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Serum irisin levels in colorectal cancer patients

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Abstract. – OBJECTIVE: There are limited studies investigating the role of irisin in colorectal cancer, and the results are diverse. The role of irisin in colorectal cancer patients was investigated in this study.

PATIENTS AND METHODS: This cross-sectional study included 53 patients diagnosed with colorectal cancer (CRC) and 87 healthy volunteers. Serum irisin, glucose, insulin, C-peptide, and whole blood hemoglobin A1c (HbA1c) levels were measured in venous blood samples taken from patients and the control group.

RESULTS: The mean serum irisin levels were significantly lower in the patient group (23.97 ± 16.94 ng/mL) than in the control group (32.71 ± 17.26 ng/mL) (p = 0.004). Serum glucose levels were 96.58 ± 15.12 mg/dL in the patient group and 81.91 ± 11.24 mg/dL in the control group. Serum glucose levels were significantly higher in the patient group than in the control group (p < 0.01). In the patient group, there was no statistically significant difference between metastasis (+) patients and metastasis (-) patients in terms of serum irisin levels (27.53 ± 18.48 ng/mL and 21.23 ± 15.43 ng/mL, respectively; p = 0.182).

CONCLUSIONS: Our study has provided new insights into the potential role of irisin in CRC. However, further studies, including in vitro, in vivo, and larger patient groups, are necessary to fully understand the potential of irisin as a biomarker or therapeutic target for CRC and other diseases.

Key Words:
Colorectal cancer, Health, Irisin, Obesity.

Introduction

Colorectal cancer (CRC) is the third most common type of cancer worldwide and is the second most common cause of cancer-related mortality in both men and women[1]. The risk of developing CRC is influenced by a combination of environmental, genetic, and nutritional factors[2,3]. Obesity is considered one of the most important preventable risk factors for CRC. The exact mechanisms by which it increases the risk of CRC are not well understood, but it is thought to be related to chronic inflammation and changes in hormone levels. Irisin is a protein (with a molecular weight of 12 kDa and containing 112 amino acids) that was first discovered in muscle tissue and is now defined as a myokine or adipo-myokine[4]. Irisin is formed by the degradation of the 206 aa fibronectin type III domain 5 (FNDC5) protein that is found in skeletal muscle by an unknown protease[5,6]. Irisin is considered a thermogenic protein, which means that it can increase the amount of heat produced by the body. This is thought to be due to its ability to promote the conversion of white fat into brown fat. Brown adipose tissue contains many more mitochondria than white adipose tissue[7]. The mitochondrial inner membranes of brown adipose tissue contain a protein called uncoupling protein-1 (UCP1) that pumps protons from the intermembrane space to the mitochondrial matrix. When UCP1 is activated, it acts as an "uncoupling protein" that uncouples the process of oxidation and phosphorylation in the mitochondria, which is the process by which energy is normally produced in the cell[5].

The hormone irisin is stimulated by peroxisome proliferator activating receptor gamma coactivator 1α (PGC-1α). PGC-1α is a transcriptional coactivator that was first described by Lin et al[8]. PGC-1α is involved in the regulation of en-

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ergy metabolism, and it works by increasing the activity of nuclear receptors such as PPAR-γ (peroxisome proliferator-activated receptor gamma). It is predominantly expressed in tissues rich in mitochondria, such as skeletal muscle, brown adipose tissue, and the heart. PGC-1α enhances fat oxidation and assists in regulating the transcriptional program for energy homeostasis by stimulating mitochondrial biogenesis. In addition, exercise increases PGC-1α expression in heart and skeletal muscle[9]. Increased expression of PGC-1α parallels increased oxidative phosphorylation in mitochondrial DNA and brown adipose tissue[10]. It has been observed[5] that mRNA expression of the FNDC5 protein increases with exercise in humans and mice.

Irisin is related to many chronic diseases, including cancer[11]. In a recent study[12], low serum irisin levels were reported in patients with CRC, and the risk of developing CRC was reduced by 78% in individuals with high irisin levels. CRC is often referred to as an "insidious" disease because it often does not show symptoms in the early stages. Cancer screening is crucial in the early detection of colorectal cancer[13]. Considering the irisin-glucose metabolism-CRC relationship and that irisin may have protective properties against the development of CRC, serum irisin levels were determined in CRC patients and healthy controls in this study.

Patients and Methods

Ethics Approval

Institutional ethics approval was obtained from the Clinical Research Ethics Committee of the Faculty of Medicine, Ataturk University, and informed consent was provided by all the participants.

Participants

The study included 53 colorectal cancer patients between the age of 18-85 years who applied to the Atatürk University Research Hospital Medical Oncology outpatient clinic and were diagnosed with colorectal cancer between July 2019 and January 2020 and 87 healthy volunteers without a history of general medical conditions. Inclusion criteria for the CRC patient group (male and female) were being diagnosed with CRC and being between the age of 18-85. The exclusion criteria for the CRC patient group were BMI <18 kg/m^2, acute inflammatory diseases, chronic rheumatological diseases, diagnosis of primary cancer other than CRC (multiple primary tumors), inflammatory bone disease, and severe organ dysfunction (noncancerous primary cause). Inclusion criteria for healthy volunteers (male and female) were 18-85 years old, not following any special diet, and volunteering to participate in the study. Exclusion criteria for healthy volunteers (men and women) were BMI <18 kg/m^2, having a known systemic disease, having a known chronic disease, continuous chronic drug use, pregnancy, breastfeeding (for women), smoking and alcohol use.

Biochemical Analysis

Venous blood samples (5 mL) were taken once after 12 h of night fasting from patients diagnosed with colorectal cancer and healthy controls. Serum was obtained from the samples taken. Additionally, each participant had a venous whole blood sample collected into a potassium ethylenediaminetetraacetic acid (EDTA) collection tube (Greiner Bio-One Vacuette® 3 ml K3E K3EDTA, Kremsmünster, Austria) for measurement of HbA1c. Serum irisin levels were obtained using the enzyme-linked immunosorbent assay (ELISA) technique following the technical methods recommended for the kit (Catalog No.: YLA-1361HU, Biont, Shangai, China). Whole blood HbA1c levels were analyzed by high-performance liquid chromatography (HPLC). The hexokinase method was used to analyze serum glucose levels on a Roche Cobas 8000 autoanalyzer (Basel, [Switzerland]). ECLIA was used to analyze serum insulin and C-peptide levels on a Roche E170 immunoassay analyzer (Basel, [Switzerland]).

Statistical Analysis

Statistical analyses were performed using the SPSS for Windows 25 package program (IBM Corp., Armonk, NY, USA). The normality of data was evaluated with the Kolmogorov-Smirnov test. Descriptive statistical methods (mean, standard deviation) were used. A *t*-test was used to compare independent groups. Pearson's correlation analysis and logistic regression analysis were performed. *p*-values lower than 0.05 at the 95% confidence interval were considered significant.

Results

The mean age of the patients was 58.58±14.90 years and that of the healthy controls was 53.67±14.47 years, and there was no significant

Table I. Demographic and clinical characteristics of patients.

Demographic/clinical characteristics	Frequency (n, %)
High red and/or processed meat consumption	43 (81.1%)
High salt intake	40 (75.4%)
High fat consumption	36 (67.9%)
Regular fruit and vegetable consumption	12 (22.6%)
Regular physical activity	7 (13.2%)
Smoking	23 (43.4%)
Alcohol consumption	5 (9.4%)
Clinical distribution	Underwent surgical resection: 6 (11.3%) Underwent surgical resection followed by chemoradiation therapy: 27 (50.9%) Underwent chemoradiation therapy:20 (37,8)
Site of primary tumor	Proximal colon: 17 (32.1%) Distal colon: 15 (28.3%) Rectum: 13 (24.5%) Colo-rectum: 4 (7.5%) Unknown: 4 (7.5%)
Metastasis	M1a/1b: 23 (43.4%)
Node stage	N0: 29 (54.7%) N1: 24 (45.3%)
Disease stage	Stage I: 27 (50.9%) Stage II: 10 (18.9%) Stage III: 7 (13.2%) Stage IV: 9 (17.0%)
Tumor grade	T1: 26 (49.1%) T2: 11 (20.8%) T3: 7 (13.2%) T4: 9 (17.0%)

difference between the groups in terms of age ($p = 0.056$). The mean diagnosis time of patients was 38.58±53.46 months. Again, there were no significant differences in terms of age and diagnosis time between male and female patients ($p > 0.05$ for both comparisons). Metastases were present in 43.4% of CRC patients. The demographic and clinical characteristics of the patients are given in Table I. The results of the biochemical data of all patients and the control group are presented in Table II.

According to Table I, serum glucose, insulin, C-peptide and whole blood HbA1c values were significantly higher in the patient group than in the control group ($p < 0.01$; $p = 0.002$; $p < 0.01$; and $p = 0.039$, respectively). Serum irisin levels were lower in the patient group than in the control group ($p = 0.004$) (Table II).

The patient group was evaluated separately as metastasis (+) and metastasis (-) within itself, and the comparisons of biochemical data in patients with metastasis (+) and metastasis (-) are given in Table III.

According to Table II, there was no significant difference between metastasis (+) and metastasis (-) patients in terms of serum glucose, insulin,

Table II. Comparisons of biochemical data of patients and control group.

	Patient Group (n=53)	Control Group (n=87)	*p*
Glucose (mg/dL)	96.58±15.12	81.91±11.24	<0.01
Insulin (nIU/mL)	12.20±8.90	8.13±6.40	0.002
C-peptide (ng/mL)	5.33±4.71	3.05±1.74	<0.01
HbA1c (%)	5.51±0.70	5.31±0.43	0.039
Irisin (ng/mL)	23.97±16.94	32.71±17.26	0.004

p: Test statistics *p*-value.

Table III. Comparisons of biochemical data in metastasis (+) and metastasis (-) patients.

	Metastasis (+) patients (n=23)	Metastasis (-) patients (n=30)	p
Glucose (mg/dL)	98.26±16.68	95.30±13.96	0.485
Insulin (nIU/mL)	13.07±9.57	11.52±8.46	0.535
C-peptide (ng/mL)	4.78±3.20	5.75±5.61	0.460
HbA1c (%)	5.61±0.88	5.43±0.52	0.357
Irisin (ng/mL)	27.53±18.48	21.23±15.43	0.182

p: Test statistics *p*-value.

C-peptide, irisin levels and whole blood HbA1c levels ($p > 0.05$ for all parameters). Correlations between biochemical data in the patient group were examined by Pearson's correlation analysis, and only moderately positive (r=0.654) and significant ($p < 0.01$) correlations were found between insulin and C-peptide in the patient group.

The effect of biochemical parameters on metastasis was evaluated by logistic regression analysis. Logistic regression analysis was performed by taking metastasis status (constant variable: no metastasis) as the dependent variable and biochemical parameters glucose, insulin, C-peptide, HbA1c, and irisin as independent variables. The regression model is not significant ($p = 0.65$) and it can explain only 10.18% of the variance in the dependent variable (Nagelkerke R^2 value: 0.1018).

Discussion

In this study, serum glucose, insulin, C-peptide, irisin levels and whole blood HbA1c levels were determined in 53 volunteers diagnosed with colorectal cancer and 87 healthy volunteers without a history of disease related to a general medical condition. Serum irisin levels were 23.97±16.94 ng/mL in the patient group and 32.71±17.26 ng/mL in the control group. There was a statistically significant difference between the serum irisin levels of the patient and control groups ($p = 0.004$).

There is a relationship between metabolic syndrome and the risk of CRC in both men and women, but this relationship is more pronounced in men[14]. Again, the link between type 2 diabetes and colorectal cancer is well known[15-17]. According to the results of a retrospective study[18], the incidence of CRC, has been found to increase in diabetic patients. At this point, irisin is an interesting molecule. Serum irisin levels in newly diagnosed type 2 diabetes patients are significantly lower than those in nondiabetic controls[19]. A decrease in irisin concentrations was found in adults diagnosed with type 2 diabetes compared to those with normal glucose tolerance. A statistically significant relationship was shown[20] between irisin and the development of type 2 diabetes. Similarly, parameters such as age, sex and BMI showed to significantly reduce irisin concentrations in adults with type 2 diabetes[19,21].

Considering the relationship between CRC-obesity and CRC-type 2 diabetes, it is vital to examine the relationship between CRC and irisin. In a study[22] using irisin antibody immunohistochemistry to compare changes in irisin expression in gastrointestinal cancers with normal tissues, the tissues were examined using 15 sections from each cancer type and 15 from control tissues before chemotherapy or radiotherapy was given to patients. Immunoreactivity of irisin was observed in the intestinal glands of the normal colon among the examined tissues. Histoscores showed markedly increased irisin immunoreactivity in colon adenocarcinoma and mucinous colon adenocarcinoma. However, the intensity of irisin immunoreactivity in colon adenocarcinoma is similar to that in mucinous adenocarcinoma. Research in literature has suggested that irisin increases in tumor tissues and may release energy due to the induction of hyperthermia, which tends to destroy cancer cells. Researchers[22] have emphasized that hyperthermia may be beneficial in cancer treatment, and additional research is needed on the potential use of irisin in cancer treatment. Researchers[5,23] have proposed two explanations for increased irisin expression in most of the gastrointestinal tract. First[23], increased irisin in cancerous tissues may be an adaptation mechanism to inhibit ATP production as a way to control cell division. The second[5] is that the increased irisin causes hyperthermia. Increased irisin increases BAT (brown adipose tissue), and increased BAT can release more heat *via* UCP1 rather than ATP. In the *in vitro* study of Moon et al[24] it was found that irisin had no effects on cell proliferation in human and mouse endometrial, colon, thyroid and esophageal cell lines, unlike metformin. Comparison of

autopsy materials from CRC patients and controls has shown[25] that irisin expression is higher in cancerous materials than in control tissues.

This study investigated serum irisin levels in colorectal cancer patients and healthy volunteers. As a result of our study, we determined that serum irisin levels were lower in CRC patients than in healthy controls. Based on these results, we think that research should be conducted on irisin as a biomarker that determines the diagnosis, treatment follow-up and prognosis of CRC. In addition, the therapeutic efficacy of irisin, especially in early-stage CRC, needs to be investigated. However, it should be noted that tumor formation and development mechanisms are quite complex and often associated with multiple factors and signaling pathways.

Limitations

Our study has some limitations. First, in addition to determining serum irisin levels by ELISA, the mRNA expression of FNDC-5 protein should be determined. Second, we could not report a relationship between body mass index values and irisin levels of patients since the body mass index values were near 20 kg/m^2 and did not show a wide range.

Conclusions

Our study will contribute to the literature investigating the relationship between cancer and irisin. Our study will also be an important source in this field, especially since only a few studies have investigated the direct relationship between CRC and irisin. In our upcoming work, we plan to find a specific receptor for irisin. We hope our study results will lead to more studies in large patient groups using irisin, a promising compound, to treat many diseases, especially cancer. Then, based on our results, we hope that therapeutic agents that can be used to treat many metabolic diseases will be developed.

Future studies should focus on larger patient groups to confirm the findings in literature and to better understand the mechanisms by which irisin may be involved in the development and progression of CRC. Additionally, *in vitro* and *in vivo* studies can help to investigate the effects of irisin on the biology of CRC and to test its potential therapeutic effects on cancer. There is also a need for studies to investigate the correlation between irisin levels and other biomarkers and clinical parameters, such as stage, grade, and overall survival of CRC patients. Furthermore, studies on the effects of lifestyle and dietary interventions on irisin levels could provide insight into ways to modulate irisin levels and potentially reduce their risk.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Ethics Approval

Institutional ethics approval was obtained from the Clinical Research Ethics Committee of the Faculty of Medicine, Ataturk University.

Informed Consent

Informed consent was provided by all the participants.

Authors' Contributions

All authors contributed to the study's conception and design. Sourcing materials, performing experiments, and analyzing data were performed by Zeynep Celik, Nurcan Kilic Baygutalp, Adil Furkan Kilic, Salim Basol Tekin, Ebubekir Bakan, Mehmet Ali Gul and Neslihan Yuce. The first draft of the manuscript was written by Zeynep Celik, Nurcan Kilic Baygutalp and Neslihan Yuce. All authors read and approved the final manuscript.

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