Characterization of the Serum Levels of Meteorin-like in Patients with Inflammatory Bowel Disease and its Association with Inflammatory Cytokines

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Research

Keywords: Meteorin-like, Inflammatory Bowel Disease, Ulcerative Colitis, Crohn's disease, Interleukin, Tumor necrosis factor, Adiponectin

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Abstract

Background : Meteorin-like (Metrnl) is a newly discovered adipokine with insulin sensitizing and anti-inflammatory properties. The relation among Metrnl, Inflammatory Bowel Disease (IBD), and obesity still remained unexplored. Methods : The present study was conducted on 54 healthy control, 42 Ulcerative Colitis (UC), and 43 Crohn's disease (CD) patients who were diagnosed by pathological examination. In all participants, serum levels of adiponectin, Metrnl, interleukin (IL)-6, and Tumor necrosis factor (TNF-α) were measured using ELISA kits.

Results: Metrnl concentration was considerably decreased in both UC (85.25±36.55 pg/mL) and CD (76.93±27.92 pg/mL) patients in comparison to control group (107.52±35.33 pg/mL). In addition, the level of adiponectin decreased in these two patient groups compared to the controls. However, IL-6 and TNF-α significantly elevated in the patient groups. Moreover, Metrnl showed an inverse correlation with body mass index (BMI) in the controls and the patients. In addition, there was an inverse correlation among Metrnl, IL-6, and TNF-α in both of the patient groups.

Conclusions : The current study is the first one reporting the decreased serum levels of Metrnl in patients with IBD, which is inversely related with BMI, TNF-α, and IL-6. These results suggested a possible relation of Metrnl with the pathogenesis of IBD, particularly through inflammatory process that need further study to dissect the possible mechanism. Keywords : Meteorin-like, Inflammatory Bowel Disease, Ulcerative Colitis, Crohn's disease, Interleukin, Tumor necrosis factor, Adiponectin

Introduction

Inflammatory Bowel Disease (IBD) is known as one of the causes of mortality in modern societies, which characterized by gastrointestinal chronic inflammation [1, 2]. Crohn’s disease (CD) and ulcerative colitis (UC) are two pathogenic form of IBD. The exact etiology of IBD is not well-understood yet, , therefore no-definitive cure has been identified [3]. In spite of previous research which showed that underweight and malnutrition have possible association in occurrence of IBD, recent studies have revealed a rising prevalence of IBD among obese patients (15%–40% are obese, and 20%–40% are overweight) in general population [4]. Recent epidemiological studies have also shown that increasing rate of IBD is parallel with the obesity prevalence [4, 5]. Moreover, hospitalization and surgery are more frequent among IBD patients with obesity [6]. Although it is not fully understood yet, scientific studies have suggested that, genetics, gut microbiome, and the immune system may play critical roles in IBD [7]. IBD (UC and CD) also displays the characteristics of chronic inflammation and metabolic syndrome, which is affectedly altered in metabolism [8, 9]. Adipocytokines are secreted by white adipose tissue affecting the gut microbiome, inflammation, and metabolism pathways [10, 11]. In a mutual way, IBD also can be considered as a risk factor for obesity by changes in the intestinal microbial metabolism [9, 12].

On the other hand, the impaired white adipose tissue (WAT) function such as abnormal adipocytokine secretion has a major and effective role on the inflammatory condition in colon tissue [12]. For example,

leptin has decreased in the serum sample obtained from the IBD patients (with or without overweight). While the levels of resistin, adiponectin, and active ghrelin have remarkably increased[13].

Meteorin-like (Metrnl, known as Subfatin) is a novel adipo-myokine that is mainly expressed in WAT, however, it was also reported that Metrnl is expressed colon epithelium. The anti-inflammatory function of Metrnl has been revealed recently[14]. Moreover, it can enhance lipid metabolism, decrease adipose inflammation, and ameliorate obesity-mediated insulin resistance (IR). Notably, in this study, Metrnl expression was higher in mesenteric WAT of the CD patients in comparison to the controls [15] . Metrnl is highly expressed in intestinal cells, white adipose tissue, and skin. In addition, Metrnl is expressed in other tissues including muscle, liver, heart, spleen, and central nervous system (CNS). Moreover, the activated macrophages produced Metrnl, which by this fact, Metrnl may connected to the inflammatory disorders such as IBD [16]. A few studies have been done on tissue Metrnl levels in IBD disease. However, up to now, no studies have been performed on investigating the association between serum Metrnl and IBD disease as well as the association among this protein, obesity, and the pro-inflammatory cytokines. Regarding to the fact that IBD is an inflammatory disease and Metrnl demonstrated anti-inflammatory activities, in this study, the serum levels of Metrnl were examined in the patients with inflammatory bowel disease and also its association with the hall-markers of inflammatory cytokines, interleukin (IL)-6, and Tumor necrosis factor (TNF-α) were evaluated.

Methods

Study population

This case-control study was conducted on 54 control subjects and 85 IBD patients including 42 CD and 43 UC who were recruited from endoscopy unit of Valiasr Hospital, Birjand, Iran. All the individuals aged between 35 and 60 years old. The patient and control groups were selected by clinical examination, radiologic, endoscopic, and pathologic criteria. Normal people without any inflammatory diseases was considered as control group and based on age, gender and BMI were matched with patient groups. The participants in case groups were included if; were diagnosed by radiologic, endoscopic examination according to clinical and pathological guideline documents, and presence of other IBD's manifestations (diarrhea, abdominal pain, rectal bleeding, and malnutrition). In addition, none of them were received medication or anti-inflammatory drugs. All of patients were diagnosed recently. All of participants signed written form. The diagnosis of UC/CD were based on established clinical and histopathological criteria. Moreover, the subjects with any history of cancer, diabetes, autoimmune diseases or active infectious disease were excluded from the study.

Anthropometric data and laboratory measurements

Demographic data and medical history were obtained by a self-questionnaire from all participants. At the beginning of examinations, weight and height were taken from participants who were wearing light clothes only, without shoes. Body mass index (BMI) was also calculated by body weight (kg) divided by height squared (m2), and studied population categorized into normal weight (BMI<25) and overweight

(BMI≥25). Afterward, systolic and diastolic blood pressures of all the participants were evaluated using a standard sphygmomanometer after 15 min resting in a sitting posture. After 12 hours of fasting, 5 ml of venous blood was obtained from all the participants and the serum was then separated by centrifugation. Subsequently, fasting blood sugar (FBS) and lipid profiles including triglycerides (TG), total cholesterol (TC), High-density lipoprotein-cholesterol (HDL-C), and Low-density lipoprotein-cholesterol (LDL-C) were measured using auto-analyzer and the commercially available kits (Pars Azmoon, Tehran, Iran).

Serum adipokine and cytokines

Circulating levels of Metrnl were evaluated using an immunoassay kit (R&D Systems, Minneapolis, USA, Cat#DY7867). Moreover, the inter-assay and intra-assay variations were calculated as 6 and 8 %, respectively. Adiponectin serum levels were measured using an ELISA kit (Adipogen, Seoul, South Korea, Cat#AG-45A-0001YEK-K101) with inter- and intra-assay variations of 4.4% and 4.6%, respectively. Afterward, the ultrasensitive ELISA kits were used to measure the serum levels of inteleukin-6 (IL-6) (R & D Systems, Minneapolis, USA, Cat# HS600B) and TNF-α (R & D Systems, Minneapolis, USA, Cat# DTA00C). Notably, the minimum detectable ranges of IL-6 and TNF-α were obtained as 0.7 and 1.6 pg/ml. Furthermore, inter and intra-assay variations of IL-6 were 9% and 7% and inter and also intra-assay variations of TNF-α were 6% and 5%, respectively.

Statistical analysis

Statistical analysis was performed using SPSS version 18. Categorical data was analyzed using chi-square test and presented in frequency and percentage. Continuous variables were also examined by student t-test and one-way ANOVA, and presented in mean and standard deviation (SD). Pearson correlation test was applied to correlation analysis. Furthermore, multinomial logistic regression was conducted to estimate the risk of diseases status according to serum levels of Metrnl.

Results

Anthropometric and biochemical measurement

The details of anthropometric and biochemical variables of the studied population are given in Table 1. In this regard, the studied groups showed no significant difference in terms of age, sex, and BMI. In addition, there were no significant difference in the frequency of normal weight and overweight between the groups. Similarly, systolic blood pressure (SBP) and diastolic blood pressure (DBP) indicated no considerable difference among these 3 groups. Although FBS illustrated no significant difference between the controls and patients with UC and CD, insulin and homeostatic model assessment for insulin resistance (HOMA-IR) were dramatically higher in the CD patients compared to the controls. It should be noted that, higher levels of insulin and HOMA-IR in the UC patients compared to the controls did not reach to the significant threshold. Furthermore, lipids profile including TG, TC, HDL-C, and LDL-C demonstrated no considerable variation between the patients and controls.

Serum levels of adipokines and cytokines

The ELISA results (**Fig.1**) showed that, adiponectin concentration considerably decreased in the patients with UC and CD compared to the controls. Furthermore, TNF-α considerably elevated in the patients with UC and CD in comparison to the controls. Moreover, the patients with CD showed higher IL-6 levels compared to the controls, while the serum levels of IL-6 did not reach to the significant threshold in the patients with UC compared to the control group. Furthermore, Metrnl serum concentration considerably diminished in the patients with UC and CD compared to the controls.

Regarding the crucial role of adipose tissue on Metrnl levels, analysis was performed according to the BMI cutoff (Overweight, BMI≥25 and normal weight, BMI<25). In this regard, Metrnl serum levels were found to be lower in all the overweight subgroups (Table 2).

Association of serum Metrnl with the risk of diseases status

Multinomial logistic regression was performed to assess the risk of diseases status according to serum levels of Metrnl. The results demonstrated a significant association between the decreased serum levels of Metrnl with the risk of UC and CD diseases. Furthermore, these associations have been adjusted for confounding factors including age, sex and BMI and the relationships remained as significant for both UC and CD diseases (Table 3).

Correlation analysis

Correlation analyses were performed in 2 subgroups, as controls and patients and the results are presented in Table 4. In the control group, Metrnl was found to be inversely correlated with BMI (Fig 2). Also, in the patient groups, Metrnl had an inverse correlation with BMI, IL-6, and TNF-α, and a positive correlation with FBG (Fig 2).

Discussion

Adipokines have several effects on the immune system through regulating the expression and secretion of various cytokines. Therefore, they can play a crucial role in inflammatory diseases like IBD, which also have a metabolic background [17]. Several studies have shown that, adipokines such as leptin, resistin, visfatin, retinol-binding protein-4, adiponectin, glucose, and insulin are deregulated in the IBD patients [13]. Metrnl is a novel adipokine, which plays a key role in inflammation and insulin resistance improvement [18]. Accordingly, this adipokine has been investigated in several metabolic and inflammatory diseases. Lee et al. showed that, the levels of Metrnl were lower in the serum samples of the type 2 diabetes mellitus (T2DM) patients [19]. Moreover, Dadmanesh et al. found lower serum levels of Metrnl in the patients with coronary artery disease and T2DM [20]. While, Chung et al. reported an increased serum level of Metrnl in the patients with T2DM [21]. Most of the previous studies determined

the tissues level of Metrnl in inflammatory disorders, so there is no data on the serum levels of Metrnl in these complications. Bridgewood et al. investigated the Metrnl in synovial tissue in the patients with Rheumatoid Arthritis, Psoriatic Arthritis, and Osteoarthritis. As a result, they found the elevated level of Metrnl in Psoriatic Arthritis [22]. To the best of knowledge, this is the first report on the serum levels of Metrnl in the IBD patients. In addition, the results show the lower serum levels of Metrnl in the IBD patients compared to the controls. However, Metrnl was not different between the patients with UC and CD. Li et al. demonstrated that, Metrnl is highly expressed in the gastrointestinal tract of normal donors as well as mice. On the other hand, they produced intestinal epithelial cell-specific knockout mice, which showed no significant serum reduction, despite the reduction of Metrnl expression in the gastrointestinal tract [16]. A recent study performed by Zuo et al. reported that Metrnl expression is higher in mesenteric adipose tissue (MAT) of the CD patients compared to the controls. They also showed that, systemic treatment of Metrnl can improve the adipocyte function, and reduce the macrophage infiltration and inflammation by acting on the peroxisome proliferator-activated receptors (PPARγ) pathway in mice. Therefore, they suggested that, upregulation of Metrnl in the MAT of the patients may be a compensatory response [14]. Regarding the inconvenient results, it seems likely that, Metrnl expression can have an organ dependent pattern; however, further longitudinal research is needed to support this hypothesis.

Furthermore, in the present study, Metrnl indicated an inverse relationship with the inflammatory cytokines in the IBD patients. Also, it was observed that, Metrnl displays a function in inflammation pathways. Zuo et al. administered the Metrnl in IL-10-/- mice and then observed a significant decrease in the score of inflammation and pro-inflammatory factors such as TNF-α, interferon (IFN)-γ, and IL-6 (12). Additionally, Zhi-yong LI et al. reported that, Metrnl plays a regulatory role in the expression of antimicrobial peptides such as islet-derived 3 gamma (Reg3g), lactotransferrin, and amyloid A-3 (SAA3) (22). Since TNF-α and IL-6 are considered as the markers of inflammation, the results suggest a relationship between Metrnl and IBD pathogenesis. In addition, adiponectin decreased in the patients groups however there were no relation between Metrnl and adiponectin that suggested a different regulation of these two adipokines.

When the population were stratified based on obesity, the serum level of Metrnl was significantly lower in obese subjects than in non-obese ones. Consistently, AlKhairi et al. reported that, Metrnl is significantly higher in the T2DM obese patients, in a way that this elevation can be explained as a compensatory response [23]. However, Zhi-Yong Li et al. showed no correlation between serum Metrnl levels and BMI [24]. As the adipose tissue is the main source of Metrnl secretion, it is expected that, BMI can affect the levels of this adipokine, and adipose tissue inflammation and dysfunction might be considered as the causes for the decrease in Metrnl levels.

Conclusion

In conclusion, the current study for the first time showed that serum levels of Metrnl have decreased in the IBD patients. Moreover, it was found that, serum level of Metrnl has a negative correlation with serum levels of TNF-α, IL-6 and BMI in the patients with IBD. Altogether, the present study demonstrated a

relationship among Metrnl, inflammation, and obesity that suggested a possible relation of Metrnl with the pathogenesis of IBD which can be considered for ameliorating the inflammatory milieu in these patients.

Study strength and limitations

The present study included a age, sex and BMI matched groups that eliminated the impact of these confounding factor on the results. On the other hand, the present study has no data on body fat distribution that could be more clinically significant than BMI. Furthermore, the cross-sectional design of the study limited us in concluding a cause and effect relationship, so further studies are needed to dissect the possible mechanism for the reported relationship.

Abbreviations

Metrnl: Meteorin-like; IBD: Inflammatory bowel disease; CD: Crohn's disease; IL-6: Interleukin 6; TNF-α: Tumor necrosis factor α; WAT: white adipose tissue; TG: triglyceride; Chol: Cholesterol; FBS: Fasting blood sugars; SD: standard deviations. T2DM: type 2 diabetes mellitus; HOMA-IR: homeostatic model assessment- insulin resistance; SBP: systolic blood pressure; DPB: diastolic blood pressure.

Declarations

Acknowledgment

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Author's contribution

Afsane Gholamrezayi & Maryam Mohamadinarab: Design and performed experiments, analyzed data and co-wrote the paper. Pegah Rahbarinejad, Shekufe Rezghi Barez , Leila Setayesh: Performed experiments; Soudabeh Fallah: super vision and revised final manuscript and confirmed; Nariman Moradi and Reza Fadaei : Design experiment, Monitoring the experiment &written the manuscript and revision; *Elham Chamani & Tahmine Tavakili: Performed Endoscopy , collected samples, supervision , financial support & corresponding Authors. All authors read and approved final manuscript.

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Availability of data and material

Additional data are available from the corresponding authors for reasonable requesting.

Ethics approval and consent to participate

The written informed consent was signed by all the participants and the research was confirmed by the Ethics Committee of Birjand University of Medical Sciences.

Consent for publication

No applicable

Competing interest

The authors declare no conflict of interest.

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Tables

Table 1. Anthropometric and biochemical characteristic of studied population.

	Control (n=54)	UC (n=43)	CD (n=42)	*P* value
BMI (kg/m2)	24.12 ± 3.56	23.45 ± 3.64	24.43 ± 4.61	0.502
Normal weight / over weight	29/25	26/16	21/22	0.473
Age (year)	39.02 ± 4.6	38.24 ± 5.32	38.74 ± 5.7	0.763
SBP (mmHg)	131.17 ± 20.79	133.40 ± 24.26	131.65 ± 23.97	0.887
DBP (mmHg)	81.63 ± 13.11	83.10 ± 14.48	83.16 ± 14.26	0.825
FBG (mg/dl)	90.26 ± 9.03	93.65 ± 13.27	94.28 ± 12.05	0.172
Insulin (µU/ml)	4.03 ± 0.31	5.66 ± 0.5	5.93 ± 0.64[a*]	0.014
HOMA-IR	0.89 ± 0.07	1.31 ± 0.14	1.43 ± 0.17[a**]	0.005
TG (mg/dl)	117.41 ± 44.83	128.76 ± 47.64	126.08 ± 38.15	0.409
TC (mg/dl)	157.22 ± 35.41	164.54 ± 47.49	162.25 ± 30.83	0.629
LDL-C (mg/dl)	94.70 ± 26.95	100.64 ± 35.84	104.93 ± 21.74	0.212
HDL-C (mg/dl)	43.53 ± 6.20	42.11 ± 9.07	41.46 ± 7.76	0.394

- Data are presented as Mean ± SD

-Independent sample t-test or Mann-Whitney U-test were used for comparison of quantitative variables

-Abbreviations: UC, ulcerative colitis; CD, Crohn's disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride.

[a] Comparison between control, UC and CD.

*$P < 0.05$.

**$P < 0.01$.

Table 2. Serum levels of Metrnl according to BMI cutoff.

Group	Normal weight	Overweight	*P**
All participants	102.27 ± 36.23	78.13 ± 31.04	<0.001
Control	117.84 ± 37.53	95.56 ± 28.90	0.019
UC	96.92 ± 35.63	66.29 ± 30.25	0.007
CD	87.40 ± 27.24	66.93 ± 25.24	0.014

- Data are presented as Mean ± SD

- Ancova test was used to assess Metrnl levels, among three groups based on BMI cutoff (Overweight, BMI≥25 and normal weight, BMI<25).

- Abbreviations: UC, ulcerative colitis; CD, Crohn's disease.

*$P < 0.05$.

Table 3. Odd ratio of diseases status according to 10 unit change in the serum levels of Metrnl.

Model	Group	Odd Ratio	95% CI	*P* value
Crude	UC	0.833	(0.735-0.944)	0.004
	CD	0.760	(0.661-0.874)	<0.001
Model 1	UC	0.794	(0.692-0.912)	0.001
	CD	0.738	(0.636-0.858)	<0.001

- Abbreviations: UC, ulcerative colitis; CD, Crohn's disease; CI, Confidence interval
- Model 1. Adjusted for age, sex and BMI.

Table4. Correlation analysis of serum Metrnl levels with anthropometric and biochemical variables.

	Metrnl	
	Control	IBD
BMI (kg/m2)	-0.298*	-0.391**
Age	0.174	-0.146
SBP	-0.026	0.032
DBP	-0.110	-0.061
FBG (mg/dl)	0.023	0.222*
Insulin (uU/ml)	-0.184	0.085
HOMA-IR	-0.175	0.127
TG (mg/dl)	0.202	-0.004
TC (mg/dl)	0.082	-0.016
LDL-C (mg/dl)	0.026	-0.061
HDL-C (mg/dl)	0.085	-0.029
Adiponectin (ug/ml)	-0.012	-0.076
TNF-alpha	0.137	-0.380**
IL-6 (pg/ml)	-0.027	-0.324**

Abbreviations: UC, ulcerative colitis; CD, Crohn's disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride.

*$P<0.05$.

$^{**}P<0.01$.

Figures

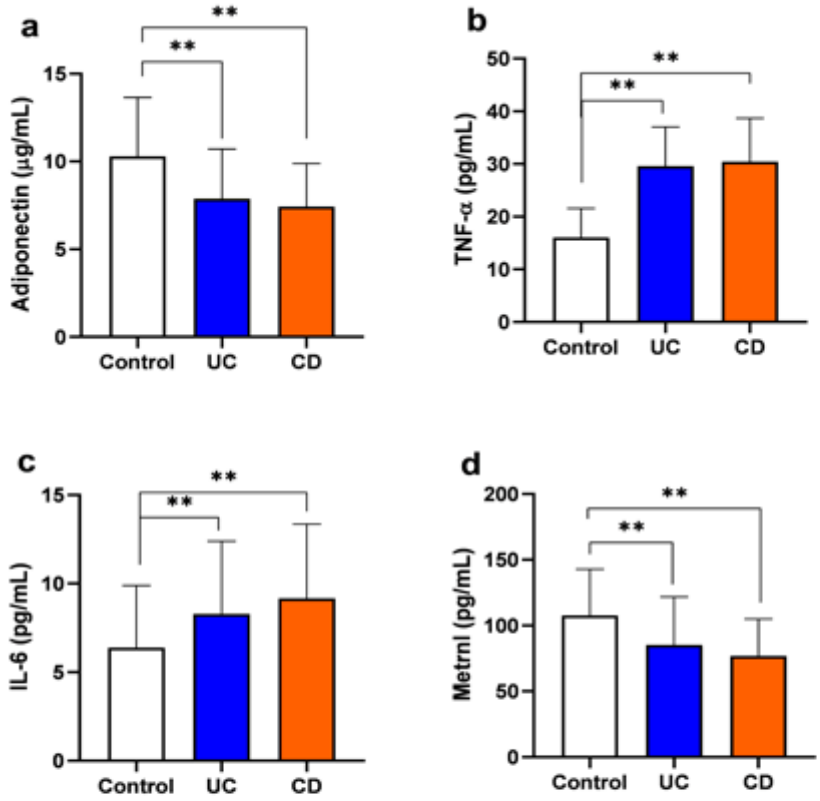

Fig.1

Figure 1

Serum levels of adipokines and cytokines. a) Serum levels of adiponectin decreased significantly in both UC and CD patients compared to controls. b) Serum levels of TNF-alpha were found to be lower in both patient groups compared to controls. c) IL-6 serum concentration indicated a considerable increase in both patient groups compared to controls. d) Metrnl serum levels demonstrated a significant decline in UC and CD patients compared to controls. CD, Crohn's disease; IL-6, Interleukin 6; TNF-α, Tumor Necrosis Factor Alpha; UC, ulcerative colitis.

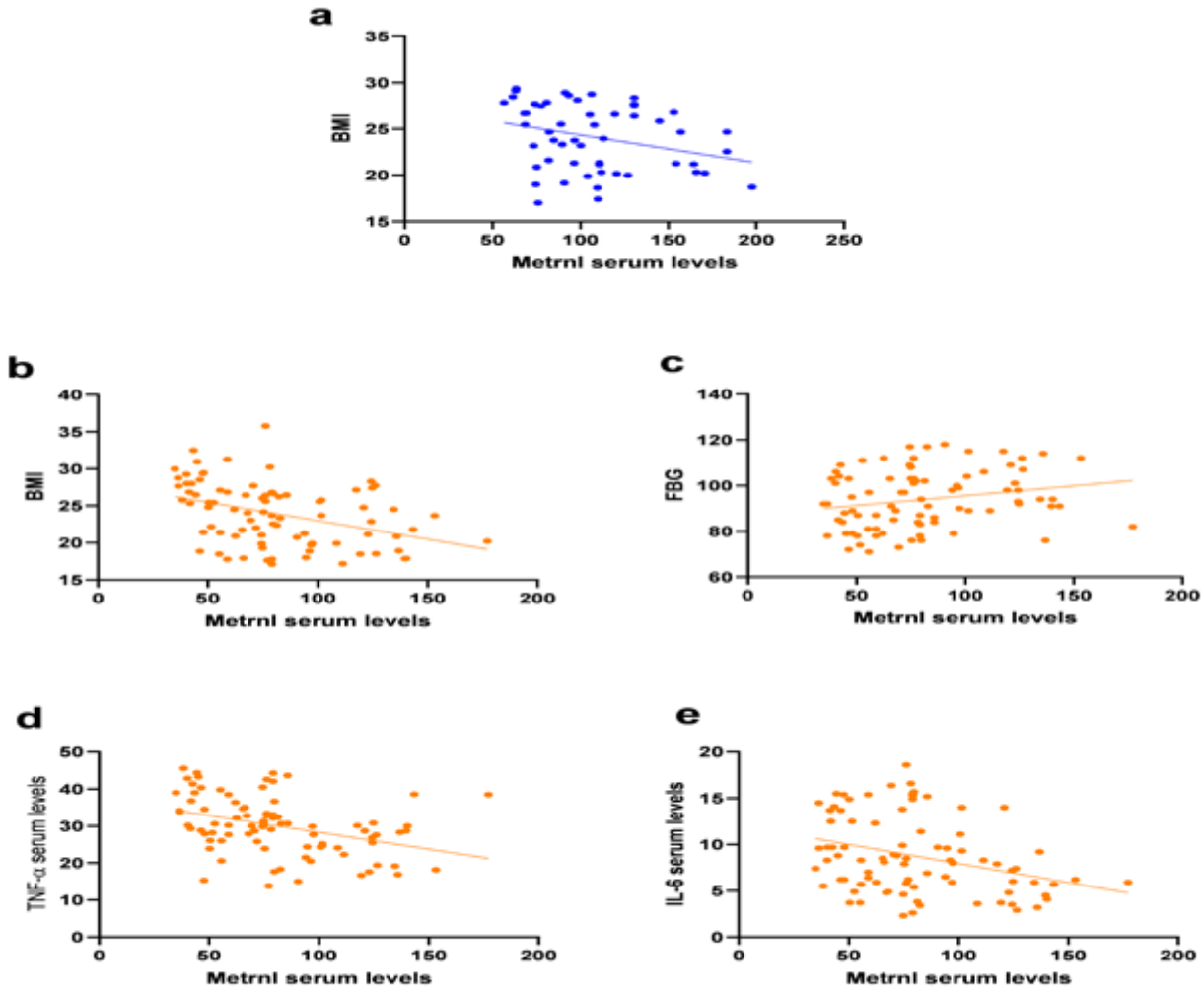

Fig.2

Figure 2

The correlation of Metrnl with a) BMI in controls, b) BMI in patients, c) FBG in patients, d) TNF-α in patients and e) IL-6 in patients. BMI, body mass index; TNF-α, Tumor Necrosis Factor Alpha; IL-6, Interleukin 6.