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Circulating ghrelin levels and susceptibility to colorectal cancer

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ABSTRACT – **Background and Objective** – Considering the association between colorectal cancer (CRC) and both insulin resistance and obesity, and the prominent role of ghrelin in these metabolic disorders, we explored whether plasma levels of ghrelin were associated with CRC. Moreover, in the patients with CRC the possible correlations between ghrelin and insulin, insulin resistance, and body mass index (BMI) as an indicator of obesity were examined. **Methods** – A total of 170 subjects, including 82 cases with CRC and 88 controls were enrolled in this study. Plasma levels of ghrelin, insulin, and glucose were measured in all the subjects using ELISA and glucose oxidase methods. Furthermore, insulin resistance was assessed by calculating HOMA-IR index. **Results** – The cases with CRC had decreased ghrelin levels (P<0.001) and a higher HOMA-IR index (P<0.001) than controls. Interestingly, when CRC patients were stratified based on tumor site, lower ghrelin levels and a higher HOMA-IR index were observed in the patients with either colon or rectal cancer vs. controls too. Additionally, there were an age and BMI-independent negative correlation between ghrelin levels and HOMA-IR (r=-0.365, P<0.05), and an age-independent negative correlation between ghrelin levels and BMI (r=-0.335, P<0.05) in the rectal subgroup. **Conclusion** – Our findings support a role for ghrelin in connection with insulin resistance and obesity in CRC susceptibility; however, it needs to be corroborated by further studies.

Keywords – Colorectal cancer; ghrelin; insulin resistance; obesity.

INTRODUCTION

Colorectal cancer (CRC) is a serious public health concern and a major cause of tumor-related death. It is the world's second most commonly diagnosed cancer in women and the third in men[1]. Previous studies have demonstrated that CRC predisposes patients to obesity and to metabolic changes such as insulin resistance and hyperinsulinemia. Furthermore, hyperinsulinemia is a putative mechanism that links obesity with CRC[2-6]. Obesity could promote cancer through both insulin resistance and inflammation[7]. Interestingly, people who suffer from type 2 diabetes mellitus, which is associated with insulin resistance and obesity, are at an increased risk of CRC[8]. In addition, CRC patients have a higher circulating level of insulin than controls, and insulin therapy might boost CRC risk[9,4].

It has also been reported that obesity, insulin secretion, and CRC are all associated with alterations in circulating levels of ghrelin. This 28-amino acid peptide is predominantly produced by the stomach with small amounts also released by the hypothalamus, pituitary, small intestine, pancreas, brain, lung, heart, and kidney. Ghrelin has a range of biological actions including regulation of appetite, energy homeostasis, modulation of insulin signaling, growth hormone release through the activation of the growth hormone secretagogue receptor type 1a (GHSR-1a), and the induction of apoptosis[10-13]. A negative association between ghrelin levels and obesity have also been shown[14,15]. Ghrelin plays a role in inhibition of glucose-stimulated insulin secretion too[16-18]. Lastly, ghrelin gene polymorphisms were significantly associated with body mass index (BMI)[19]. Despite the biological plausibility, however, the results of the association between CRC and both ghrelin gene polymorphisms[20-22] and serum ghrelin levels[23-27] in the previous epidemiological studies have been conflicting.

These observations led us to investigate whether abnormalities in circulating levels of ghrelin were associated with CRC risk. The possible correlations between ghrelin and insulin, insulin resistance, and BMI in the CRC patients were also studied.

METHODS

Participants

One hundred seventy Iranian and genetically unrelated participants, including eighty two cases with CRC (age range, 34–88 years) and eighty eight controls (age range, 36–81 years) were recruited into this study between May 2016 and March 2018. This hospital

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based case-control study was multicenter research and the centers were as follows: (1) School of Medicine, Qom University of Medical Sciences (QUMS) (2) Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences (3) Colorectal Research Center, Iran University of Medical Sciences (4) Internal Medicine Department, Semnan University of Medical Sciences (5) Faculty of Science, University of Mohaghegh Ardabili. Both the case and control groups were recruited from people who were undergoing colonoscopy either due to miscellaneous gastrointestinal symptoms (like change in bowel habits, constipation, chronic diarrhea, hematochezia, unexplored weight loss and abdominal pain) or owing to the high risk for CRC. Having said that, however, the cases were patients who had a positive pathology report for CRC. Instead, the controls were selected from those participants whose colonoscopy results were negative for malignancy and different kinds of polyps including adenomatous and hyperplastic polyps. The subjects' demographic, anthropometric, and clinical characteristics were collected in a self-reported manner. The formula for BMI calculation was weight in kilograms (kg) divided by height in meters squared (m^2). Informed consent was obtained from all participants before entering the study. This research was also approved by the Ethics Committee of QUMS and was conducted in line with the Declaration of Helsinki and subsequent amendments.

Biochemical measurements

Blood samples were collected in EDTA vials after a twelve hour overnight fast, and the plasma shortly afterwards was separated by centrifugation at 4ºC and then stored at -80ºC until use. Concentrations of ghrelin, insulin, and glucose in all the plasma samples were measured and we used HOMA-IR index [fasting insulin (μU/mL) × fasting glucose (mmol/L)]/22.5 to calculate insulin resistance[28]. Plasma concentrations of ghrelin, insulin, and glucose were determined by ELISA (Phoenix Europe, GmbH, Germany), ELISA (Mercodia, Sweden), and glucose oxidase (Pars Azmoon Co., Iran) methods respectively. The intra- and inter assay CV, respectively, were 4.7 and 8.0% for glucose, 5.1 and 8.7% for insulin, and 5.1 and 7.2% for ghrelin.

Statistical methods

All the statistical analyses were conducted using SPSS software package (SPSS version 25.0 for Windows, Chicago, IL) and *P* values <0.05 were regarded as statistically significant. To compare variables between different groups, we used chi-square (χ^2) test, *t*-test, or logistic regression analysis when appropriate. To adjust confounding factors (age, BMI, gender, smoking history, regular non-steroidal anti-inflammatory drug (NSAID) use, and family history of CRC), logistic regression analysis was employed too. Furthermore, the Kolmogorov-Smirnov goodness-of-fit test was used to explore the normality of distribution of the continuous variables. We examined the possible correlations between continuous variables using Pearson correlation coefficients. And finally, partial correlation analysis was applied to investigate independent relations.

RESULTS

General characteristics of the populations studied and their statistical significance are provided in TABLE 1. The cases with CRC

TABLE 1. Characteristics of the cases with colorectal cancer (CRC) and the controls.

Variables[a]	Controls (n=88)	Cases (n=82)	*P*-value[b]	*P*-value[c]	*P*-value[d]
Age (years)	57.7 (8.2)	64.8 (12.0)	<0.001	–	–
BMI (kg/m^2)	24.1(1.9)	26.7 (2.3)	<0.001	–	–
Gender					
Men	47 (53.4)	43 (52.4)			
Women	41 (46.6)	39 (47.6)	0.899	–	–
Smoking history					
No	57 (64.8)	48 (58.5)			
Former	14 (15.9)	20 (24.4)			
Current	17 (19.3)	14 (17.1)	0.669	–	–
Regular NSAID use					
No	63 (71.6)	59 (71.9)			
Yes	25 (28.4)	23 (28.1)	0.958	–	–
Family history of colorectal cancer					
No	80 (90.9)	61 (74.4)			
Yes	8 (9.1)	21 (25.6)	0.004	–	–
Tumor site					
Colon	–	47 (57.3)			
Rectum	–	35 (42.7)	–	–	–
Metastasis					
No	–	63 (76.9)			
Yes	–	19 (23.1)	–	–	–
Glucose (mmol/L)	6.1 (1.1)	7.5 (1.4)	<0.001	<0.001	<0.001
Insulin (μU/mL)	5.4 (1.3)	5.8 (1.2)	0.052	0.462	0.543
HOMA-IR	1.4 (0.3)	1.8 (0.4)	<0.001	<0.001	<0.001
Ghrelin (pg/mL)	356.8 (77.5)	250.3 (100.1)	<0.001	<0.001	<0.001

NSAID: non-steroidal anti-inflammatory drug. [a] Variables presented as mean (SD) or number (%). [b] Unadjusted *P*-values. [c] Adjusted *P*-values for age and BMI. [d] Adjusted *P*-values for age, BMI, gender, smoking history, regular NSAID use, and family history of colorectal cancer.

were older (P<0.001) and had a higher BMI (P<0.001) compared with the controls. The cases were also more likely to have a positive family history of CRC (P=0.004). Nevertheless, no significant difference between the cases and controls in terms of their gender, smoking history, or regular NSAIDs use was observed.

As shown in TABLE 1, the cases with CRC had lower plasma levels of ghrelin (P<0.001), higher plasma levels of glucose (P<0.001), and a higher HOMA-IR index (P<0.001) than the controls. These differences also remained significant after adjustment for age and BMI (P<0.001), as well as after adjustment for age, BMI, gender, smoking history, regular NSAID use, and family history of CRC (P<0.001). Interestingly, after the stratification of the cases with CRC by tumor site, we again observed lower levels of ghrelin (P<0.001), higher levels of glucose (P<0.01), and a higher HOMA-IR index (P<0.01) in the patients with colon cancer or in patients with rectal cancer compared with the controls.

Calculation of the Pearson correlation coefficients showed that plasma ghrelin levels were negatively correlated with HOMA-IR (r=-0.365, P<0.05) and BMI (r=-0.335, P<0.05) in the rectal subgroup (TABLE 2). Partial correlation analyses showed that these correlations were age and BMI-independent.

DISCUSSION

In the present study, we found that the cases with CRC had a lower plasma ghrelin level and a higher Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) index compared with the controls. The same results were observed when we compared colon cancer patients or rectal cancer patients with the controls. Additionally, in the rectal cancer subgroup plasma ghrelin levels were negatively correlated with both HOMA-IR and BMI.

The inconsistent results concerning the association between ghrelin levels and CRC risk[23-27] can be ascribed to differences in genetic makeup, dietary habits and statistical methods, small sample size, and false positive results. The findings of our study are in concordance with those of studies that showing lower ghrelin levels in the patients with CRC than in the controls[23-26] whereas others found no association[27]. Surprisingly enough, although the report by Sundkvist et al.[27] was comparable in many respects to the study by Murphy et al.[26], their findings were completely different. Sundkvist et al.[27] could not replicate the strong association between ghrelin levels and CRC risk which was found by Murphy et al.[26]. Hence the role of ghrelin in the pathogenesis of CRC is

TABLE 2. Pearson correlation coefficients between different variables in the controls (n=88) as well as in the cases with colorectal cancer (CRC) (n=82), colon cancer (n=47), and rectal cancer (n=35).

		Age	BMI	Glucose	Insulin	HOMA-IR	Ghrelin
Control							
	Age	1					
	BMI	-0.12	1				
	Glucose	-0.069	0.112	1			
	Insulin	0.060	0.121	0.194	1		
	HOMA-IR	-0.078	0.109	0.817***	0.317**	1	
	Ghrelin	0.110	-0.115	0.182	-0.101	-0.201	1
CRC							
	Age	1					
	BMI	0.016	1				
	Glucose	-0.012	-0.026	1			
	Insulin	-0.002	-0.033	-0.028	1		
	HOMA-IR	0.031	-0.176	0.832***	0.005	1	
	Ghrelin	0.084	0.042	-0.009	-0.091	-0.158	1
Colon							
	Age	1					
	BMI	-0.183	1				
	Glucose	0.129	-0.014	1			
	Insulin	0.052	0.036	-0.243	1		
	HOMA-IR	0.157	-0.158	0.889***	-0.148	1	
	Ghrelin	0.106	0.066	0.012	-0.092	-0.080	1
Rectal							
	Age	1					
	BMI	0.248	1				
	Glucose	-0.209	0.020	1			
	Insulin	-0.049	-0.019	0.268	1		
	HOMA-IR	-0.098	-0.063	0.706***	0.163	1	
	Ghrelin	0.132	-0.335*	-0.074	-0.173	-0.365*	1

HOMA-IR: Homeostatic Model Assessment of Insulin Resistance. *P<0.05; **P<0.01; ***P<0.001.

still controversial. Very interestingly, even after the stratification of CRC patients by tumor site, we again observed lower levels of ghrelin in the patients with colon cancer than in the controls and lower levels of ghrelin in the patients with rectal cancer than in the controls. A possible hypothesis for lower levels of ghrelin in the patients with CRC is that the tumor might damage the X/A-like cells of the stomach somehow and as a consequence of this, the secretion of ghrelin has been reduced. An alternative hypothesis is obesity-related hyperinsulinemia and the inhibitory effect of insulin on ghrelin secretion from the X/A-like cells of the stomach's fundus[29]. In line with the latter hypothesis, Saad et al. have shown that insulin might be an independent modulator of circulating ghrelin levels, and insulin, directly or indirectly, mediates the effects of nutritional status and chronic energy balance on ghrelin levels[30].

The molecular mechanism through which ghrelin might affect CRC susceptibility is still unknown. Nonetheless, some previous studies have shown that ghrelin can have a role in the pathogenesis of CRC at least through obesity, insulin resistance, or inflammation. On the one hand, the high insulin level found in obesity and CRC increases the bioavailability and expression of IGF1, which in turn raises cell proliferation and diminishes apoptosis[3,4,31]. On the other hand, in accordance with previous studies, our findings indicated that the ghrelin level is inversely associated with BMI[14,15] and CRC[23-26], and ghrelin level which is increased after weight loss[32]. Ghrelin is expressed in pancreatic islet beta-cells[33]; it plays a major role in the modulation of insulin signaling pathway and inhibition of glucose-stimulated insulin secretion[16-18]. Previous studies have also reported a significant negative correlation between ghrelin levels and insulin resistance[34,35] which is in agreement with the present study. A negative correlation between ghrelin and insulin levels has been found too[36]. However, the underlying mechanism of association between ghrelin and insulin resistance is still unknown, and whether low circulating ghrelin level is the cause or the consequence of insulin resistance has not yet been clarified. Inflammation as a known risk factor for CRC is the other possible mechanism linking ghrelin with CRC pathogenesis. Ghrelin has anti-inflammatory and antioxidant activities[37,38] and it suppresses the expression of inflammatory cytokines[39]. And lastly, the gastrointestinal peptide hormone ghrelin also directly affects apoptosis and cell proliferation and cell survival. Ghrelin activates apoptosis in human colorectal carcinoma cells (HCT116 cells) via inhibiting the ubiquitin-proteasome system and autophagy induction[13]. Ghrelin treatment increases the intracellular levels of the proteasome substrates p27 and IkBa gradually. This, in turn, results in the nuclear accumulation of inactive NFkB/IkBa complex and the inhibition of NFkB-dependent survival genes expression, triggering apoptosis[40,41]. Treatment with ghrelin also causes accumulation of p53 protein, a key regulator of cell cycle and apoptosis, in response to proteasome inhibition, which in turn may induce autophagy. Therefore, our finding that the lower ghrelin concentration is a possible marker of increased CRC risk seems to be in line with the above notions. Nevertheless, previous reports have also demonstrated that ghrelin has both proliferative and anti-proliferative properties which is dependent on the cell type. Ghrelin has a role in the proliferation of intestine and colon cancer cells via the PI3K/Akt and ERK 1/2 signaling pathways. Ghrelin also inhibits the proliferation of lung, breast, and prostate carcinoma cells, and induces the proliferation of adipocytes, cardiomyocytes, and osteoblastic cells[42].

A number of potential limitations of this study should be addressed when interpreting its findings. One limitation was the modest sample size that precluded us from performing sub-analyses. Another limitation due to budget limitations was lack of information on circulating levels of other obesity and insulin resistance-related hormones besides insulin and ghrelin, such as leptin and adiponectin, which might had modified our findings. The other limitation was that our study was somehow incomplete because we just tested the circulating ghrelin levels without investigating the function of its gene and protein. Notwithstanding these limitations, our study was well designed and it gave interesting information that were consistent with previous publications too.

CONCLUSION

In summary, our findings corroborate the hypothesis that ghrelin could be related to the metabolic changes in the patients with CRC and have a role in the pathogenesis of CRC. These observations are relevant from a scientific standpoint; however, further studies are required to confirm them.

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Authors' contribution

Asadi A: performing the experiments; analysis and interpretation of data; coordination responsibility; drafting of manuscript. Farahani H: acquisition of data; acquisition of the financial support; supervising the project; drafting of manuscript. Mahmoudi T: study conception and design; analysis and interpretation of data; drafting of manuscript. Tabaeian SP: acquisition of data; survey execution; drafting of manuscript. Rezamand G: acquisition of data; survey execution; drafting of manuscript. Mohammadbeigi A: performing statistical analyses; drafting of manuscript. Dabiri R: acquisition of data; drafting of manuscript. Nobakht H: acquisition of data; drafting of manuscript. Rezvan S: acquisition of data; drafting of manuscript. Mohammadi F: acquisition of data; drafting of manuscript.

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RESUMO – Contexto e Objetivo – Considerando a associação entre câncer colorretal (CCR), a resistência à insulina, à obesidade e o papel proeminente da grelina nessas doenças metabólicas, foi explorado se os níveis plasmáticos de grelina estavam associados ao CCR. Além disso, nos pacientes com CCR foram pesquisadas as possíveis correlações entre a grelina, insulina, resistência insulínica e índice de massa corporal (IMC) como indicadores de obesidade. **Métodos** – Foram incluídos neste estudo 170 indivíduos, sendo 82 com CRC e 88 controles. Os níveis plasmáticos de grelina, insulina e glicose foram medidos em todos os sujeitos utilizando métodos ELISA e glicose oxidase. Além disso, a resistência à insulina foi avaliada pelo cálculo do índice HOMA-IR. **Resultados** – Os pacientes com CRC apresentaram redução dos níveis de grelina (P<0,001) e maior índice HOMA-IR (P<0.001) do que os controles. Curiosamente, quando os pacientes com CRC foram estratificados com base no local do tumor, níveis mais baixos de grelina e maior índice de HOMA-IR foram observados nos indivíduos com câncer de cólon ou retal versus controles também. Além disso, houve uma correlação negativa entre idade e IMC independente entre os níveis de grelina e HOMA-IR (r=-0,365, P<0,05) e uma correlação negativa independente da idade entre os níveis de grelina e IMC (r=-0,335, P<0,05) no subgrupo retal. **Conclusão** – Nossos achados apoiam o papel da grelina em relação à resistência à insulina e à obesidade na suscetibilidade do CRC; no entanto, ela precisa ser corroborada por estudos posteriores.

Palavras-chave – Câncer colorretal; grelina; resistência à insulina; obesidade.

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