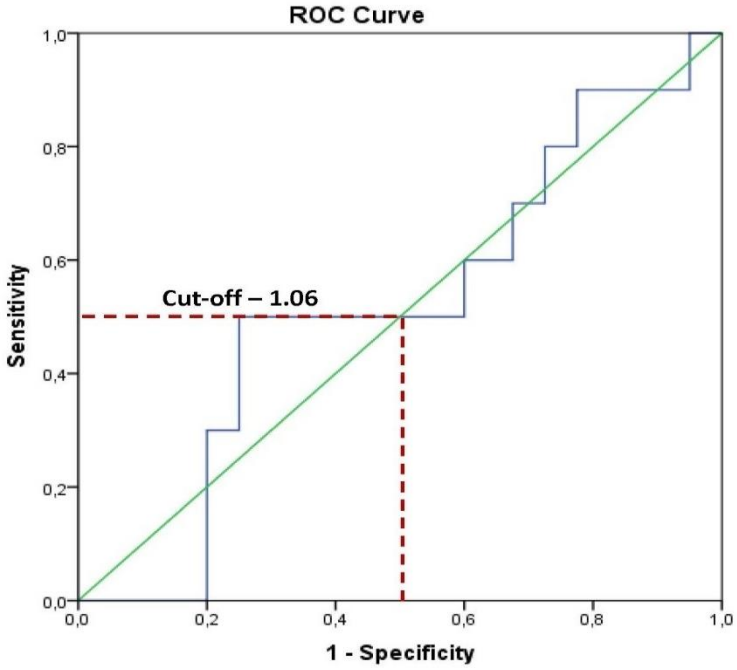

Fig. 5. ROC curve analysis to determine the threshold value of hsa-miR-146a-5p expression to discriminate IBD patients with obesity

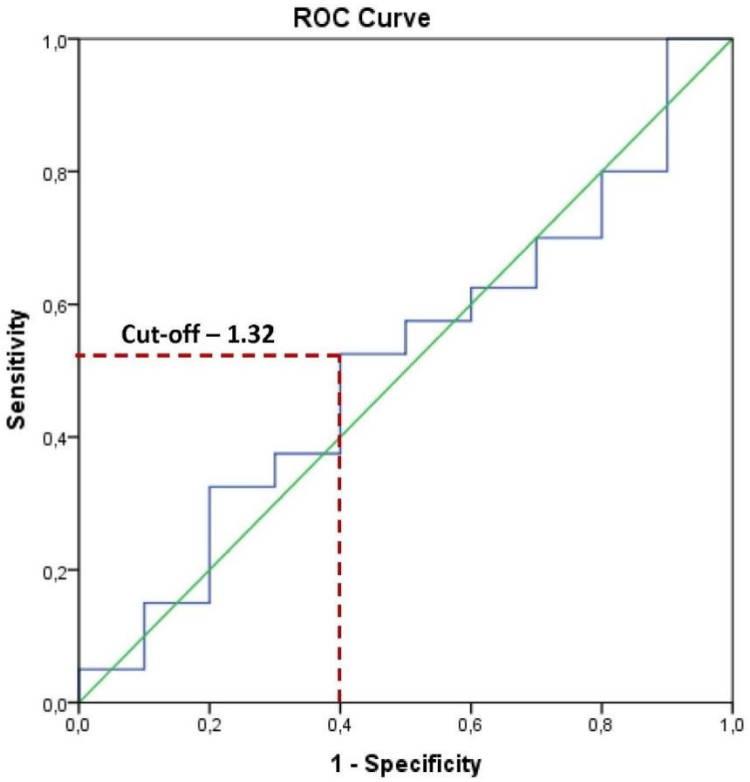

Fig. 6. ROC curve analysis to determine the threshold value of hsa-miR-155-5p expression to discriminate IBD patients with obesity

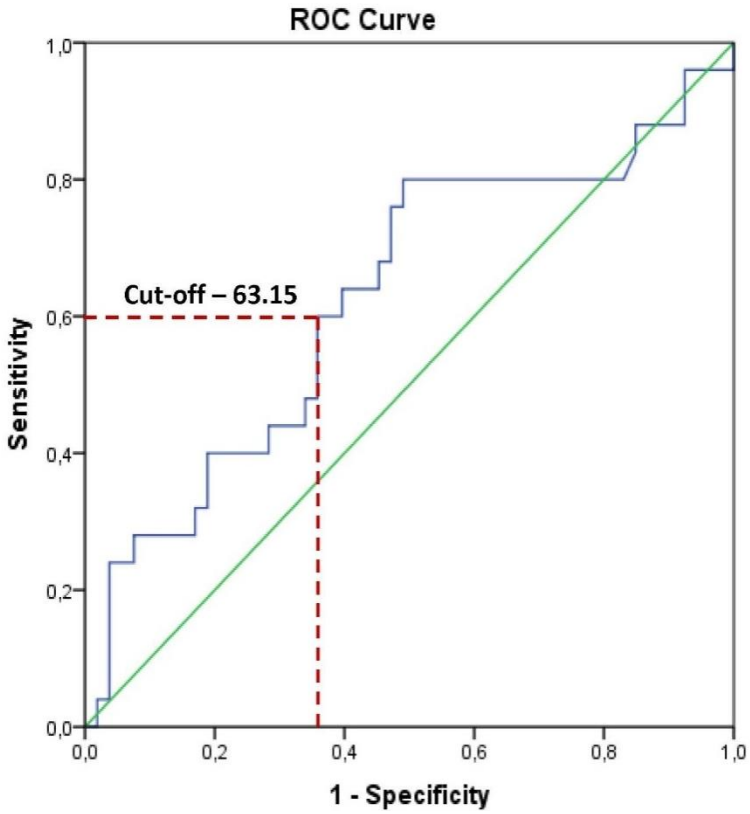

Fig. 7. ROC curve analysis to determine the threshold value of human Lipocalin-2/NGAL expression to distinguish IBD patients with obesity

4.2 Anthropometric characteristics in Crohn's disease and ulcerative colitis patients with obesity

There was a weak to moderate-без това correlation between BMI and age in CD patients (r=0.245; p=0.047), whereas no similar correlation was observed in UC patients.

On the other hand, BMI was found to correlate with disease activity in UC patients (r=0.292; p=0.045). No similar correlation was found in CD patients.

There was a linear strong correlation between BMI and waist circumference in both CD and UC patients (r=0.903; p<0.001 for CD and r=0.941; p<0.001 for UC, respectively) (Fig. 8).

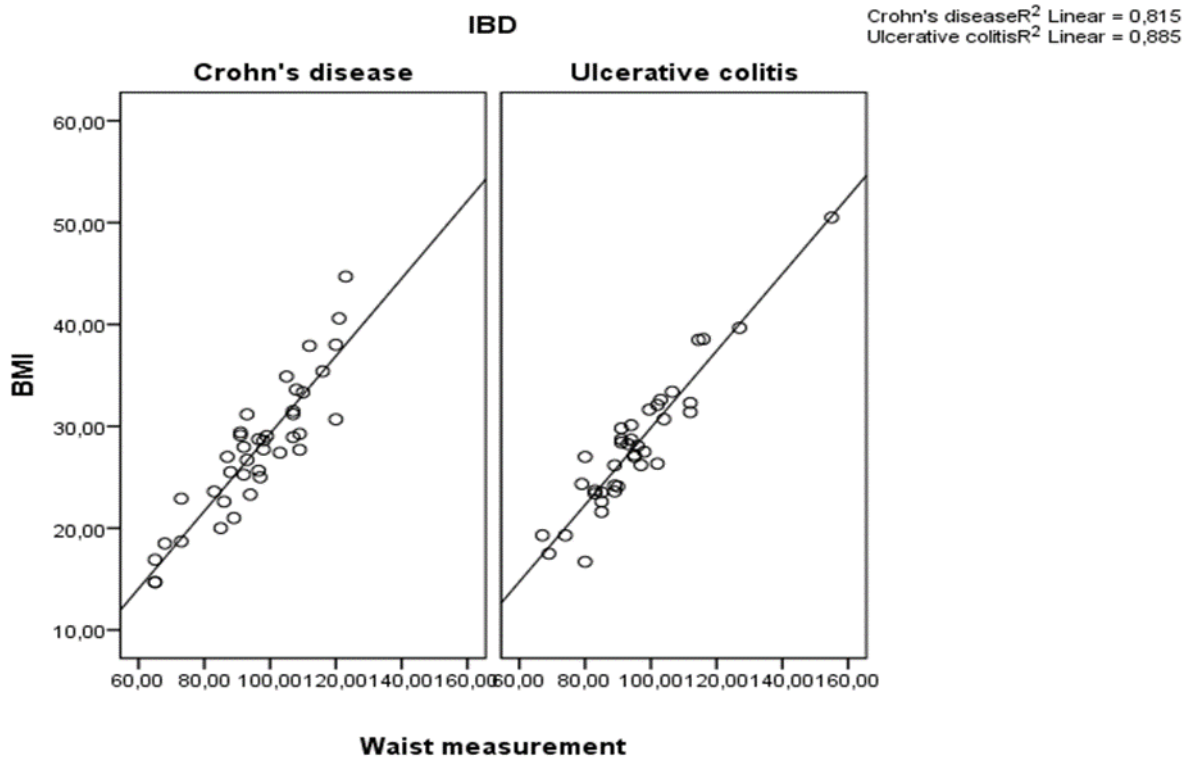

Fig. 8. Correlation analysis between BMI and waist circumference in CD and UC patients

A strong orthogonal relationship was also found between BMI and abdominal fold size in both groups of patients (r=0.773; p<0.001 for CD and r=0.834; p<0.001 for UC, respectively) (Fig. 9).

There was no correlation between BMI and fasting blood glucose, but there was a moderate orthogonal relationship between BMI and triglyceride levels in UC patients (r=0.312; p=0.043), whereas the correlation was weak in those with CD (r=0.221; p=0.024) (Fig. 10).

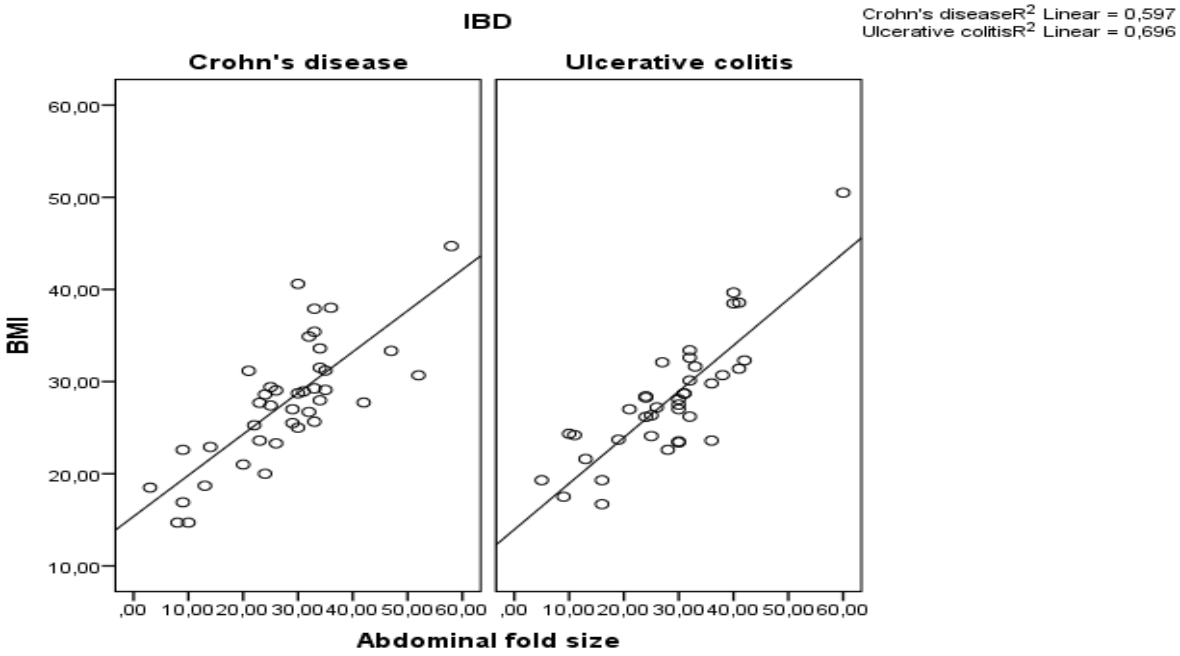

Fig. 9. Correlation analysis between BMI and abdominal fold size in CD and UC patients

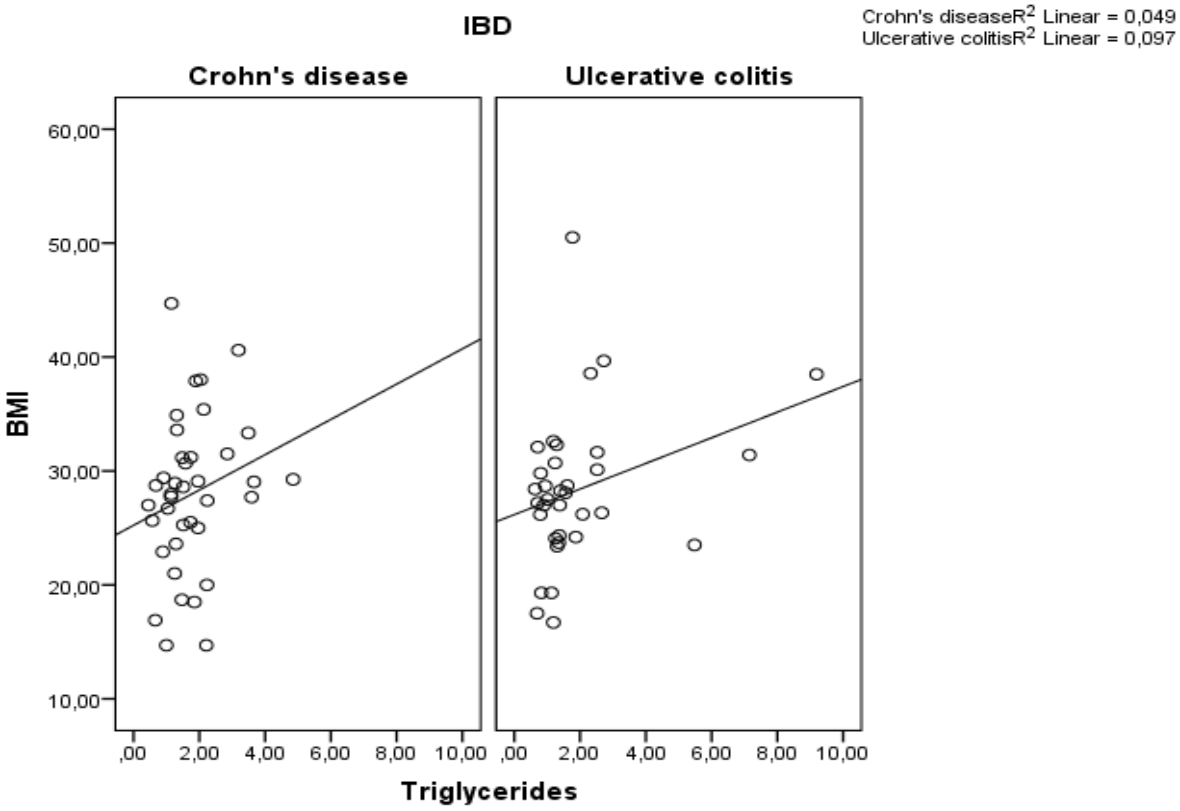

Fig. 10. Correlation analysis between BMI and Triglycerides levels in CD and UC patients

BMI was moderately correlated with total cholesterol in both groups of patients, with a stronger correlation observed in CD patients compared with UC patients (r=0.332; p=0.05 for CD and r=0.465; p=0.003 for UC, respectively) (Fig. 11).

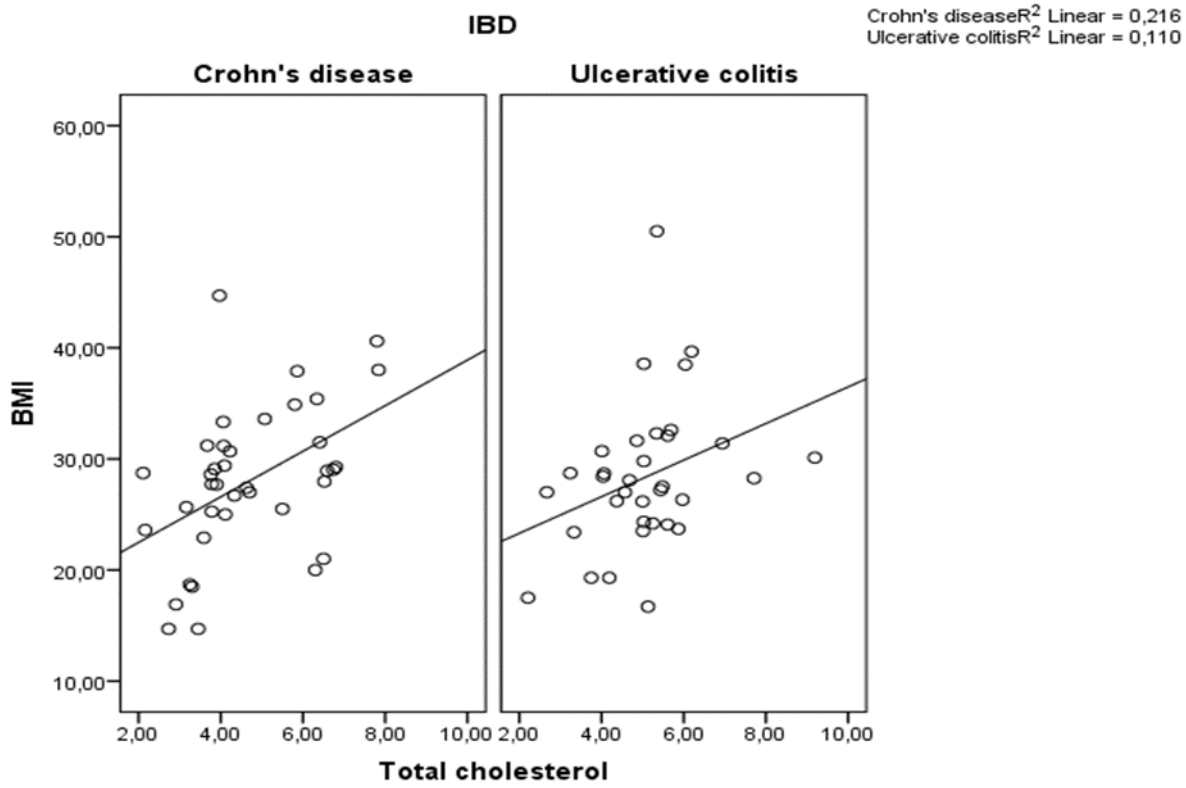

Fig. 11. Correlation analysis between BMI and total cholesterol levels in CD and UC patients

In the present study, there was no correlation between HDL-cholesterol and BMI, but a weak to moderate orthogonal relationship was observed in the correlations between BMI and LDL-cholesterol (r = 0.219; p = 0.037 for UC and r = 0.354; p = 0.038 for CD, respectively) (Fig. 12).

BMI correlated moderately and directly with VLDL-cholesterol levels in both UC and CD patients (r=0.465; p=0.042 for UC and r=0.424; p=0.039 for CD, respectively) (Fig. 13).

Examination of the relationship of another anthropometric parameter, waist circumference, and age in IBD patients showed a straightforward moderate relationship in UC patients (r=0.323; p=0.048) and a weak relationship in CD patients (r=0.267; p=0.046) (Fig. 14).

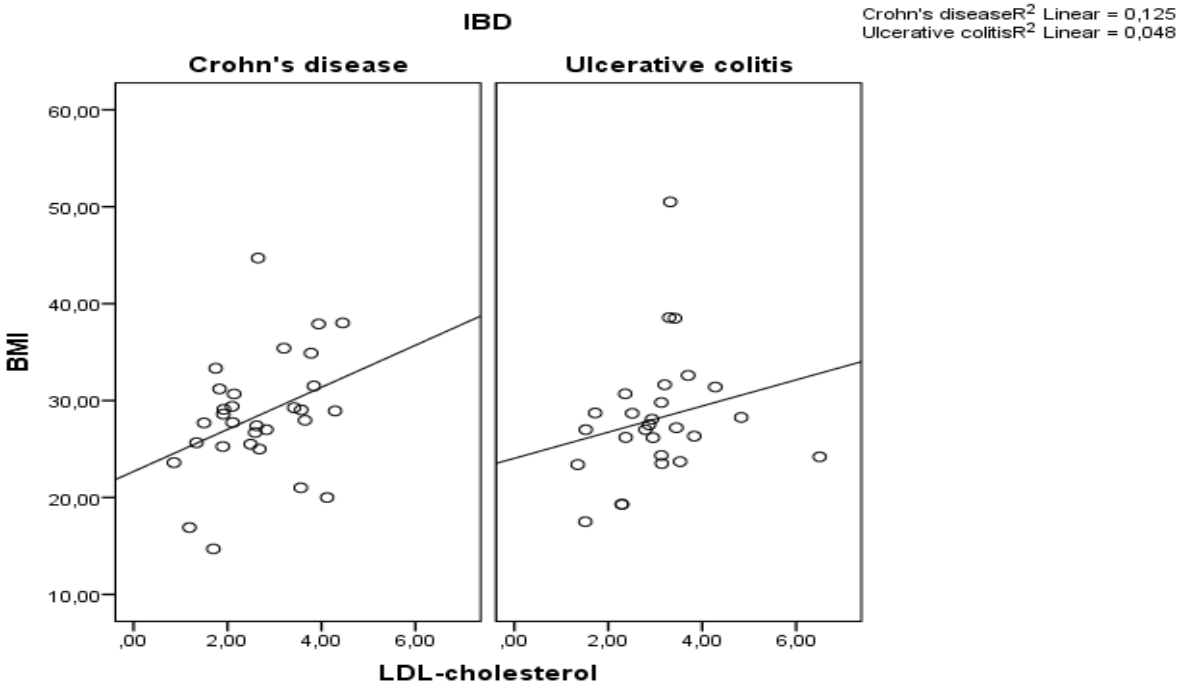

Fig. 12. Correlation analysis between BMI and LDL-cholesterol levels in CD and UC patients

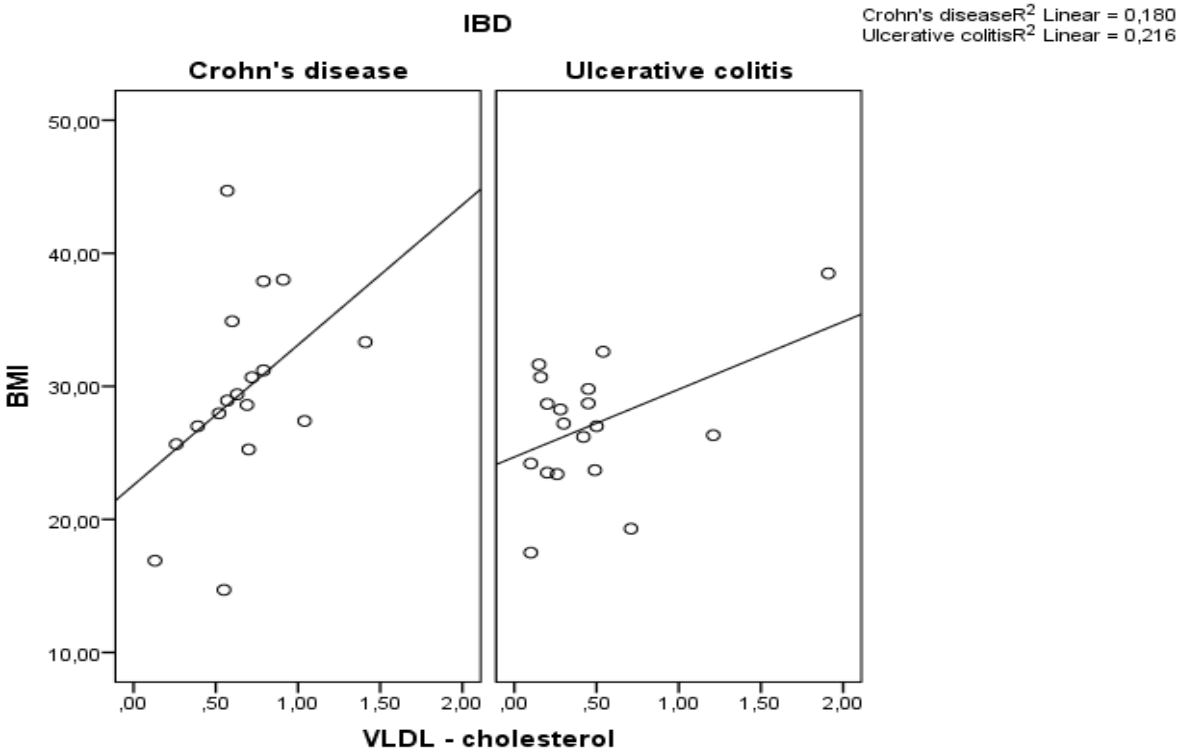

Fig. 13. Correlation analysis between BMI and VLDL-cholesterol levels in CD and UC patients

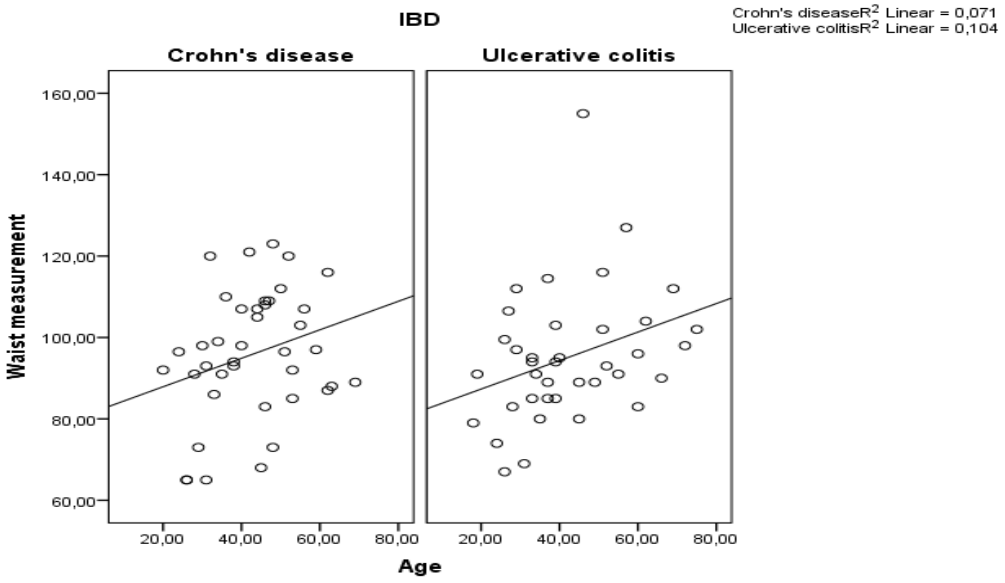

Fig. 14. Correlation analysis between waist circumference and age in CD and UC patients

No correlation was found between waist circumference and disease activity and duration.

A strong orthogonal relationship was observed when analysing the association between waist circumference and abdominal fold size in both UC and CD patients (r=0.864; p<0.001 for UC and r=0.817; p<0.001 for CD, respectively) (Fig. 15).

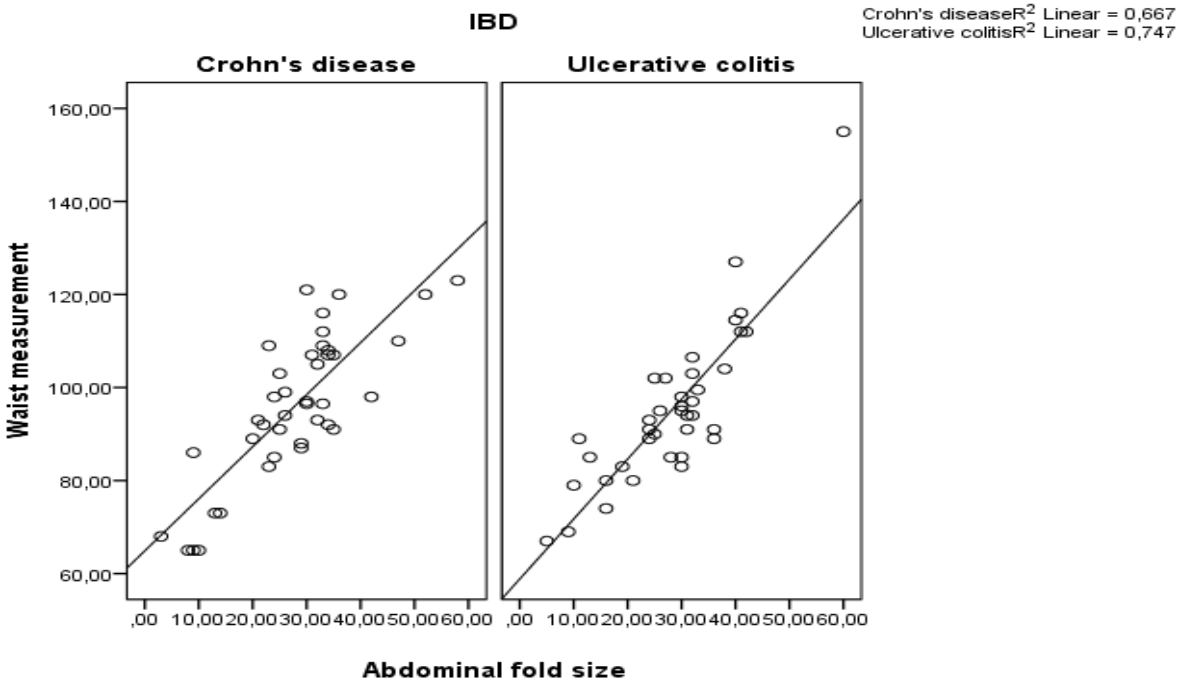

Fig. 15. Correlation analysis between waist circumference and abdominal fold size in CD and UC patients

No correlation was found between waist circumference and fasting blood glucose, fibrinogen and CRP levels.

Examination of the relationship between triglyceride levels and waist circumference showed a moderate, directly proportional relationship in both UC patients (r=0.335; p=0.043) and those with CD (r=0.376; p=0.020) (Fig. 16).

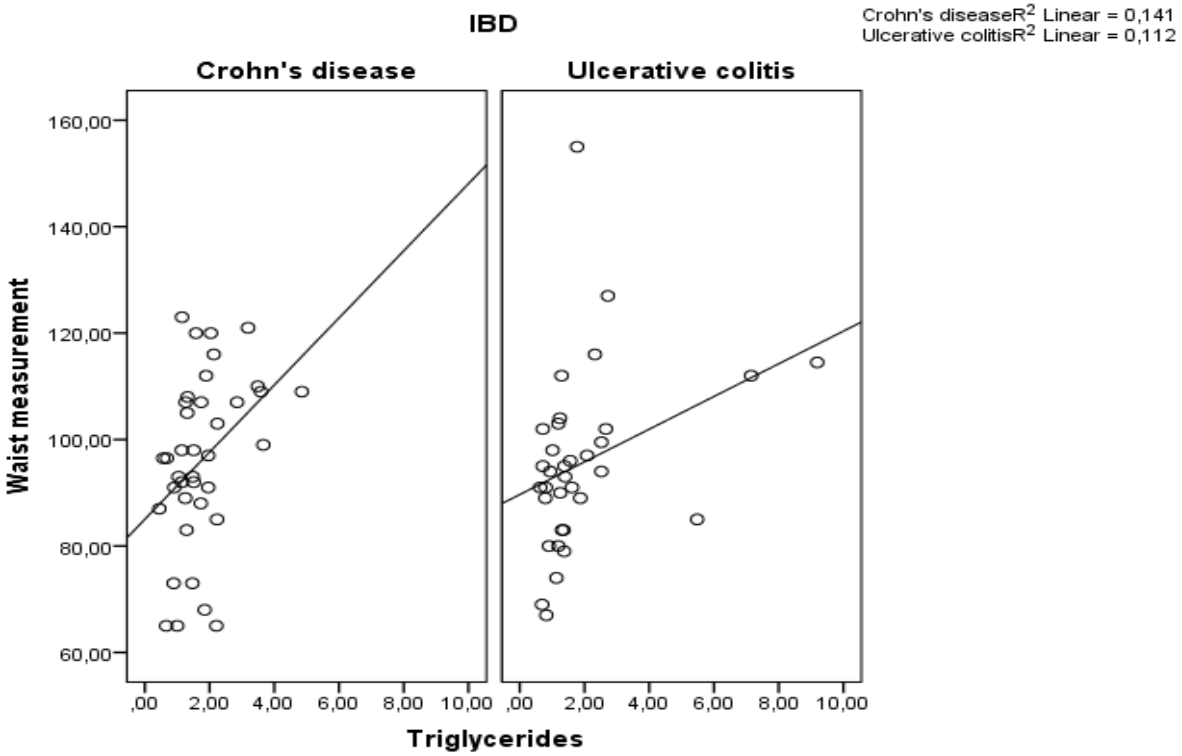

Fig. 16. Correlation analysis between waist circumference and triglyceride levels in CD and UC patients

On the other hand, there was a moderate orthogonal relationship between waist circumference and total cholesterol levels in UC patients (r=0.381; p=0.026), whereas the relationship was significant in CD patients (r=0.524; p=0.001) (Fig. 17).

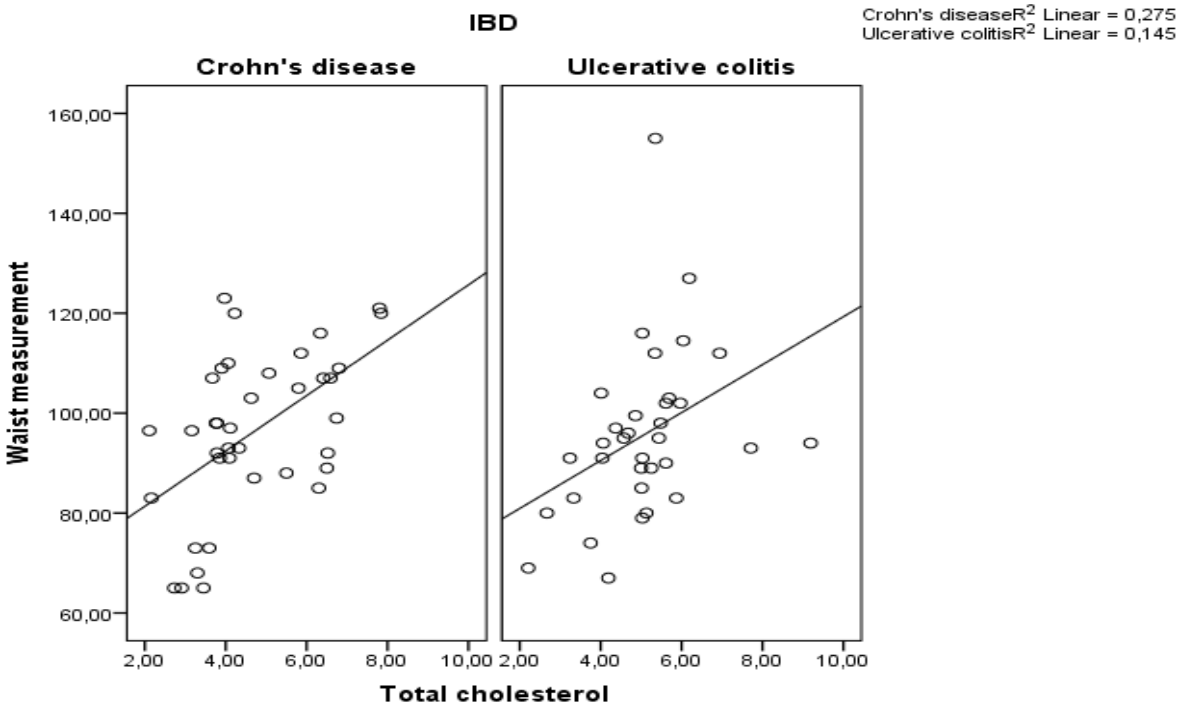

Fig. 17. Correlation analysis between waist circumference and total cholesterol levels in CD and UC patients

There was no correlation between waist circumference and LDL-cholesterol, whereas a moderate orthogonal correlation was observed with LDL-cholesterol in both child IBD patient groups studied (r=0.303; p=0.024 for UC and r=0.376; p=0.040 for CD, respectively) (Fig. 18).

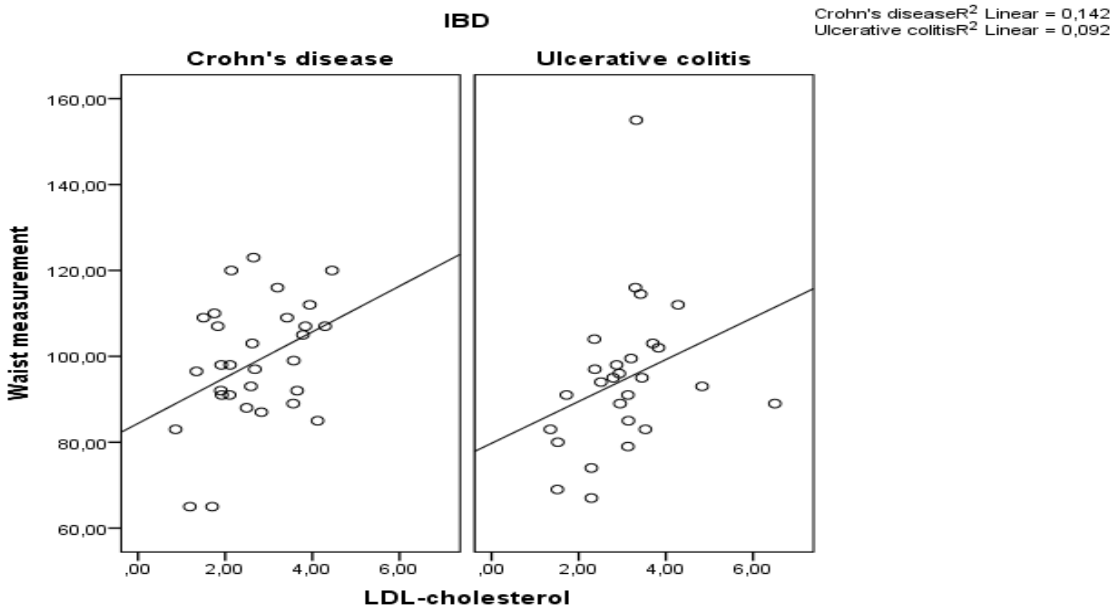

Fig. 18. Correlation analysis between waist circumference and LDL-cholesterol levels in CD and UC patients

Waist circumference correlated linearly significantly with VLDL-cholesterol in CD and UC patients (r=0.502; p=0.034 for UC and r=0.539; p=0.025 for CD, respectively) (Fig. 19)

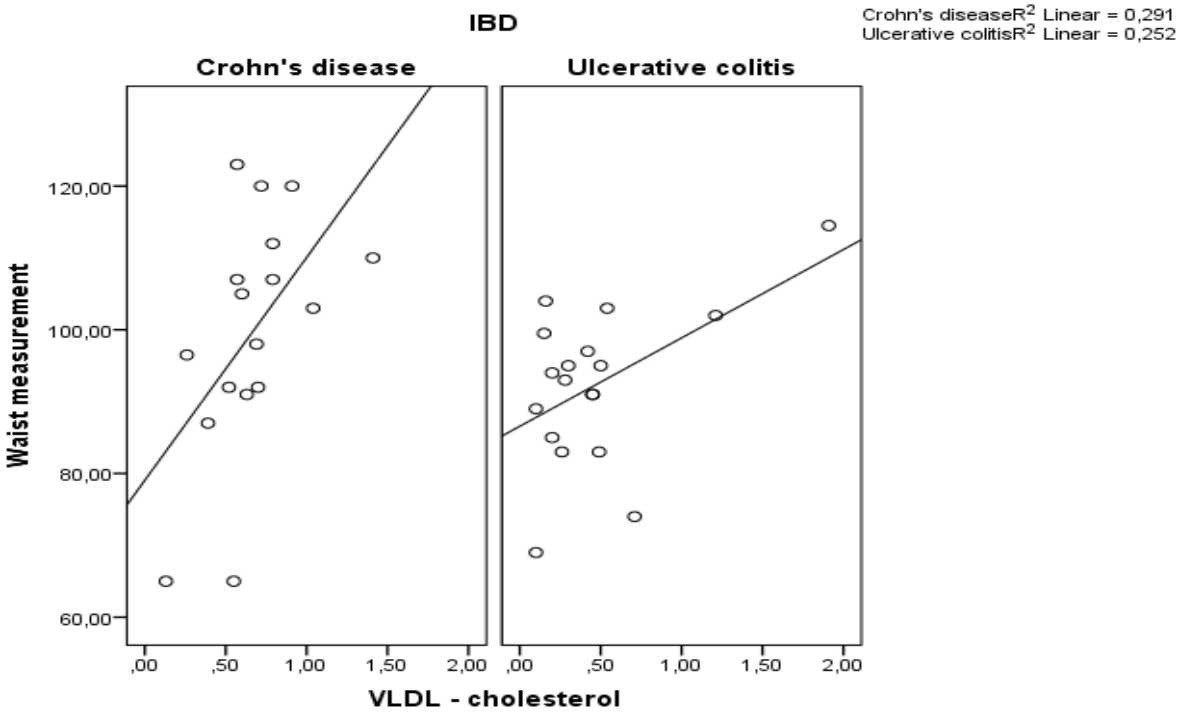

Fig. 19. Correlation analysis between waist circumference and VLDL-cholesterol levels in CD and UC patients

The third anthropometric measure, the waist/hip ratio, which was used in the present analysis correlated moderately orthogonally with age in both UC and CD patients (r=0.379; p=0.019 for UC and r=0.398; p=0.011 for CD, respectively) (Fig. 20).

Waist-to-height ratio showed a strong correlation with BMI in both groups of IBD patients (r=0.931; p<0.001 for UC and r=0.915; p<0.001 for CD, respectively) (Fig. 21).

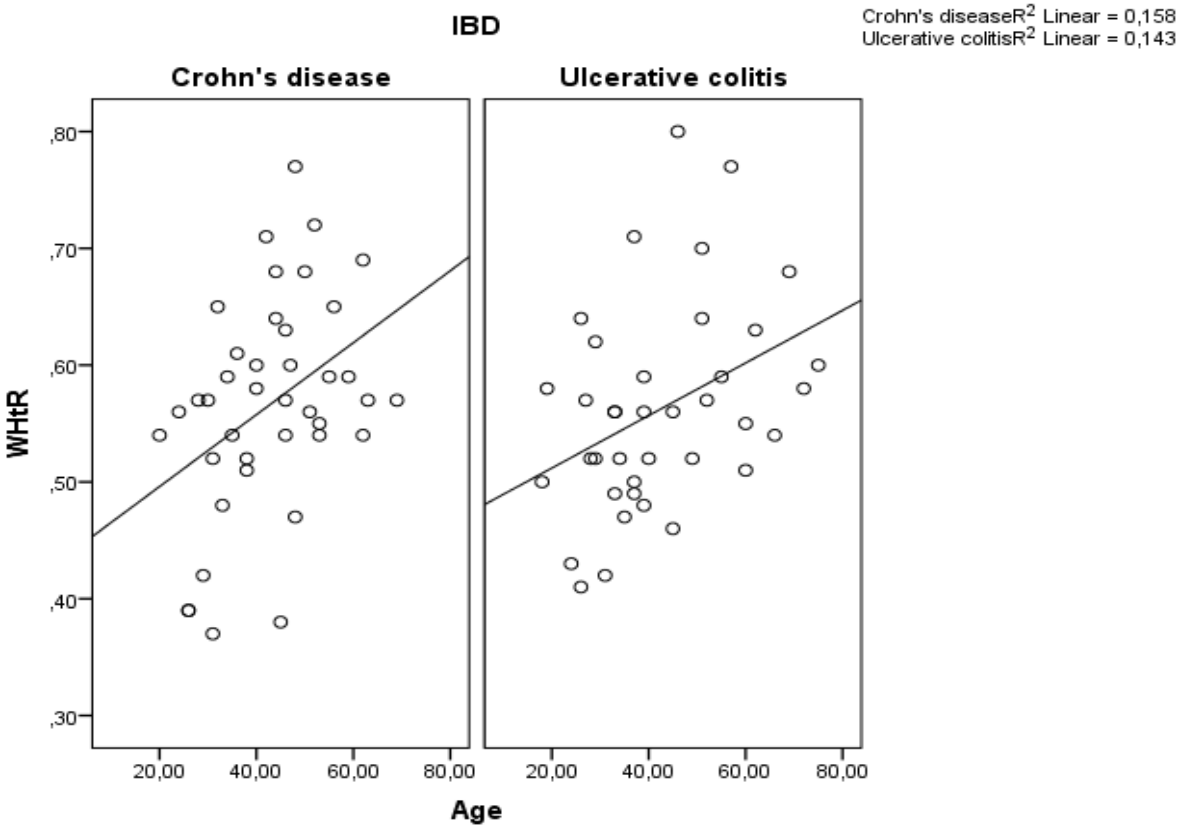

Fig. 20. Correlation analysis between waist/height ratio and age in CD and UC patients

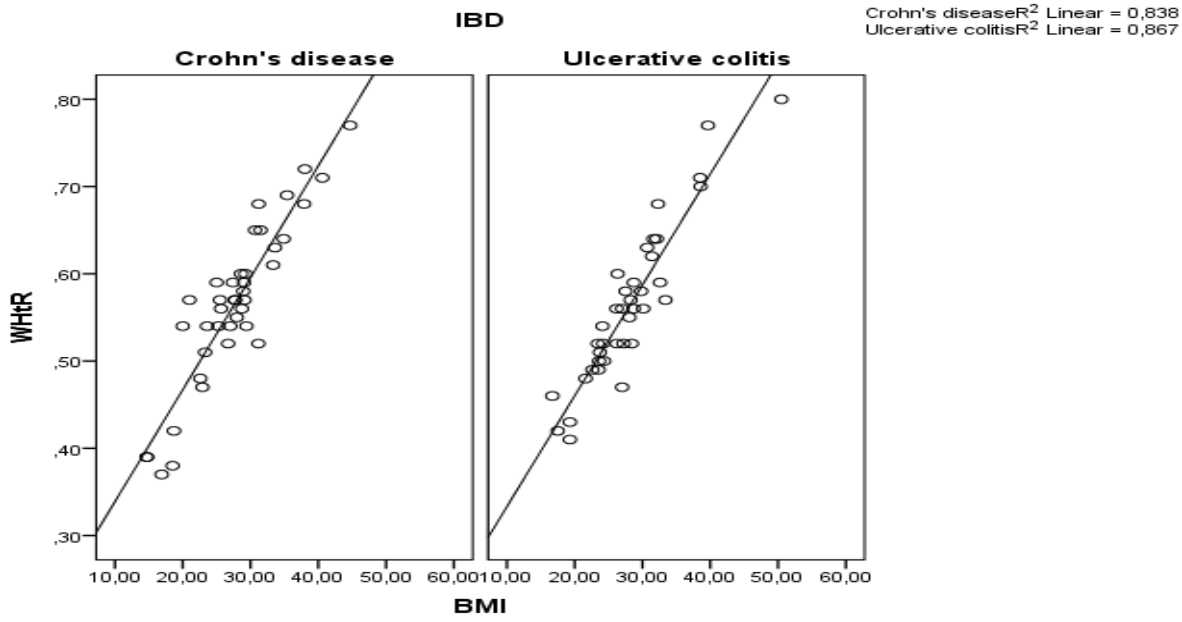

Fig. 21. Correlation analysis between waist/height ratio and BMI in CD and UC patients

A strong orthogonal relationship was also observed between waist-to-height ratio and waist circumference (r=0.938; p<0.001 for UC and r=0.943; p<0.001 for CD, respectively)

(Fig. 22), and with the size of the skinfold in the studied patient groups (r=0.828; p<0.001 for UC and r=0.818; p<0.001 for CD, respectively) (Fig. 23).

There was no relationship between waist/height ratio and fasting blood glucose, fibrinogen and CRP.

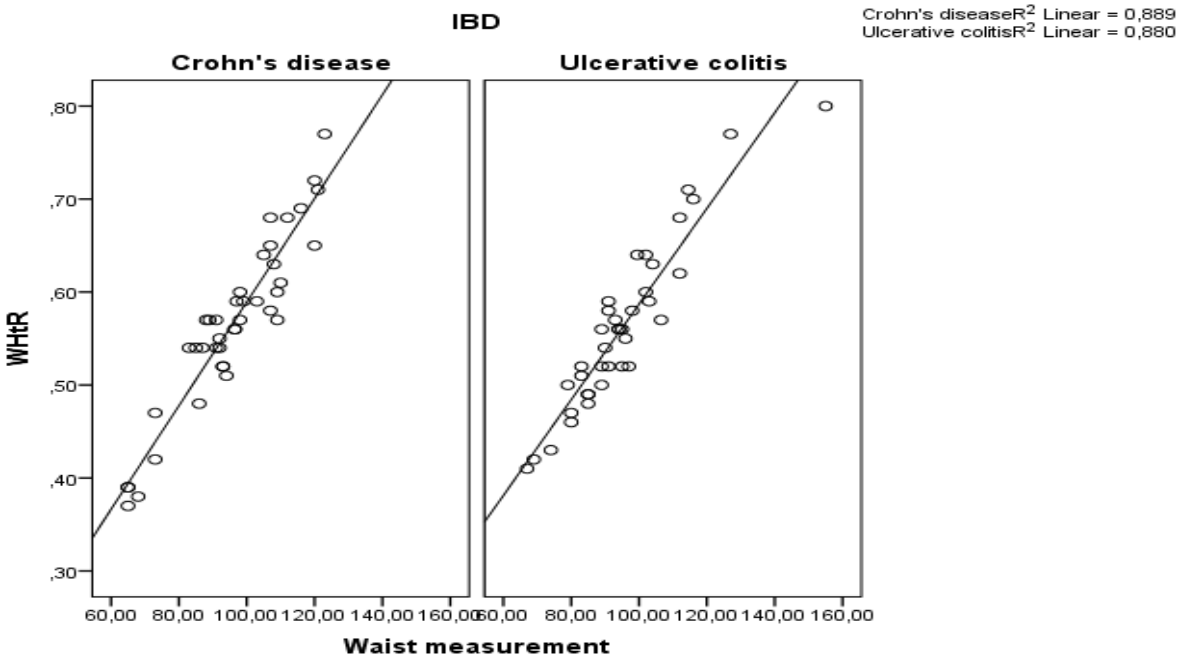

Fig. 22. Correlation analysis between waist/height ratio and waist measurement in patients with CD and UC

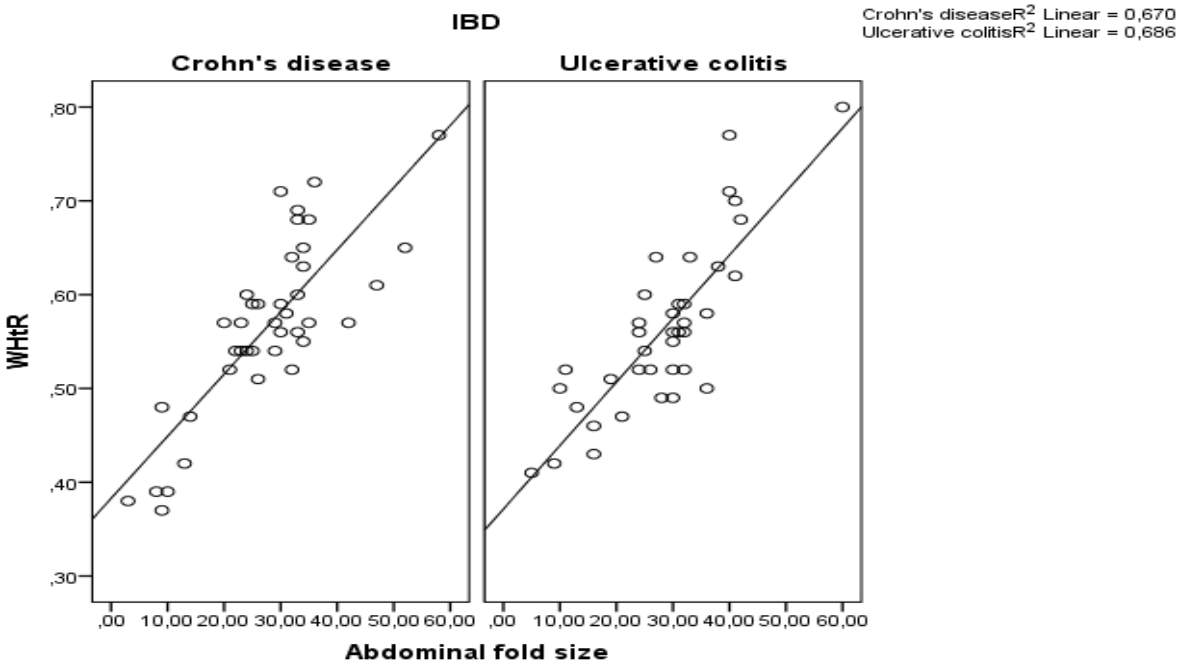

Fig. 23. Correlation analysis between waist/height ratio and abdominal skinfold size in CD and UC patients

Waist-to-height ratio correlated moderately with triglycerides in UC patients (r=0.344; p=0.046) and weakly in CD patients (r=0.279; p=0.05) (Fig. 24).

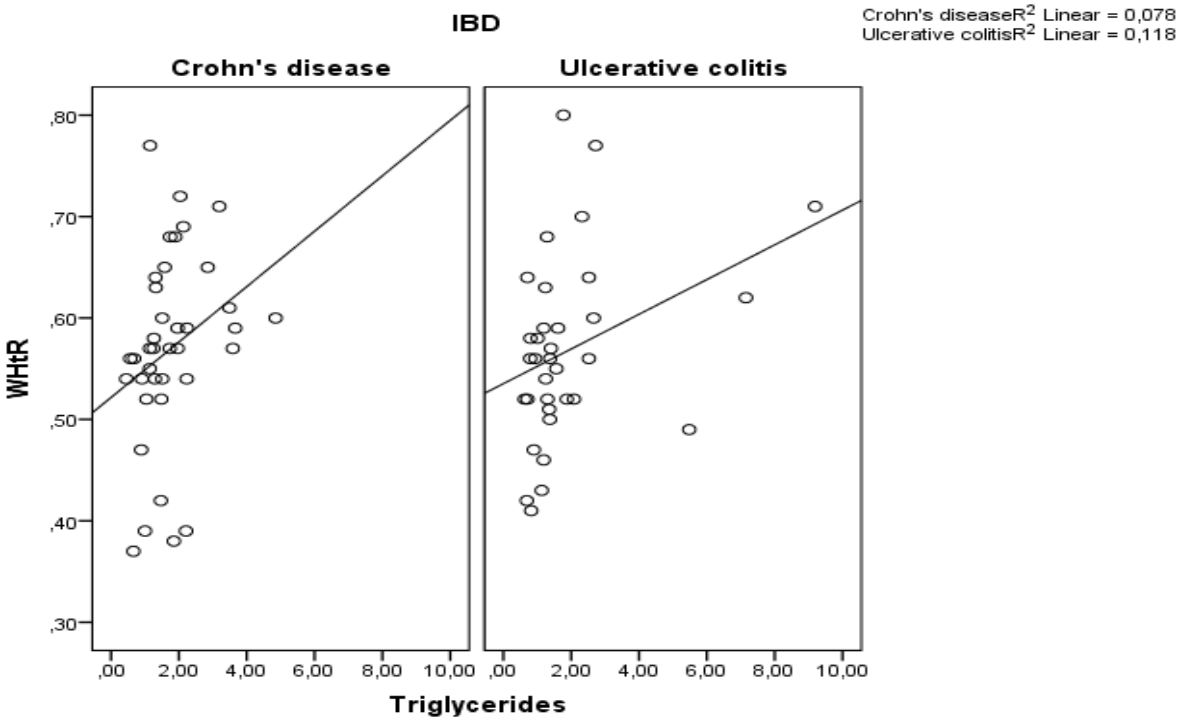

Fig. 24. Correlation analysis between waist/height ratio and triglycerides in CD and UC patients

Waist-to-height ratio correlated moderately with total cholesterol in UC patients (r=0.385; p=0.024) and significantly in CD patients (r=0.540; p<0.001) (Fig. 25). There was no correlation between waist-to-height ratio and HDL-cholesterol and LDL-cholesterol.

Waist-to-height ratio correlated moderately orthogonally with VLDL-cholesterol in UC patients and CD (r=0.491; p=0.039 for UC and r=0.466; p=0.049 for CD, respectively) (Fig. 26).

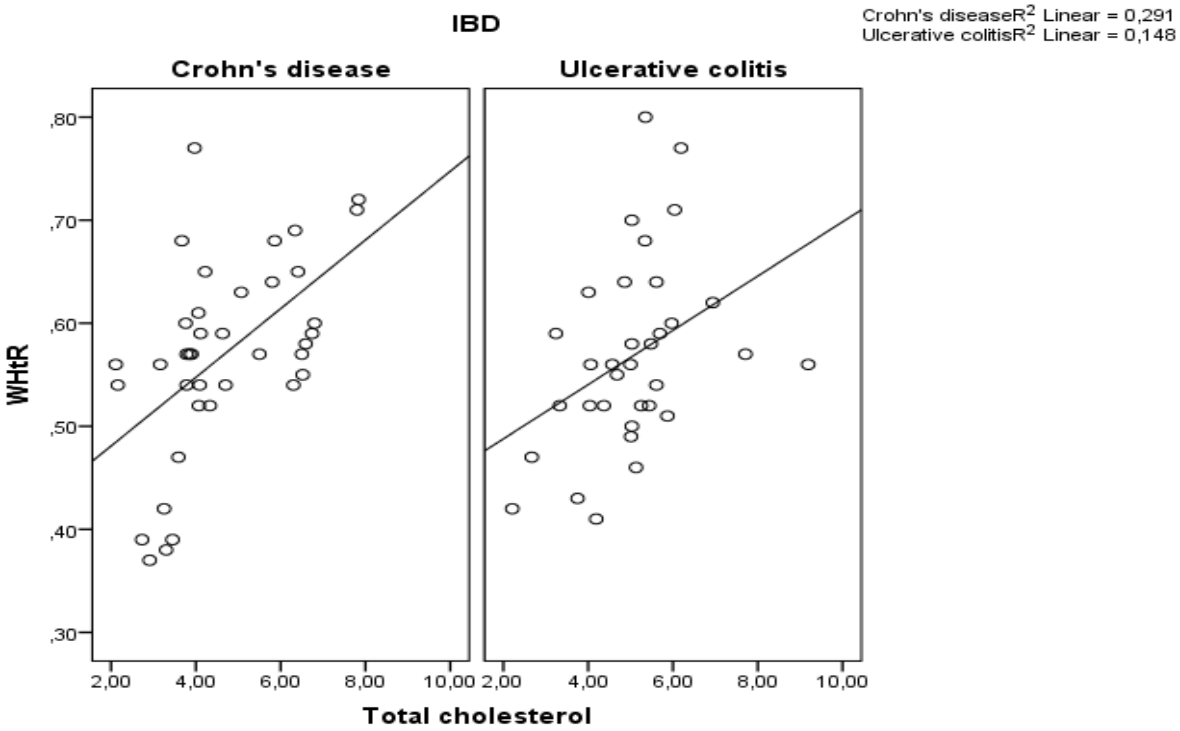

Fig. 25. Correlation analysis between waist/height ratio and total cholesterol in CD and UC patients

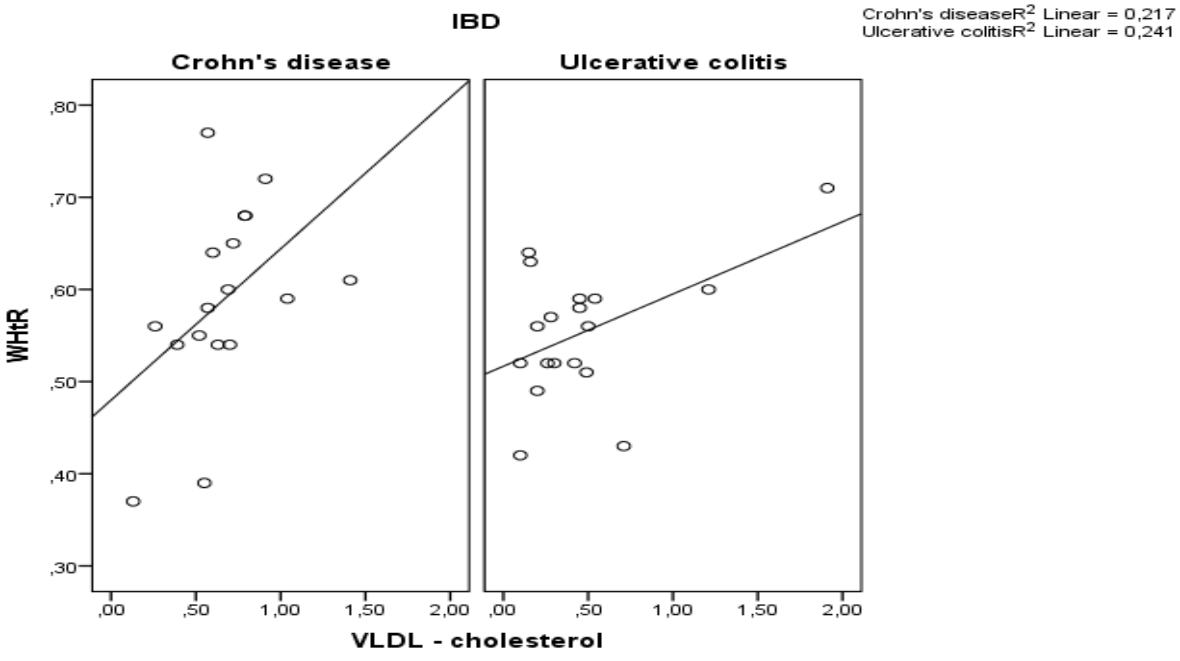

Fig. 26. Correlation analysis between waist/height ratio and VLDL-cholesterol in CD and UC patients

The fourth anthropometric index that is considered in this work is the size of the skinfold. The investigation of its relationship with gender in IBD patients showed that there was a moderate correlation only in UC patients (r=0.388; p=0.016).

There was no correlation between the size of the skinfold and age and disease duration, or with fasting blood glucose levels in the two groups of patients studied.

Examination of the relationship between skinfold size and disease activity showed that in UC patients there was a moderate correlation between the two indices in active disease (r=0.408; p=0.011), whereas CD patients also showed a similar trend, but the correlation was weak (r=0.265; p=0.048).

There was a moderate orthogonal correlation between skinfold size and triglycerides (r=0.364; p=0.035) in UC patients, whereas no correlation was observed between the two factors (Fig. 27).

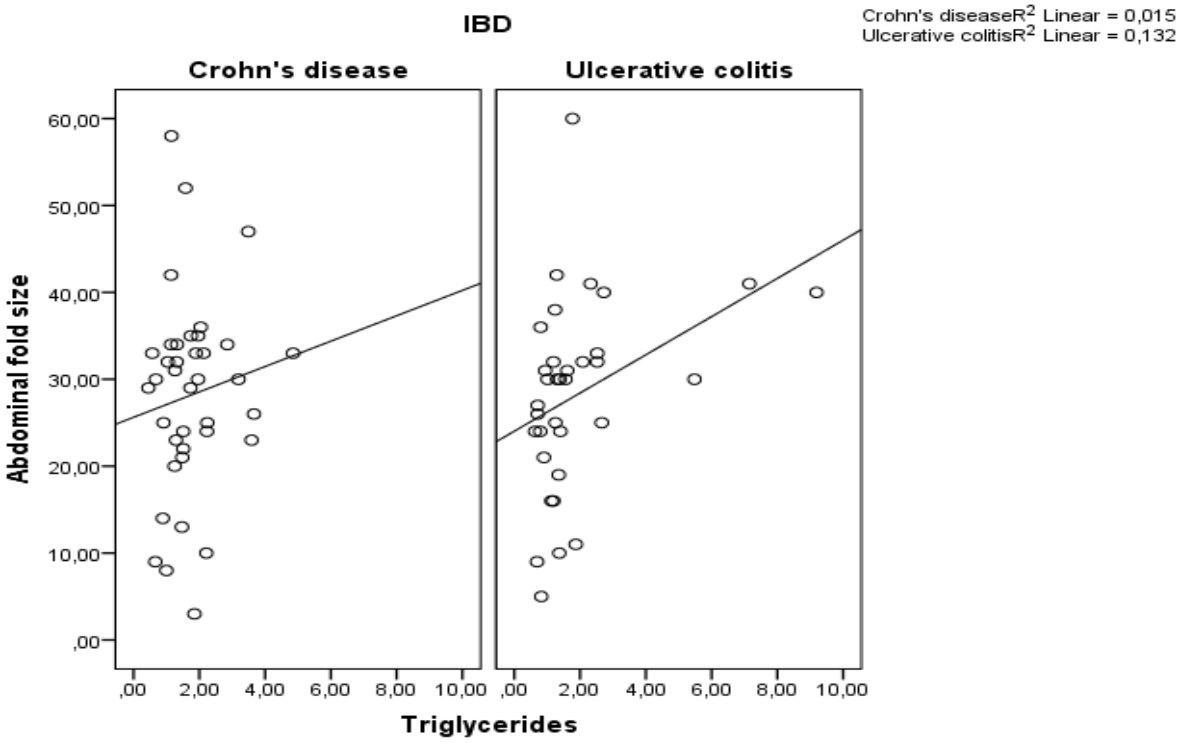

Fig. 27. Correlation analysis between the size of the abdominal skin fold and triglycerides in the CD and UC patients

Total cholesterol correlated weakly with skinfold size in both UC and CD patients, and the results were not statistically significant (r=0.279; p=0.110 for UC and r=0.274; p=0.096 for CD, respectively) (Fig. 28).

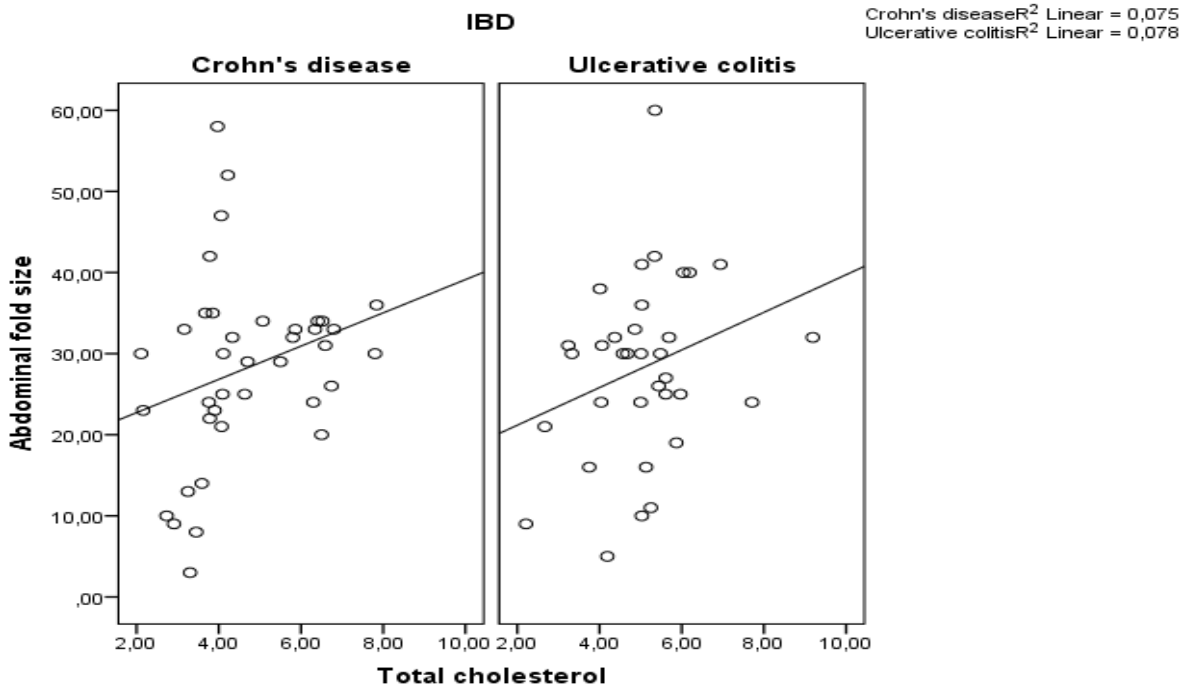

Fig. 28. Correlation analysis between the size of the abdominal skin fold and total cholesterol in the CD and UC patients

There was an inverse correlation between the size of the abdominal skinfold and HDL - cholesterol (r= -0.343; p=0.047) in UC patients (Fig. 29).

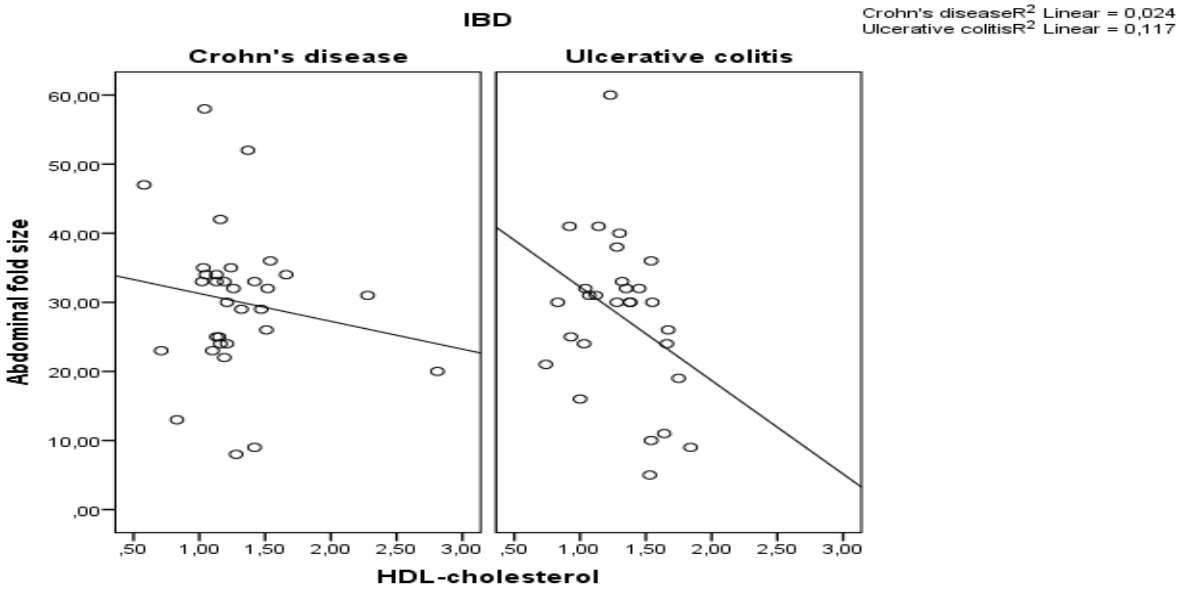

Fig. 29. Correlation analysis between the size of the abdominal skin fold and HDL-cholesterol in the CD and UC patients

Skinfold size did not correlate with LDL-cholesterol, VLDL-cholesterol, fibrinogen and CRP levels.

4.3. Expression study of a panel of miRNAs in CD and UC patients according to BMI

On Tab. 3 shows the expression of the studied miRNAs in CD and UC patients according to normal BMI and BMI ≥ 25 kg/m2. As a comparative analysis was performed between the two groups.

Table 3. Comparative expression analysis of a panel of circulating miRNAs in UC and CD patients according to BMI

miRNAs	**Cut-off**	**BMI in the norm(n=10)**	**BMI ≥ 25 kg/m2 (n=40)**	**P value**	**Expression BMI in the norm**	**Expression BMI ≥ 25 kg/m2**
hsa-miR-17-5p	0.99	1.04 ± 0.79	1.53 ± 1.69	< 0.05	6↓/ 4↑	17↓/ 23↑
hsa-miR-29a-5p	1.54	2.06 ± 1.53	1.89 ± 2.01	> 0.05	5↓/ 5↑	25↓/ 15↑
hsa-miR-142-3p	1.15	1.34 ± 0.73	1.14 ± 0.68	> 0.05	4↓/ 6↑	24↓/ 16↑
hsa-miR-146a-5p	1.06	1.15 ± 0.60	1.36 ± 1.28	> 0.05	5↓/ 5↑	20↓/ 20↑
hsa-miR-155-5p	1.32	2.61 ± 3.47	2.70 ± 3.28	> 0.05	6↓/ 4↑	19↓/ 21↑

Increased expression of hsa-miR-17-5p carries an approximately 2-fold increased likelihood of overweight and obesity in IBD patients (OR=2.029 (0.495-8.329) p<0.05).

Table 4 shows the mean serum expression data of hsa-miR-17-5p according to the studied parameters. The results showed elevated levels of the studied hsa-miR-17-5p in patients aged > 40 years (1.89 for age > 40 years to 0.89 for age < 40 years, respectively), with obesity (1.53 for obese patients to 1. 04 for patients in normal), with abdominal girth above normal (1.55 for patients with abdominal girth above normal to 0.94 for patients with normal waist circumference, respectively) and in activity (1.66 for patients in activity to 1.23 for patients in remission, respectively) (p<0.05).

There was a moderate orthogonal relationship between serum hsa-miR-17-5p expression and BMI in patients with active disease (r=0.333; p<0.05).

An orthogonal moderate correlation was also found between hsa-miR-17-5p and abdominal girth (r=0.394; p<0.05) in CD and UC patients in activity.

Another correlation that was found in patients in remission was between hsa-miR-17-5p and age, where a moderate, tending to strong orthogonal relationship was observed (r=0.482; p=0.013).

Table 4. Average value of hsa-miR-17-5p

Indicator		Serum expression of hsa-miR-17-5p	P value
Age	< 40 years	0.89±0.56	0.021
	> 40 years	1.89±1.97	
Gender	Male	1.23±0.75	0.406
	Female	1.61±2.02	
BMI groups	Norm	1.04±0.79	0.038
	Obesity	1.53±1.69	
Waist measurement (cm)	norm	0.75±0.41	0.027
	94-102/80-88	1.29±0.81	
	>102/>88	1.67±1.91	
Waist/height ratio	< 0.5	0.65±0.52	0.012
	> 0.5	1.54±1.63	
Activity/Remission	Activity	1.66±2.06	0.034
	Remission	1.23±0.89	
IBD	Crohn's disease	1.27±0.83	0.421
	Ulcerative colitis	1.56±1.99	

* The statistically significant difference ($p<0.05$) is marked in gray

A comparative analysis of the expression of circulating hsa-miR-17-5p according to both the studied parameters and patients' disease is presented in Table 5. Significant difference in miRNA expression in CD patients was found with respec-in regard to age, gender and abdominal girth. On the other hand, there was a significant difference in CD patients with respect-in regard to age, BMI, abdominal girth and presence of activity or remission.

A significant difference in expression according to the studied parameters was found in patients over 40 years, male, obese, with abdominal girth above the norm and in activity (Table 5).

Table 5. Mean value of hsa-miR-17-5p according to IBD

Indicator		**Crohn's disease (n=23)**	**Ulcerative colitis (n=27)**	**P value**
Age	< 40 years	1.00±0.52	0.91±0.67	**>0.05**
	> 40 years	1.45±0.95	2.39±2.74	**< 0.05**
	P value	**< 0.05**	**< 0.001**	
Gender	Male	0.99±0.44	1.45±0.94	**< 0.05**
	Female	1.59±1.05	1.62±2.51	**>0.05**
	P value	**< 0.05**	**> 0.05**	
BMI groups	norm	1.26±1.11	0.94±0.72	**>0.05**
	Obesity	1.28±0.82	1.78±2.26	**<0.05**
	P value	**>0.05**	**< 0.01**	
Waist measurement	norm	0.96±0.59	0.62±0.26	**>0.05**
	94-102/80-88	1.04±0.46	1.48±0.97	**<0.05**
	>102/>88	1.41±0.92	1.97±2.64	**<0.05**
	P value	**<0.05**	**<0.01**	
Waist/height ratio	< 0.05	0.63±0.18	0.66±0.57	**>0.05**
	>0.05	1.33±0.82	1.80±2.21	**<0.05**
	P value	**<0.05**	**<0.01**	
Activity/Remission	Activity	1.35±0.74	1.87±2.64	**<0.05**
	Remission	1.22±0.91	1.24±0.91	**>0.05**
	P value	**>0.05**	**<0.05**	

Fig. 30 presents a comparative analysis of the mean expression values of hsa-miR-17-5p versus activity of CD and UC and gender. As the results showed the presence of significant difference in both CD and UC patients ($p<0.05$).

Fig. 31 presents a comparative analysis of the mean expression values of hsa-miR-17-5p versus CD and UC activity and age. As the results showed the presence of significant difference in both CD and UC patients ($p<0.05$). There was a significantly higher expression in UC patients over 40 years of age.

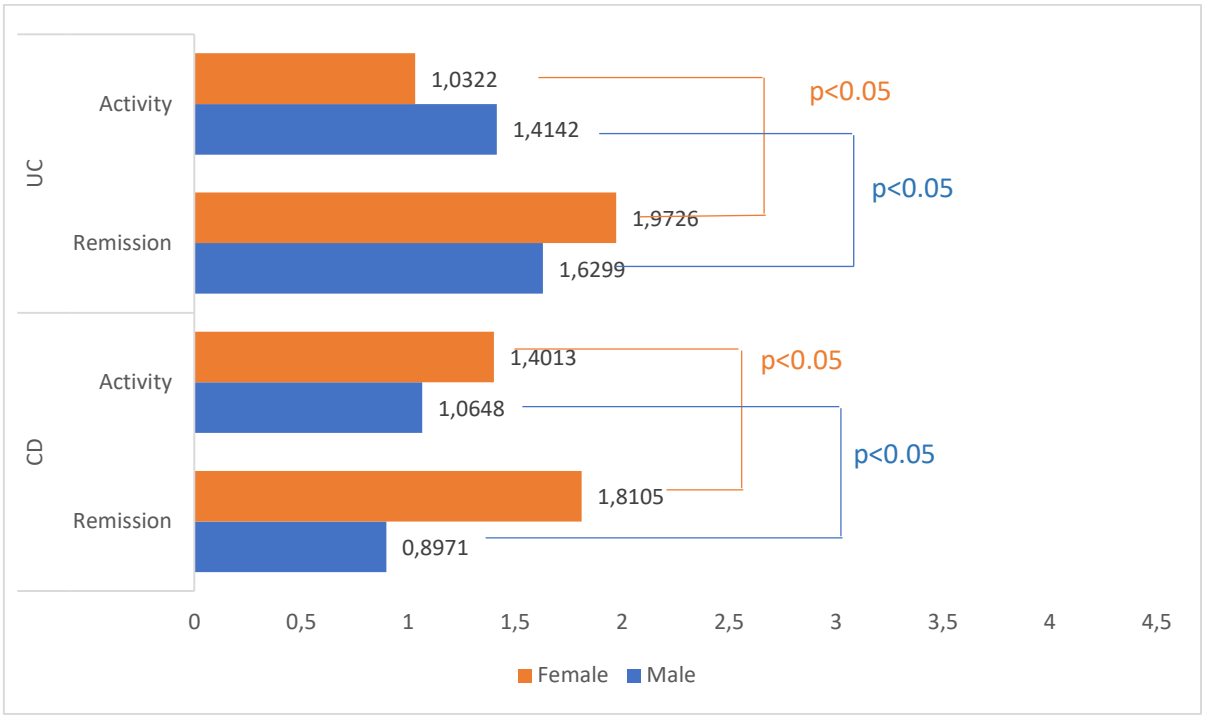

Fig. 30. Expression of hsa-miR-17-5p in relation to CD and UC activity and sex

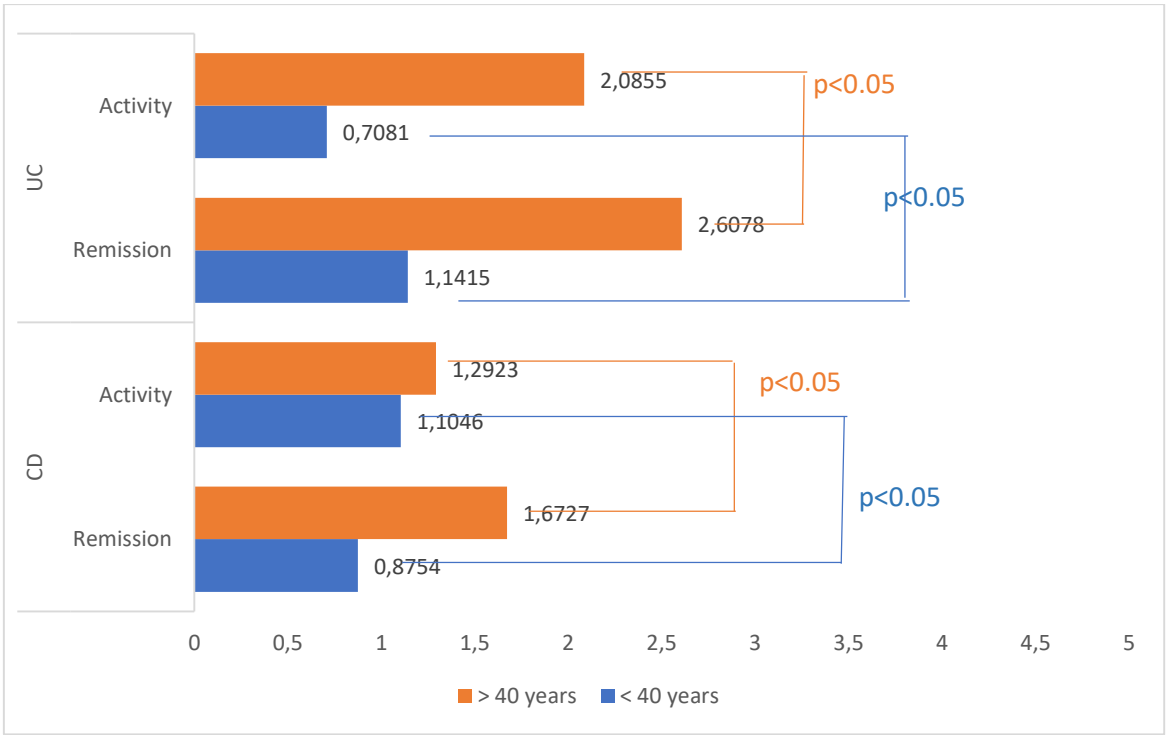

Fig. 31. Hsa-miR-17-5p expression in relation to CD and UC activity and age

Fig. 32 shows a comparative analysis of the mean expression values of hsa-miR-17-5p versus the activity of CD and UC and BMI. As the results showed the presence of significant difference only in UC patients (p<0.01). There was no significant difference in the expression of activity group in CD patients, and there were no patients with normal BMI in the remission group.

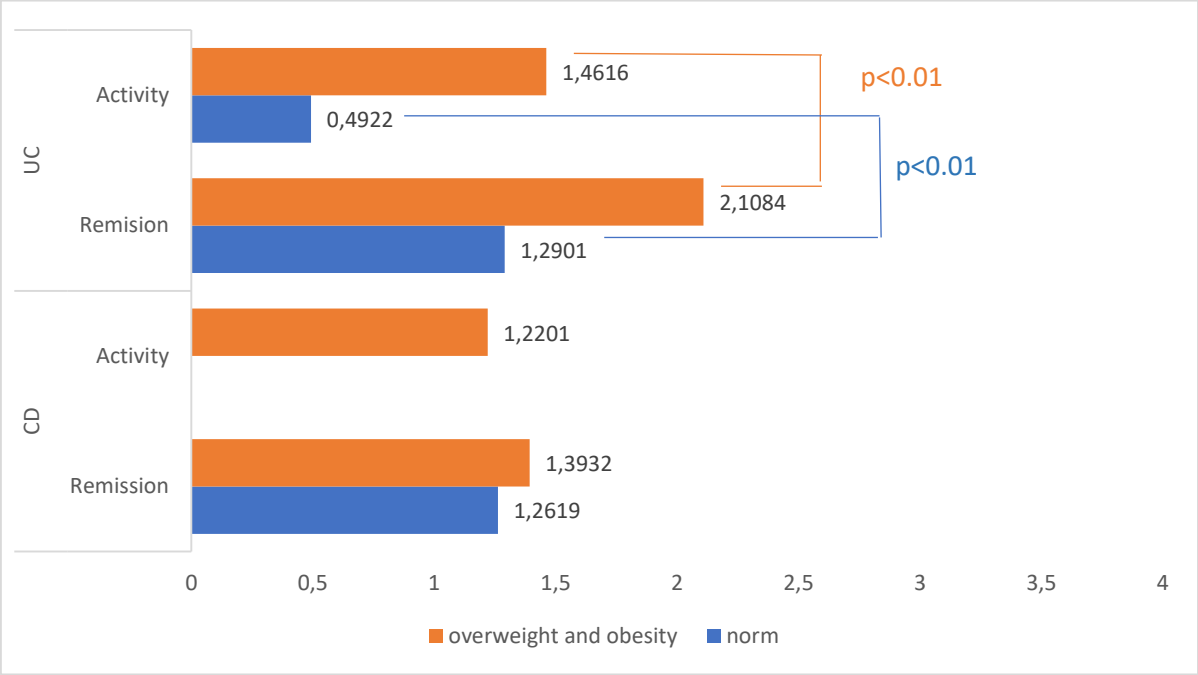

Fig. 32. Expression of hsa-miR-17-5p in relation to CD and UC activity and BMI

Table 6. Expression of hsa-miR-17-5p according to the investigated indicators and the cut-off

Indicator	**Expression cut-off**	**< 0.99 (n=21)**	**> 0.99 (n=29)**	**P value**
Age	Mean ± SD	37.00±12.88	47.55±13.83	0.009
Gender	Male	10	11	> 0.05
	Female	13	16	> 0.05
Weight	Mean ± SD	78.90±17.43	88.98±24.99	< 0.05
BMI	Mean ± SD	27.99±6.59	30.19±5.99	> 0.05
BMI groups	Norm	6	15	< 0.001
	Obesity	4	25	< 0.001
Waist measurement	Mean ± SD	95.71±15.36	101.14±14.82	>0.05
Abdominal skinfold size (mm)	Mean ± SD	28.26±12.27	30.78±10.60	>0.05
Waist/height ratio	Mean ± SD	0.57±0.09	0.59±0.07	>0.05
IBD	Crohn's disease	11	12	> 0.05
	Ulcerative colitis	10	17	0.031
Activity/Remission	Activity	9	12	> 0.05
	Remission	15	14	> 0.05

* The statistically significant difference (p<0.05) is marked in gray

Table 6 shows a comparative analysis of the studied parameters according to the established threshold value of hsa-miR-17-5p expression. Significant difference in expression according to the threshold value was found with respect to age, weight, BMI and UC.

On tab. 7 presents the mean serum expression data of hsa-miR-29a-5p according to the studied parameters. The results showed elevated levels of the studied hsa-miR-29a-5p in patients over 40 years of age (1.87 for age > 40 years to 1.66 for age < 40 years, respectively), normal weight patients (1.89 for obese patients to 2. 06 for normal patients), with waist circumference between 94-102 cm for men and 80-88 cm for women, in activity (2.44 for patients in activity to 1.48 for patients in remission, respectively) ($p<0.05$), and in CD patients (2.29 for CD patients to 1.59 for UC patients; $p<0.05$).

Table 7. Mean value of hsa-miR-29a-5p

Indicator		**Serum expression of hsa-miR-29a-5p**	**P value**
Age	< 40 years	1.66±1.65	0.630
	> 40 years	1.87±2.12	
Gender	Male	1.87±1.63	0.839
	Female	1.98±2.15	
BMI group	Norm	2.06±1.53	0.810
	Obesity	1.89±2.01	
Waist measurement (cm)	Norm	2.18±1.53	0.634
	94-102/80-88	2.29±2.05	
	>102/>88	1.72±1.97	
Waist/height ratio	< 0.5	2.19±1.73	0.696
	> 0.5	1.88±1.95	
Activity/Remission	Activity	2.44±2.53	0.046
	Remission	1.48±1.01	
IBD	Crohn's disease	2.29±2.38	0.016
	Ulcerative colitis	1.59±1.30	

* The statistically significant difference ($p<0.05$) is marked in gray

There was a moderate inverse correlation between serum hsa-miR-29a-5p expression and BMI (r=-0.397; p=0.044) (Fig. 33), and with waist circumference (r=-0.414; p=0.035) in UC patients (Fig. 34). A similar trend was observed with respect-in regard to the association between serum hsa-miR-29a-5p expression and waist-to-height ratio, where a moderate inverse correlation (r=-0.417; p=0.034) was found in UC patients (Fig. 35).

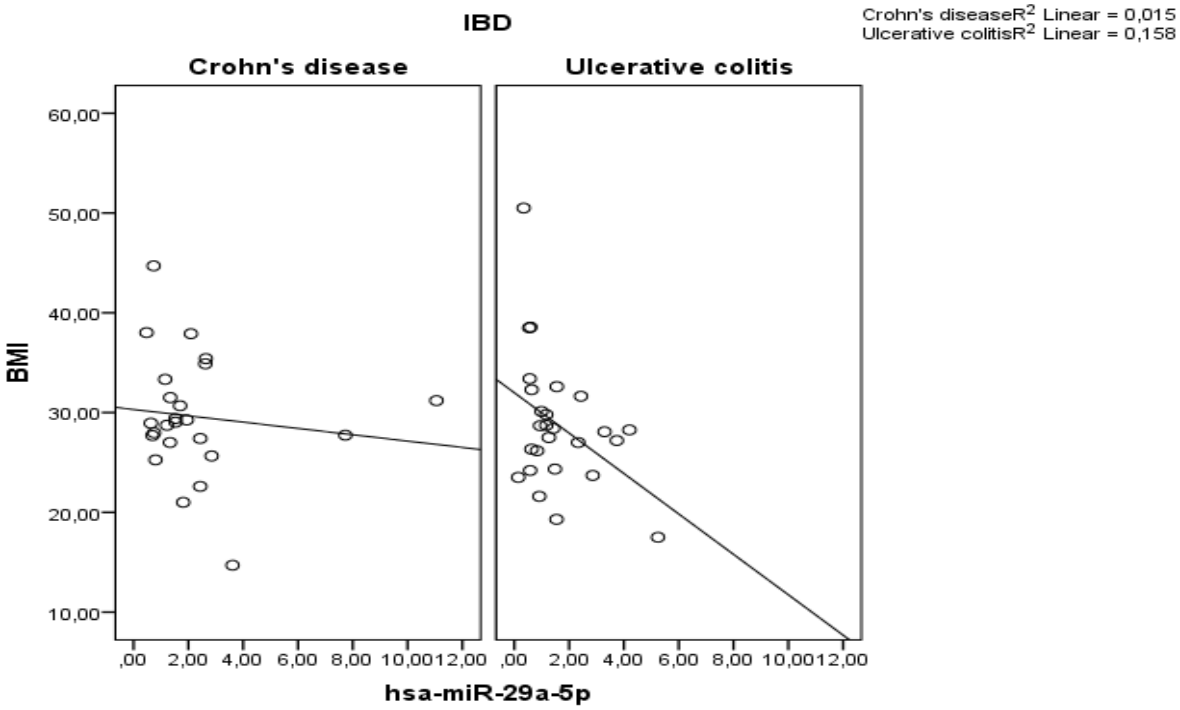

Fig. 33. Correlation analysis of the relationship between hsa-miR-29a-5p expression and BMI according to CD and UC

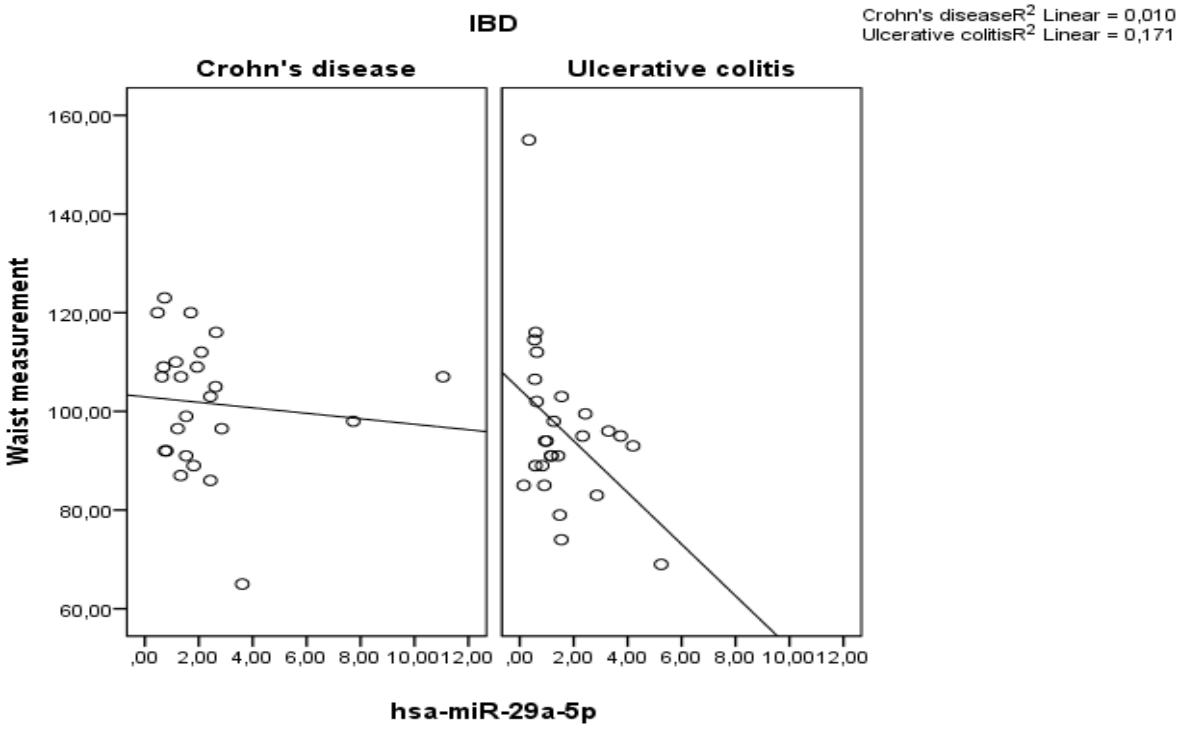

Fig. 34. Correlation analysis of the relationship between hsa-miR-29a-5p expression and waist measurement according to CD and UC

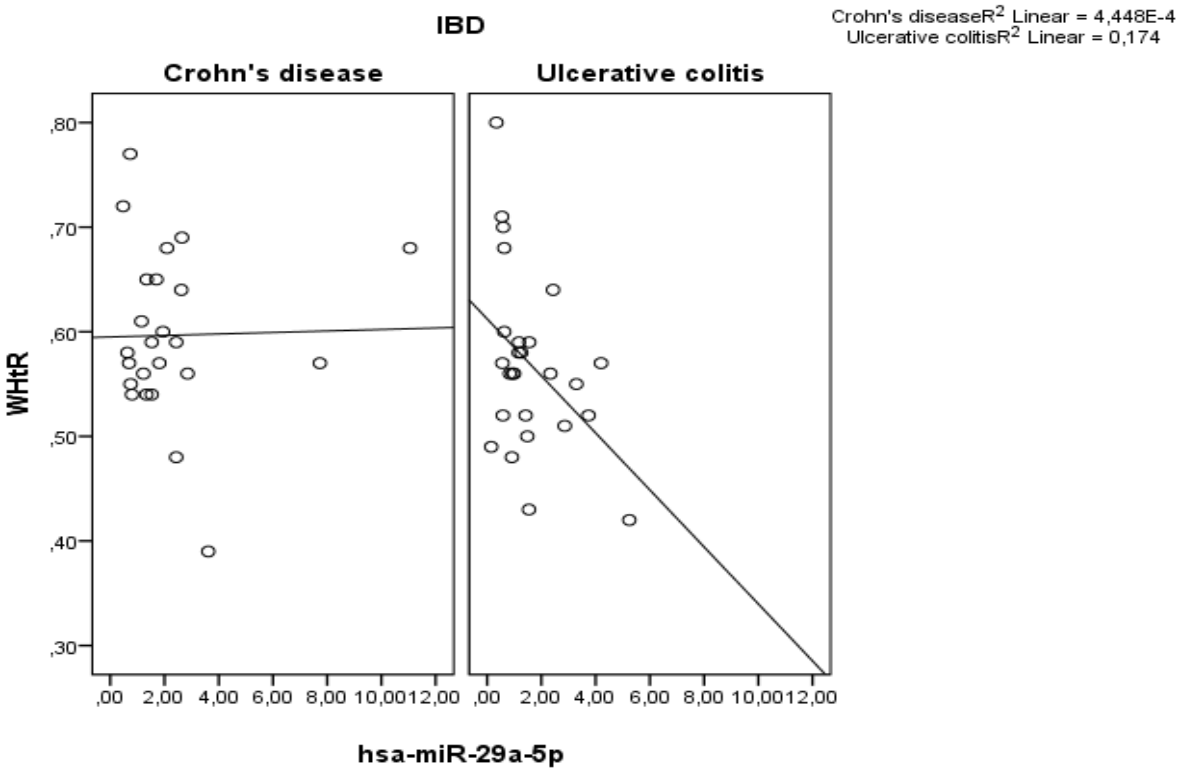

Fig. 35. Correlation analysis of the relationship between hsa-miR-29a-5p expression and waist/height ratio according to CD and UC

Another correlation that was found in UC patients was between circulating hsa-miR-29a-5p expression and skinfold size, where a moderate inverse correlation was observed (r=-0.396; p=0.045) (Fig. 36).

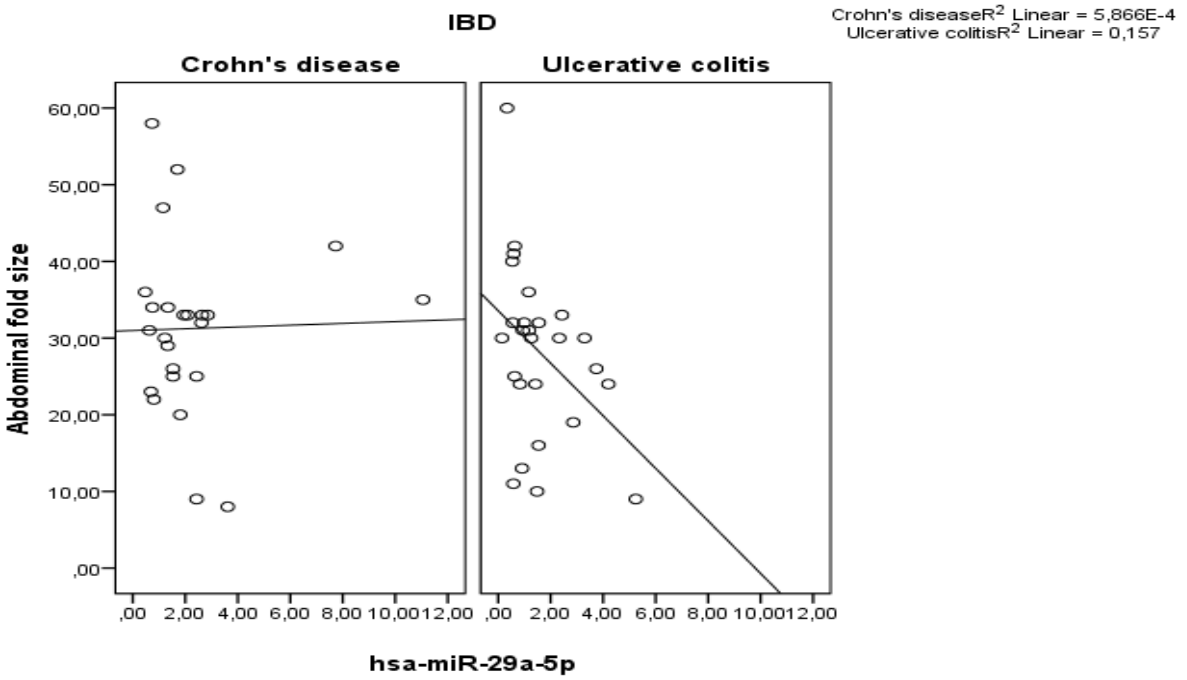

Fig. 36. Correlation analysis of the relationship between hsa-miR-29a-5p expression and abdominal skinfold size according to CD and UC

A comparative analysis of the expression of circulating hsa-miR-29a-5p according to both the studied parameters and the patients' disease is presented in Table 8. Significant difference in miRNA expression in CD patients was found with respect to disease activity and waist-to-height ratio. On the other hand, there was a significant difference in CD patients with respect to waist circumference and presence of activity or remission.

Table 8. Mean value of hsa-miR-29a-5p according to IBD

Indicator		**Crohn's disease (n=23)**	**Ulcerative colitis (n=27)**	**P value**
Age	< 40 years	2.39±2.09	1.66±1.33	**<0.05**
	> 40 years	2.22±1.64	1.49±1.32	**< 0.05**
	P value	**> 0.05**	**>0.05**	
Gender	Male	2.39±2.96	1.69±1.41	**< 0.05**
	Female	2.21±1.88	1.43±1.17	**< 0.05**
	P value	**> 0.05**	**> 0.05**	
BMI groups	Norm	2.62±0.92	1.82±1.74	**< 0.05**
	Obesity	2.25±2.53	1.51±1.15	**<0.05**
	P value	**>0.05**	**> 0.05**	
Waist measurement	Norm	2.28±1.41	2.13±1.75	**>0.05**
	94-102/80-88	2.93±2.76	1.83±1.43	**<0.05**
	>102/>88	2.09±2.49	1.28±1.06	**<0.05**
	P value	**>0.05**	**<0.05**	
Waist/height ratio	< 0.05	3.02±0.83	1.86±1.97	**<0.01**
	>0.05	2.23±2.47	1.53±1.15	**<0.05**
	P value	**<0.01**	**>0.05**	
Activity/Remission	Activity	1.75±0.94	1.23±1.03	**>0.05**
	Remission	2.93±3.34	2.00±1.51	**<0.05**
	P value	**<0.05**	**<0.05**	

Fig. 37 presents a comparative analysis of the mean expression values of hsa-miR-29a-5p versus CD and UC activity and gender. As the results showed the presence of significant difference in both CD and UC patients ($p<0.05$). A common trend emerged, both diseases showed an increase in serum hsa-miR-29a-5p expression with disease activity.

Fig. 38 presents a comparative analysis of the mean expression values of hsa-miR-29a-5p versus CD and UC activity and age. As the results showed the presence of significant difference in both CD and UC patients ($p<0.05$). BC patients showed significantly higher expression regardless of age.

There was no significant difference in hsa-miR-29a-5p expression according to the obesity score and the presence of activity and remission.

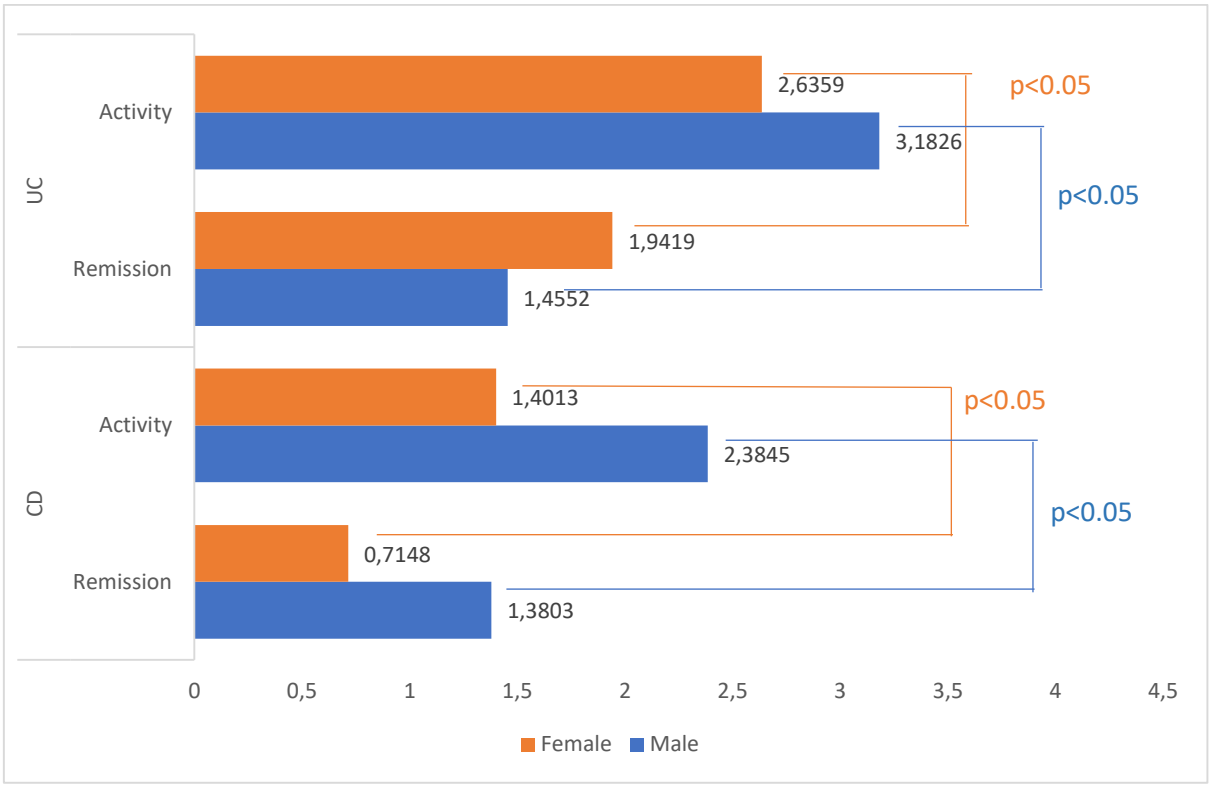

Fig. 37. Expression of hsa-miR-29a-5p in relation to CD and UC activity and gender

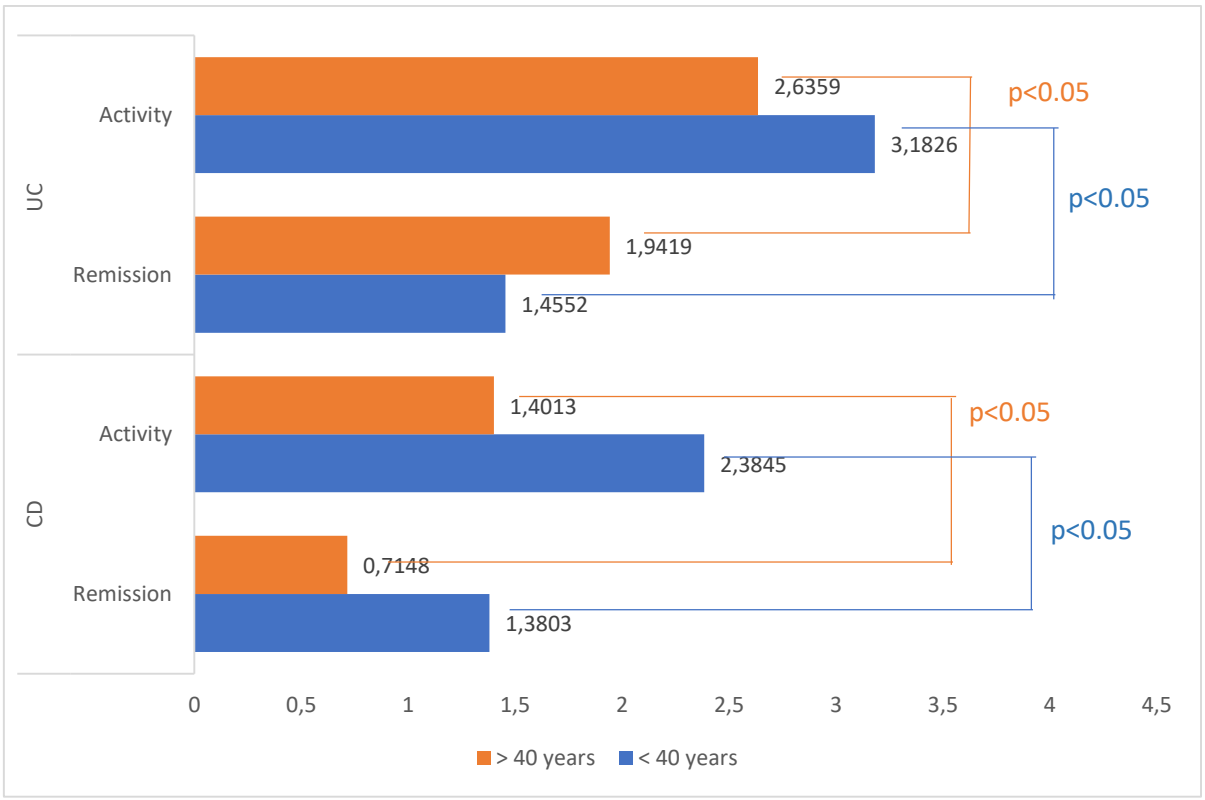

Fig. 38. Expression of hsa-miR-29a-5p in relation to CD and UC activity and age

On Table 9 presents a comparative analysis of the studied parameters according to the established threshold value of hsa-miR-29a-5p expression. A significant difference in

expression according to the threshold value was found with respect to male sex, BMI ≥ 25 kg/m2, UC patients and achieved remission.

Table 9. Expression of hsa-miR-29a-5p according to the investigated indicators and the cut-off

Indicator	**Expression cut-off**	**< 1.54 (n=30)**	**> 1.54 (n=20)**	**P value**
Age	Mean ± SD	43.46±14.41	42.82±13.08	>0.05
Gender	Male	14	9	< 0.05
	Female	16	11	> 0.05
Waist	Mean ± SD	87.79±24.73	80.52±18.33	> 0.05
BMI	Mean ± SD	30.12±6.57	27.72±5.82	> 0.05
BMI groups	Norm	5	5	> 0.05
	Obesity	25	15	< 0.05
Waist measurement	Mean ± SD	99.98±15.98	97.00±13.98	>0.05
Abdominal skinfold size (mm)	Mean ± SD	30.77±11.64	27.90±10.95	>0.05
Waist/height ratio	Mean ± SD	0.59±0.08	0.57±0.08	>0.05
IBD	Crohn's disease	12	12	> 0.05
	Ulcerative colitis	22	8	< 0.01
Activity/Remission	Activity	13	10	> 0.05
	Remission	17	10	< 0.05

* The statistically significant difference ($p<0.05$) is marked in gray

On Table 10 presents the mean serum expression data of hsa-miR-142-3p according to the studied parameters. The results showed elevated levels of the studied hsa-miR-142-3p in patients over 40 years of age (1.58 for age > 40 years to 1.31 for age < 40 years, respectively), with normal BMI (1.34 for normal patients to 1. 14 for obese patients), with abdominal girth above normal (1.38 for patients with abdominal girth above normal to 0.93 for patients with normal waist circumference, respectively), and in remission (1.21 for patients in remission to 1.15 for patients in activity, respectively).

Another correlation that was found in active patients was between hsa-miR-142-3p and age, where a moderate orthogonal relationship was observed (r=0.344; p=0.018).

Table 10. Mean value of hsa-miR-142-3p

Indicator		**Serum expression of hsa-miR-142-3p**	**P value**
Age	< 40 years	1.31±0.48	0.640
	> 40 years	1.58±0.49	
Gender	Male	1.11±0.52	0.480
	Female	1.25±0.82	
BMI groups	Norm	1.34±0.73	0.414
	Obesity	1.14±0.68	
Waist measurement	Norm	0.93±0.54	0.372
	94-102/80-88	1.38±0.47	
	>102/>88	1.17±0.78	
Waist/height ratio	< 0.5	1.07±0.64	0.636
	> 0.5	1.21±0.70	
Activity/Remission	Activity	1.15±0.62	0.796
	Remission	1.21±0.76	
IBD	Crohn's disease	1.02±0.43	0.355
	Ulcerative colitis	1.33±0.85	

On Tab. 11 presents a comparative analysis of the expression of circulating hsa-miR-142-3p according to both the studied parameters and the patients disease. A significant difference in miRNA expression in CD patients was found in the waist/height ratio. On the other hand, no significant difference was found in the examined parameters in UC patients.

A significant difference in expression according to the studied parameters was found in patients with waist circumference above normal and waist/height ratio < 0.05 (Table 11).

Table 11. Mean value of hsa-miR-142-3p according to IBD

Indicator		**Crohn's disease (n=23)**	**Ulcerative colitis (n=27)**	**P value**
Age	< 40 years	1.30±0.48	1.33±0.48	**>0.05**
	> 40 years	1.43±0.51	1.73±0.47	**> 0.05**
	P value	**>0.05**	**>0.05**	
Gender	Male	1.23±0.44	1.50±0.52	**> 0.05**
	Female	1.55±0.52	1.50±0.53	**>0.05**
	P value	**> 0.05**	**> 0.05**	
BMI групи	Norm	1.33±0.58	1.71±0.49	**>0.05**
	Obesity	1.38±0.49	1.42±0.51	**>0.05**
	P value	**>0.05**	**> 0.05**	
Waist measurement	Norm	1.33±0.57	1.20±0.45	**>0.05**
	94-102/80-88	1.20±0.44	2.00±0.00	**<0.05**
	>102/>88	1.44±0.51	1.36±0.49	**>0.05**
	P value	**>0.05**	**>0.05**	
Waist/height ratio	< 0.05	1.00±0.00	1.60±0.55	**<0.05**
	>0.05	1.41±0.50	1.47±0.51	**>0.05**
	P value	**<0.05**	**>0.05**	
Activity/Remission	Activity	1.36±0.51	1.50±0.52	**>0.05**
	Remission	1.38±0.51	1.50±0.52	**>0.05**
	P value	**>0.05**	**>0.05**	

Fig. 39 presents a comparative analysis of the mean expression values of hsa-miR-142-3p versus CD and UC activity and gender. Despite the variation in the mean serum levels of the miRNA examined, there was no significant difference in either disease activity or disease itself between the two sexes. It can be said that a slight increase in hsa-miR-142-3p expression was observed in female CD patients. On the other hand, in UC patients, miRNA expression was higher in patients in remission in males (1.4765 for males and 1.3404 for females, respectively), whereas in patients in activity in females (1.3916 for females and 1.2073 for males, respectively).

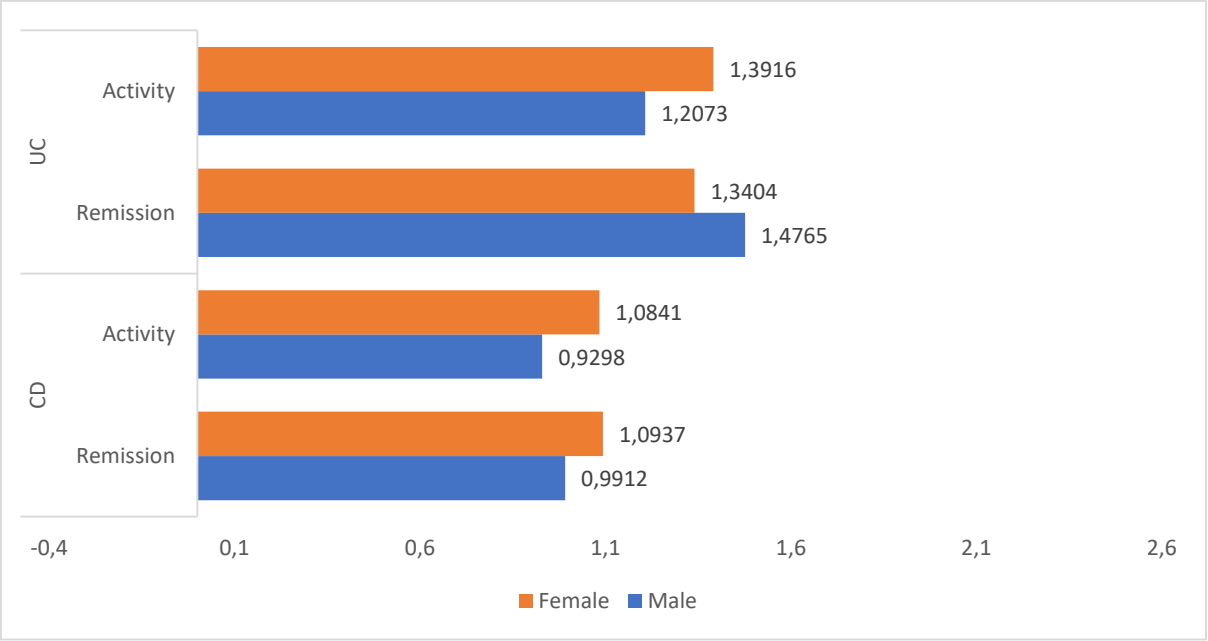

Fig. 39. Expression of hsa-miR-142-3p in relation to CD and UC activity and gender

Fig. 40 presents a comparative analysis of the mean expression values of hsa-miR-142-3p versus CD and UC activity and age. As the results showed the presence of a significant difference only in UC patients >40 years ($p<0.05$). A significantly higher expression was observed in UC patients >40 years.

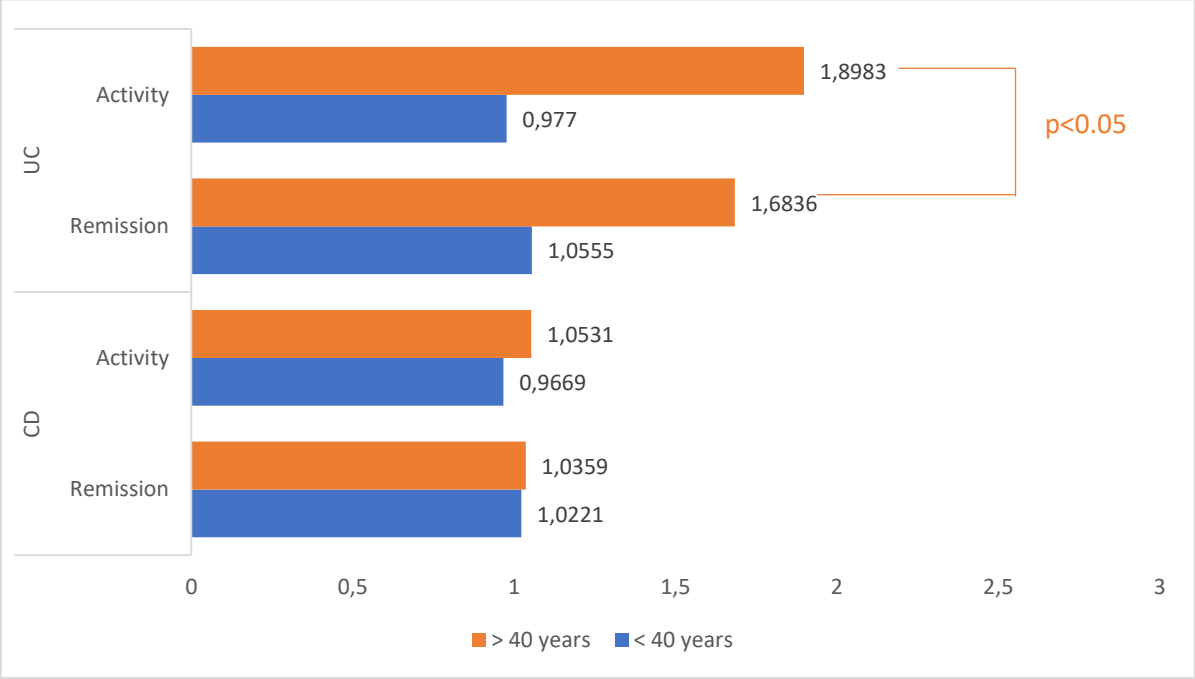

Fig. 40. Expression of hsa-miR-142-3p in relation to CD and UC activity and age

Fig. 41 shows a comparative analysis of the mean expression values of hsa-miR-142-3p versus CD and UC activity and BMI. As the results showed the presence of significant difference only in overweight and obese CD patients ($p<0.01$) and in normal BMI in CD patients ($p<0.01$).

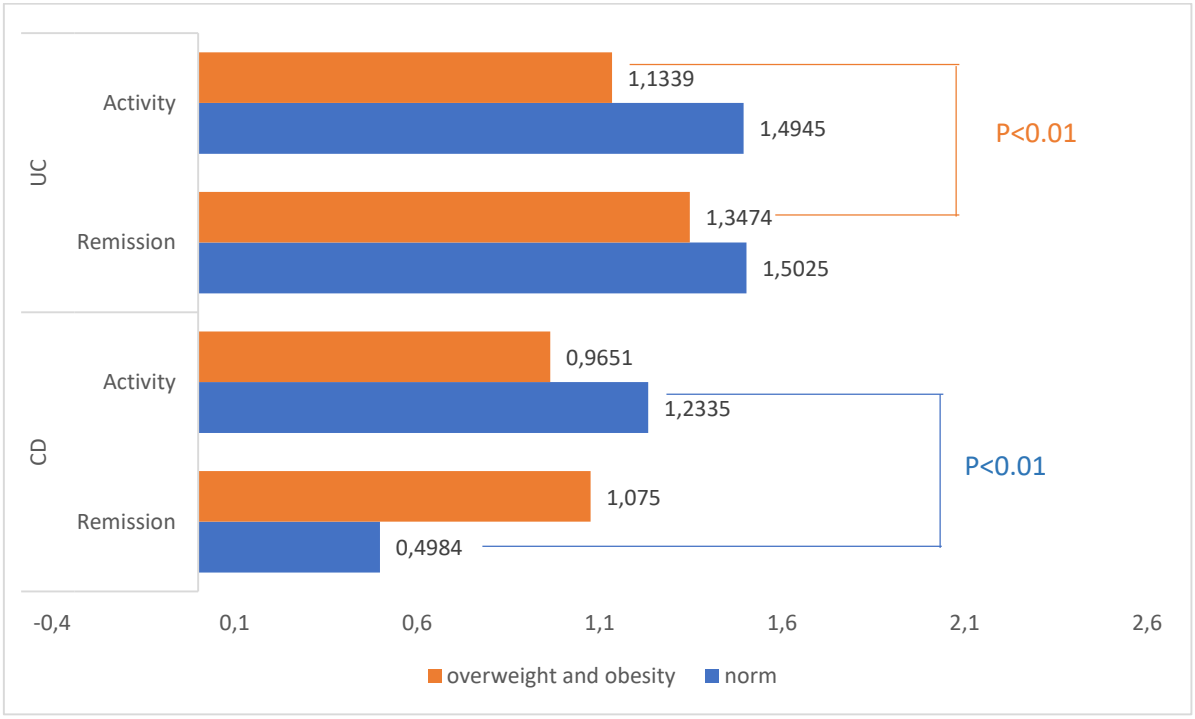

Fig. 41. Expression of hsa-miR-142-3p in relation to CD and UC activity and BMI

On Table 12 presents a comparative analysis of the studied parameters according to the established threshold value of hsa-miR-142-3p expression. Significant difference in expression according to the threshold value was found with respect-in regard to age, male sex, weight, and CD ($p<0.05$). A uniform distribution was observed in females. For BMI, waist circumference, skinfold size, and waist-to-height ratio, there was no significant difference in the results according to the threshold value of hsa-miR-142-3p.

Table 12. Expression of hsa-miR-142-3p according to the investigated indicators and the cut-off

Indicator	**Expression cut-off**	**< 1.15 (n=21)**	**> 1.15 (n=29)**	**P value**
Age	Mean ± SD	39.70±12.09	46.83±16.27	0.035
Gender	Male	15	8	< 0.05
	Female	13	14	> 0.05
Weight	Mean ± SD	78.90±17.43	88.98±24.99	< 0.05
BMI	Mean ± SD	27.99±6.59	30.19±5.99	> 0.05
BMI groups	Norm	4	6	>0.05
	Obesity	24	16	>0.05

Waist measurement	Mean ± SD	98.11±13.78	99.66±17.00	>0.05
Abdominal skinfold size (mm)	Mean ± SD	30.18±11.28	28.91±11.66	>0.05
Waist/height ratio	Mean ± SD	0.58±0.08	0.58±0.08	>0.05
IBD	Crohn's disease	15	9	< 0.05
	Ulcerative colitis	13	13	> 0.05
Activity/Remission	Activity	13	10	> 0.05
	Remission	15	12	> 0.05

* The statistically significant difference ($p<0.05$) is marked in gray

The comparative analysis of mean serum expression levels of has-miR-146a-5p showed significantly higher levels in patients aged 40 years and older (1.72) and in patients with waist circumference above normal (1.44). Despite no statistically significant difference, higher expression of has-miR-146a-5p was observed in male, obese, active and ulcerative colitis patients (Table 13).

Table 13. Average value of hsa-miR-146a-5p

Indicator		**Serum expression of hsa-miR-146a-5p**	**P value**
Age	< 40 years	0.89±0.46	0.012
	> 40 years	1.72±1.48	
Gender	Male	1.35±1.49	0.856
	Female	1.29±0.86	
BMI groups	Norm	1.16±0.60	0.633
	Obesity	1.36±1.28	
Abdominal circumference	Norm	0.64±0.24	0.050
	94-102/80-88	1.92±1.86	
	>102/>88	1.26±0.86	
Waist/height ratio	< 0.05	0.90±0.53	0.318
	>0.05	1.38±1.24	
Activity/Remission	Activity	1.41±1.43	0.623
	Remission	1.24±0.91	
IBD	Crohn's disease	1.15±0.88	0.358
	Ulcerative colitis	1.46±1.38	

* The statistically significant difference ($p<0.05$) is marked in gray

There was a moderate orthogonal relationship between serum hsa-miR-146a-5p expression and BMI in CD patients (r=0.330; p<0.05).

A moderate orthogonal relationship was also found between hsa-miR-146a-5p and waist circumference in CD patients (r=0.324; p<0.05).

On Table 14 shows a comparative analysis of hsa-miR-146a-5p expression according to the selected parameters in the two groups of patients. The results showed a significant difference in serum miRNA levels according to age, sex and waist circumference in CD patients and according to age, sex, BMI and waist circumference in UC patients. It makes impression that, the expression of hsa-miR-146a-5p was higher in UC patients.

Table 14. Mean value of hsa-miR-146a-5p according to IBD

Indicator		**Crohn's disease (n=23)**	**Ulcerative colitis (n=27)**	**P value**
Age	< 40 years	0.85±0.37	0.91±0.51	>0.05
	> 40 years	1.34±1.07	2.15±1.80	< 0.05
	P value	**< 0.05**	**< 0.001**	
Gender	Male	0.84±0.35	1.91±2.02	< 0.05
	Female	1.49±1.16	1.15±0.57	>0.05
	P value	**< 0.05**	**< 0.05**	
BMI groups	Norm	1.16±0.71	1.16±0.61	>0.05
	Obesity	1.15±0.92	1.57±1.56	<0.05
	P value	**>0.05**	**< 0.05**	
Waist measurement	Norm	0.85±0.19	0.51±0.18	>0.05
	94-102/80-88	2.08±2.96	1.81±0.71	>0.05
	>102/>88	1.31±1.03	1.21±0.64	>0.05
	P value	**<0.05**	**<0.05**	
Waist/height ratio	< 0.05	0.75±0.13	0.96±0.63	>0.05
	>0.05	1.47±1.59	1.30±0.75	>0.05
	P value	**<0.05**	**<0.05**	
Activity/Remission	Activity	1.14±0.81	1.59±1.75	<0.05
	Remission	1.15±0.97	1.31±0.87	>0.05
	P value	**>0.05**	**>0.05**	

Fig. 42 presents a comparative analysis of the mean expression values of hsa-miR-146a-5p versus CD and UC activity and gender. As the results showed the presence of a significant difference in UC patients ($p<0.01$). Overexpression of circulating miRNA was observed in males with active stage of UC.

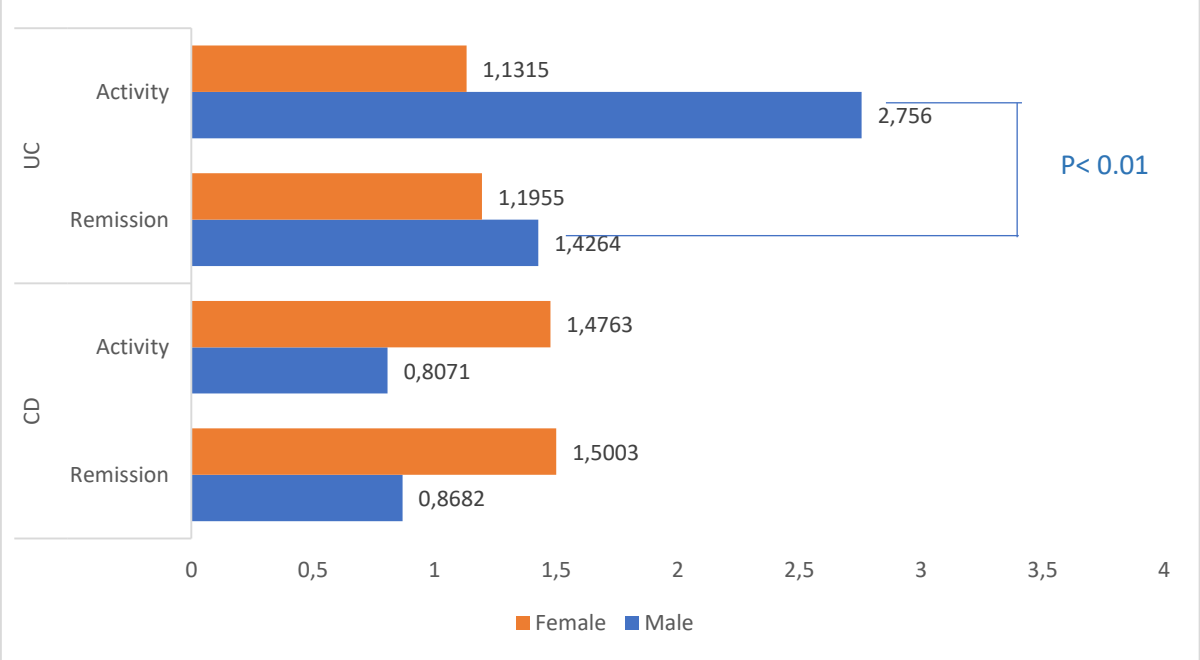

Fig. 42. Expression of hsa-miR-146a-5p in relation to CD and UC activity and gender

Fig. 43 presents a comparative analysis of the mean expression values of hsa-miR-146a-5p versus CD and UC activity and age. As the results showed the presence of significant difference in both CD and UC patients ($p<0.05$). There was a significantly higher expression in UC patients over 40 years of age.

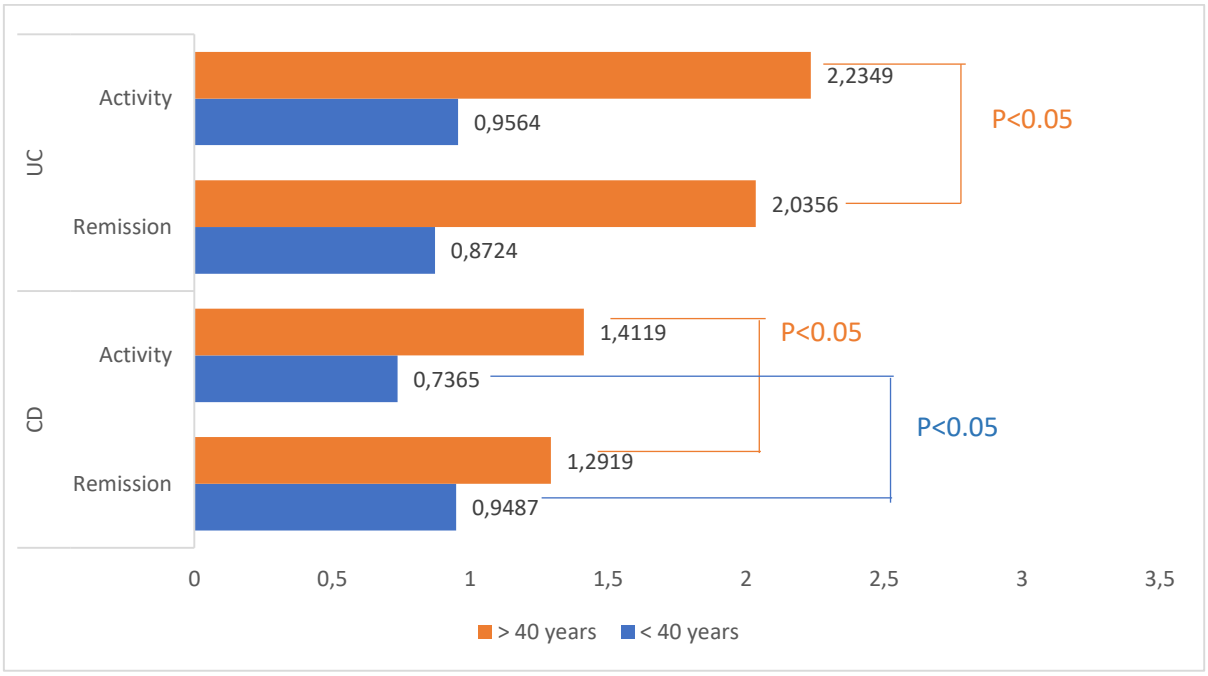

Fig. 43. Expression of hsa-miR-146a-5p in relation to CD and UC activity and age

Fig. 44 shows a comparative analysis of the mean expression values of hsa-miR-146a-5p versus the activity of CD and UC and BMI. As the results showed the presence of significant difference only in the UC patients ($p<0.01$). There was no significant difference in the expression of activity group in CD patients, and there were no patients with normal BMI in the remission group.

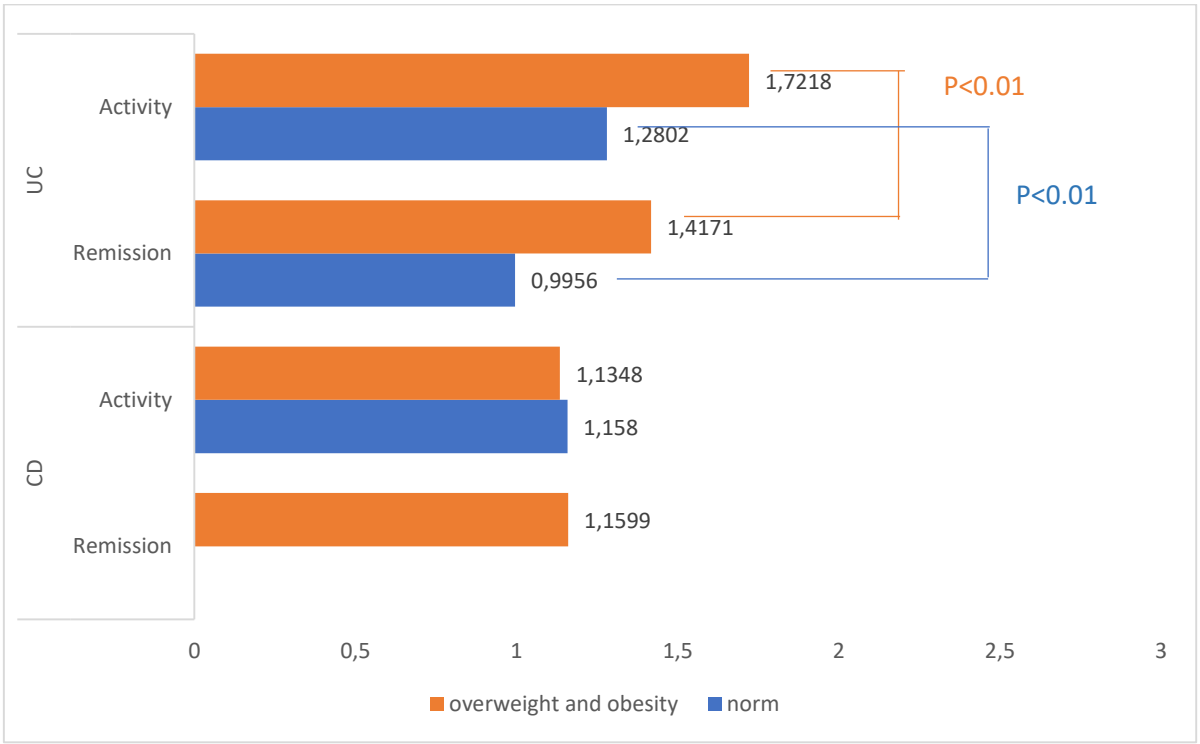

Fig. 44. Expression of hsa-miR-146a-5p in relation to CD and UC activity and BMI

Fig. 45 presents a comparative analysis of the mean expression values of hsa-miR-146a-5p versus CD and UC activity and waist circumference. As the results showed the presence of significant difference in UC patients ($p<0.05$). There was a significant difference in the expression of the activity group ($p<0.01$) and no patients with normal waist circumference in the remission group.

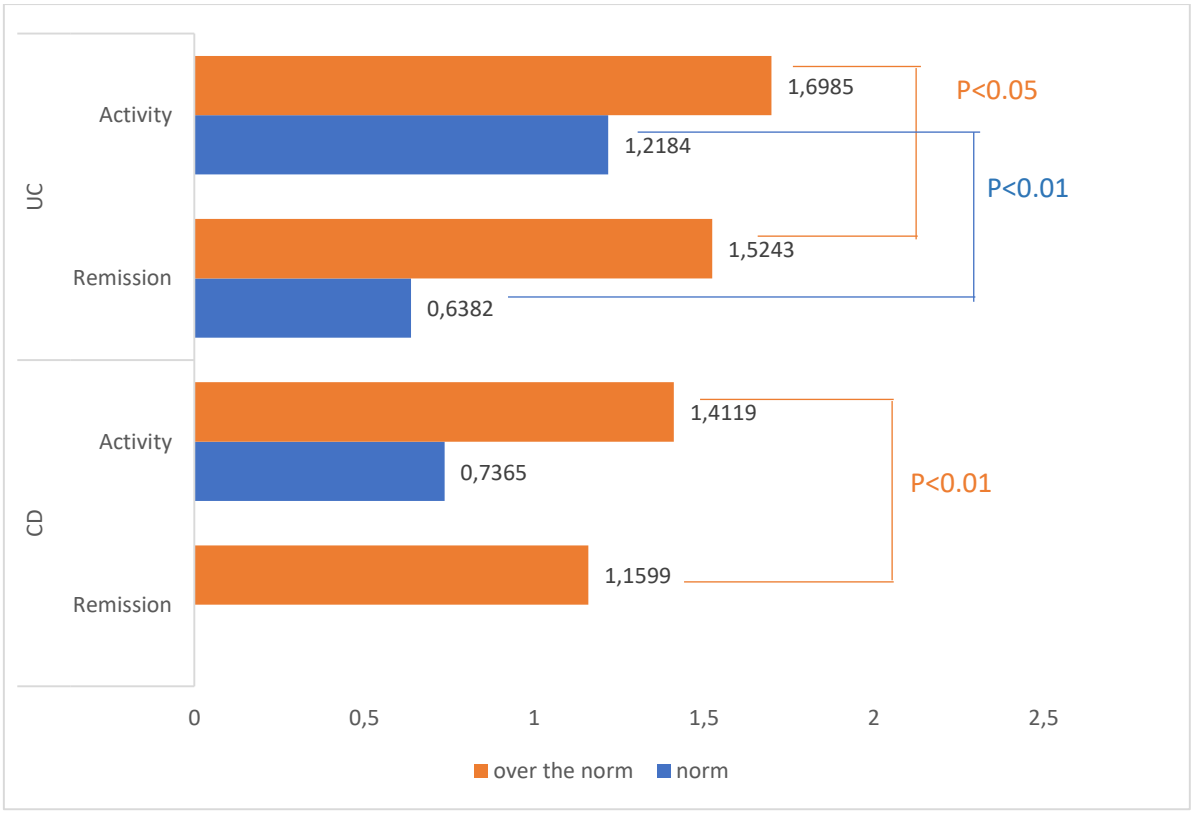

Fig. 45. Expression of hsa-miR-146a-5p in relation to CD and UC activity and waist circumference

On Table 15 presents a comparative analysis of the studied parameters according to the established threshold value of hsa-miR-146a-5p expression. Significant difference in expression according to the threshold value was found with respect to age, and IBD.

Table 15. Expression of has-miR-146a-5p according to the investigated indicators and the cut-off

Indicator \ Cut-off of hsa-miR-146a-5p		< 1.06 (n=25)	> 1.06 (n=25)	P value
Age	Mean ± SD	37.00±12.68	49.04±13.56	0.003
Gender	Male	13	10	>0.05
	Female	12	15	>0.05
Weight	Mean ± SD	80.40±16.78	89.10±26.69	>0.05
BMI	Mean ± SD	28.22±6.11	30.31±6.40	>0.05
BMI groups	Norm	5	5	>0.05
	Obesity	20	20	>0.05
Waist measurement	Mean ± SD	95.96±14.15	101.76±15.81	>0.05
Size of the abdominal skinfold (mm)	Mean ± SD	28.28±11.91	30.96±10.84	>0.05
Waist/height ratio	Mean ± SD	0.57±0.09	0.59±0.08	>0.05
IBD	Crohn's disease	15	8	< 0.05
	Ulcerative colitis	10	17	< 0.05
Activity/Remission	Activity	11	13	>0.05
	Remission	14	12	>0.05

* The statistically significant difference ($p<0.05$) is marked in gray

On table 16 the data on the average serum expression of hsa-miR-155-5p according to the studied parameters are presented. The results showed elevated levels of the studied hsa-miR-155-5p in patients over 40 years of age (2.72 for age > 40 years to 1.99 for age < 40 years, respectively), with obesity (2.70 for obese patients to 2. 61 for normal patients), with abdominal girth above normal (2.95 for patients with abdominal girth above normal to 1.47 for patients with normal waist circumference, respectively), and in remission (3.35 for patients in remission to 1.90 for patients in activity, respectively). An orthogonal moderate correlation was also found between hsa-miR-155-5p and waist circumference in UC patients ($r=0.351$; $p<0.05$).

Table 16. Mean value of hsa-miR-155-5p

Indicator		Serum expression of hsa-miR-155-5p	P value
Age	< 40 years	1.99±2.18	0.276
	> 40 years	2.72±3.72	
Gender	Male	2.99±3.57	0.542
	Female	2.42±3.07	
BMI groups	Norm	2.61±3.47	0.939
	Obesity	2.70±3.28	
Waist measurement	Norm	1.47±0.76	0.532
	94-102/80-88	2.83±3.24	
	>102/>88	2.95±3.69	
Waist/height ratio	< 0.5	2.01±1.67	0.562
	> 0.5	2.79±3.48	
Activity/Remission	Activity	1.90±2.37	0.120
	Remission	3.35±3.81	
IBD	Crohn's disease	2.76±3.50	0.163
	Ulcerative colitis	2.62±3.14	

On table 17 presents a comparative analysis of the expression of circulating hsa-miR-155-5p according to both the studied parameters and the patients' disease. A significant difference in miRNA expression was found in CD patients with respect-in regard to age, sex, BMI, waist circumference and waist-to-height ratio. On the other hand, there was a significant difference in CD patients with respect-in regard to gender, BMI and waist/height ratio.

Table 17. Mean value of hsa-miR-155-5p according to IBD

Indicator		**Crohn's disease (n=23)**	**Ulcerative colitis (n=27)**	**P value**
Age	< 40 years	1.70±0.48	1.40±0.51	**<0.05**
	> 40 years	1.42±0.51	1.36±0.50	**>0.05**
	P value	**<0.05**	**>0.05**	
Gender	Male	1.62±0.51	1.60±0.51	**>0.05**
	Female	1.45±0.52	1.25±0.44	**<0.05**
	P value	**<0.05**	**<0.05**	
BMI groups	Norm	2.00±0.11	1.14±0.37	**<0.05**
	Obesity	1.48±0.51	1.47±0.51	**>0.05**
	P value	**<0.05**	**<0.05**	
Waist measurement	Norm	2.00±0.59	1.20±0.44	**<0.05**
	94-102/80-88	1.60±0.55	1.57±0.53	**>0.05**
	>102/>88	1.44±0.51	1.36±0.49	**>0.05**
	P value	**<0.05**	**>0.05**	
Waist/height ratio	< 0.05	2.00±0.18	1.20±0.44	**<0.05**
	>0.05	1.50±0.51	1.43±0.51	**>0.05**
	P value	**<0.05**	**<0.05**	
Activity/Remission	Activity	1.54±0.52	1.33±0.49	**<0.05**
	Remission	1.54±0.52	1.43±0.51	**>0.05**
	P value	**>0.05**	**>0.05**	

A significant difference in expression according to the studied parameters was found in patients < 40 years old, female, BMI in the normal range, waist circumference in the normal range, waist/height ratio < 0.05 and in activity (table 17).

Fig. 46 presents a comparative analysis of the mean expression values of hsa-miR-155-5p versus CD and UC activity and gender. As the results showed the presence of significant difference in both CD and UC patients ($p<0.01$). Male patients in remission with UC were found to overexpress the miRNA studied (7.34).

Fig. 47 presents a comparative analysis of the mean expression values of hsa-miR-155-5p versus CD and UC activity and age. As the results showed the presence of significant difference in both CD and UC patients ($p<0.05$). Significantly higher expression was observed in UC patients over 40 years in remission (4.37), and similar results were observed in CD patients under 40 years in remission (4.02).

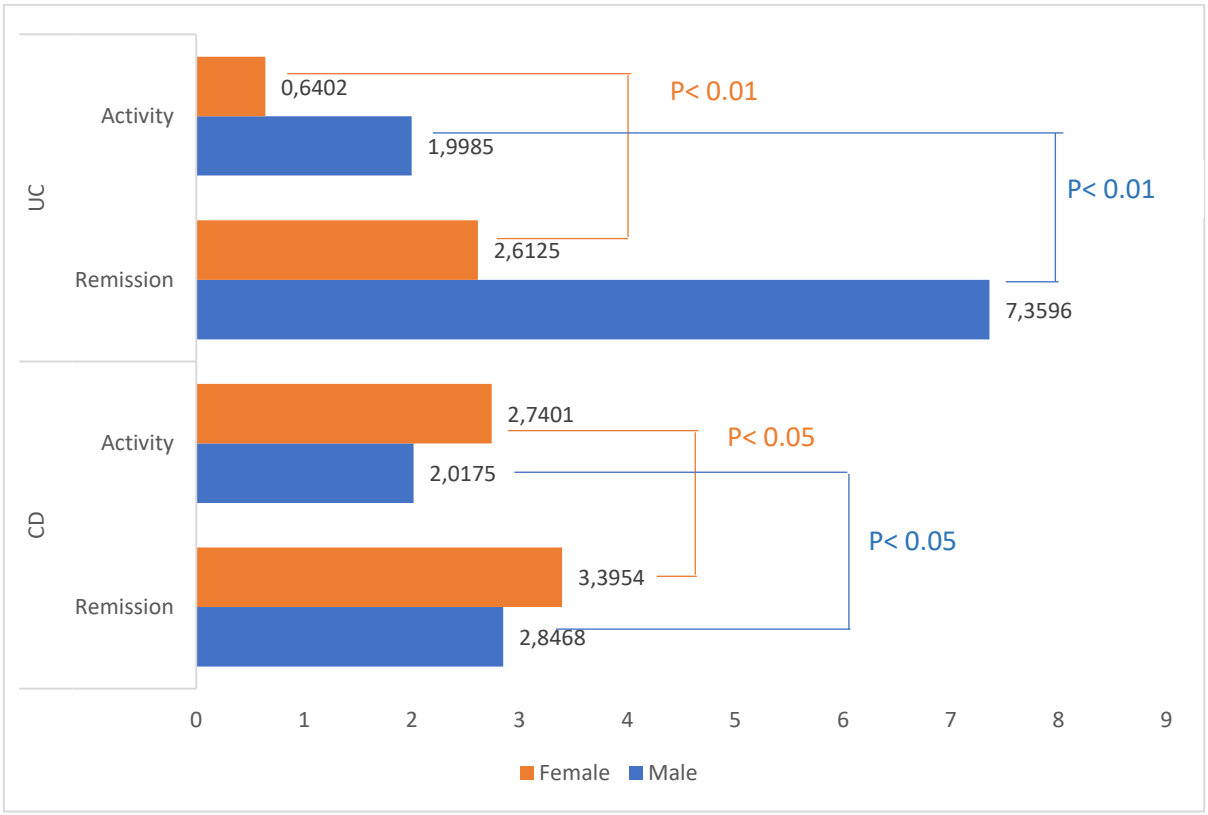

Fig. 46. Expression of hsa-miR-155-5p in relation to CD and UC activity and gender

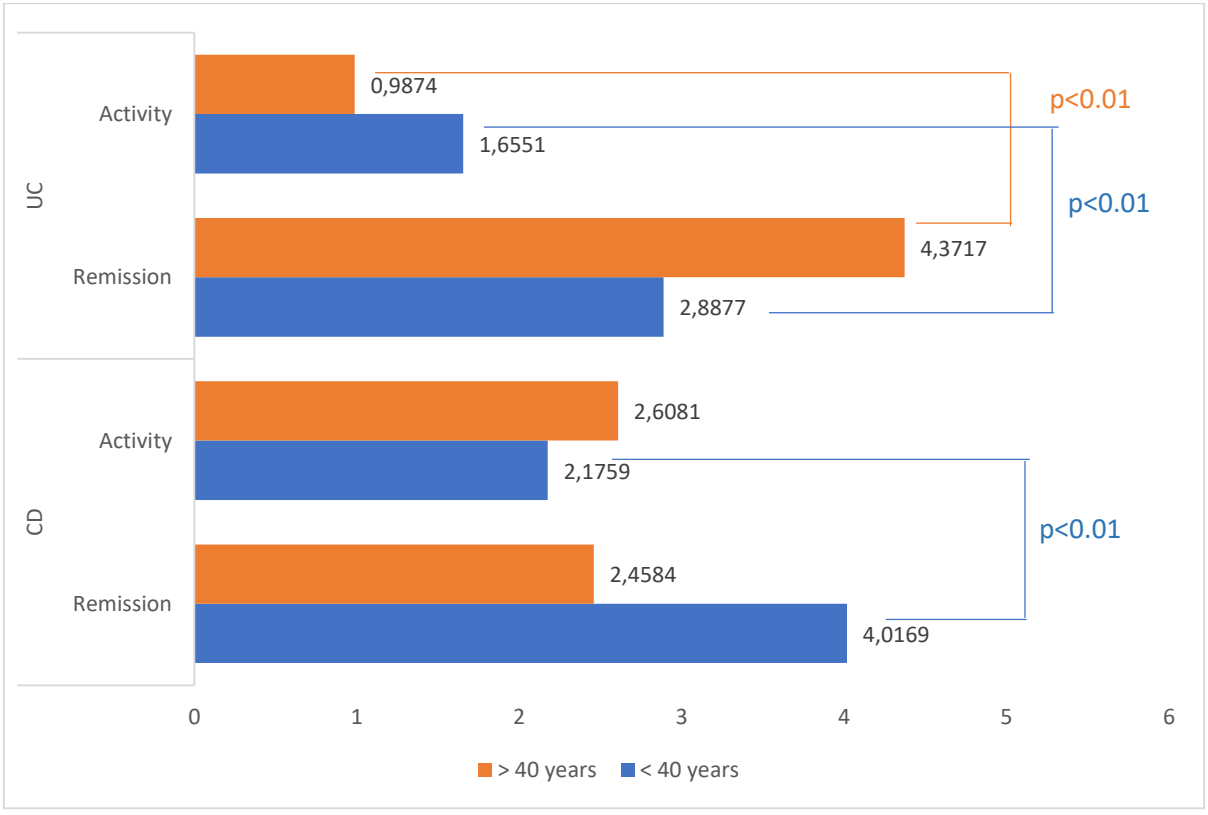

Fig. 47. Expression of hsa-miR-155-5p in relation to CD and UC activity and age

On table 18 presents a comparative analysis of the studied parameters according to the established threshold value of hsa-miR-155-5p expression. No significant difference in expression according to the threshold value was found with respect to the examined indicators.

Table 18. Expression of hsa-miR-155-5p according to the investigated indicators and the cut-off

Cut-off of hsa-miR-155-5p **Indicator**		**< 1.32 (n=25)**	**> 1.32 (n=25)**	**P value**
Age	Mean ± SD	45.04±14.19	41.16±14.41	>0.05
Gender	Male	16	11	>0.05
	Female	9	14	>0.05
Weight	Mean ± SD	82.65±16.71	87.12±27.24	>0.05
BMI	Mean ± SD	28.74±5.46	29.57±7.19	>0.05
BMI groups	Norm	6	4	>0.05
	Obesity	19	21	>0.05
Waist measurement	Mean ± SD	97.86±14.00	99.72±16.43	>0.05
Size of the abdominal skinfold (mm)	Mean ± SD	27.72±10.43	31.52±12.12	>0.05
Waist/height ratio	Mean ± SD	0.58±0.08	0.59±0.08	>0.05
IBD	Crohn’s disease	11	13	> 0.05
	Ulcerative colitis	14	12	> 0.05
Activity/Remission	Activity	13	10	>0.05
	Remission	12	15	>0.05

4.4 Study of human Lipocalin-2/NGAL expression in patients with Crohn's disease and ulcerative colitis

On table 19 the data on the mean value of serum human Lipocalin - 2/NGAL expression according to the studied parameters are presented. The results showed elevated human Lipocalin - 2/NGAL levels in patients < 40 years (76.14 for age < 40 years to 61.90 for age > 40 years, respectively), BMI in the normal range (81.11 for normal subjects to 63.59 for obese subjects, respectively), with waist circumference in the normal range (80.53 for patients with waist circumference in normal to 61.37 for patients with waist circumference above normal) and in activity (83.90 for patients in activity to 56.61 for patients in remission, respectively).

Table 19. Average value of human Lipocalin - 2/NGAL

Indicator		Lipocalin 2 serum expression	P value
Age	< 40 г.	76.14±42.89	0.135
	> 40 г.	61.90±40.34	
Gender	Male	69.40±40.16	0.968
	Female	69.02±44.21	
BMI groups	Norm	81.11±44.28	0.086
	Obesity	63.59±40.11	
Waist measurement (cm)	Norm	80.53±39.85	0.208
	94-102/80-88	75.08±40.37	
	>102/>88	61.37±43.03	
Waist/height ratio	< 0.5	84.09±39.22	0.099
	> 0.5	65.05±42.13	
Activity/Remission	Activity	83.90±44.13	0.004
	Remission	56.61±36.06	
IBD	Crohn's disease	75.96±45.80	0.146
	Ulcerative colitis	62.09±36.89	

* The statistically significant difference ($p<0.05$) is marked in gray

There was a weak inverse correlation between human Lipocalin - 2/NGAL expression and BMI in IBD patients (r=-0.237; p=0.036) (Fig. 48).

There was a moderate inverse correlation between human Lipocalin - 2/NGAL expression and waist circumference in CD patients (r=-0.324; p=0.041) (Fig. 49).

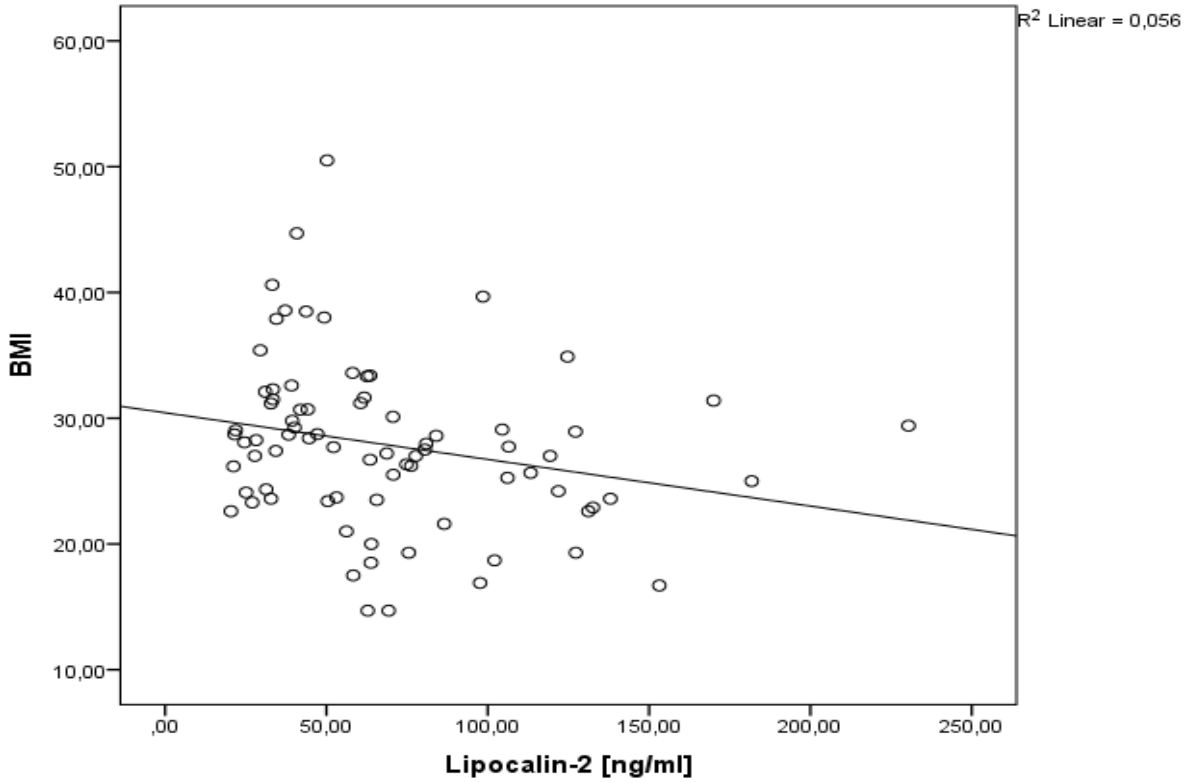

Fig. 48. Correlation between human Lipocalin - 2/NGAL and BMI in IBD patients

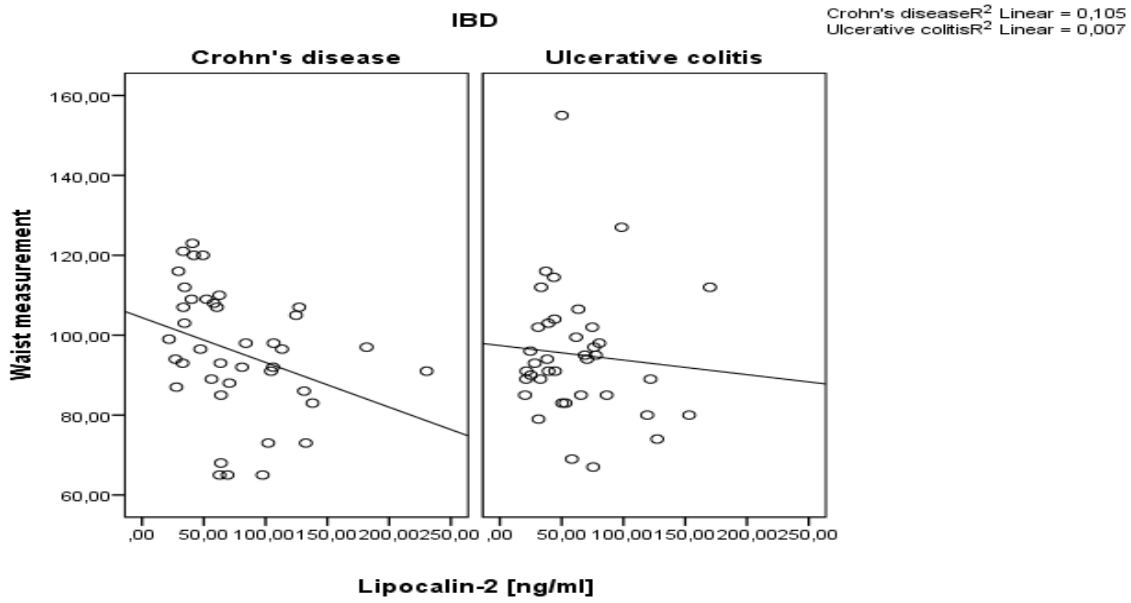

Fig. 49. Correlation dependence between human Lipocalin - 2/NGAL and waist measurement in IBD patients

A weak, tending to moderate, inverse correlation was also found with respect to serum human Lipocalin - 2/NGAL levels and waist-to-height ratio in CD patients (r=-0.277; p=0.037) (Fig. 50).

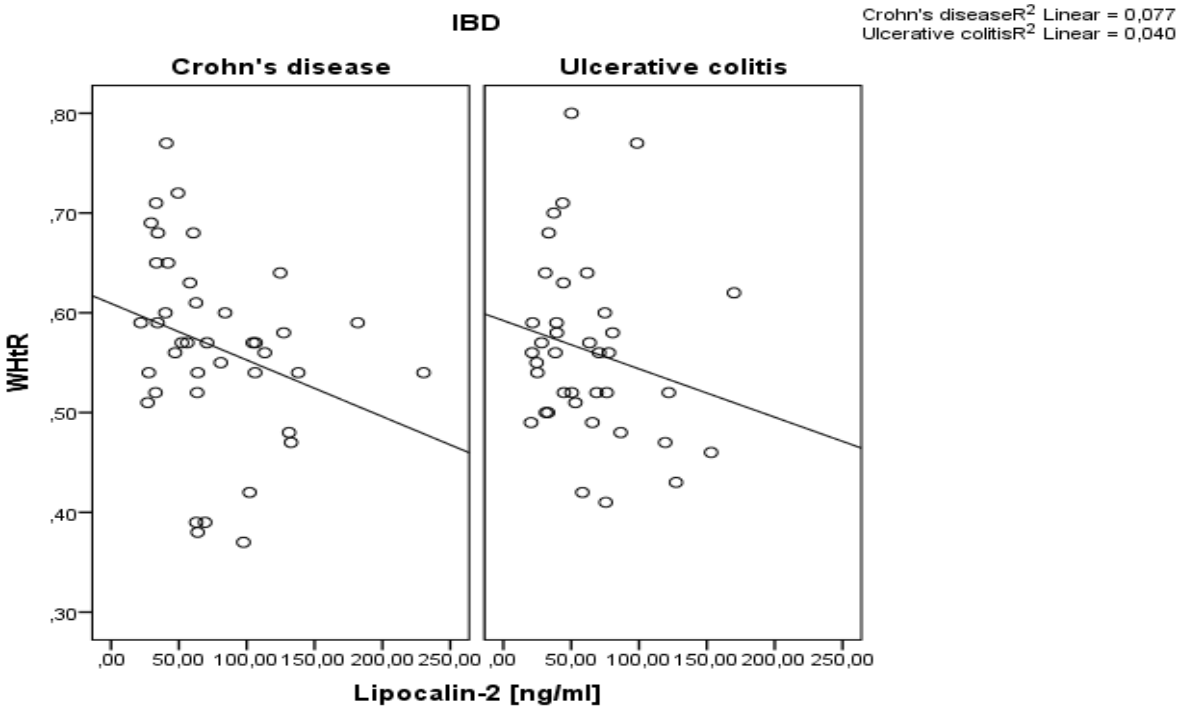

Fig. 50. Correlation between human Lipocalin - 2/NGAL and waist/height ratio in IBD patients

Table 20 shows a comparative analysis of human Lipocalin - 2/NGAL expression according to the studied parameters as well as according to the patients' disease. A significant difference in Lipocalin 2 expression in CD patients was found with respect to all examined parameters. On the other hand, in UC patients, there was a significant difference with respect to age, BMI, waist circumference, waist/height ratio and presence of activity or remission.

Table 20. Mean value of human Lipocalin - 2/NGAL according to IBD

Indicator		**Crohn's disease (n=40)**	**Ulcerative colitis (n=38)**	**P value**
Age	< 40 years	88.04±49.15	66.41±35.22	**<0.05**
	> 40 years	66.07±41.08	56.16±39.44	**<0.05**
	P value	**<0.05**	**<0.05**	

Gender	Male	71.77±41.41	66.14±39.47	**<0.05**
	Female	81.08±51.41	59.15±35.54	**<0.05**
	P value	**<0.05**	**>0.05**	
BMI groups	Norm	93.86±44.63	69.34±42.21	**<0.05**
	Obesity	68.29±44.89	58.33±34.12	**<0.05**
	P value	**<0.05**	**<0.05**	
Waist measurement	Norm	92.11±28.48	70.10±46.89	**<0.05**
	94-102/80-88	85.68±53.35	64.49±19.11	**<0.05**
	>102/>88	65.37±47.25	56.75±38.30	**>0.05**
	P value	**<0.05**	**<0.05**	
Waist/height ratio	< 0.05	94.20±30.13	77.02±44.65	**<0.05**
	>0.05	72.09±47.94	56.76±32.99	**<0.05**
	P value	**<0.05**	**<0.05**	
Activity/Remission	Activity	89.26±48.67	77.21±38.17	**<0.05**
	Remission	62.66±39.54	51.10±32.50	**<0.05**
	P value	**<0.05**	**<0.05**	

Fig. 51 presents a comparative analysis of mean human Lipocalin - 2/NGAL expression values versus CD and UC activity and gender. As the results showed the presence of significant difference in both CD and UC activity ($p<0.05$).

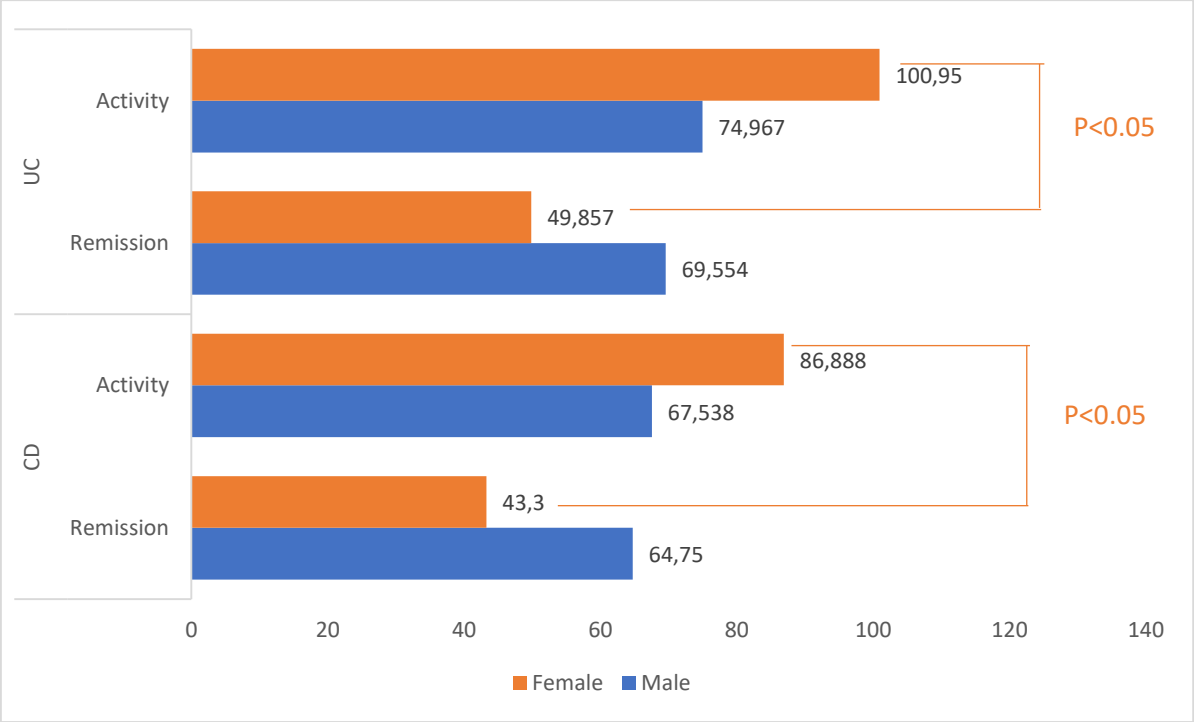

Fig. 51. Human Lipocalin - 2/NGAL expression in relation to CD and UC activity and sex

Fig. 52 presents a comparative analysis of mean human Lipocalin - 2/NGAL expression values versus CD and UC activity and age. As the results showed the presence of significant difference in both CD and UC patients ($p<0.05$). Significantly higher expression was observed in UC patients up to 40 years with activity.

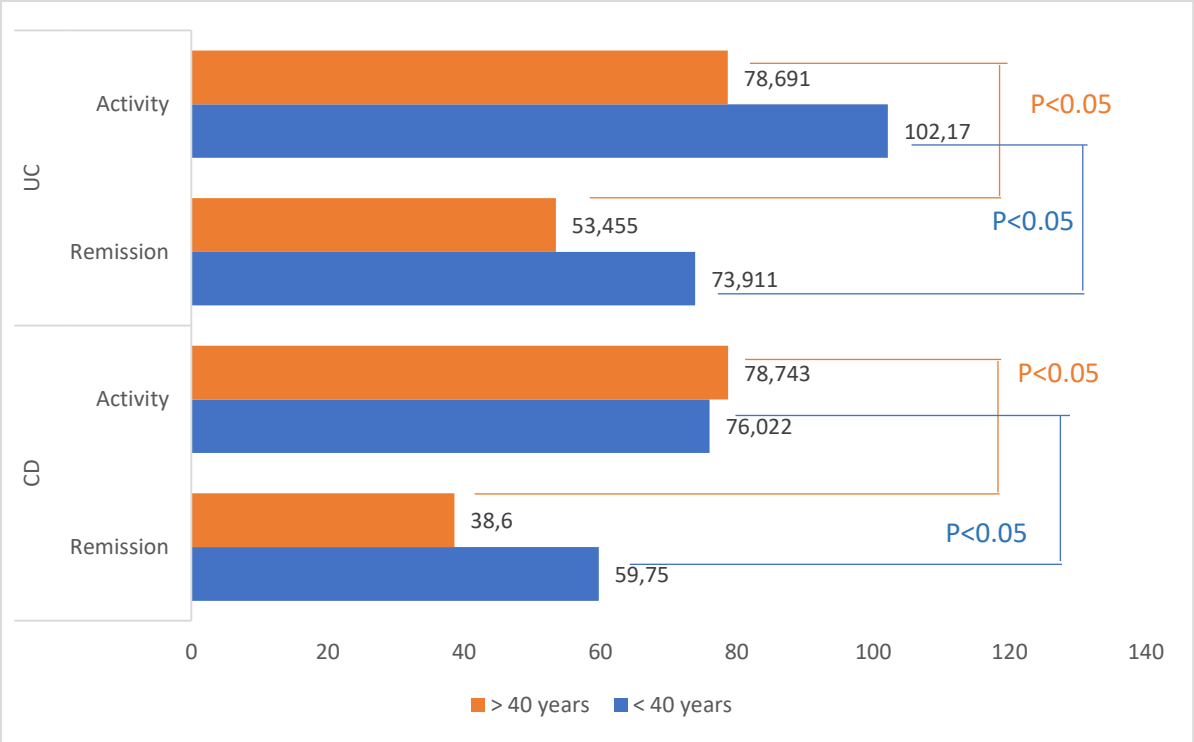

Fig. 52. Human Lipocalin - 2/NGAL expression in relation to CD and UC activity and age

Fig. 53 presents a comparative analysis of the mean human Lipocalin - 2/NGAL expression values versus CD and UC activity and BMI. As the results showed the presence of significant difference in both UC and CD patients ($p<0.05$). UC patients in normal BMI showed significantly higher expression.

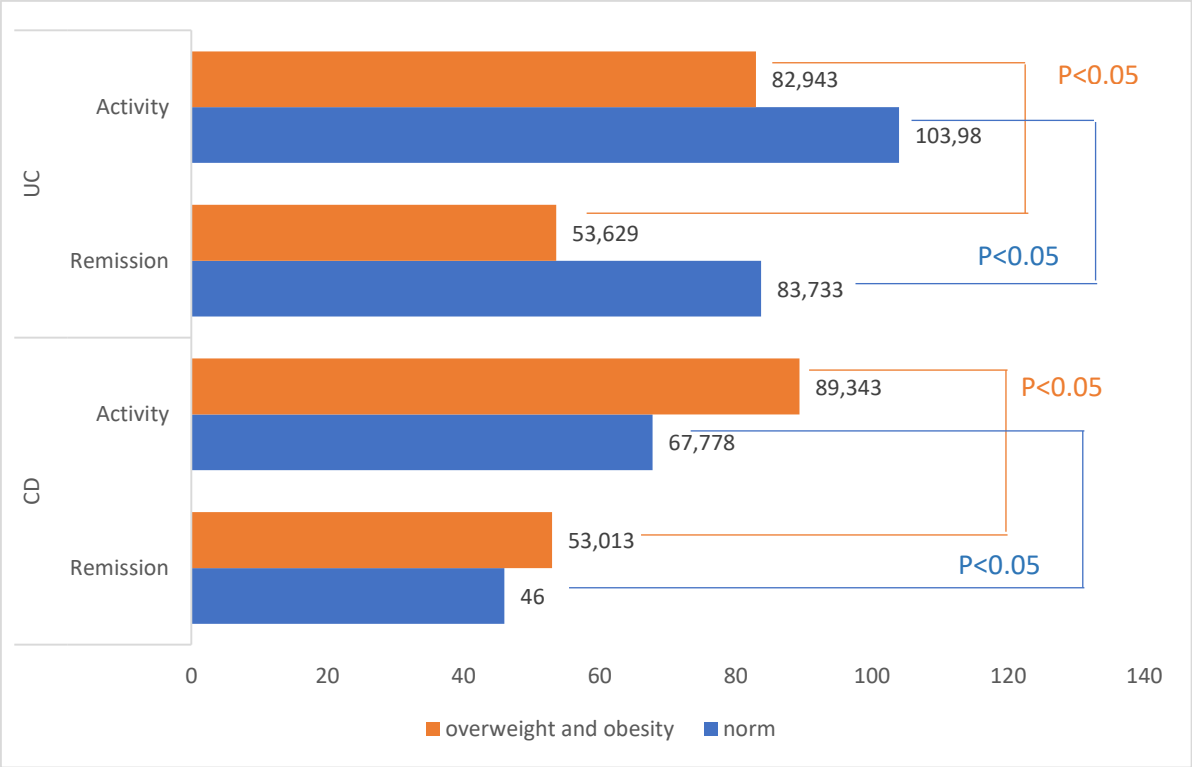

Fig. 53. Human Lipocalin - 2/NGAL expression in relation to CD and UC activity and BMI

Table 21 shows a comparative analysis of the studied parameters according to the established threshold value of human Lipocalin - 2/NGAL expression. Significant difference in expression according to the threshold value was found in terms of weight, BMI ≥ 25 kg/m2, waist circumference, skinfold size, waist-to-height ratio, activity and remission.

Table 21. Expression of human Lipocalin - 2/NGAL according to the investigated indicators and the cut-off

Indicator	**Expression cut-off**	**< 63.15 (n=42)**	**> 63.15 (n=36)**	**P value**
Age	Mean ± SD	44.83±13.25	39.92±13.49	>0.05
Gender	Male	20	18	>0.05
	Female	22	18	>0.05
Weight	Mean ± SD	86.15±24.27	74.28±18.13	0.018
BMI	Mean ± SD	30.01±6.83	25.36±5.32	0.001
BMI groups	Norm	10	15	>0.05
	Obesity	32	21	<0.05
Waist measurement (cm)	Mean ± SD	100.42±16.20	89.81±13.77	0.003
Abdominal skinfold size (mm)	Mean ± SD	31.07±10.86	24.22±10.52	0.006
Waist/height ratio	Mean ± SD	0.59±0.09	0.53±0.08	0.001
IBD	Crohn's disease	10	20	>0.05
	Ulcerative colitis	22	16	>0.05

Activity/Remission	Activity	13	23	<0.05
	Remission	29	13	<0.05

* The statistically significant difference ($p<0.05$) is marked in gray

4.5. Assessment of inflammation in IBD patients with obesity by comparing some anthropometric parameters, levels of a panel of circulating miRNAs and serum human Lipocalin - 2/NGAL expression

To assess inflammation in IBD patients with obesity using the expression of a panel of miRNAs and human Lipocalin - 2/NGAL, we calculated threshold values for the markers and indices examined: according to elevated levels of CRP > 5 mg/l (Table 22), FCP > 50 μg/g (Table 23), CDAI > 150 (Table 24) and endoscopic Mayo score (Table 25) to characterize activity in CD and UC.

Table 22. Threshold value of circulating miRNAs and serum human Lipocalin -2 /NGAL in individuals with CRP > 5 mg/l

miRNAs	Cut-off	AUC 95% CI	P value	Sensitivity/ Specificity
hsa-miR-17-5p	1.12	0.615 (0.456-0.774)	< 0.05	54.2%/56.0%
hsa-miR-29a-5p	1.45	0.543 (0.377-0.708)	< 0.05	48.0%/50.0%
hsa-miR-142-3p	0.94	0.535 (0.371-0.699)	< 0.05	54.2%/52.0%
hsa-miR-146a-5p	1.06	0.545 (0.380-0.710)	< 0.05	54.2%/56.0%
hsa-miR-155-5p	1.44	0.503 (0.338-0.667)	< 0.05	50.0%/52.0%
human Lipocalin - 2/NGAL	59.45	0.687 (0.566-0.808)	< 0.05	62.2%/63.2%

Table 23. Threshold value of circulating miRNAs and serum human Lipocalin - 2/NGAL in individuals with FCP > 50 μg/g

miRNAs	Cut-off	AUC 95% CI	P value	Sensitivity/ Specificity
hsa-miR-17-5p	1.27	0.642 (0.469-0.815)	< 0.05	60.0%/64.7%
hsa-miR-29a-5p	1.38	0.536 (0.356-0.717)	< 0.05	52.0%/52.9%
hsa-miR-142-3p	0.94	0.619 (0.436-0.802)	< 0.05	64.0%/64.7%
hsa-miR-146a-5p	1.06	0.659 (0.471-0.847)	< 0.05	64.0%/64.7%
hsa-miR-155-5p	1.44	0.544 (0.361-0.726)	< 0.05	60.0%/64.7%
human Lipocalin - 2/NGAL	57.25	0.589 (0.424-0.754)	< 0.05	58.3%/60.0%

Table 24. Threshold value of circulating miRNAs and serum human Lipocalin - 2/NGAL in subjects with CDAI > 150

miRNAs	Cut-off	AUC 95% CI	P value	Sensitivity/ Specificity
hsa-miR-17-5p	1.13	0.667 (0.257-1.000)	< 0.05	66.7%/66.7%
hsa-miR-29a-5p	2.26	0.556 (0.067-1.000)	< 0.05	66.7%/66.7%
hsa-miR-142-3p	1.08	0.556 (0.217-0.894)	< 0.05	55.6%/66.7%
hsa-miR-146a-5p	1.04	0.556 (0.136-0.975)	< 0.05	66.7%/66.7%
hsa-miR-155-5p	0.73	0.667 (0.347-0.986)	< 0.05	66.7%/66.7%
human Lipocalin - 2/NGAL	61.70	0.560 (0.266-0.854)	< 0.05	65.0%/80.0%

Table 25. Threshold value of circulating miRNAs and serum human Lipocalin - 2/NGAL in subjects with Mayo score ≥ 2

miRNAs	Cut-off	AUC 95% CI	P value	Sensitivity/ Specificity
hsa-miR-17-5p	1.28	0.673 (0.453-0.893)	< 0.05	58.3%/64.3%
hsa-miR-29a-5p	1.22	0.768 (0.578-0.957)	< 0.05	66.7%/71.4%
hsa-miR-142-3p	1.21	0.601 (0.366-0.836)	< 0.05	58.3%/64.3%
hsa-miR-146a-5p	1.25	0.524 (0.296-0.752)	< 0.05	50.0%/50.0%
hsa-miR-155-5p	1.16	0.542 (0.311-0.772)	< 0.05	50.0%/50.0%
human Lipocalin - 2/NGAL	51.75	0.742 (0.574-0.910)	< 0.05	60.9%/64.3%

Fig. 54 presents a comparative analysis of the mean serum hsa-miR-17-5p expression according to CRP levels in patients with normal BMI and those with overweight and obesity.

The results showed that at CRP levels > 5mg/l, both normal and those with BMI > 25kg/m2 had higher expression compared to patients with CRP < 5mg/l (p<0.05).

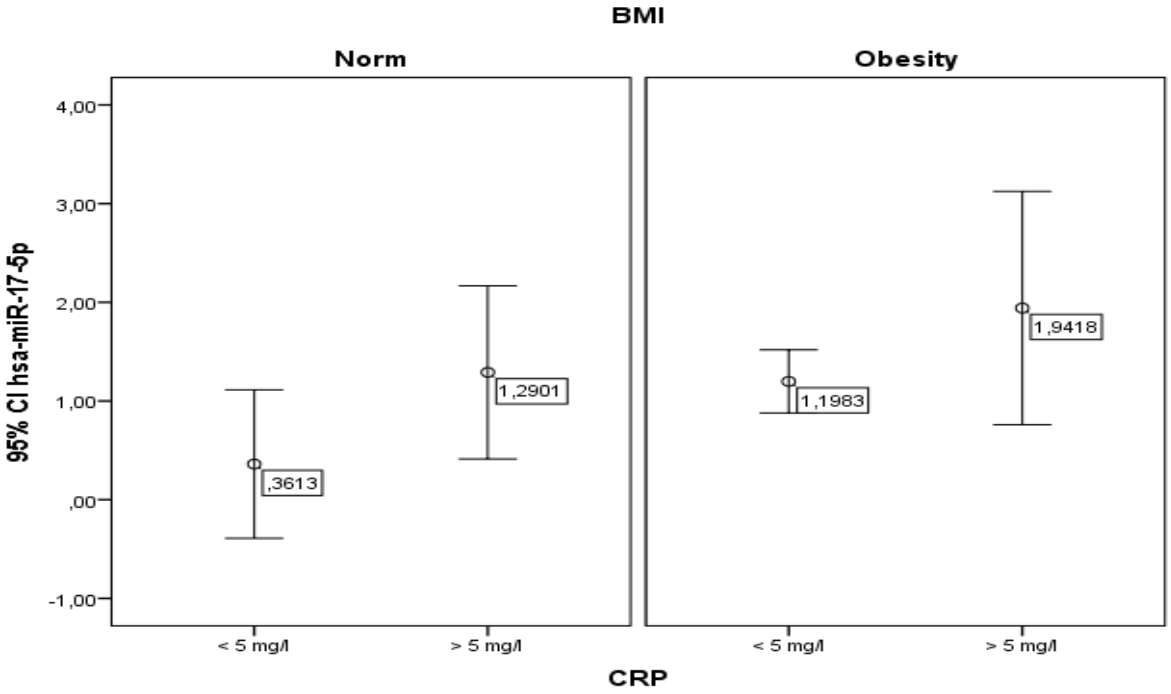

Fig. 54. Comparative analysis of hsa-miR-17-5p expression according to CRP and BMI levels

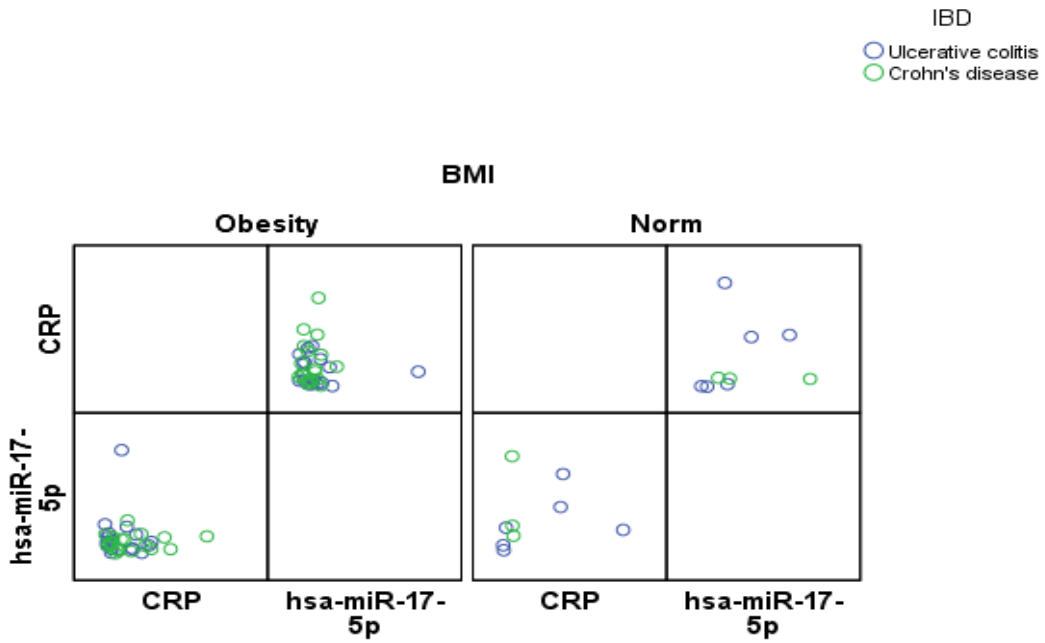

Fig. 55. Correlation analysis between hsa-miR-17-5p expression and serum CRP levels according to BMI in CD and UC patients

The results presented in fig. 55 show that there is a strong correlation between hsa-miR-17-5p expression and serum CRP levels in IBD patients with BMI > 25kg/m2 (r=0.856; p<0.001 for UC patients and r=0.922; p<0.001 for CD patients, respectively).

Fig. 56 presents a comparative analysis of the mean serum hsa-miR-29a-5p expression according to CRP levels in patients with normal BMI and those with overweight and obesity. The results showed that at CRP levels > 5mg/l in both normal and those with BMI > 25kg/m2, there was a decrease in expression compared to patients with CRP < 5mg/l, which was more pronounced in overweight and obese patients (p<0.05).

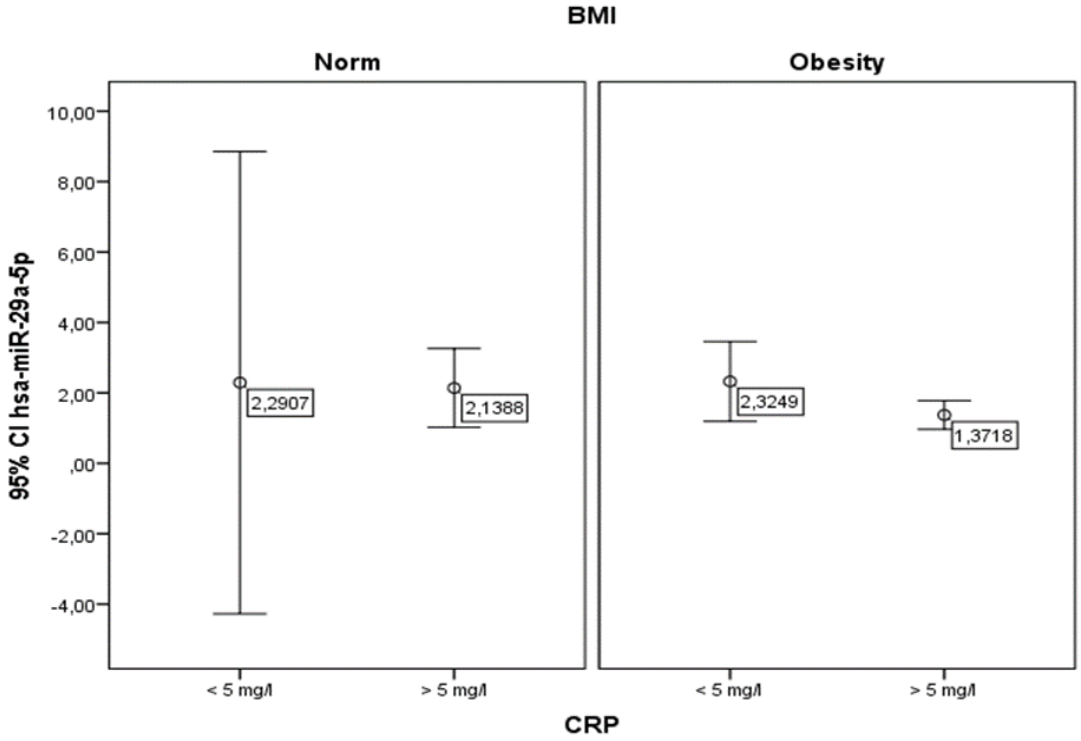

Fig. 56. Comparative analysis of hsa-miR-29a-5p expression according to CRP and BMI levels

The results presented in fig. 57 show that there is a strong correlation between hsa-miR-29a-5p expression and serum CRP levels in IBD patients with BMI > 25kg/m2 (r=0.843; p<0.001 for UC patients and r=0.845; p<0.001 for CD patients, respectively). A strong correlation between hsa-miR-29a-5p expression and serum CRP levels in overweight and obese patients was also found in healthy controls (r=0.921; p<0.001).

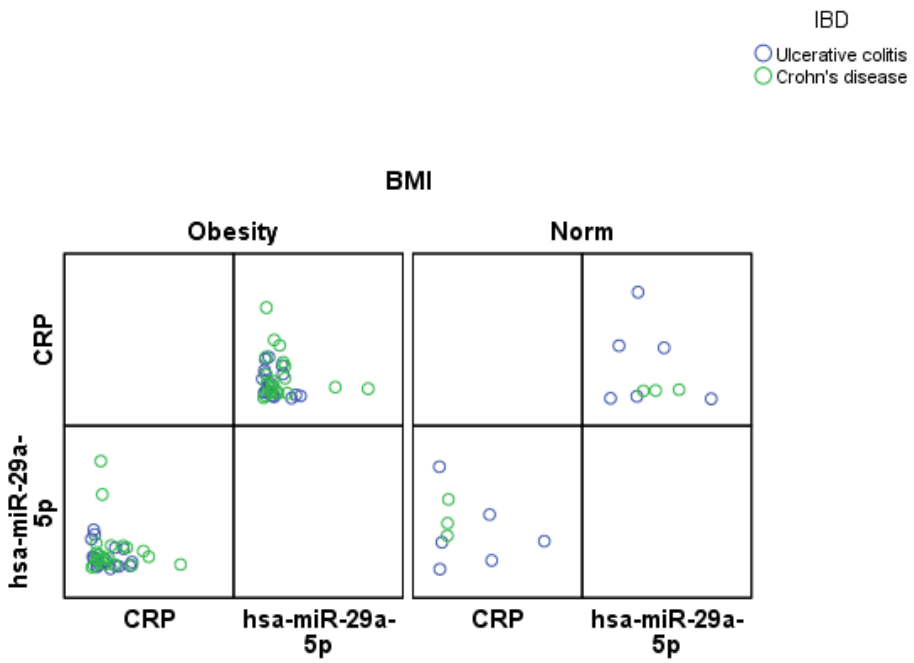

Fig. 57. Correlation analysis between hsa-miR-29a-5p expression and serum CRP levels according to BMI in CD and UC patients

Fig. 58 presents a comparative analysis of the mean serum hsa-miR-142-3p expression according to CRP levels in patients with normal BMI and those with overweight and obesity. The results showed that at CRP levels > 5mg/l, both normal and those with BMI > 25kg/m2 had slightly increased expression compared to patients with CRP < 5mg/l without statistical significance.

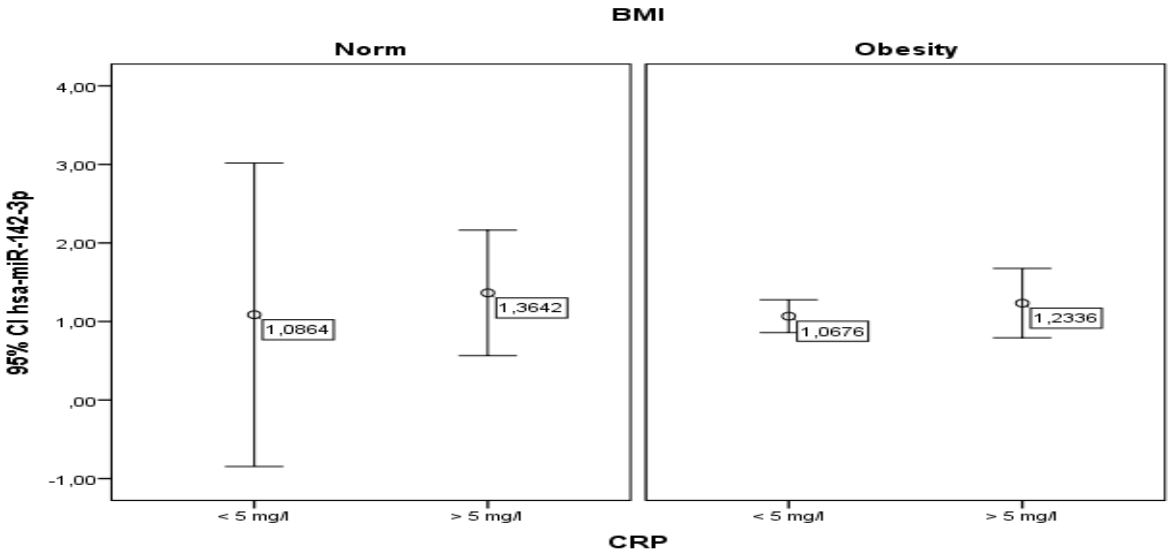

Fig. 58. Comparative analysis of hsa-miR-142-3p expression according to CRP and BMI levels

The results presented in fig. 59 show that there is a strong correlation between hsa-miR-142-3p expression and serum CRP levels in IBD patients with BMI > 25kg/m2 ($r=0.926$; $p<0.001$ for UC patients and $r=0.842$; $p<0.001$ for CD patients, respectively). A strong correlation between hsa-miR-142-3p expression and serum CRP levels in overweight and obese patients was also found in healthy controls ($r=0.764$; $p<0.001$).

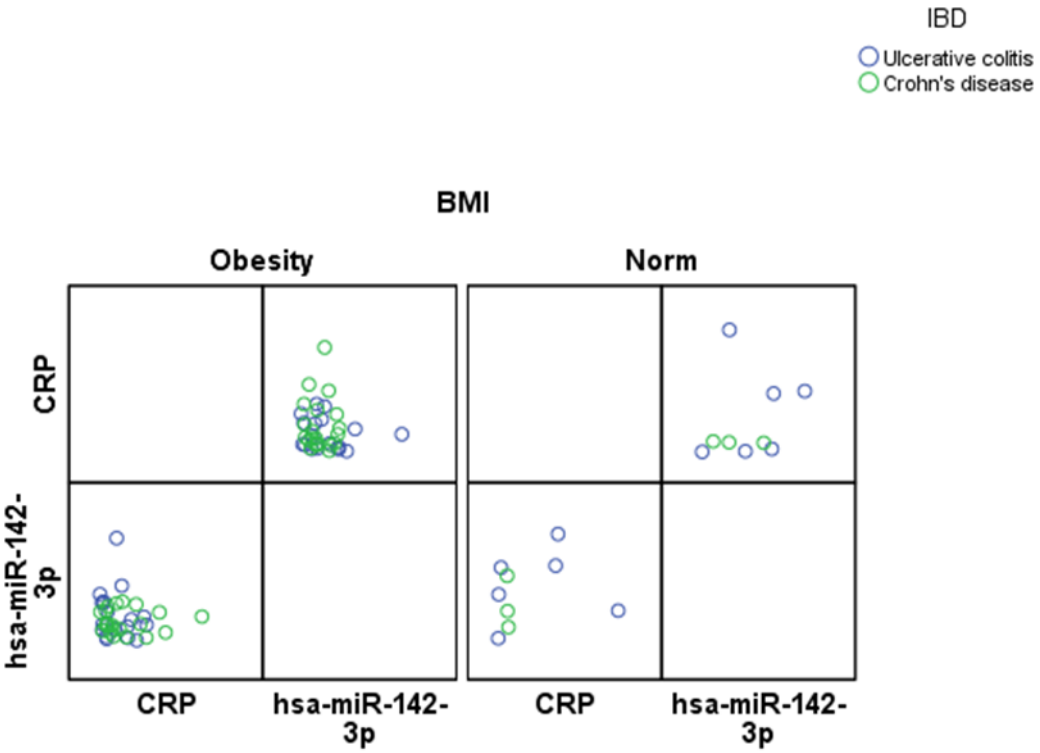

Fig. 59. Correlation analysis between hsa-miR-142-3p expression and serum CRP levels according to BMI in CD and UC patients

Fig. 60 presents a comparative analysis of the mean serum hsa-miR-146a-5p expression according to CRP levels in patients with normal BMI and those with overweight and obesity. The results showed that at CRP levels > 5mg/l, normal patients had a slight increase in the expression of the miRNA under study, while those with BMI > 25kg/m2 had a slightly decreased expression compared to patients with CRP < 5mg/l without statistical significance.

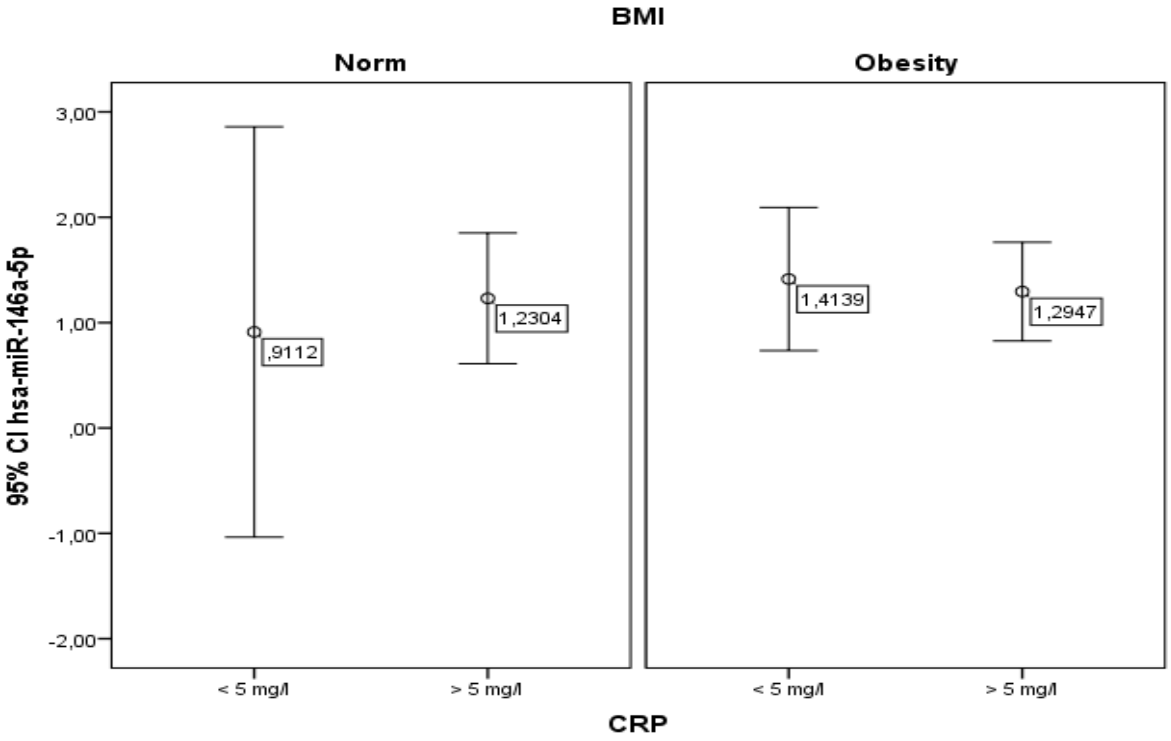

Fig. 60. Comparative analysis of hsa-miR-146a-5p expression according to CRP and BMI levels

The results presented in fig. 61 show that there is a strong correlation between hsa-miR-146a-5p expression and serum CRP levels in IBD patients with BMI < 25kg/m2 (r=0.960; p<0.001 for UC patients and r=0.911; p<0.001 for CD patients, respectively).

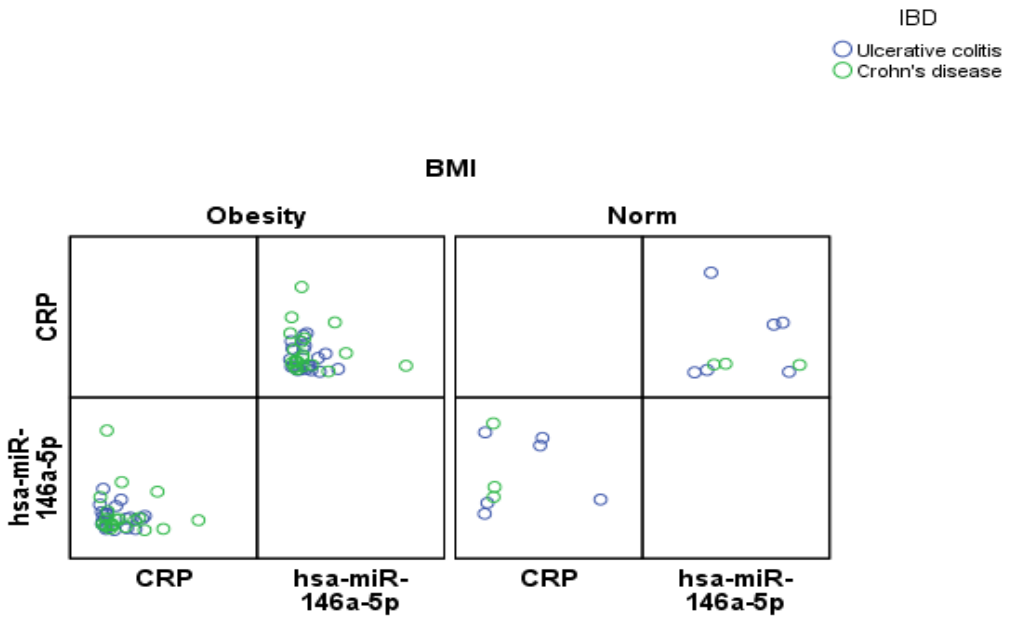

Fig. 61. Correlation analysis between hsa-miR-146a-5p expression and serum CRP levels according to BMI in CD and UC patients

Fig. 62 presents a comparative analysis of the mean serum hsa-miR-155-5p expression according to CRP levels in patients with normal BMI and those with overweight and obesity. The results showed that at CRP levels > 5mg/l, normal patients had a slight increase in the expression of the miRNA under study, while those with BMI > 25kg/m2 had a slightly decreased expression compared to patients with CRP < 5mg/l without statistical significance.

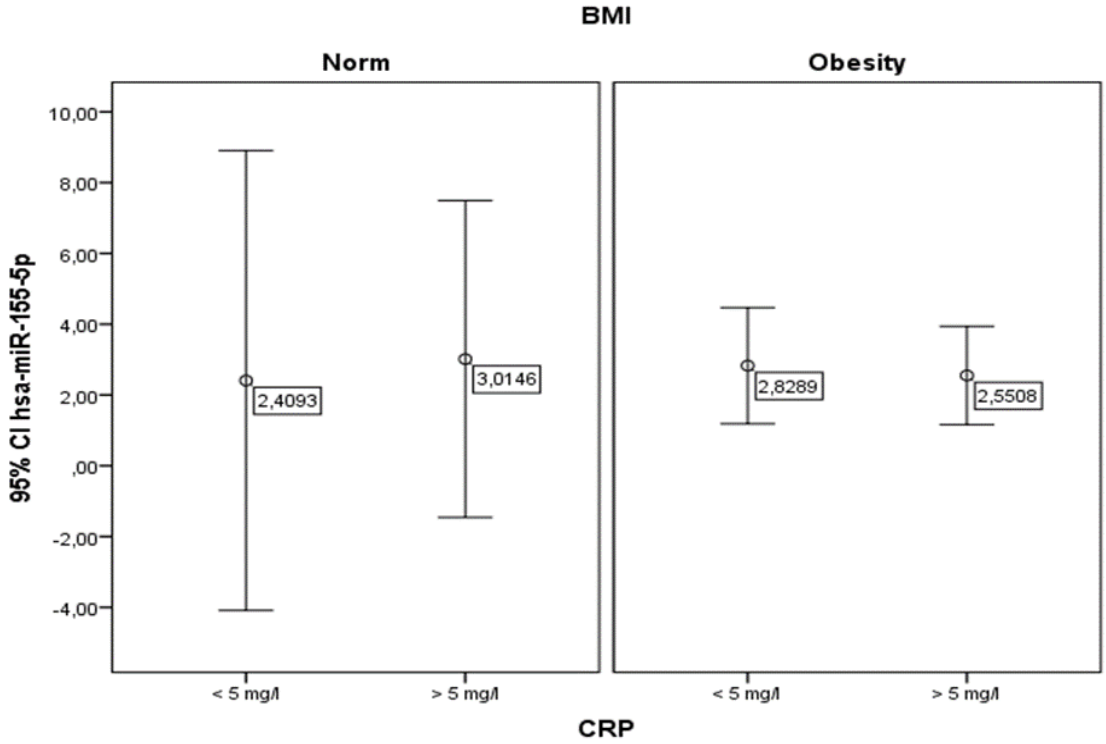

Fig. 62. Comparative analysis of hsa-miR-155-5p expression according to CRP and BMI levels

The results presented in fig. 63 show that there is a strong correlation between hsa-miR-155-5p expression and serum CRP levels in IBD patients with BMI < 25kg/m2 ($r=0.854$; $p<0.001$ for UC patients and $r=0.950$; $p<0.001$ for CD patients, respectively). A strong correlation between hsa-miR-155-5p expression and serum CRP levels in normal weight patients was also found in healthy controls ($r=0.974$; $p<0.001$).

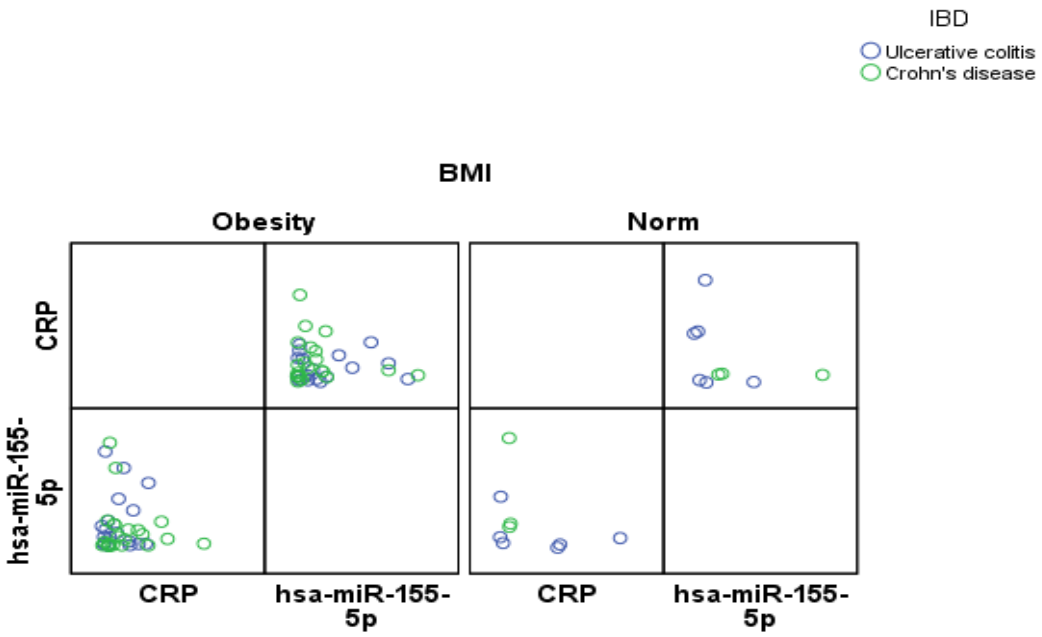

Fig. 63. Correlation analysis between hsa-miR-155-5p expression and serum CRP levels according to BMI in CD and UC patients

Fig. 64 presents a comparative analysis of mean serum human Lipocalin – 2/NGAL expression according to CRP levels in patients with normal BMI and those with overweight and obesity. The results showed that at CRP levels > 5mg/l, both normal and those with BMI > 25kg/m2 had higher expression compared to patients with CRP < 5mg/l ($p<0.05$).

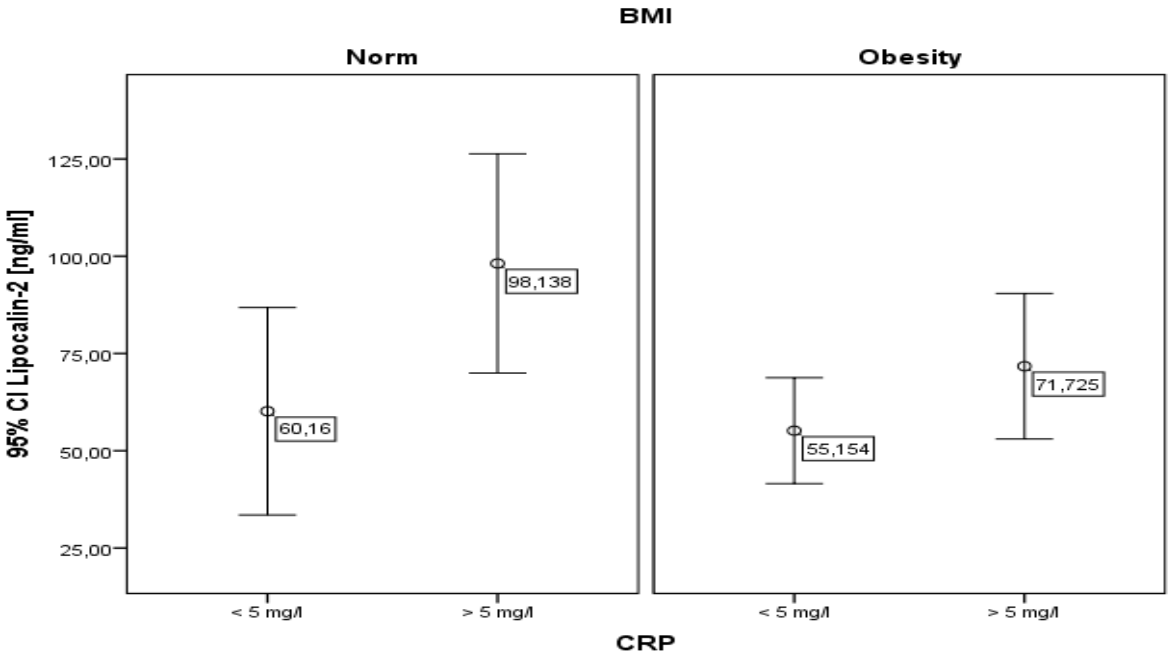

Fig. 64. Comparative analysis of human Lipocalin – 2/NGAL expression according to CRP and BMI levels

The results presented in fig. 65 show that there is a strong correlation between human Lipocalin – 2/NGAL expression and serum CRP levels in IBD patients with BMI < 25kg/m2 (r=0.948; p<0.001 for UC patients and r=0.860; p<0.001 for CD patients, respectively).

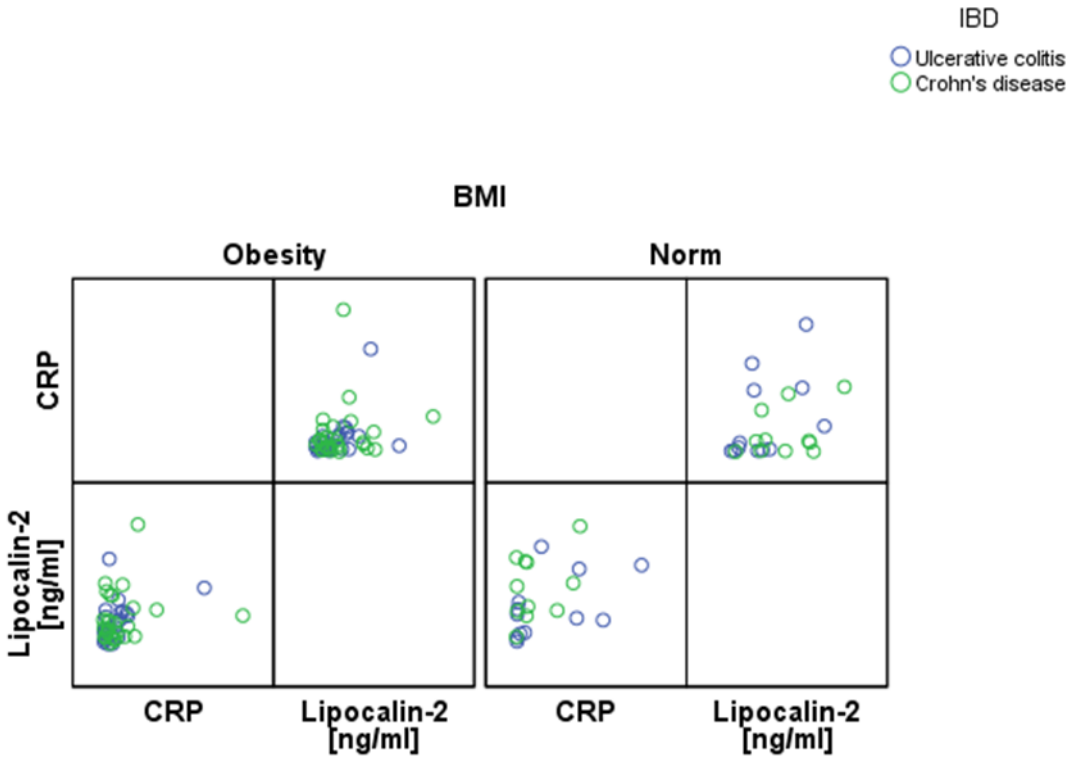

Fig. 65. Correlation analysis between human Lipocalin – 2/NGAL expression and serum CRP levels according to BMI in CD and UC patients

Fig. 66 presents a comparative analysis of mean serum hsa-miR-17-5p expression according to FCP levels in patients with normal BMI and those with overweight and obesity. The results showed that at FCP levels > 50 µg/g, patients with BMI > 25 kg/m2 had higher expression compared to patients with FCP < 50 µg/g (p<0.05).

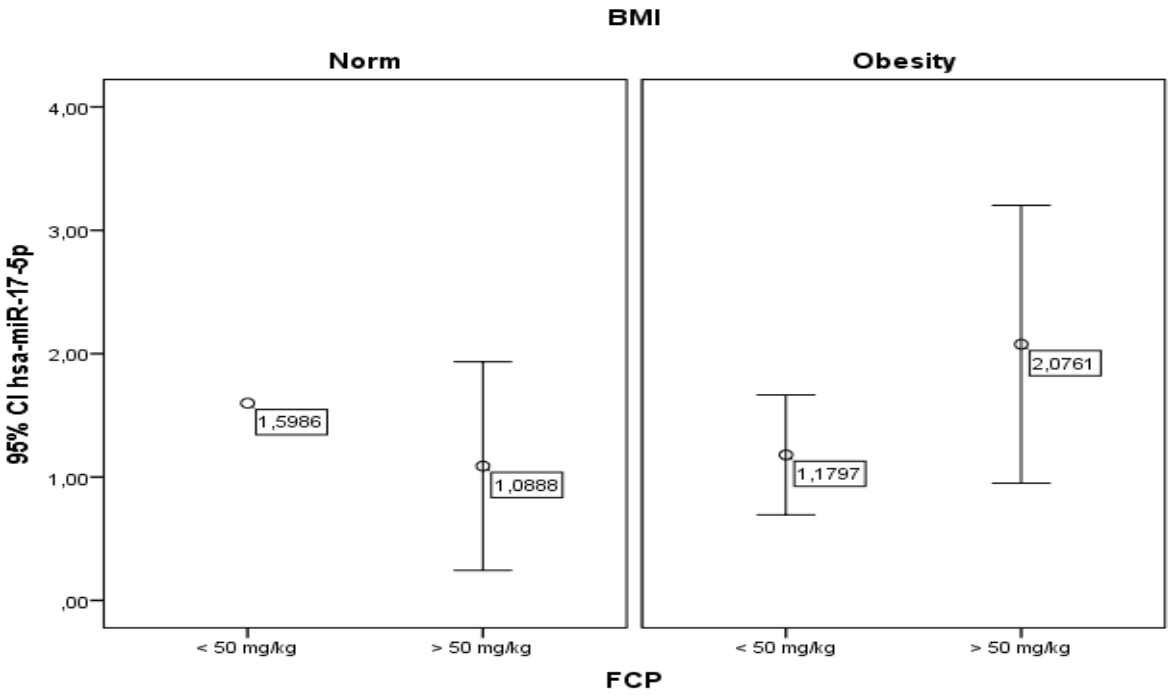

Fig. 66. Comparative analysis of hsa-miR-17-5p expression according to FCP and BMI levels

The results presented in fig. 67 show that there is a strong correlation between hsa-miR-17-5p expression and serum FCP levels in IBD patients with BMI > 25kg/m2 ($r=0.793$; $p<0.001$ for UC patients and $r=0.916$; $p<0.001$ for CD patients, respectively).

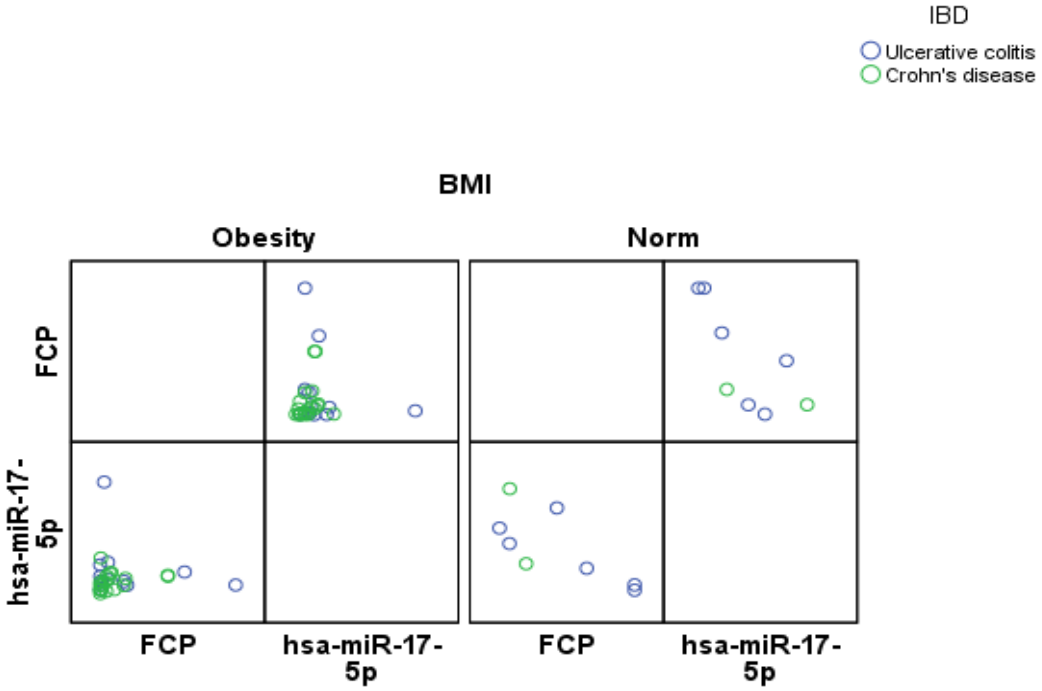

Fig. 67. Correlation analysis between hsa-miR-17-5p expression and serum FCP levels according to BMI in CD and UC patients

Fig. 68 presents a comparative analysis of the mean serum hsa-miR-29a-5p expression according to FCP levels in patients with normal BMI and those with overweight and obesity. The results showed that at FCP levels > 50 µg/g, patients with BMI > 25 kg/m2 had decreased expression compared to patients with FCP < 50 µg/g.

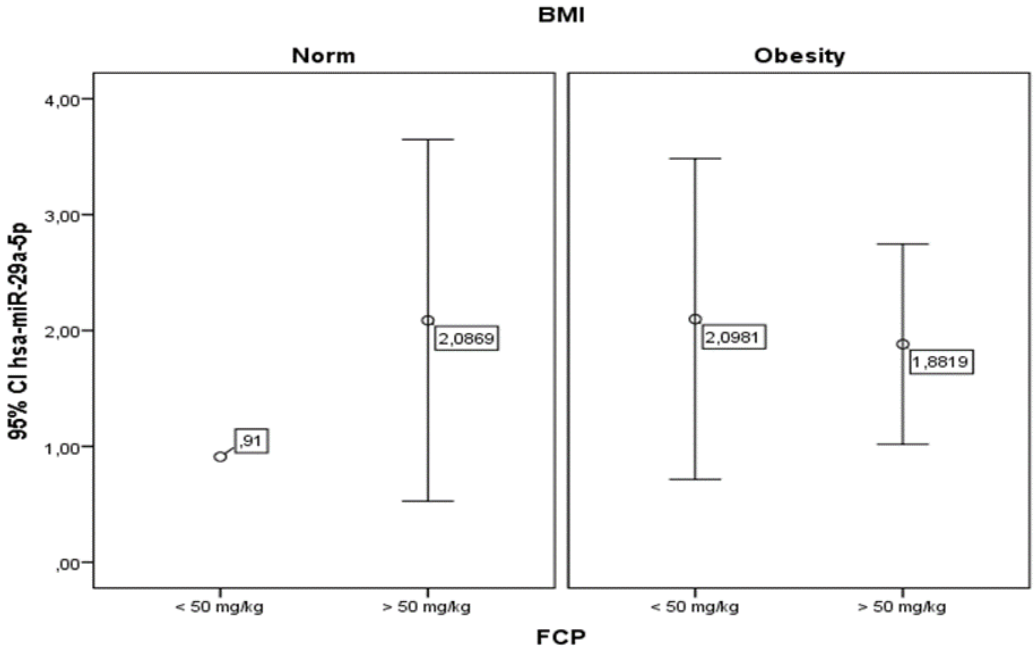

Fig. 68. Comparative analysis of hsa-miR-29a-5p expression according to FCP and BMI levels

The results presented in fig. 69 show that there is a strong correlation between hsa-miR-29a-5p expression and serum FCP levels in IBD patients with BMI < 25kg/m2 ($r=0.778$; $p<0.001$ for UC patients and $r=0.845$; $p<0.001$ for CD patients, respectively). A strong correlation between hsa-miR-29a-5p expression and serum FCP levels in normal weight patients was also found in healthy controls ($r=0.921$; $p<0.001$).

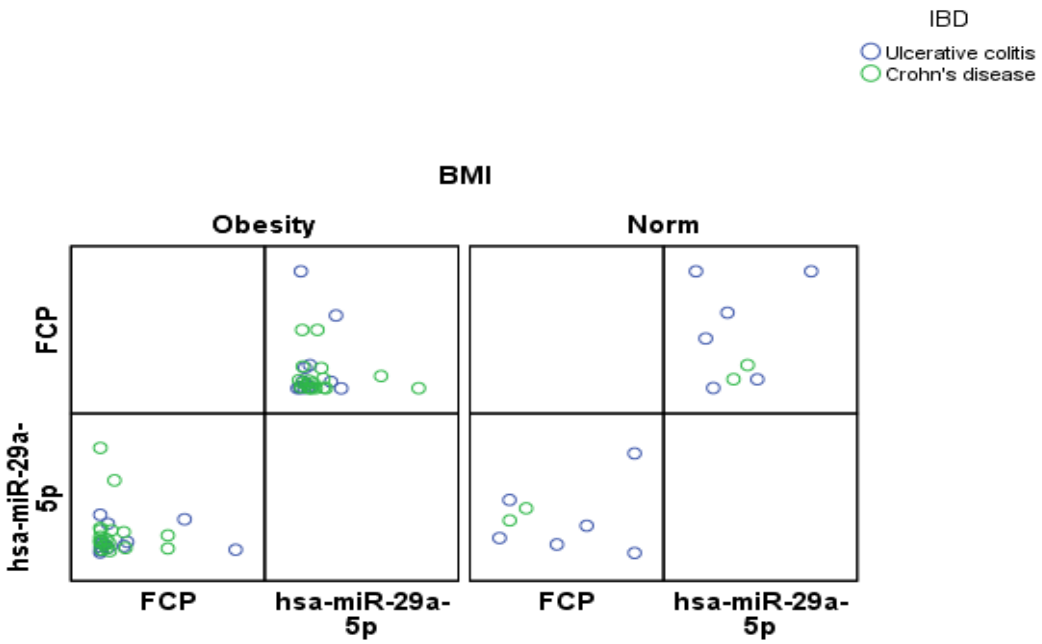

Fig. 69. Correlation analysis between hsa-miR-29a-5p expression and serum FCP levels according to BMI in CD and UC patients

Fig. 70 presents a comparative analysis of the mean serum hsa-miR-142-3p expression according to FCP levels in patients with normal BMI and those with overweight and obesity. The results showed that at FCP levels > 50 µg/g, patients with BMI > 25 kg/m2 had higher expression compared to patients with FCP < 50 µg/g.

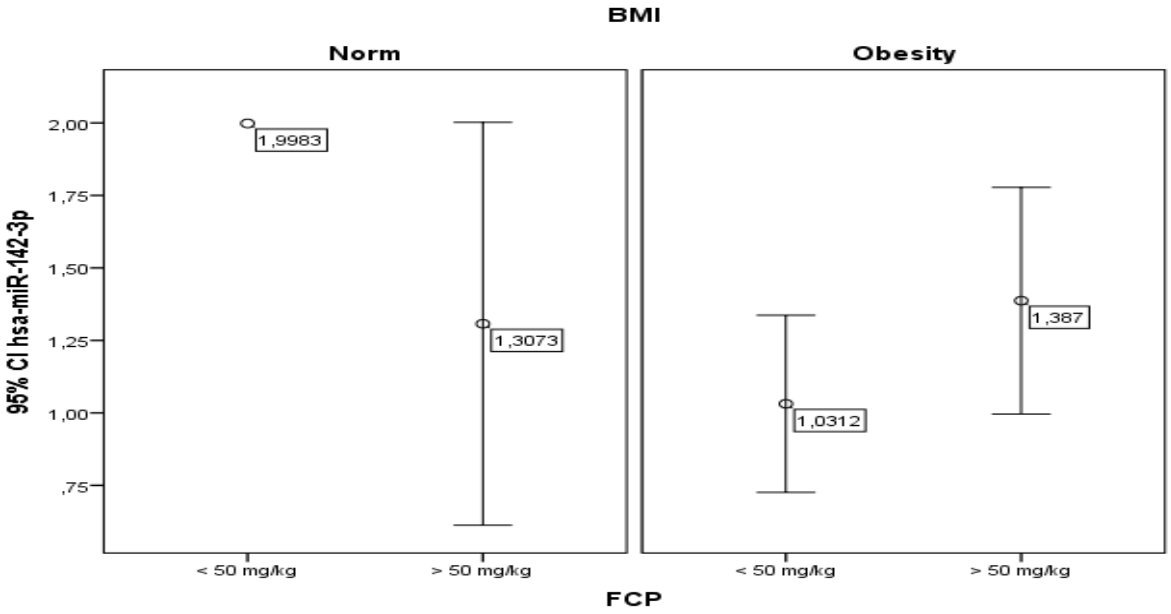

Fig. 70. Comparative analysis of hsa-miR-142-3p expression according to FCP and BMI levels

The results presented in fig. 71 show that there is a strong correlation between hsa-miR-142-3p expression and serum FCP levels in IBD patients with BMI > 25kg/m2 (r=0.926; p<0.001 for UC patients and r=0.842; p<0.001 for CD patients, respectively). A strong correlation between hsa-miR-142-3p expression and serum FCP levels in overweight and obese patients was also found in healthy controls (r=0.764; p<0.001).

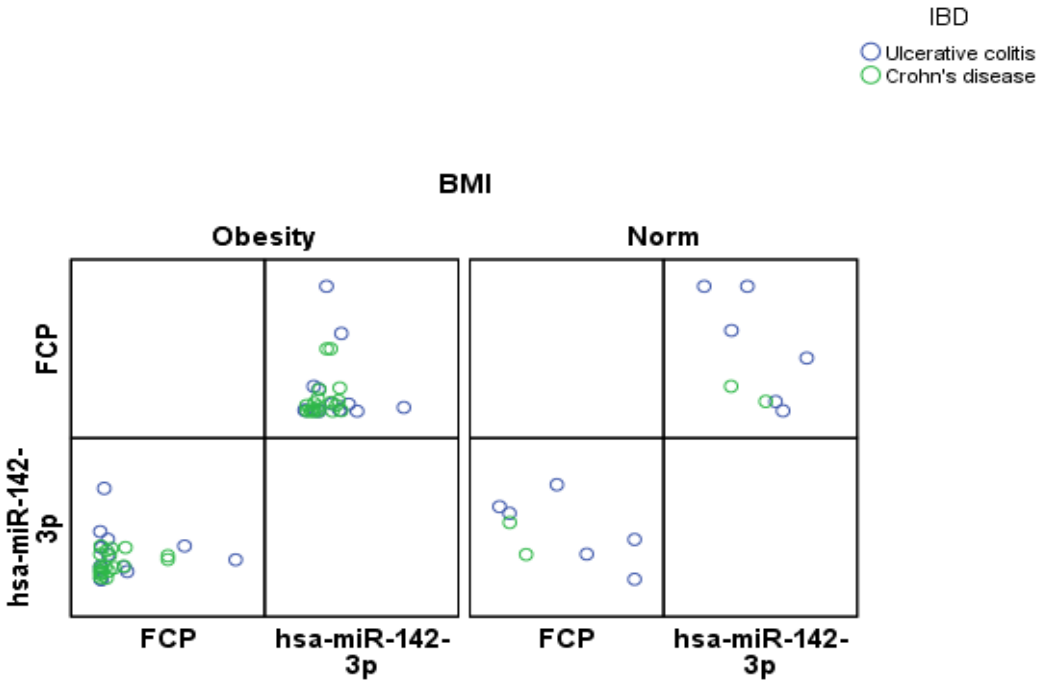

Fig. 71. Correlation analysis between hsa-miR-142-3p expression and serum FCP levels according to BMI in CD and UC patients

Fig. 72 presents a comparative analysis of the mean serum hsa-miR-146a-5p expression according to FCP levels in patients with normal BMI and those with overweight and obesity. The results showed that at FCP levels > 50 µg/g, patients with BMI > 25 kg/m2 had higher expression compared to patients with FCP < 50 µg/g.

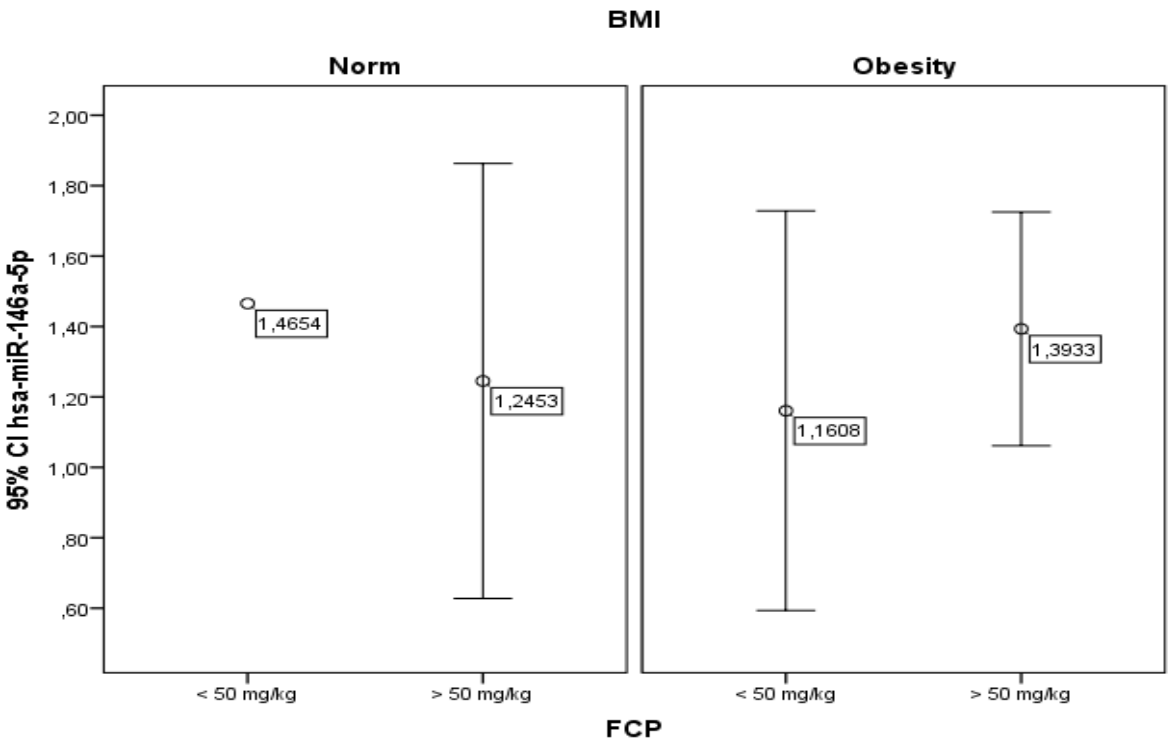

Fig. 72. Comparative analysis of hsa-miR-146a-5p expression according to FCP and BMI levels

The results presented in fig. 73 show that there is a strong correlation between hsa-miR-146a-5p expression and serum FCP levels in IBD patients with BMI > 25kg/m2 ($r=0.960$; $p<0.001$ for UC patients and $r=0.911$; $p<0.001$ for CD patients, respectively).

Fig. 74 presents a comparative analysis of the mean serum hsa-miR-155-5p expression according to FCP levels in patients with normal BMI and those with overweight and obesity. The results showed that at FCP levels > 50 μg/g, patients with BMI > 25 kg/m2 had decreased expression compared to patients with FCP < 50 μg/g, with no statistically significant difference.

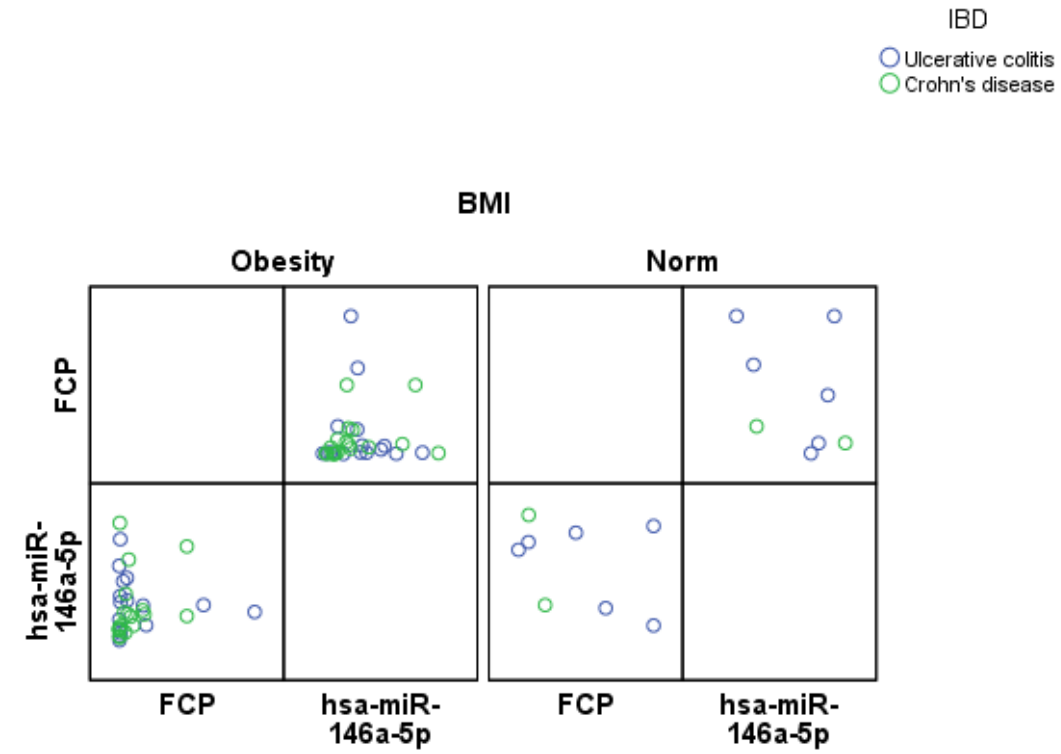

Fig. 73. Correlation analysis between hsa-miR-146a-5p expression and serum FCP levels according to BMI in CD and UC patients

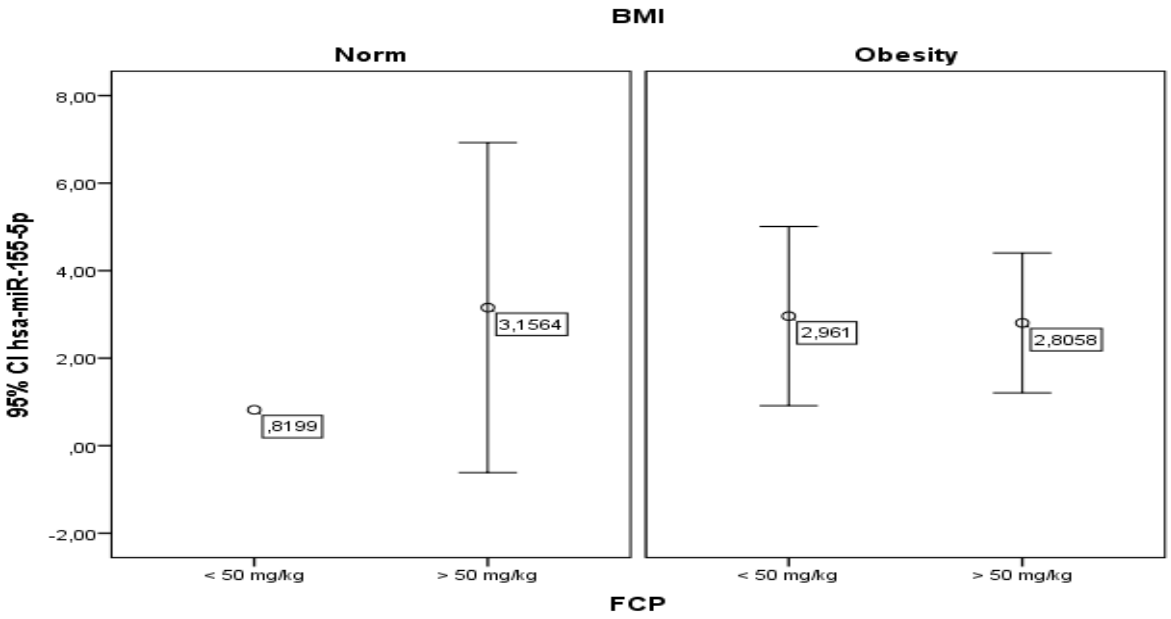

Fig. 74. Comparative analysis of hsa-miR-155-5p expression according to FCP and BMI levels

Fig. 75 presents a comparative analysis of mean serum human Lipocalin – 2/NGAL expression according to FCP levels in patients with normal BMI and those with overweight and obesity. The results showed that at FCP levels > 50 µg/g, patients with BMI > 25 kg/m2

had a decrease in expression compared to patients with FCP < 50 μg/g, whereas patients with normal BMI had a significant increase in serum levels of the marker (p<0.05).

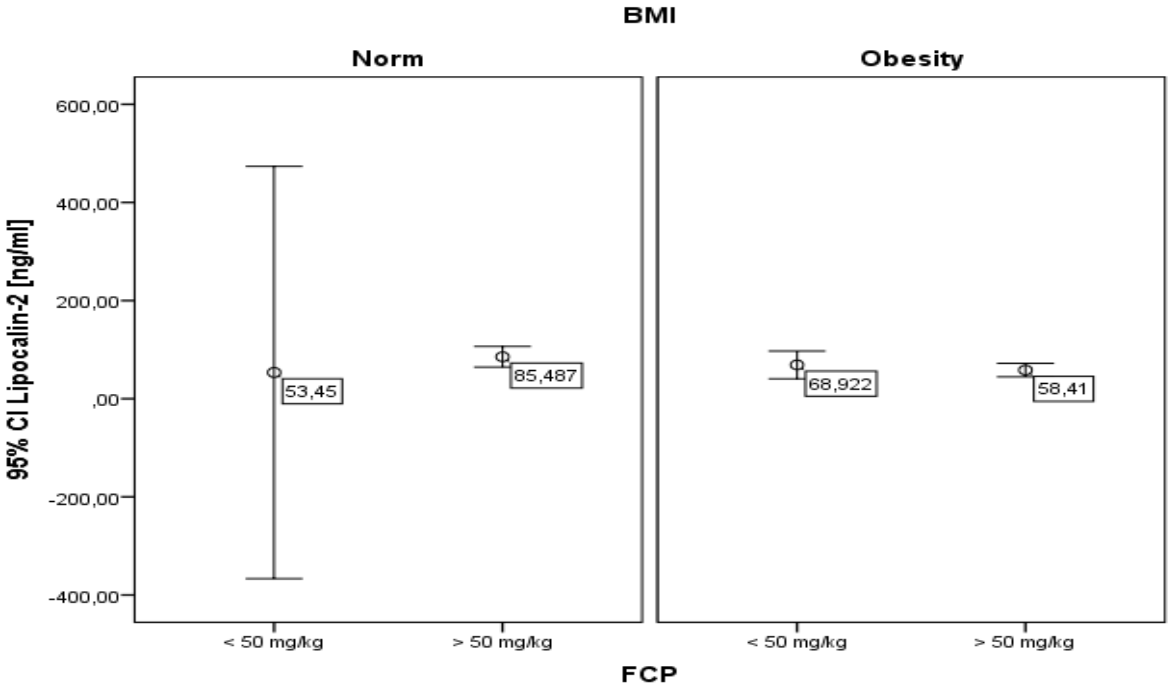

Fig. 75. Comparative analysis of human Lipocalin – 2/NGAL expression according to FCP and BMI levels

The results presented in fig. 76 show that there is a strong correlation between human Lipocalin 2 expression and serum FCP levels in IBD patients with BMI > 25kg/m2 (r=0.899; p<0.001 for UC patients and r=0.816; p<0.001 for CD patients, respectively).

Fig. 77 presents a comparative analysis of the mean serum hsa-miR-17-5p expression according to CDAI in overweight and obese patients. The results showed that CDAI > 150 in patients with BMI >25kg/m2 had higher expression compared to patients with CDAI < 150 (p<0.05).

There was a strong correlation between hsa-miR-17-5p expression and CDAI in CD patients with BMI >25kg/m2 (r=0.964; p<0.001).

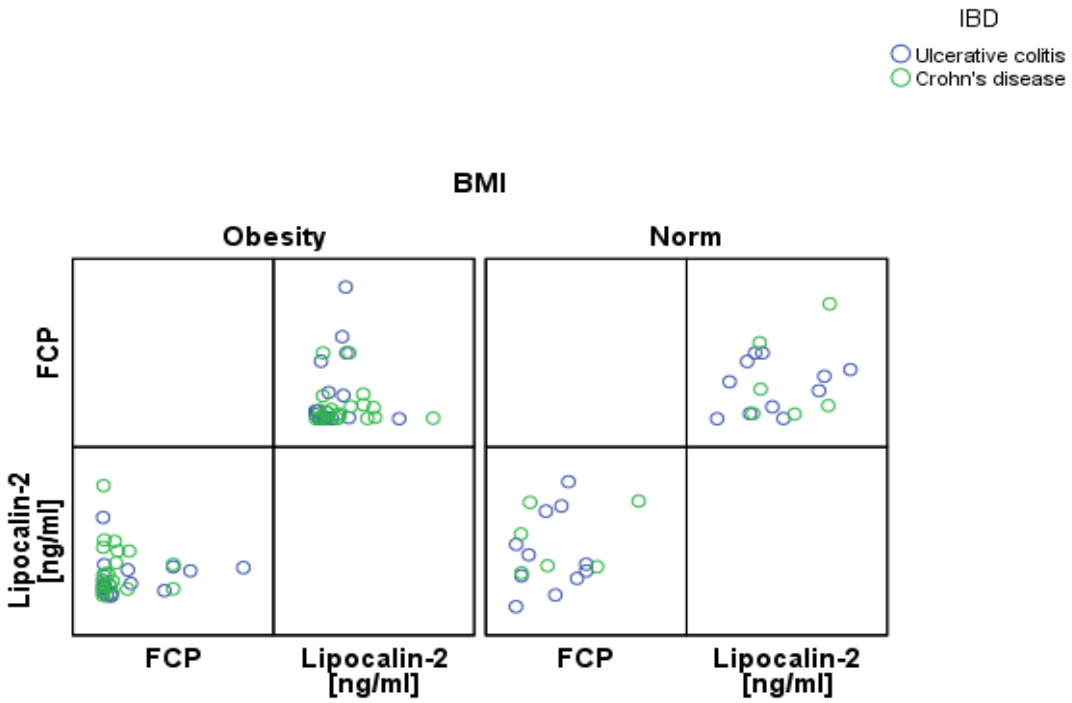

Fig. 76. Correlation analysis between human Lipocalin – 2/NGAL expression and serum FCP levels according to BMI in CD and UC patients

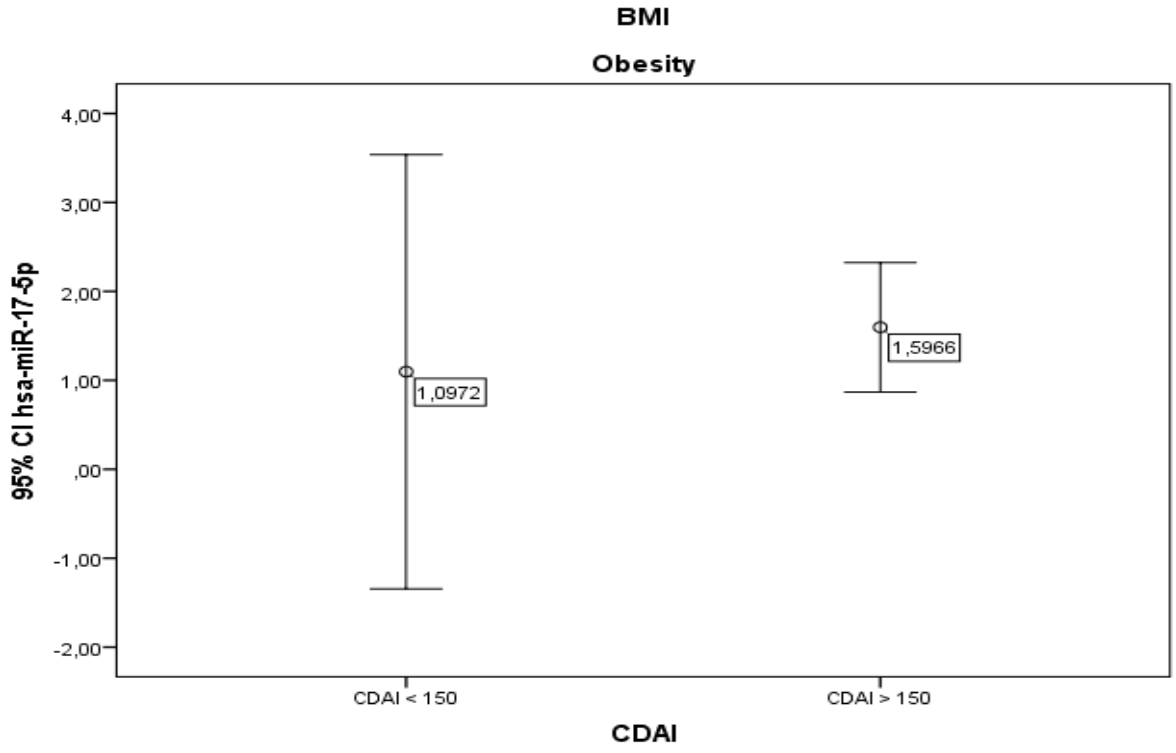

Fig. 77. Comparative analysis of hsa-miR-17-5p expression according to CDAI in overweight and obesity patients

Fig. 78 presents a comparative analysis of the mean serum expression levels of hsa-miR-29a-5p according to CDAI in overweight and obese patients. The results showed that

CDAI < 150 in patients with BMI >25kg/m2 had higher expression compared to patients with CDAI > 150 (p<0.01).

There was a strong correlation between hsa-miR-29a-5p expression and CDAI in CD patients with BMI > 25kg/m2 (r=-0.845; p<0.001).

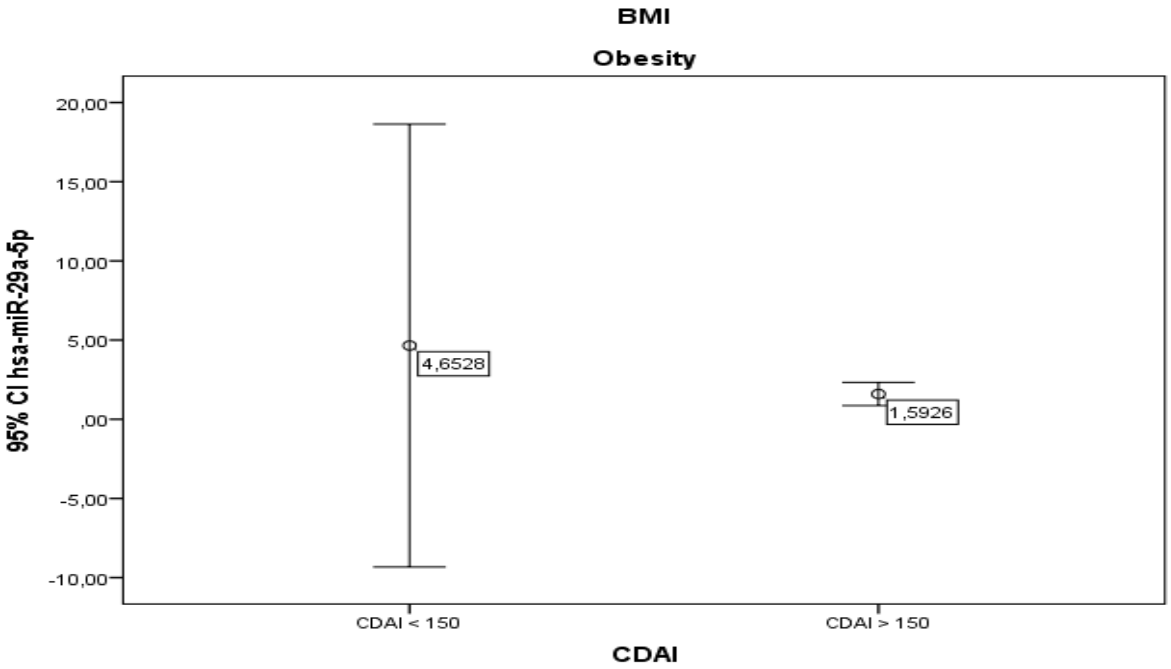

Fig. 78. Comparative analysis of hsa-miR-29a-5p expression according to CDAI in overweight and obesity patients

Fig. 79 presents a comparative analysis of mean serum hsa-miR-142-3p expression according to CDAI in overweight and obese patients. The results showed that at CDAI > 150, patients with BMI > 25kg/m2 had higher expression compared to patients with CDAI < 150, without a statistically significant difference. There was no correlation between serum hsa-miR-142-3p expression and CDAI at BMI > 25kg/m2.

Fig. 80 presents a comparative analysis of the mean serum expression levels of hsa-miR-146a-5p according to CDAI in overweight and obese patients. The results showed that there was no statistically significant difference. There was no correlation between serum hsa-miR-146a-5p expression and CDAI in BMI > 25kg/m2.

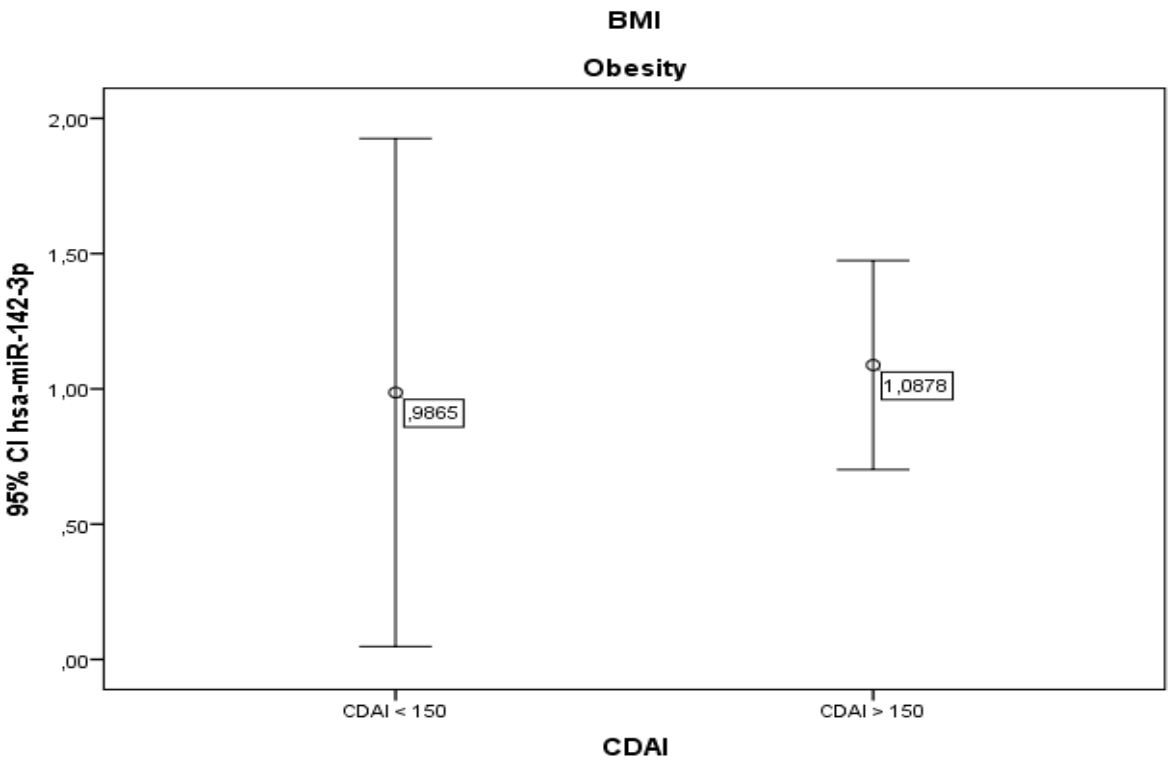

Fig. 79. Comparative analysis of hsa-miR-142-3p expression according to CDAI in overweight and obesity patients

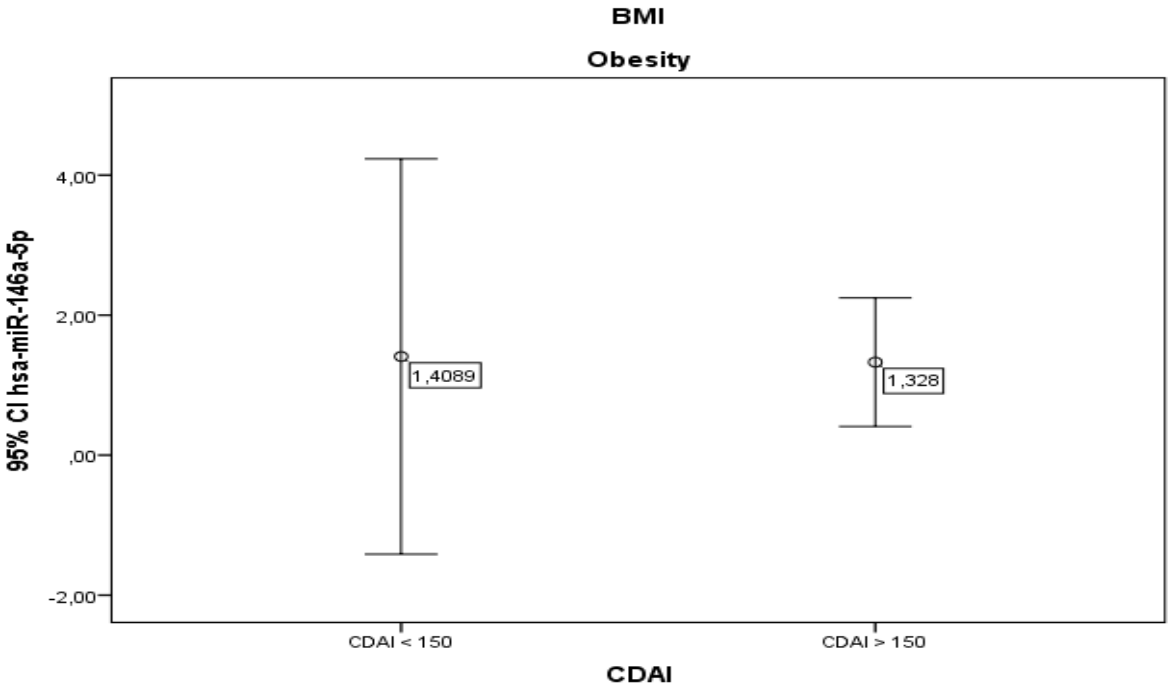

Fig. 80. Comparative analysis of hsa-miR-146a-5p expression according to CDAI in overweight and obesity patients

Fig. 81 presents a comparative analysis of the mean serum expression levels of hsa-miR-155-5p according to CDAI in overweight and obese patients. The results showed that CDAI > 150 in patients with BMI >25kg/m2 had higher expression compared to patients with CDAI < 150 ($p<0.05$). There was a strong correlation between serum hsa-miR-155-5p expression and CDAI at BMI > 25kg/m2 ($r=0.714$; $p<0.001$).

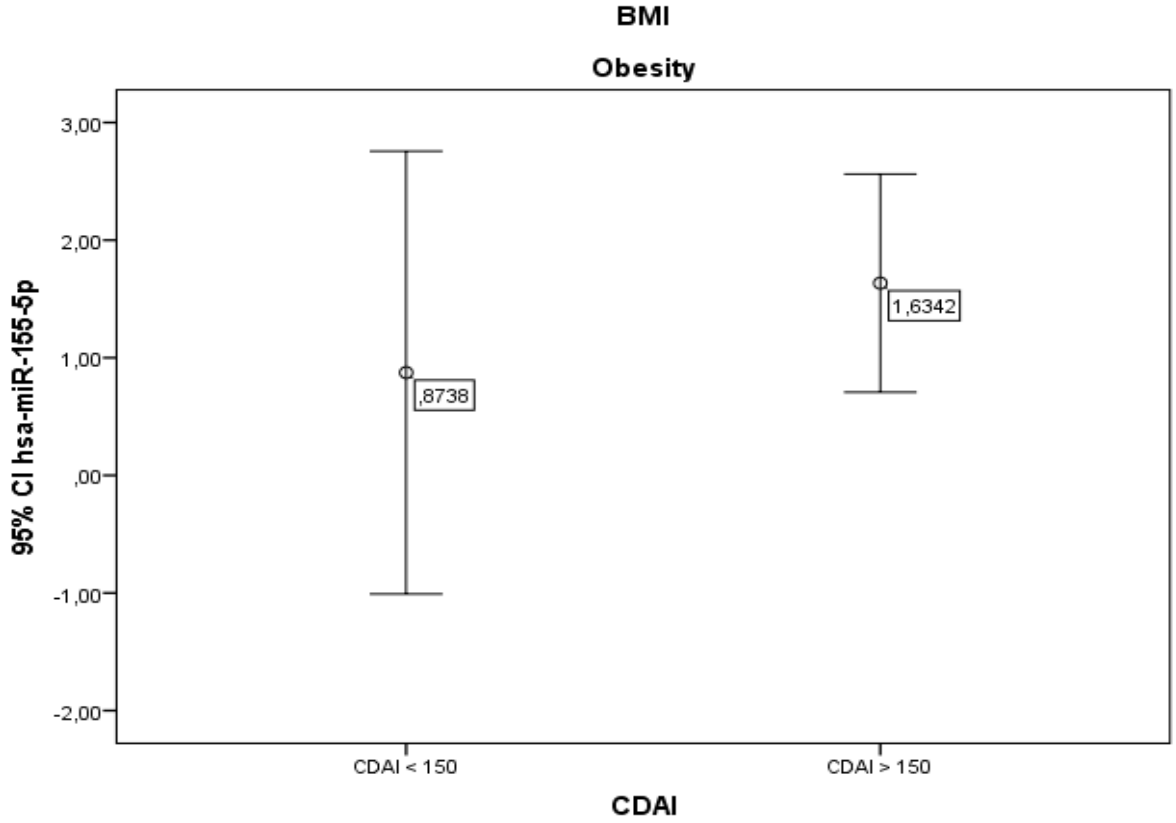

Fig. 81. Comparative analysis of hsa-miR-155-5p expression according to CDAI in overweight and obesity patients

Fig. 82 presents a comparative analysis of mean serum human Lipocalin – 2/NGAL expression values according to CDAI in overweight and obese patients. The results showed that CDAI > 150 in patients with BMI >25kg/m2 had higher expression compared to patients with CDAI < 150 ($p<0.05$). There was a strong correlation between serum human Lipocalin – 2/NGAL expression and CDAI at BMI > 25kg/m2 ($r=0.899$; $p<0.001$).

Fig. 83 presents a comparative analysis of the mean serum hsa-miR-17-5p expression according to the Mayo score in overweight and obese patients. The results showed that Mayo score 3 patients with BMI>25kg/m2 had higher expression compared to Mayo score 1 patients ($p<0.05$).

There was a strong correlation between hsa-miR-17-5p expression and Mayo score in UC patients with BMI >25kg/m2 ($r=0.687$; $p<0.01$).

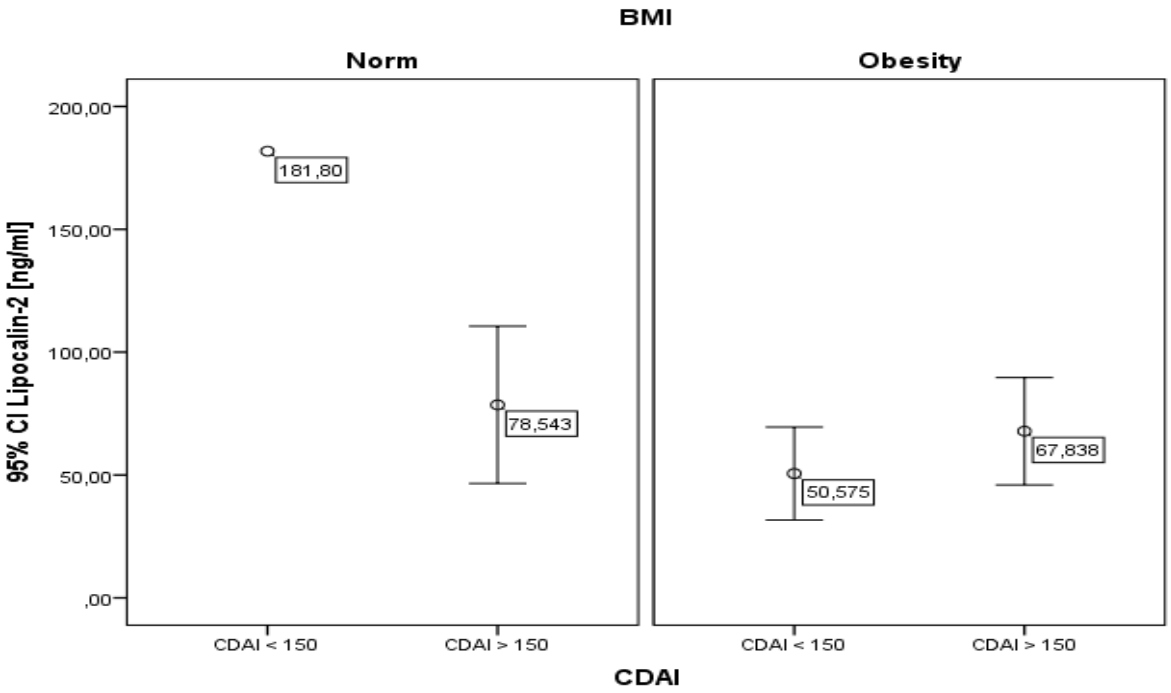

Fig. 82. Comparative analysis of human Lipocalin – 2/NGAL expression according to CDAI in overweight and obesity patients

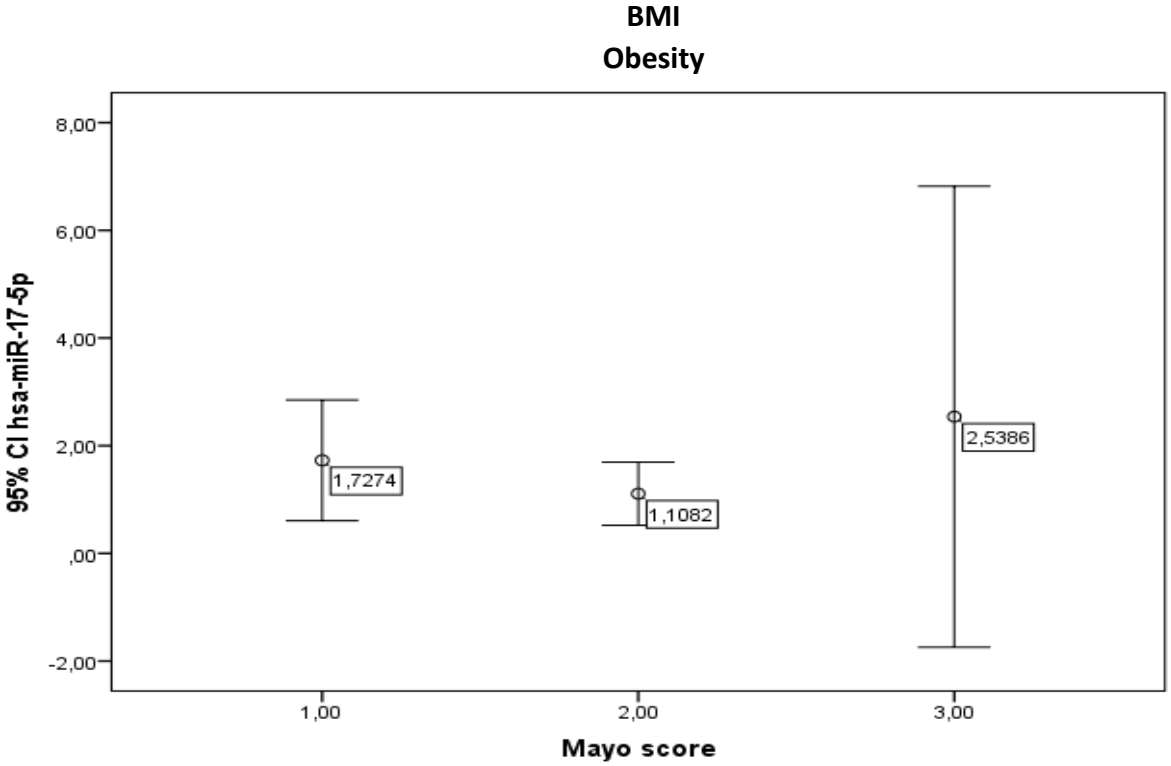

Fig. 83. Comparative analysis of hsa-miR-17-5p expression according to Mayo score in overweight and obesity patients

Fig. 84 presents a comparative analysis of the mean serum hsa-miR-29a-5p expression according to the Mayo score in overweight and obese patients. The results showed that Mayo score 3 patients with BMI>25kg/m2 had significantly lower expression compared to Mayo score 1 patients ($p<0.05$).

There was a moderate correlation between hsa-miR-29a-5p expression and Mayo score in UC patients with BMI >25kg/m2 ($r=-0.301$; $p<0.01$).

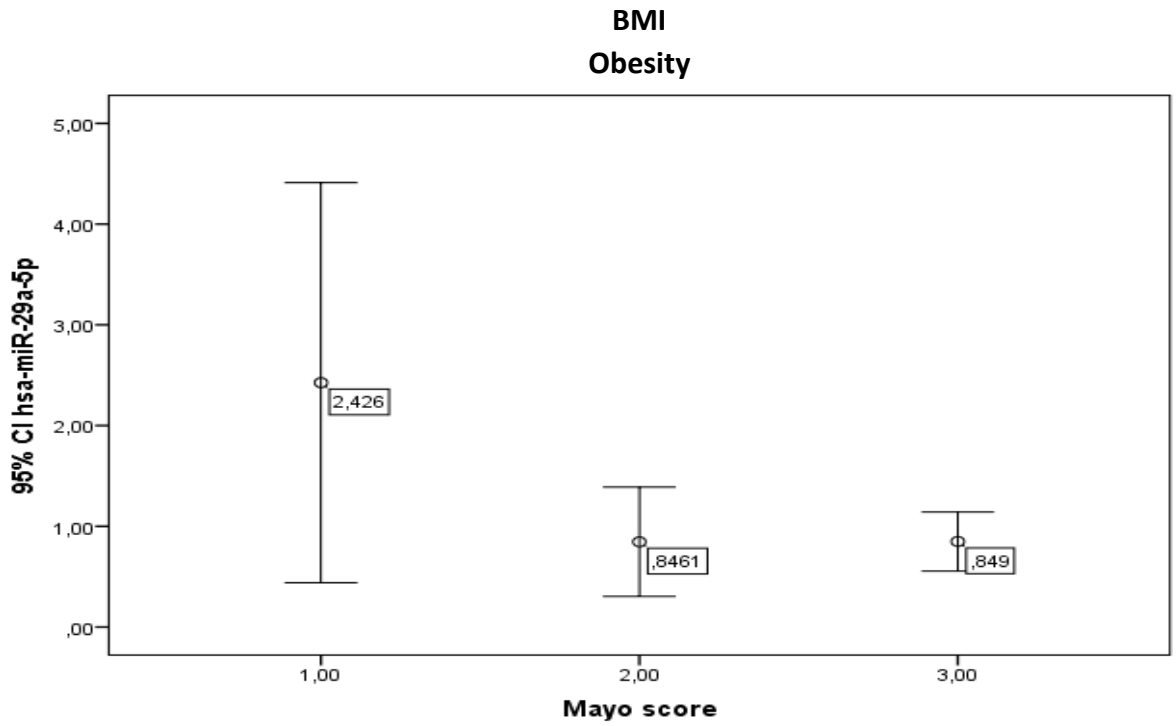

Fig. 84. Comparative analysis of hsa-miR-29a-5p expression according to Mayo score in overweight and obesity patients

Fig. 85 shows a comparative analysis of the mean serum hsa-miR-142-3p expression according to the Mayo score in overweight and obese patients. The results showed that there was no significant difference in the expression of the studied miRNA according to Mayo score.

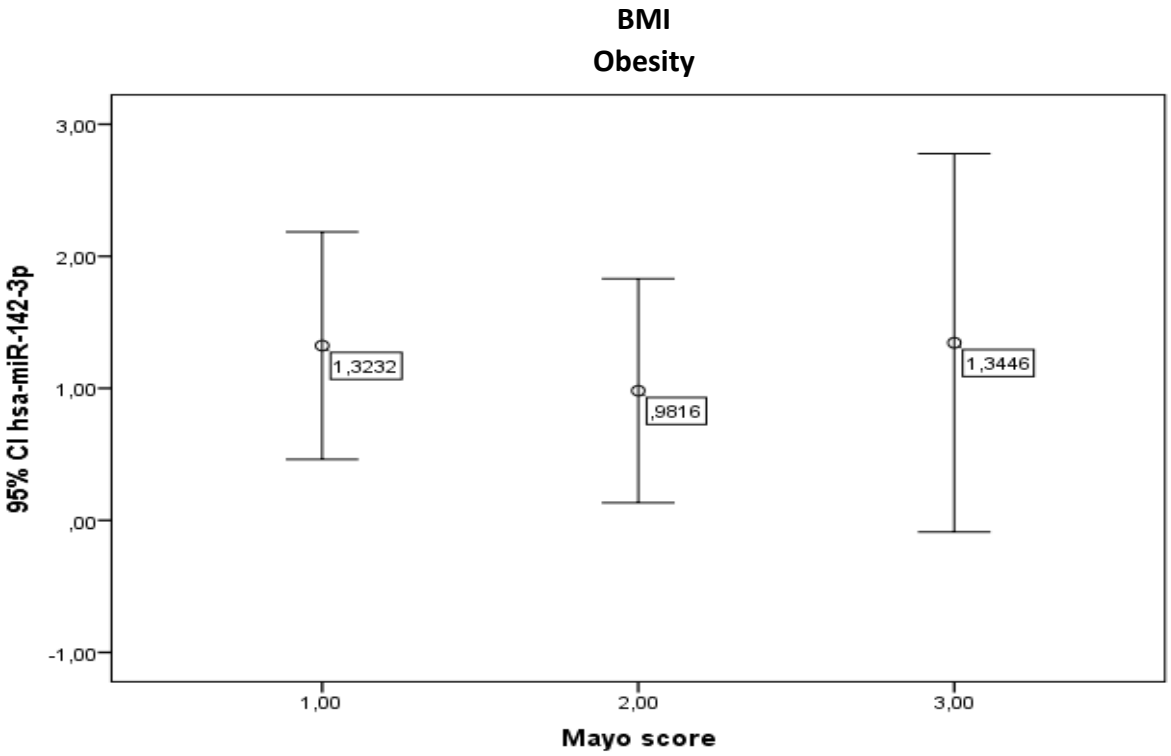

Fig. 85. Comparative analysis of hsa-miR-142-3p expression according to Mayo score in overweight and obesity patients

Fig. 86 shows a comparative analysis of the mean serum expression of hsa-miR-146a-5p according to Mayo score in overweight and obese patients. The results showed that there was no significant difference in the expression of the studied miRNA according to Mayo score.

Fig. 87 shows a comparative analysis of the mean serum expression of hsa-miR-155-5p according to the Mayo score in overweight and obese patients. The results showed that patients with Mayo score 2 had increased expression of the studied miRNA (5.16) compared to patients with Mayo score 1 and Mayo score 3 (2.87 and 2.85, respectively) ($p<0.01$).

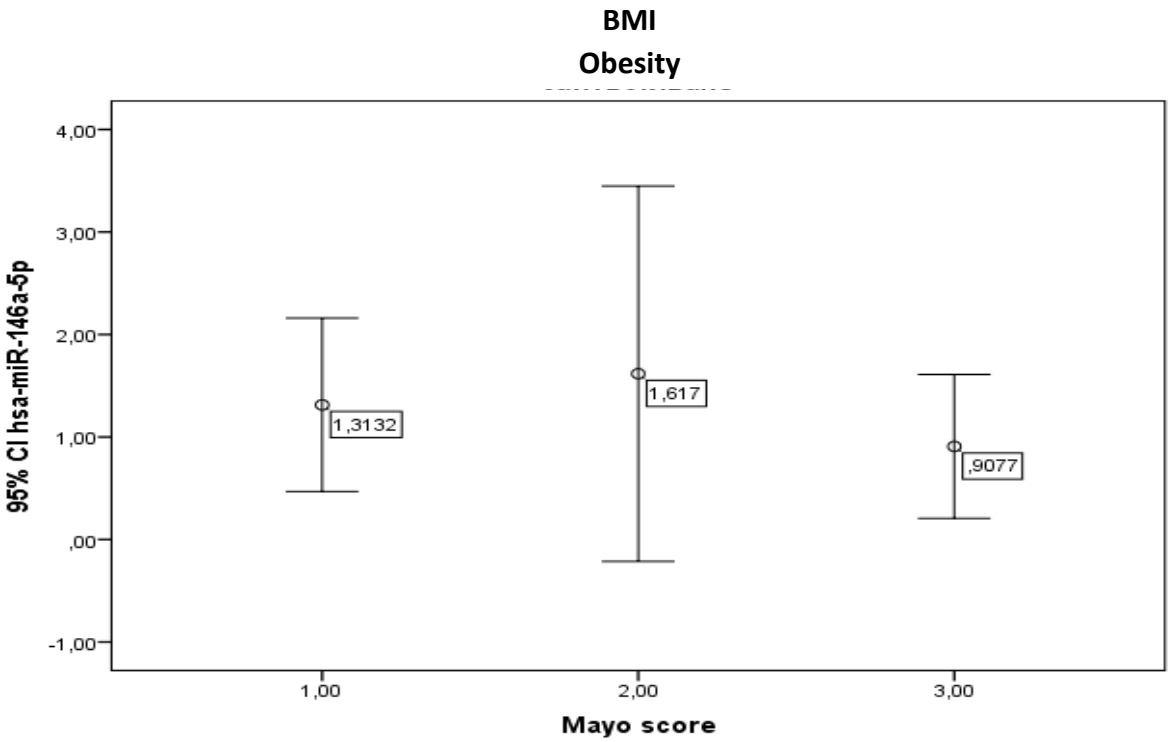

Fig. 86. Comparative analysis of hsa-miR-146a-5p expression according to Mayo score in overweight and obesity patients

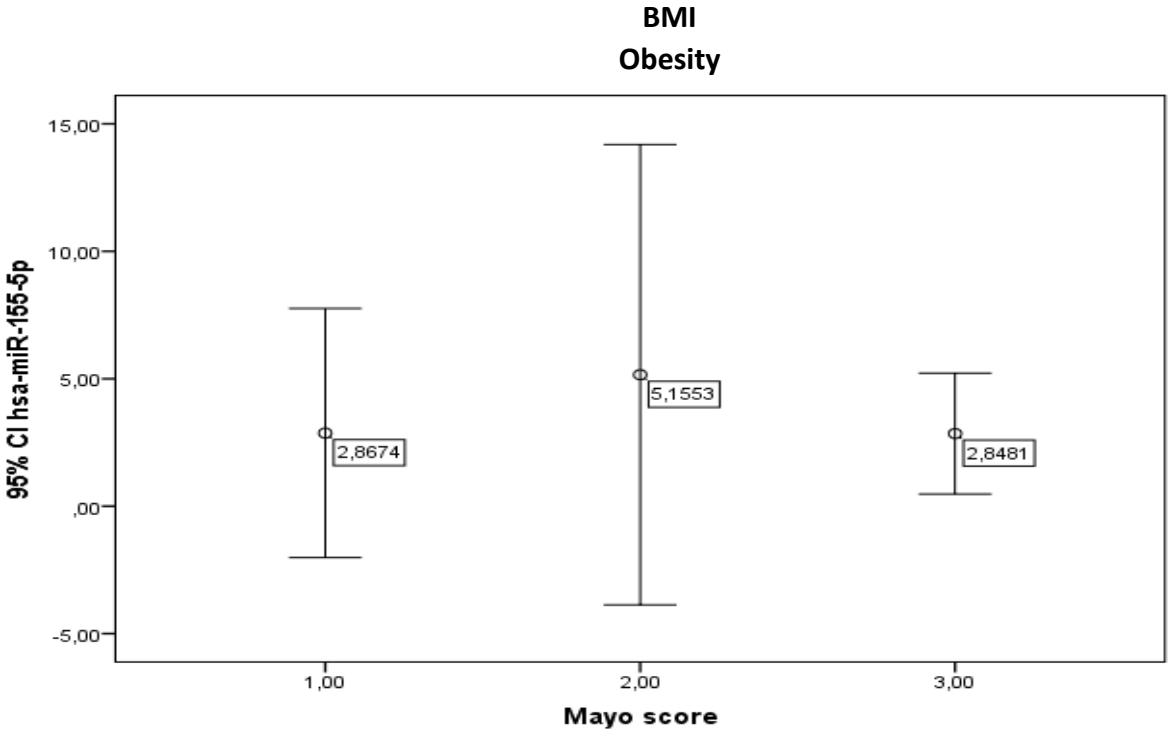

Fig. 87. Comparative analysis of hsa-miR-155-5p expression according to Mayo score in overweight and obesity patients

Fig. 88 presents a comparative analysis of the mean serum human Lipocalin – 2/NGAL expression according to the Mayo score in overweight and obese patients. The results showed that patients with Mayo score 3 had increased expression of the studied miRNA (62.99) compared to patients with Mayo score 1 (50.54) ($p<0.05$). There was a trend towards increased-increasing the expression of human Lipocalin – 2/NGAL with increasing Mayo score in UC patients.

There was a linear moderate correlation between serum human Lipocalin – 2/NGAL expression and Mayo score in overweight and obese UC patients ($r=0.344$; $p=0.037$).

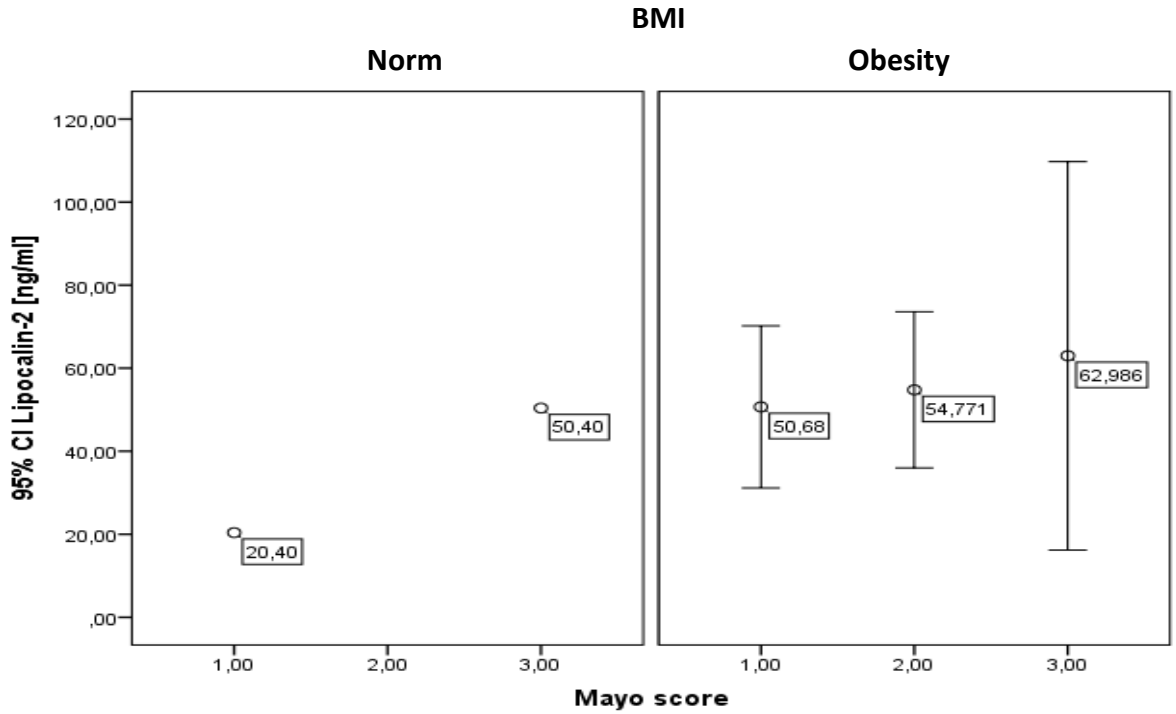

Fig. 88. Comparative analysis of human Lipocalin – 2/NGAL expression according to Mayo score in overweight and obesity patients

4.6. Estimation of circulating levels of the panel of miRNAs tested and serum expression of human Lipocalin – 2/NGAL according to CD and UC activity and conventional or biologic treatment performed

Table 26 shows the mean serum expression values of the panel of miRNAs and human Lipocalin – 2/NGAL tested compared with the threshold values of the tested markers according to BMI and the biological treatment performed.

The results showed that the variation of the expression of the studied markers according to the threshold value for BMI and anti-TNF treatment was in the upward direction. Patients on antiintegrin treatment also showed an increase in expression, except for hsa-miR-29a-5p, where the mean value was below the threshold value.

Table 26. The cut-off and mean values of the serum expression of the panel of studied miRNAs and human Lipocalin – 2/NGAL according to the performed biological treatment

miRNAs	**Cut-off BMI**	**anti-TNF**	**Anti-integrins**	**Direction of change relative to the cut-off**
hsa-miR-17-5p	0.99	1.55±1.87	1.41±1.05	↑/↑
hsa-miR-29a-5p	1.54	1.96±2.28	1.16±0.60	↑/↓
hsa-miR-142-3p	1.15	1.23±0.73	1.36±0.91	↑/↑
hsa-miR-146a-5p	1.06	1.41±1.33	1.21±0.74	↑/↑
hsa-miR-155-5p	1.32	2.66±3.29	3.85±5.15	↑/↑
human Lipocalin - 2/NGAL	63.15	65.03±33.93	99.07±58.32	↑/↑

On the other hand, table 27 shows the direction in the expression of the panel of miRNAs and human Lipocalin-2/NGAL under biological treatment in obese patients according to disease activity. The results showed that patients with anti-TNF treatment activity had a decrease in the expression of hsa-miR-17-5p, hsa-miR-146a-5p and hsa-miR-155-5p. An increase in expression was observed in human Lipocalin-2/NGAL, and hsa-miR-29a-5p was overexpressed. On the other hand, hsa-miR-142-3p remained unchanged in both active and remission patients. Patients in remission showed decreased expression of hsa-miR-29a-5p and human Lipocalin-2/NGAL. Increased expression was found in hsa-miR-17-5p and hsa-miR-146a-5p, whereas overexpression was found in hsa-miR-155-5p.

Table 27. Study of the expression of the panel of miRNAs and human Lipocalin - 2/NGAL in biological treatment in patients with obesity

miRNAs	anti-TNF		Anti-integrins	
	Activity	**Remission**	**Activity**	**Remission**
hsa-miR-17-5p	↓	↑	↑	↓
hsa-miR-29a-5p	↑↑	↓	↑	↓
hsa-miR-142-3p	NS	NS	↑	↓
hsa-miR-146a-5p	↓	↑	↑	↓
hsa-miR-155-5p	↓	↑↑	↑↑	↓
human Lipocalin - 2/NGAL	↑	↓	↑↑	↓

Patients on antiintegrin treatment who were in remission showed a decrease in the expression of all miRNAs and human Lipocalin – 2/NGAL examined and an increase in expression activity. It is striking that hsa-miR-155-5p and human Lipocalin – 2/NGAL had overexpression.

Table 28 shows the mean serum expression values of the panel of miRNAs and human Lipocalin – 2/NGAL tested compared with the threshold values of the tested markers according to BMI and conventional treatment. The results showed that the variation of the expression of the examined markers according to the threshold value for BMI and the treatment with 5-ASA was in the upward direction. Patients on corticosteroid treatment also showed an increase in expression, except for hsa-miR-29a-5p, where the mean value was below the threshold value. On the other hand, patients on immunosuppressant treatment showed an increase in expression for hsa-miR-17-5p, hsa-miR-29a-5p, and human Lipocalin – 2/NGAL and a decrease in expression for hsa-miR-142-3p, hsa-miR-146a-5p, and hsa-miR-155-5p, according to BMI threshold values.

Table 28. The threshold and mean values of the serum expression of the panel of studied miRNAs and human Lipocalin – 2/NGAL according to the performed conventional treatment

miRNAs	Cut-off BMI	5-ASA	Corticosteroids	Immunosuppressants	Direction of change relative to the cut-off
hsa-miR-17-5p	0.99	1.47±1.74	1.44±0.77	1.36±0.78	↑/↑/↑
hsa-miR-29a-5p	1.54	1.95±1.94	1.52±1.09	1.57±0.90	↑/↓/↑
hsa-miR-142-3p	1.15	1.22±0.73	1.28±0.54	0.99±0.46	↑/↑/↓
hsa-miR-146a-5p	1.06	1.32±1.24	1.32±0.73	0.95±0.23	↑/↑/↓
hsa-miR-155-5p	1.32	2.55±3.28	3.34±3.91	1.30±0.92	↑/↑/↓
human Lipocalin - 2/NGAL	63.15	66.35±39.98	67.61±41.67	68.91±52.84	↑/↑/↑

Table 29 shows the direction of expression of the panel of miRNAs and human Lipocalin-2/NGAL under conventional treatment in obese patients according to disease activity. The results showed that patients on treatment with 5-ASA in activity had an increase in the expression of hsa-miR-17-5p, hsa-miR-29a-5p and human Lipocalin - 2/NGAL and a decrease in hsa-miR-146a-5p and hsa-miR-155-5p. With unchanged expression in both active and remission patients, hsa-miR-142-3p remained. Patients on 5-ASA treatment in remission showed decreased expression of hsa-miR-17-5p, hsa-miR-29a-5p and human Lipocalin – 2/NGAL and increased expression of hsa-miR-146a-5p, with hsa-miR-155-5p overexpressed.

Table 29. Study of the expression of the panel of miRNAs and human Lipocalin - 2/NGAL in conventional treatment in patients with obesity

miRNAs	5-ASA		Corticosteroids		Immunosuppressants	
	Activity	**Remission**	**Activity**	**Remission**	**Activity**	**Remission**
hsa-miR-17-5p	↑	↓	↑	↓	↑	↓
hsa-miR-29a-5p	↑	↓	↑	↓	NS	NS
hsa-miR-142-3p	NS	NS	↑	↓	↑	↓
hsa-miR-146a-5p	↓	↑	↓	↑	↑	↓
hsa-miR-155-5p	↓	↑↑	↓	↑↑	↓	↑
human Lipocalin - 2/NGAL	↑	↓	↑	↓	↑	↓

Patients on active corticosteroid treatment showed increased expression of hsa-miR-17-5p, hsa-miR-29a-5p, hsa-miR-142-3p and human Lipocalin – 2/NGAL and decreased expression of hsa-miR-146a-5p and hsa-miR-155-5p. Patients on corticosteroid treatment who were in remission were found to have increased expression of hsa-miR-146a-5p, with hsa-miR-155-5p overexpressing and the remaining miRNAs and human Lipocalin – 2/NGAL having decreased expression.

No change in hsa-miR-29a-5p expression was detected in patients on immunosuppressant treatment, either in active or in remission patients. Decreased expression was observed for hsa-miR-155-5p, whereas expression was increased for the remaining miRNAs and human Lipocalin – 2/NGAL. In patients in remission, increased expression was present in hsa-miR-155-5p, whereas expression was decreased in the remaining miRNAs and human Lipocalin – 2/NGAL.

V. DISCUSSION

5.1. Anthropometric characteristics in patients with Crohn's disease and ulcerative colitis with obesity

The global prevalence of obesity has reached epidemic proportions and is one of the leading public health problems of the 21st century. Increasing levels of obesity among IBD patients have been documented, paralleling those in the general population. Data from cross-sectional and single-center cohorts suggest that the prevalence of obesity (according to BMI ≥30) in IBD patients is between 15% and 40%, with this percentage further increasing with the addition of overweight individuals (BMI 25 to <30). Various studies have found that obesity negatively affects the clinical course of IBD and may increase the burden of the disease on various aspects of the patient's life, as well as on the effectiveness of ongoing treatment and postoperative outcomes [100, 200, 201, 202, 267].

In September 2022, the Joint Guideline on Clinical Nutrition and Metabolism in Gastrointestinal and Liver Diseases was released with a total of 19 recommendations for IBD patients with special attention to the atropometric assessment tools that should now be used to characterize these patients and the importance of combating obesity through various methods [28].

Even before these recommendations were published, episodic research in this area and the impact obesity has on various aspects of IBD patients lives. This determined the need to study obesity among IBD patients in the Bulgarian population and its influence on different types of therapy, assessed by means of anthropometric measurements. Before these recommendations were even published, episodic studies in this area focused on the impact that obesity has on various aspects of IBD patients' lives. The lack of a systematic analysis and assessment of overweight and obesity in the IBD patient population has determined the need to investigate them using anthropometric measurements, new biomarkers, and to systematize their impact on different types of therapy.

Our results should encourage clinicians to treat obesity in IBD patients as an active problem, as this may help improve clinical outcomes.

Further studies are needed to consider obesity as a dynamic process in the course of the disease and to select the appropriate moments for impact and limitation within a personalized approach.

In their study, Ortega et al. (2013) in a study of obese patients (32 men) found that there was a directly proportional moderate relationship between serum expression of miR - 142-3p and BMI ($r=0.43$; $p<0.001$) and waist circumference in cm ($r=0.43$; $p<0.001$) [230]. Compared with his results, in the present study, no correlation was found between miR 142-3p expression and BMI, as well as waist circumference, which may be explained by the influence of immune-mediated inflammation combined with meta-inflammation on miRNAs expression and anthropometric parameters .

In another study by Hijmans et al. (2018) found that serum expression of miR-146 was moderately inversely correlated with BMI ($r=-0.33$; $p<0.05$), with no correlation with waist circumference [125]. The examination of the relationship between miR-146 expression and BMI and waist circumference in the present study did not show a significant relationship, which proves that in IBD patients, not only the distribution of adipose tissue affects the level of the studied miRNA.

The few published scientific studies in the field of obesity assessment in IBD patients revealed the need to deepen the study in this direction not only by means of the assessment scale of anthropometric studies, but also by means of modern biomarkers such as miRNAs and human Lipocalin - 2/NGAL.

5.2. Examining the expression of a panel of miRNAs in patients with Crohn's disease and ulcerative colitis according to BMI

In the present study, 5 miRNAs were identified that showed altered expression in CD and UC patients with and without obesity.

The functional role of miR-17-5p as a critical regulator of molecular mechanisms involved in insulin secretion as well as proliferation and adaptation of pancreatic β-cells to metabolic stress is known [56]. In the present study, the increased expression of miR-17-5p in CD and UC patients and obesity is contrary to a study by Heneghan et al. [123] who demonstrated decreased expression of miR-17-5p in obese patients, which correlated negatively with BMI. In another study by Ramzan et al. [253] found decreased expression and negative correlation between miR-17-5p and metabolic syndrome and waist circumference. In this regard, the authors suggest the potential role of miR-17-5p in increased central obesity, as a major factor involved in the progression of the metabolic syndrome. On the other hand, our results correspond with those of Karolina et al. [146] who found increased expression of miR-17-5p in patients with metabolic syndrome (1.93±0.19 for Karolina et al. and 1.53±1.69 in the present study, respectively).The decreased expression of miR-17-5p in the group of Heneghan et al., Karolina et al. explained by the fact that metabolic syndrome was studied in patients with type 2 diabetes mellitus, which means that miR-17-5p expression in patients with metabolic syndrome or obesity is affected not only by the early differentiation of adipocyte mass, but also by the accompanying patient's illness.

In mouse models placed on the high-fat diet, an increase in miR-29a expression was observed in myocytes, leading to impairment of insulin signaling by reducing the expression of insulin receptor substrate 1 (IRS1) [341].

Thompson et al. [301] conducted a study of 20 miRNAs potentially involved in NAFD and found that 15 of the investigated miRNAs had a significant change in their expression ($p < 0.05$) in the plasma of children with obesity and NAFD compared to normal-weight and non-implantation controls NAFD.

One of these miRNAs, mir-29-3p, whose expression was 2.81 times higher in obese children compared to the control group (<0.0001). In the present study, the expression of miR-29a-5p was also decreased in healthy controls (1.38±1.58), but in the background of IBD the expression was increased (1.92±1.91) and increased expression was observed in patients with IBD and normal weight (2.06± 1.53), while in obese patients it decreased (1.89±2.01) but remained higher compared to that of healthy controls.

Other authors also found altered expression of miR-29a in obesity and metabolic syndrome [121, 316]. Our results in the obese and IBD patients regarding miR-29a-5p expression relative to healthy controls confirm those of Roncarati et al. [258] who found increased plasma expression of the investigated mir in patients with obesity and chronic heart disease. In his study, JA Deiuliis found decreased expression of miR-29b in obese patients in peripheral blood [79], and these results converged with our results comparing the expression of miR-29a-5p in IBD patients with and without obesity. Other authors who found similar results are Zampetaki et al [348], found decreased expression of miR-29b in plasma in patients with diabetes mellitus compared to healthy controls.

Increased expression of miR-142-3p has been reported in children and adults with obesity compared to individuals with normal body weight [13, 231]. The present study found a similar trend in CD patients, where the expression of miR-142-3p in obese patients was 1.03, while in those with normal weight it was 0.98. On the other hand, it was found that in patients with UC and normal body weight, there was an increased expression of the studied miRNA (1.49), compared to those with obesity (1.27).

Another study also found increased expression of circulating miR-142-3p in obese subjects compared to normal weight controls [248].

In a study by Ortega et al. [231] among 80 individuals found that miR-142-3p expression correlated directly with BMI, with normal-weight individuals having mean circulating miRNA expression levels of 0.26, which increased to 0.53 in obese individuals (30≤ BMI<40) to 0.83 in persons with morbid obesity (BMI≥40). A similar trend was observed in the CD patients in the present study. In the same study, the relationship between the expression of mirR-142-3p and some indicators, which are shown in the table, was evaluated. 30 were compared with the results of the present study.

Table 30. Comparative analysis of the results of the study of Ortega et al. and the present study – correlation analysis of the expression of miR-142-3p

Indicator	**Ortega et al. (2013) [231]**	**Moneva-Petrova M. (2023)**
Age (years)	-0.24 (p=0.03)	0.07 (p=0.741)
BMI	0.43 (p<0.001)	0.26 (p=0.05)
Waist measurement (cm)	0.39 (p<0.001)	0.165 (p=0.027)
Fasting blood sugar	0.22 (p=0.05)	0.194 (p=0.038)

According to the results presented in table 30 it can be said that in the present study (Moneva - Petrova, 2023) a weaker correlation was observed between the expression of miR-142-3p and the considered indicators, maintaining the same trend as in Ortega et al. (2013) except for age.

In the literature, miR-146b is in the group of anti-adipogenic miRNAs [347]. Chartoumpekis et al. found increased expression of some miRNAs including miR-146a, miR-146b during the development of obesity in mouse models [52]. Cui et al. investigated the risk of obesity and the later development of diabetes mellitus and found that miR-146a, miR-146b were overexpressed in obese children [71]. These results are also confirmed in the present study where the expression of miR-146a is increased in IBD and obese patients (1.36 in obese patients and 1.15 in normal weight patients, respectively).

In his study, Al-Rawaf evaluated the expression of miR-146a and found decreased serum levels in obese adolescents, which correlated strongly with BMI and waist/height ratio [13].

In the present study, a weak correlation was found between miR-146a and BMI and waist/height ratio. In two other studies by Ahn et al. [10] and Chen et al. [55] found increased expression of miR-146b, which was associated with visceral and subcutaneous adipose tissue in overweight and obese subjects. In the present study, visceral adipose tissue was examined by waist circumference, which correlated moderately directly with miR-146a expression ($r=0.350$; $p<0.05$). Subcutaneous adipose tissue in the waist area was assessed by measuring the abdominal skinfold, and no association was found with miR-146a expression. In contrast to the present study, in that of Huang et al. [133] found hsa-miR-146b-3p, hsa-miR-146b-5p in subcutaneous adipose tissue of obese patients. Two other authors found that miR-146a expression was significantly lower in obese and overweight patients compared to the normal BMI group [126, 280].

miR-155 is a multifunctional miRNA that is associated with the regulation of various immune-mediated processes, such as hematopoiesis [225], innate immunity [226], B-cell and T-cell differentiation [155] and is one of the most studied miRNAs, involved in obesity as it plays a role in adipogenesis, adipocyte function and inflammation [145, 186, 206, 351]. Induction of miR-155 expression is mediated by TNF-α in adipocytes and in white adipose tissue, explaining the role of this miRNA in obesity-mediated inflammation [145]. In obese individuals, increased expression of miR-155 was observed in adipose tissue, which correlated significantly directly with BMI [145]. The present study also found increased expression of miR-155 in patients with IBD and obesity (2.70), compared to the expression of the miR-155 in healthy controls (1.47). On the table 31 a comparative analysis of miR-155 expression in IBD patients is presented.

Table 31. Comparative analysis of miR-155 expression in IBD patients

Study group	**Atanassova A. (2021) [1]**		**Moneva-Petrova M. (2023)**	
	BMI<24.9 kg/m2	**BMI>25.0 кg/m2**	**BMI<24.9 кg/m2**	**BMI>25.0 кg/m2**
Healthy controls	1.47±1.29	-	1.47±1.29	-
CD patients	2.73±3.07	2.24±1.86	5.46±5.22	2.37±3.18
UC patients	1.25±0.87	1.49±0.92	1.39±1.81	3.07±3.44

According to the presented results, in both studies, a different expression of miR-155 was observed in CD and UC patients, indicating that this miRNA responds differently in the pathogenesis of obesity in the two diseases. In CD patients, increased expression was observed in individuals of normal body weight, whereas in UC patients, increased expression was observed in obesity, these results confirming those already described in the literature. In this regard, Kim et al. [151] in the study of obese individuals and healthy controls found that miR-155 expression was increased between 1.5 and 2-fold in obese individuals in both serum and tissues. In another study by Thompson et al. [301] who conducted a study of 20 miRNAs potentially involved in NAFD and found that 15 had a significant change in their expression ($p < 0.05$) in the plasma of children with obesity and NAFD compared to normal weight and non-NAFD controls found that miR-155 was increased 2.63-fold in obese children compared to controls. Another group of authors found that miR-155 expression correlated directly with subcutaneous adipose tissue [154]. These results are confirmed in the present study, where a weak to moderate directly proportional relationship was found between circulating levels of miR-155 and the size of the abdominal skinfold ($r=0.282$; $p=0.047$).

5.3. Study of the expression of human Lipocalin - 2/NGAL in patients with Crohn's disease and ulcerative colitis

Lipocalin-2 (LCN2) is a secreted glycoprotein that is involved in various chronic inflammatory processes. Higher plasma levels of LCN2 have been found in patients with obesity [20, 48] and other related diseases characterized by a metabolic inflammatory pattern, including type 2 diabetes mellitus (T2DM), nonalcoholic fatty liver disease (NAFD), and cardiac - vascular disorders [6, 324]. LCN2 is also highly expressed in the colon, where it is synthesized by epithelial cells, especially under inflammatory conditions. During such processes, LCN2 may favor the transformation of the gut microbiota into one with anti-inflammatory activity [213].

In a study by Currò et al. [72] found that patients with metabolic syndrome had higher levels of lipocalin 2 compared to healthy controls. A more detailed analysis including assessment of marker expression according to BMI found that at BMI > 30 there was a decrease in LCN2 expression. Similar results were observed in the present study, where patients with IBD and normal body weight had higher expression levels than those with obesity (81.1 for normal weight patients vs. 63.59 for obese patients, respectively).

5.4. Assessment of inflammation in obese IBD patients by matching some anthropometric parameters, levels of a panel of circulating miRNAs and serum expression of human Lipocalin - 2/NGAL

In one clinical trial, LCN2 was investigated as a possible biomarker to assess activity in IBD. Faecal LCN2 can detect endoscopic UC activity with a cut-off of 6700 ng/g and a sensitivity and specificity of 82 and 80%, respectively, not so different from the 86.4% sensitivity and 80.0% specificity with which faecal calprotectin does the same at levels higher than 250 μg/g. [40]

Serum LCN2 can distinguish endoscopically active from inactive UC with a threshold value of 43.6 ng/ml, with higher sensitivity (96%) but lower specificity (54%) than fecal LCN 2 [38]. In the present study, a strong correlation was established between the serum levels of human Lipocalin 2 and inflammation markers, such as CRP and FCP, as well as with the assessment scales for assessing activity - CDAI and Mayo score. These results support the data from other studies that human Lipocalin 2 correlates with the activity of both diseases and can be used as a new reliable non-invasive biomarker.

5.5. Evaluation of the circulating levels of the panel of studied miRNAs and the serum expression of human Lipocalin - 2/NGAL according to the activity of CD and UC and the conventional or biological treatment performed

The following tables present a comparative analysis of the expression of some miRNAs according to treatment behavior in IBD patients with obesity in the two single-point cross-sectional studies that were performed at different time intervals. A. Atanassova's 2021 study was conducted before the COVID-19 pandemic, while the current study was conducted entirely in an epidemic setting.

According to the results presented in the table 32 in the comparative analysis of the expression of some miRNAs according to 5-ASA treatment in IBD patients with obesity, a difference in the expression direction of hsa-miR-29a-5p and hsa-miR-142-3p was observed in activity and remission. This indicates the need for additional studies to monitor the dynamics of the expression of miRNAs in IBD patients with obesity. On the other hand, hsa-miR-155-5p showed identical expression in both studies.

Table 32. Comparative analysis of the expression of some miRNAs according to the treatment with 5-ASA in IBD patients with obesity

miRNAs	Atanassova A. (2021) [1]		Moneva-Petrova M. (2023)	
	Activity	**Remission**	**Activity**	**Remission**
hsa-miR-29a-5p	↓	↑	↑	↓
hsa-miR-142-3p	↓	↑	NS	NS
hsa-miR-155-5p	↓	↑	↓	↑↑

According to the results presented in the table 33 it is seen that hsa-miR-29a-5p has the same direction of expression in the corticosteroid-treated patients in activity and remission in both studies. A similar trend was also observed in the expression of hsa-miR-142-3p, with the difference that in A. Atanassova's study in 2021, the miRNA in question was overexpressed in active patients. A difference in the direction of expression in the two studies was found for hsa-miR-155-5p, which was overexpressed in the patients in activity in the study of A. Atanassova (2021), while in the present study it was overexpressed in the patients in remission.

Table 33. Comparative analysis of the expression of some miRNAs according to corticosteroid treatment in IBD patients with obesity

miRNAs	Atanassova A. (2021) [1]		Moneva-Petrova M. (2023)	
	Activity	**Remission**	**Activity**	**Remission**
hsa-miR-29a-5p	↑	↓	↑	↓
hsa-miR-142-3p	↑↑	↓	↑	↓
hsa-miR-155-5p	↑↑	↓	↓	↑↑

According to the results presented in the table 34 it is seen that the expression of hsa-miR-29a-5p does not change in the patients treated with immunosuppressants in the present study, while in that of A. Atanassova, a decrease in expression was observed in patients in activity and a decrease in those in remission. A similar trend was observed in the expression of hsa-miR-142-3p with no difference in the two studies. A difference in the direction of expression in the two studies was found for hsa-miR-155-5p, which had increased expression in the active patients in the study by A. Atanassova (2021), while in the present study the expression was decreased.

Table 34. Comparative analysis of the expression of some miRNAs according to the treatment with immunosuppressants in IBD patients with obesity

miRNAs	Atanassova A. (2021) [1]		Moneva-Petrova M. (2023)	
	Activity	**Remission**	**Activity**	**Remission**
hsa-miR-29a-5p	↓	↑	NS	NS
hsa-miR-142-3p	↑	↓	↑	↓
hsa-miR-155-5p	↑	↓	↓	↑

According to the results presented in the table 35 it is seen that the expression of hsa-miR-142-3p does not change in the patients on biological treatment with anti-TNF in the present study, while in that of A. Atanassova an overexpression was observed in the patients in activity and a decrease in those in remission. A similar trend was observed in the expression of hsa-miR-29a-5p, which was overexpressed in the active patients in the present study. A difference in the direction of expression in the two studies was found for hsa-miR-155-5p, which had increased expression in the active patients in the study by A. Atanassova (2021), while in the present study the expression was decreased, with overexpression also observed in patients in remission.

Table 35. Comparative analysis of the expression of some miRNAs according to the implementation of biological treatment with anti-TNF in IBD patients with obesity

miRNAs	Atanassova A. (2021) [1]		Moneva-Petrova M. (2023)	
	Activity	**Remission**	**Activity**	**Remission**
hsa-miR-29a-5p	↑	↓	↑↑	↓
hsa-miR-142-3p	↑↑	↓	NS	NS
hsa-miR-155-5p	↑	↓	↓	↑↑

As a summary of the results, profiles of overweight and obese CD and UC patients in disease activity and remission are presented (Fig. 89, Fig. 90, Fig. 91 and Fig. 92). The direction of expression of the investigated markers is shown relative to patients with normal BMI.

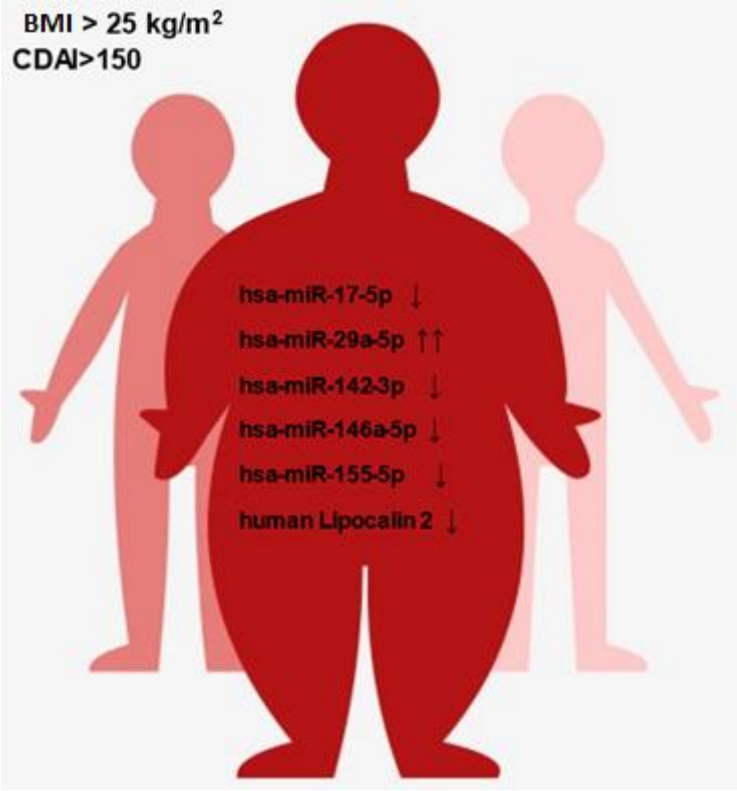

Fig. 89. Profile of a CD patient with overweight and obesity in the activity stage

Fig. 90. Profile of a UC patient with overweight and obesity in the activity stage

Fig. 91. Profile of a CD patient with overweight and obesity in remission

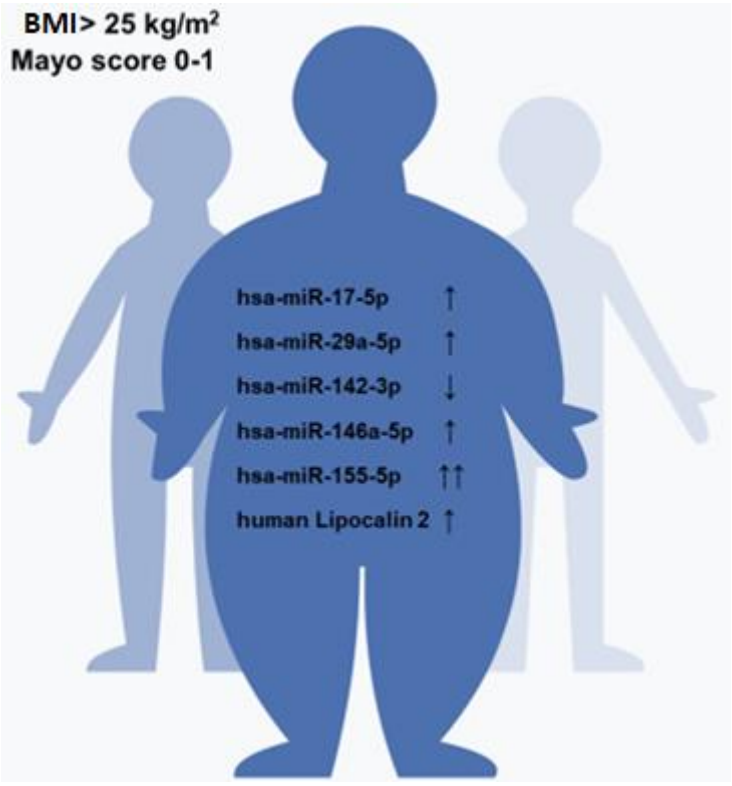

Fig. 92. Profile of a UC patient with overweight and obesity in remission

CONCLUSIONS

1. A strong orthogonal relationship was found between BMI, waist circumference, abdominal fold size and waist-to-hip ratio in IBD patients.
2. A variable degree of correlation was also found between anthropometric parameters (BMI, waist circumference, abdominal skinfold size and waist/hip ratio) and laboratory parameters of the complete lipid profile (total cholesterol, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol, triglycerides).
3. The expression of the panel of miRNAs considered according to anthropometric indices for the assessment of obesity differed in CD and UC patients.
4. Patients with BMI ≥ 25 kg/m2 showed increased expression of hsa-miR-17-5p, hsa-miR-146a-5p and hsa-miR-155-5p, whereas hsa-miR-29a-5p, hsa-miR-142-3p and human Lipocalin 2/NGAL were down-regulated.5. Overexpression of hsa-miR-17-5p was associated with patient age > 40 years, obesity, waist circumference above the norm for both sexes, waist circumference/height ratio > 0.5, disease activity, and UC.
6. Decreased expression of hsa-miR-29a-5p was associated with male sex, obesity, UC patients, and achieved remission.
7. Decreased expression of hsa-miR-142-3p was observed in age > 40 years, male gender and CD patients.
8. Increased expression of hsa-miR-146a-5p was found in age < 40 years, obesity, waist circumference above the norm for both sexes, active disease and UC.
9. Overexpression of hsa-miR-155-5p is seen in female gender, obesity, CD patients and achieved remission.

10. Decreased human Lipocalin 2/NGAL expression is associated with obesity, waist circumference above normal, skinfold size, UC patients, and achieved remission.
11. Obesity in IBD patients on biologic treatment is a risk factor for not achieving remission as assessed by hsa-miR-29a-5p and hsa-miR-155-5p expression relative to FCP threshold values.
12. In BC patients, obesity as measured by BMI is a risk factor for not achieving remission as assessed by the expression of hsa-miR-146a-5p and hsa-miR-155-5p relative to their threshold values at CDAI>150.
13. In patients with UC on biologic therapy, BMI<25 kg/m2 and Mayo score <2 are prerequisites for achieving remission, as assessed by serum expression of hsa-miR-17-5p, hsa-miR-29a-5p, hsa-miR-146a-5p, and hsa-miR-155-5p relative to their threshold values at Mayo score ≥2.
14. Assessment of obesity and activity of the two diseases under conventional therapy with 5 ASA, immunosuppressants and/or corticosteroids showed no significant correlation with the panel of miRNAs studied and the level of human Lipocalin 2/NGAL.

CONCLUSION

Obesity is an increasingly common comorbid condition in the IBD population, although, as indicated in the literature review, its clinical significance in the pathogenesis, natural history, and treatment outcomes of IBD is controversial among the few available studies.

First, most available data come from retrospective studies measuring BMI at different time frames during the course of the disease. Adiposity is a dynamic measurement and undoubtedly undergoes a range of changes over the course of an individual's disease, another limiting factor in the analysis and comparison of different studies.If obesity affects disease outcomes, it is unclear when this effect is strongest. Both the retrospective nature of the studies and the variable time frames in which obesity was assessed make it difficult to investigate causality. This necessitates the need for new prospective studies. The growing frequency of overweight and obesity among IBD patients from the Bulgarian population determined the need and relevance of research on this comorbidity, which was unjustifiably neglected until recently.

The use of classical anthropometric assessment scales such as body mass index, waist circumference, waist circumference/height ratio, as well as abdominal skinfold measurement with a caliper are not sufficient to convey the complex clinical picture of overweight and obesity in these patients, nor can reveal the diverse impact on the phenotypic expression of Crohn's disease or ulcerative colitis, the activity of both diseases, as well as the impact on classic assessment scales such as CDAI, Mayo and/or non-invasive biomarkers C-reactive protein, faecal calprotectin to assess remission achieved and ongoing treatment.

Application of a selected panel of miRNAs involved in both immune system control, barrier epithelial function in ulcerative colitis and Crohn's disease, and human Lipocalin – 2/NGAL levels changing during activity and remission of both diseases under different therapeutic regimens have proven to be innovative, useful modalities that open new perspectives in personalized therapy and monitoring in these diseases.Their application in the present study made it possible to differentiate active disease and achieved remission in the course of biological therapy, immunosuppressive or conventional. As the first study in this field, the present study provides the snapshot of the expression of selected miRNAs and human Lipocalin – 2/NGAL and their correlation with classical anthropometric assessment scales, lipid status, CRP, FCP, CDAI and Mayo score.

For future studies, it would be useful to focus on prospective evaluation of UC and CD, improved control of different therapeutic regimens, and assessment of overweight and obesity using markers reflecting visceral adipose tissue and its influence on disease behavior and phenotypic expression .

CONTRIBUTIONS

Contributions of a theoretical nature

1. For the first time in Bulgaria anthropometric characterization of patients with IBD and obesity is performed.
2. For the first time in Bulgaria, the use of miRNAs and human Lipocalin – 2/NGAL in patients with IBD and obesity is reported in detail and comprehensively.

Contributions of a practical-applied nature

1. Threshold values for differentiating miRNAs and human Lipocalin – 2/NGAL expression according to BMI, CRP, FCP, CDAI, Mayo score were determined.
2. A specific profile of obese CD and UC patients based on the expression of miRNAs and human Lipocalin – 2/NGAL was developed.

Contributions of an original nature

1. For the first time in Bulgaria, a panel of miRNAs and human Lipocalin – 2/NGAL has been investigated to assess IBD patients with obesity.
2. For the first time in Bulgaria, the expression of miRNAs and human Lipocalin – 2/NGAL was described in relation to the administered therapy and BMI in IBD patients.

DISSERTATION RELATED PUBLICATIONS

1. Moneva M., Atanassova A. Obesity in patients with chronic inflammatory bowel diseases. Collection of reports. THIRD INTERNATIONAL CONFERENCE Health care - contribution to the quality of life 07-08 JUNE 2021. 454-460
2. Moneva M., Atanassova A. Metabolic syndrome in patients with chronic inflammatory bowel diseases. Collection of reports. THIRD INTERNATIONAL CONFERENCE Health care - contribution to the quality of life 07-08 JUNE 2021. 461-474
3. Moneva M., Atanassova A. ANTHROPOMETRIC ASSESSMENT OF OBESITY AMONG IBD PATIENTS. Science & Research, Volume VI, 2022, Number 1: MEDICAL BIOLOGY STUDIES, CLINICAL STUDIES, SOCIAL MEDICINE AND HEALTH CARE, 34-38
4. Moneva M., Atanassova A. INFLUENCE OF OBESITY ON THE COURSE OF INFLAMMATORY BOWEL DISEASE. Scripta Scientifica Medica, 2022;54, suppl. 1:44-47

PARTICIPATION IN SCIENTIFIC FORUMS

1. Moneva M., A. Atanassova. Obesity in patients with chronic inflammatory bowel disease. THIRD INTERNATIONAL CONFERENCE Health care - contribution to the quality of life 07-08 JUNE 2021 - with report
2. Moneva M., A. Atanassova. Metabolic syndrome in patients with chronic inflammatory bowel disease. THIRD INTERNATIONAL CONFERENCE Health care - contribution to the quality of life 07-08 JUNE 2021 - with report
3. Moneva M., Atanassova A. INFLUENCE OF OBESITY ON THE COURSE OF INFLAMMATORY BOWEL DISEASE. Alumni club – with report
4. Moneva M., Atanassova A. Study of miRNA 146a-5p as a new biomarker for the diagnosis of obesity in patients with chronic inflammatory bowel diseases. Anniversary scientific conference on the topic: Traditions and future in medical education, Medical College - Sofia, 21.03.2023 - accepted abstract for participation with a report and full-text publication in the collection of reports from the conference.
5. Moneva M., Atanassova A. Study of miRNA 17-5p expression in patients with chronic inflammatory bowel diseases. Anniversary scientific conference on the topic: Traditions and future in medical education, Medical College - Sofia, 21.03.2023 - accepted abstract for participation with a report and full-text publication in the collection of reports from the conference.