

The Relationship between Circulating Irisin and Oxidative Stress in Gastric and Colorectal Cancer Patients

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Abstract

Objective: Gastric and colorectal cancers are two obesity-related cancers. Irisin is a dipo-myokine with an important role in the body's energy homeostasis. Oxidative Stress has a crucial role in tumorigenesis. Therefore, this study aims to investigate the association of circulating irisin with oxidative stress in gastric and colorectal cancer patients. **Methods:** A case-control study involving 62 gastric and colorectal cancers and 22 healthy individuals was carried out. Serum irisin and Total Antioxidant Capacity were measured by sandwich enzyme-linked immunosorbed assay (ELIZA) kits. Total Oxidative Stress (TOS) was measured using colorimetric methods and oxidative stress index (OSI) was also calculated. **Results:** Serum irisin decreased significantly ($p < 0.0001$) in gastric and colorectal cancer cases compared to healthy individuals. The TOS and OSI levels increased significantly ($p < 0.0001$) in gastric and colorectal cancer cases compared to healthy individuals. No significant correlation was found in terms of irisin, TOS, TAC, and OSI in gastric and colorectal cancer cases and control groups. **Conclusion:** Circulating irisin decreases and oxidative stress increases in gastric and colorectal cancers. There is no correlation between irisin and oxidative stress. The mechanism by which irisin is associated with oxidative stress is still not clear.

Keywords: Colorectal cancer- gastric cancer- Irisin- oxidative stress

Asian Pac J Cancer Prev, **23** (8), 2649-2654

Introduction

Gastric and colorectal cancers are two lethal cancers as the fifth and third most prevalent cancers (Sung et al., 2021). They are considered as obesity-related cancers (Bianchini et al., 2002; Kubo and Corley, 2006). It is well-known that physical exercises can have health benefits in the form of minimizing the risk of variety of malignancies (Canniotto et al., 2021; Friedenreich et al., 2017; Holmes et al., 2005; Willer, 2003; Xu and Rogers, 2020). Several hormones are secreted by muscles during physical exercise and one of these hormones is irisin. Irisin is an exercise-inducible myokine with a significant role in the regulation of body energy homeostasis (Boström et al., 2012). Studies on human and mouse samples have shown that circulating irisin increases after physical exercise (Boström et al., 2012; Huh et al., 2012; Rashid et al., 2020). Furthermore, Rashid et al., (2020) showed that irisin can have a potential role in the improvement of insulin resistance after six months of moderate physical exercise. Recently, many studies have investigated the role of irisin in cancer and controversial results have been reported. Some studies have attempted to prove that irisin is a biomarker (Aslan et al., 2020; Provatopoulou et al., 2015; Shahidi et al., 2020; Temur and Rashid, 2021). Studies on cell line models have reported that irisin treatment at

physiological and pharmacological concentrations did not affect endometrial, esophageal, thyroid, and colon cancer cell colonies (Moon and Mantzoros, 2014). On the other hand, it was shown that irisin treatment reduced the number of malignant breast cancer cell lines, MCF-7, and induced apoptosis, suggesting that irisin could have a therapeutic role (Gannon et al., 2014). In addition, it was shown that irisin reduced invasiveness of lung cancer cells through inhibiting epithelial-mesenchymal transition (Shao et al., 2017). Even though these studies have concluded that irisin could have potential therapeutic benefits in cancer treatment, the protective mechanism of irisin is still poorly understood.

Oxidative Stress (OS) happens as a result of imbalance between antioxidants and oxidants caused by harmful stimulants. This imbalance between oxidants and antioxidants leads to harmful damages to biomolecules (Đuračková, 2010). It is well-established that OS has a crucial role in tumorigenesis of different carcinoma (Eroglu et al., 2013; Feng et al., 2012; Srivastava et al., 2009; Wang et al., 2011). Many studies have assessed one or a group of oxidants and antioxidants; however, evaluation of total oxidative stress and total antioxidants are rare.

Several studies have shown that irisin can be linked to oxidative stress in metabolic disease. In this context,

it is reported that irisin is positively associated with total oxidative stress in gestational diabetes mellitus (Beyazit et al., 2020). In-vitro studies have reported that adding irisin to murine macrophages increases expression of antioxidant enzymes, suggesting a potential antioxidant role of irisin (Mazur-Bialy et al., 2018). However, few studies have explored the relationship between serum irisin and oxidative stress in cancer patients. Thus, the objective of this study was to investigate whether or not circulating irisin is associated with oxidative stress in gastric and colorectal cancers patients.

Materials and Methods

Study design

A case-control study involving 62 new diagnostic gastric and colorectal cancers (20 gastric cancer, 42 colorectal cancer patients) and 22 healthy individuals was carried out. The study was conducted at Oncology Teaching hospital in Baghdad from 10th October 2020 to 2nd June 2021. All participants expressed their consent to participate before blood sample collection. The protocol of the study was approved (999) by the ethics committee of the Ministry of Health in Baghdad, Iraq. The cancer patients were diagnosed using an endoscope. Patients with other metabolic diseases such as diabetes mellitus, obesity, cardiovascular disease, blood pressure, and drinking and smoking habits were excluded. Cancer patients and control individuals were matched in terms of Body Mass Index, BMI, and gender.

Biochemical analysis

The blood samples (5ml) were drawn from peripheral venous tissue of new diagnostic patients before operation and after overnight fasting. The samples were centrifuged at 1200 rpm for 10 min and the serum samples were separated and stored at -20°C . Irisin was measured using sandwich enzyme-linked immunosorbed assay (ELISA) kit (Mybiosource, USA). Total oxidative stress (TOS) was determined by oxidation of Fe+2 to Fe+3 via oxidative species found in the serum samples. Under acidic conditions, Fe+3 formed a color complex with xylenol orange. The concentration of oxidants was measured at 570 nm and at 800nm depending on the intensity of solution's color (Erel, 2005). Total antioxidant capacity (TAC) was determined using sandwich ELISA kit (Bioassay Technology laboratory, China). Oxidative stress index was calculated through dividing TOS by TAS.

Statistical analysis

Normality was tested using the Kolmogorov-Smirnov (KS) test. Normally distributed data were presented as mean \pm standard deviation and significant differences were tested using t-test. Non-normal distributed data were presented as median (25th-25th) percentile and significant differences were tested using Mann-Whitney test. Category variables were expressed as percentage and significant differences were tested using Chi-square test. Pearson and spearman correlations were performed to assess correlation coefficient between the variables which were normally distributed and non-normally distributed

respectively (P value <0.05). These statistical tests were performed in GraphPad Prism 8.

Results

Clinical characteristic of patients

As listed in Table 1, demographic characterizations such as age and sex were studied. There were significant differences between the age of the gastric and colorectal cancer patients compared to the control ($p=0.008$ and $p=0.0001$ respectively). No significant difference was observed in terms of gender.

Additionally, anthropometric characterizations including weight, height, body mass index (BMI), waist and hip circumferences, and waist to hip ratio (WHR) were tested. There were significant differences in terms of weight between gastric and colorectal cancers patients compared to the control ($p=0.014$, $p=0.024$) respectively. There were no smoking and alcohol drinking subjects in both patients and control groups. All cancer patients were in the metastasis stage.

Irisin and oxidative stress level

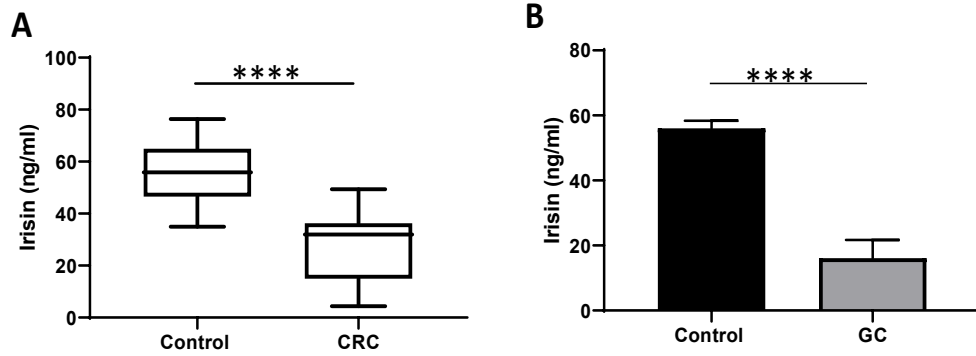
As shown in Figures 1A and B, irisin level significantly decreased in both gastric and colorectal cancer patients ($p<0.0001$). In contrast, total oxidative stress (TOS) level increased significantly in gastric and colorectal cancers patients compared to the control group ($p<0.0001$) (Figures 1C and D). No significant difference was observed in terms of total antioxidant capacity (TAC) between gastric and colorectal cancers patients and the controls ($p=0.361$, $p=0.708$ respectively) (Figures 1E and F). Oxidative stress index was high in both gastric and colorectal cancer patients compared to the control group ($p<0.0001$).

Relationship between Irisin and oxidative stress

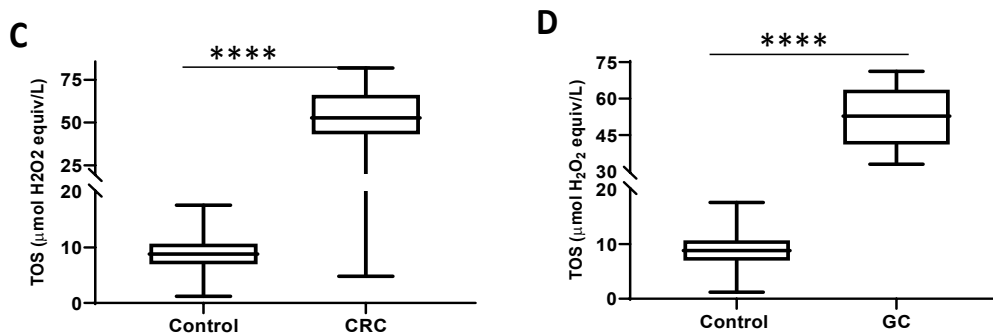
To investigate the relationship between irisin and oxidative stress in gastric and colorectal cancer patients, Pearson's coefficient correlation was performed (Table 2). No significant correlation was found between irisin and TOS in gastric and colorectal cancer patients ($r=-0.129$, $p=0.609$; $r=-0.048$, $p=0.762$). In addition, there was no significant correlation between irisin and TAC in gastric and colorectal cancer patients ($r=-0.243$, $p=0.317$; $r=0.019$, $p=0.903$ respectively). Irisin level in gastric and colorectal cancers did not correlate with OSI ($r=0.259$, $p=0.277$; $r=-0.031$, $p=0.486$ respectively).

Discussion

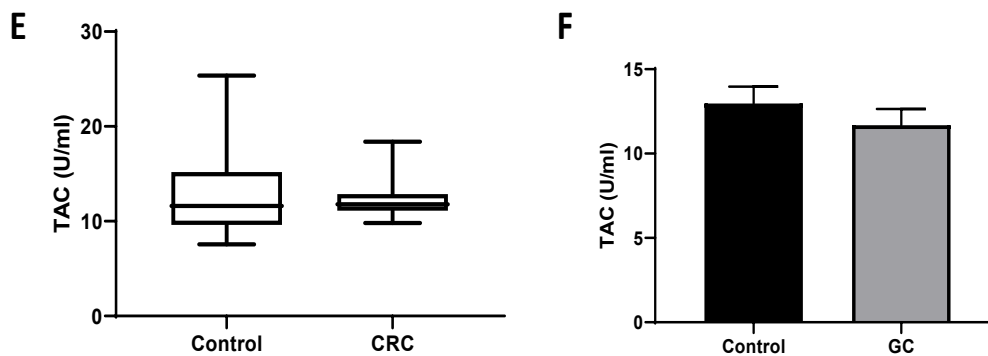
It is well-established that during physical exercise muscles secrete myokines. These myokines may have a beneficial role in reducing cancer risk (Fournier et al., 2014; Moore et al., 2016; Williams, 2014). Irisin is an exercise-induced myokine that has a role in converting white adipose tissue to brown adipose tissue. In addition, it is known that OS has a crucial role in tumorigenesis. However, there has been no study on exploring the association between irisin and OS in gastric and colorectal cancer patients.



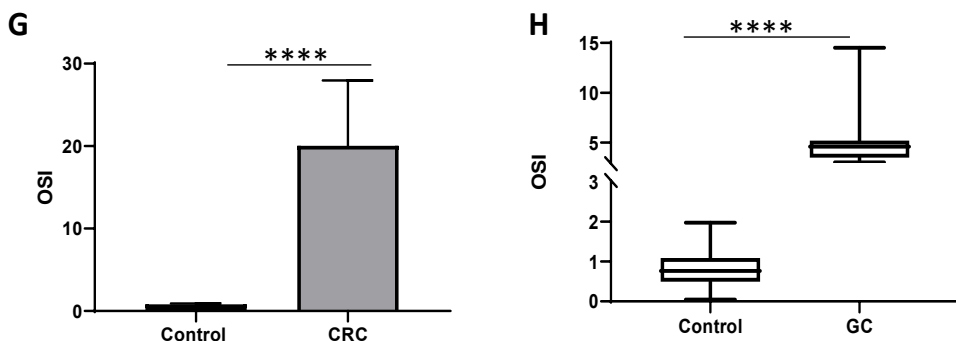
Comparison of irisin in gastric, colorectal cancers and control.



Comparison of TOS in gastric, colorectal



Comparison of TAC in gastric, colorectal cancers and control.



Comparison of OSI in gastric, colorectal cancers and control.

Figure 1. Comparison of Irisin, TOS, TAC and OSI in Gastric, Colorectal Cancers and Control. Normal distributed results express as mean \pm SD and present in histogram. Non-normal distributed results express as median (min-max) and present in box and whiskers graph.

Table 1. Demographic and Anthropometric Characterization of Individual

Demographic Variables	Gastric Cancer (n=20)	Colorectal cancer (n= 42)	Control (n=22)	P-value
Age	52.3±14.52	54.57±11.16	41.32±11.12	^a 0.008 ^b 0.0001
Sex				^{a,b} NS
Male	10 (50%)	21 (50%)	11 (50%)	
Female	10 (50%)	21 (50%)	11 (50%)	
Anthropometric				
Weight (kg)	68.7±13.28	70.83±13.43	78.73±11.89	^a 0.014
Height (m)	1.66±0.08	1.65±0.11	1.71±0.08	^a NS ^b NS
BMI (kg/m ²)	25.01±5.18	26.02±5.13	26.95±3.34	^{a,b} NS
BMI status				^{a,b} NS
Normal weight	10 (50%)	16 (38%)	9 (41%)	
Overweight	7 (53%)	17 (41%)	10 (45%)	
Obese	3 (15%)	9 (21%)	3 (14%)	
Waist circumference, WC, (cm)	36.95±4.14	36.69±4.16	36.96±2.64	^{a,b} NS
Hip circumference, HC, (cm)	38.15±3.36	38.47±4.07	41±3.46	^{a,b} NS
WHR	0.97±0.09	0.955±0.08	0.97±0.06	^{a,b} NS
Smoking	0 (0%)	0 (0%)	0 (0%)	-
Alcohol drinking	0 (0%)	0 (0%)	0 (0%)	-
Metastasis	20 (100%)	42 (100%)	0 (0%)	-

^a, P-value when compare between gastric cancer and control

Table 2. Correlation Coefficient of Irisin with Oxidative Stress Parameters

Oxidative stress parameters	r	P-value
TOS (µmolH ₂ O ₂ equiv./L)		
GC	-0.129	0.609
CRC	-0.048	0.762
TAC (U/ml)		
GC	-0.243	0.317
CRC	0.019	0.903
OSI		
GC	0.256	0.277
CRC	-0.031	0.486

TOS, Total Oxidative Stress; TAC, Total Antioxidant capacity; GC, Gastric Cancer; CRC, Colorectal Cancer; OSI, Oxidative Stress Index

Studies on irisin levels in cancer have reported controversial results. In tissue cancer, it was found that irisin expression increased in gastrointestinal cancer (Aydin et al., 2016), breast, ovarian, cervical, and endometrial cancers (Kuloglu et al., 2016) and hepatocellular carcinoma (Gaggini et al., 2017). In contrast, other studies have found that serum irisin decreases in breast cancer (Provatopoulou et al., 2015; Zhang et al., 2018), colorectal cancer (Zhu et al., 2018), and hepatocellular carcinoma (Zhang et al., 2019). These studies are consistent with our results, which showed a significant decrease in circulating irisin in gastric and colorectal cancer patients.

Studies have shown that OS has a crucial role in carcinogenesis. In the present study, the level of OS and

OSI were significantly higher in gastric and colorectal cancer patients compared to the control. This result is consistent with Wu et al., (2017). Studies have found that TAC level in cancer patients is significantly lower than that of healthy individuals (Wu et al., 2017); however, our results indicated that TAC slightly decreased in gastric and colorectal cancer patients compared to the control group. These discrepancy results could be due to antibody validation of TAC assays.

Irisin has a crucial role in maintaining glucose homeostasis and reducing inflammation (Boström et al., 2012; Mazur-Bialy et al., 2017; Perakakis et al., 2017; Rashid et al., 2020; Zhu et al., 2015) which is accompanied by minimized ROS. This suggests that there is a potential association between irisin and ROS in cancer patients. Shahidi et al., (2020) investigated serum irisin and antioxidants, malondialdehyde, thiol group and superoxide dismutase in gastric cancer; however, the relationship between irisin and these antioxidants was not tested. Moreover, studying individuals' oxidants as in Shahidi's study may not be enough to clarify the oxidant level in serum and the relationship with irisin. Thus, this study is the first study to explore relationships between irisin and total oxidative stress and total antioxidant capacity. No correlation was observed between irisin and OS, TAC, and OSI in gastric and colorectal cancer groups. The absence of correlations could be due to unknown parameters that mediate irisin and oxidative stress systems in the body. Further investigations are needed to explore the link between irisin and the oxidative stress system.

There were some limitations in this study, including the small sample size and small percentage of obese

subjects. Despite these limitations, it appears important to investigate parameters that can clarify the role of irisin in oxidative stress in cancer patients.

In conclusion, circulating irisin decreases and causes high oxidative stress in gastric and colorectal cancers. There is no correlation between irisin and oxidative stress. The mechanism by which irisin is associated with oxidative stress is still not clarified. Nevertheless, it seems worth exploring a parameter that links irisin and oxidative stress in larger populations.

Author Contribution Statement

Ahmed Abd Temur: patients' sample collection and doing all clinical assays.

Farah A. Rashid: designing the study, analyzing data statistically and writing.

Acknowledgments

The authors thank Al-Nahrain University and Ministry of Health for approving this work. There is no funder for this work. There are no conflicts of interest regarding the publication of this article. Patient's sample and biochemical measurement were performed by Ahmed Abd Temur. Designing study, statistical analysis of results and writing this work was done by Farah A. Rashid.

Approval

The study was approved as a part of an approved student thesis at Al-Nahrain University / college of science in Baghdad, Iraq.

Ethical Declaration

The protocol of the study was approved (999) by committee in the Ministry of Health

Conflict of Interest

There are no conflicts of interest regarding the publication of this article.

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