

High levels of leptin and non-high molecular weight-adiponectin in patients with colorectal cancer: Association with chemotherapy and common genetic polymorphisms

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Abstract. Colorectal cancer (CRC) is the third most frequently diagnosed cancer worldwide. Leptin and adiponectin are hormones produced by adipose tissues, which exhibit opposing effects on tumor growth. Leptin promotes tumor development and metastasis, whereas adiponectin attenuates this. The aim of the present study was to assess the possible association between leptin and adiponectin [both high molecular weight (HMW) and non-HMW fractions] levels with CRC, CRC response to chemotherapy, and to study the relationship between LEPR (rs6588147), ADIPO (rs266729), LEP (rs2167270), and ADIPO (rs822369) polymorphisms and CRC. A total of 32 blood samples collected from CRC patients were analyzed to identify the serum levels of leptin and adiponectin, and the presence of CRC related polymorphisms. A total of 25 healthy subjects were recruited in the control group. Serum levels of leptin and adiponectin were detected using ELISA whereas DNA from patients and controls was amplified and analyzed using PCR-restriction fragment length polymorphism assay. The results showed that the levels of leptin and non-HMW adiponectin were significantly higher in CRC patients compared with the controls ($P < 0.05$). In addition, HMW adiponectin was significantly higher in patients receiving chemotherapy. The association between LEPR (rs6588147), ADIPO (rs266729), LEP (rs2167270) and ADIPO (rs822369)

polymorphisms and CRC was not significant ($P > 0.05$). In conclusion, higher leptin and non-HMW adiponectin levels may be associated with increased CRC. Chemotherapy may positively influence the levels of HMW adiponectin. No association between LEPR (rs6588147), ADIPO (rs266729), LEP (rs2167270) and ADIPO (rs822369) polymorphisms with CRC was found.

Introduction

Colorectal cancer (CRC) refers to a cancer that develops in the colon or in the rectum (1). CRC is characterized by an uncontrollable growth of cells in the terminal regions of the digestive system. CRC is the third most frequently diagnosed malignancy and the fourth leading cause of cancer-related death worldwide (2). In Jordan, the statistics show that CRC is the second most frequently diagnosed cancer and the second leading cause of cancer-related death in both males and females (3).

Obesity has been shown to contribute to an increased risk of cardiovascular disease and type 2 diabetes mellitus (DM) (4-6). Additionally, obesity was identified as a risk factor in the development of cancer and cancer-related death (7-9). According to the American Cancer Society, excess body weight is hypothesized to be responsible for ~8% of all types of cancer in the United States, as well as ~7% of all cancer deaths (10). Overweight teenagers with colon cancer have a 2x risk of mortality in adulthood (11).

Adipose tissue dysfunction is one hypothesis that may link obesity and CRC due to secretion of several hormones called adipokines, the most important of which are leptin and adiponectin (12). Leptin, the product of the *ob* gene, is a 16 kDa peptide cytokine, which is produced exclusively by adipose tissues. Leptin primarily regulates food intake and energy expenditure (13), and its concentration is positively correlated with the body mass index (BMI), and is thus higher in obese individuals (14). Several experimental studies have shown that leptin has a mitogenic, antiapoptotic and tumorigenic effect on different cancer cell lines, including colorectal, lung, breast, thyroid, pancreatic and uterine cancer cells (15-17). In addition, in CRC tissues, it was found that leptin and its receptor

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Abbreviations: CRC, colorectal cancer; BMI, body mass index; HMW, high-molecular weight; DM, Diabetes mellitus; HTN, hypertension

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were significantly upregulated compared with normal tissues and its upregulation was associated with a more advanced tumor phenotype (18,19).

Adiponectin is a protein with a molecular weight of 30 kDa which is derived primarily from adipocytes (20). Its anti-atherogenic and anti-inflammatory properties, as well as its ability to sensitize cells to insulin are considered the most important functions of adiponectin (21). Adiponectin circulates in the blood in different forms including as a trimer, hexamer and high-molecular weight (HMW) complexes. These forms were found to have different biological activities (22). For example, HMW adiponectin affects insulin sensitivity, whereas the anti-inflammatory activity of the non-HMW is its prominent effects (23).

An inverse relationship between obesity, insulin resistance, type 2 DM and the serum levels of adiponectin has been identified (24). Obesity and DM are associated with a higher incidence of CRC (25). Studies have found that lower levels of total adiponectin are associated with an increased risk of colorectal cancer (26,27). Conversely, other studies have found higher levels of total adiponectin in patients with CRC compared with the control (28,29). Thus, additional studies are required to highlight the association between adiponectin levels and CRC.

The aims the present study were to determine the relationship between leptin and adiponectin levels on the risk of CRC and response to chemotherapy. Furthermore, the association between LEPR (rs6588147), ADIPO (rs266729), LEP (rs2167270) and ADIPO (rs822369) polymorphisms and CRC occurrence were investigated.

Materials and methods

Ethical approval. The present study was approved by the Institutional Review Board of Jordan University of Science and Technology (approval no. 80/2012). Written informed consent was obtained from all study participants.

Study population and blood collection. Blood samples were collected at King Abdullah University Hospital, including 32 CRC patients who were histopathologically diagnosed with colorectal adenocarcinoma; 13 of these were female and 19 were male. The median age was 52 years old (age range, 21-75 years). Additionally, 25 healthy volunteers were recruited of which, 5 were female and 20 were male. The median age was 30 years old (age range, 18-59 years). Samples of control individuals were chosen from fully healthy subjects with a similar BMI. Exclusion criteria were: i) A previous gastrointestinal tract surgery; ii) inflammatory bowel disease (ulcerative colitis or Crohn's disease); iii) patients with a cancer in a different organ; iv) acute or chronic infections; v) familial adenomatous polyposis; vi) autoimmune disease; vii) rheumatoid arthritis; viii) malnutrition; ix) other primary cachectic states (such as congestive pulmonary disease or cirrhosis); or x) lack of consent to participate in the study. Data regarding the histopathological diagnosis, staging of CRC patients, whether patients started on chemotherapy and the number of cycles given, were retrieved from the medical records. The CRC patients were classified according to

Table I. Primer sequences.

Genotype	Primer, 5'-3'
LEP R (rs6588147)	
Forward	GCATATGCCTGTGTGCAGTT
Reverse	GCAGTTCTGGATCAGTGCAA
ADIPO (rs266729)	
Forward	CATCAGAATGTGTGGCTTGC
Reverse	AGAAGCAGCCTGGAGAACTG
LEP (rs2167270)	
Forward	GATCGGGCCGCTATAAGAG
Reverse	GCATCCCTCCTGACTCAGTT
ADIPO (rs822369)	
Forward	CTGAAGTTTGGGGACAAACAG
Reverse	CTTTTCTGCTGGGAAACAGG

the Tumor-Node-Metastasis staging system and Dukes's classification (30,31).

Blood samples, which were collected from all patients and controls in the morning, were split equally in to two different tubes (plain and EDTA tubes). The serum blood obtained from the plain tubes was allowed to coagulate at room temperature for 30 min, and then centrifuged at 1,968 x g for 10 min at 4°C. The supernatant was separated from the samples and transferred into PCR tubes (100 µl), and stored at -20°C until required. The EDTA blood samples were stored at -8°C until required.

Biochemical analysis. Serum concentrations of leptin, and adiponectin (total and HMW) were measured using ALPCO Immunoassay kits using ELISA. Leptin levels were determined according to the manufacturer's protocol (Leptin ELISA kit; cat. no. 11-LEPHU-E01; sensitivity, 0.5 ng/ml; ALPCO). Plasma concentrations of adiponectin (total and HMW) were analyzed according to manufacturer's protocol [Adiponectin (Multimeric) ELISA kit; cat. no. 47-ADPHU-E01; sensitivity, 0.019 ng/ml, ALPCO].

DNA extraction and genotyping. The DNA was extracted from the EDTA blood samples of CRC patients and control participants according to the manufacturer's protocol (Wizard Genomic DNA Purification kit; Promega corporation). The LEPR (rs6588147), ADIPO (rs266729), LEP (rs2167270) and ADIPO (rs822369) polymorphisms were analyzed by PCR-restriction fragment length polymorphism assay. The sequences of the primers were designed according to their sequence gene and purchased from Integrated DNA Technologies, Inc. (Table I).

Amplification of the 194 and 163 bp PCR products for LEPR (rs6588147) and ADIPO (rs266729), respectively, was performed using the following thermocycling conditions: Initial denaturation at 95°C for 5 min; followed by 35 cycles of 95°C, 54°C and 72°C for 90 sec each; and a final extension at 72°C for 7 min. Amplification of the 176 and 214 bp PCR products for LEP (rs2167270) and ADIPO (rs822369), respectively, was performed as follows: Initial denaturation

Table II. Demographic and biomarkers in controls vs. CRC patients.

Characteristic	Value	Controls	CRC patients	P-value
Sex, n (%)				0.096
Male	39 (68.4)	20 (35.1)	19 (33.3)	
Female	18 (31.6)	5 (8.8)	13 (22.8)	
Age, years	52.85±11.51	52.80±11.46	54.10±13.58	0.001 ^b
Body mass index, kg/m ²	25.90±4.28	24.31±3.26	27.26±4.62	0.01 ^a
Leptin, ng/ml	10.17±8.64	7.13±7.59	12.35±8.79	0.026 ^a
Total adiponectin, ng/ml	3,459.84±1,911.42	3,051.21±1,162.42	3,753.54±2,278.68	0.181
HMW adiponectin, ng/ml	924.81±1,326.86	1,175.84±1,206.90	744.39±1,397.40	0.238
Non-HMW adiponectin, ng/ml	0.244±.264	1,875.36±1,079.18	3,009.15±1,856.58	0.011 ^a

^aP<0.05, ^bP<0.01. Data are presented as the mean ± standard deviation unless otherwise is indicated. HMW, high molecular weight.

Table III. Association between biomarkers and characteristics of patients.

Characteristic (n)	Leptin	Total adiponectin	HMW adiponectin	Non-HMW adiponectin
Sex				
Male (19)	10.91±8.95	3,714.77±2,615.12	674.55±1,553.16	3,040.22±2,206.16
Female (13)	14.46±8.46	3,810.20±1,774.69	846.46±1,186.19	2,963.74±1,264.91
Age, years ^b				
<40 (4)	15.48±8.69	5,244.59±1,310.39	1,859.1±1,671.46 ^a	3,385.49±537.07
40-60 (14)	12.36±7.27	2,706.97±1,429.06	310.08±290.80	2,396.88±1,327.58
>60 (11)	13.69±10.78	4,126.87±2,803.74	382.49±565.81	3,744.37±2,592.83
Body mass index, kg/m ^{2b}				
<24.99 (8)	9.47±9.01	4,348.27±3,561.70	1,038.84±1,359.09	3,309.42±3,163.05
25-29.99 (13)	12.34±7.82	3,274.84±1,466.06	362.27±390.93	2,912.56±1,360.60
>30 (8)	19.16±8.51	3,277.26±1,529.37	390.93±515.41	2,993.47±1,175.47

^aP<0.05. ^bData missing for 3 patients. Data are presented as the mean ± standard deviation. HMW, high molecular weight.

at 95°C for 5 min; followed by 35 cycles of 95°C, 53°C and 72°C for 90 sec each; and a final extension at 72°C for 7 min. Amplification was performed on a Kyrattec SuperCycler SC200 and the corresponding included software (software version 3.0.1, Hardware Version 3; Kyrattec).

Statistical analysis. Data are presented as the mean ± standard deviation. SPSS version 25 (IBM, Corp.) was used for statistical analysis. A χ^2 test was used to assess differences amongst categorical groups, whereas a Student's t-test (two-tailed) was used to compare differences between continuous variables. A one-way ANOVA followed by a Bonferroni post-hoc test was used to compare differences between >2 groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Demographics and biomarkers. There was no significant difference in the demographics between the patients and control groups. Plasma concentrations of leptin were significantly higher in the CRC group compared with the control

group (P<0.05). Compared with the controls, non-HMW adiponectin levels were significantly higher in CRC patients. However, serum concentrations of both total and HMW adiponectin were not significantly different between the two groups (Table II).

Table III shows that the sex, age and BMI variations amongst CRC patients were not significantly associated with leptin, total adiponectin, HMW adiponectin or non-HMW adiponectin levels. Nevertheless, in patients aged <40 years, HMW adiponectin was significantly higher compared with patients >40 years.

Comorbidities of CRC patients. The majority of the patients enrolled in the present study were free of any chronic disease (56.3%), whereas 4 patients (12.5%) had DM either alone (1 patient, 3.1%) or with another comorbidity, such as hypertension (HTN), chronic kidney disease (CKD) or hyperlipidemia (3 patients, 9.4%). HTN was observed in (7 patients, 21.9%), of whom 5 patients (15.6%) had HTN alone, whereas 2 patients (6.3%) had HTN and ischemic heart disease. The data of the 3 patients with comorbidities was not recorded.

Table IV. Association between biomarkers and duration of diagnosis, stages, chemotherapy and the number of cycles of chemotherapy in patients.

Parameter	Leptin	Total adiponectin	HMW adiponectin	Non-HMW adiponectin
Duration of diagnosis, years (n)				
0-1 (7)	13.05±9.25	2,455.18±888.06	269.66±155.98	2,185.52±890.47
1-2 (14)	12.61±8.51	3,456.60±1,779.24	608.90±1,128.98	2,847.69±1,365.38
2-5 (7)	12.86±8.58	5,050.55±3,296.51	776.62±683.90	4,273.93±3,093.65
>5 (1)	27.6	3,338.96	136.36	3,202.59
Disease stage (n)				
Stage I (3)	12.76±6.57	2,706.25±1,206.27	59.88±21.63	2,669.32±1,236.92
Stage II (5)	17.94±9.17	3,252.59±1,795.73	407.11±637.04	2,845.48±1,674.51
Stage III (9)	10.04±7.47	3,090.12±1,697.61	724.42±1,309.33	2,365.70±902.13
Chemotherapy (n)				
Yes (13) ^b	16.07±8.72	4,024.19±1,613.17	921.17±1,174.39 ^a	3,103.01±1,111.27
No (16)	11.03±8.22	3,247.31±2,579.27	250.60±241.65	2,996.71±2,419.77
Treatment cycles (n)				
1-3 (3)	26.40±3.79	2,287.80±192.22	174.61±128.66	2,113.20±97.41
4-6 (5)	7.34±5.20	6,857.99±2,983.28	1,389.12±1,630.81	5,468.86±3,253.69
≥7 (5)	17.86±6.36	3,642.25±1,367.20	916.47±684.18	2,910.86±8,36.91

^aP<0.05. HMW, high molecular weight. ^bThe majority of patients received FOLFOX4 chemotherapy regimen except one patient who received FOLFOX4 plus bevacizumab. Data are presented as the mean ± standard deviation. FOLFOX4, Oxaliplatin, Leucovorin, 5-Fluorouracil.

Table V. Incidence of polymorphisms in control vs. patients.

Genotype	Total samples, n (%)	Controls, n (%)	CRC patients, n (%)	P-value
LEPR (rs6588147)				0.317
AA	29 (53.7)	14 (25.9)	15 (27.8)	
AG	20 (37.0)	6 (11.1)	14 (25.9)	
GG	5 (9.3)	3 (5.6)	2 (3.7)	
ADIPO (rs266729)				0.541
CC	30 (55.6)	11 (20.4)	19 (35.2)	
CG	19 (35.2)	10 (18.5)	9 (16.7)	
GG	5 (9.3)	2 (3.7)	3 (5.6)	
LEP (rs2167270)				0.795
GG	21 (38.9)	8 (14.8)	13 (24.1)	
GA	23 (42.6)	11 (20.4)	12 (22.2)	
AA	10 (18.5)	4 (7.4)	6 (11.1)	
ADIPO (rs822396)				0.152
AA	10 (18.5)	7 (13.0)	3 (5.6)	
AG	11 (20.4)	4 (7.4)	7 (13.0)	
GG	33 (61.1)	12 (22.2)	21 (38.9)	

CRC, colorectal cancer.

Association between biomarkers and diagnosis, duration, disease stage and chemotherapy cycles. The CRC patients who were on chemotherapy had significantly higher levels of HMW adiponectin compared with those who did not take chemotherapy (Table IV). For the duration of diagnosis, disease stage, chemotherapy cycles and biomarkers

were not significant different between the control and CRC patients.

Incidence of polymorphisms. There was no significant difference between CRC patients and control subjects in the frequency of LEPR (rs6588147), ADIPO (rs266729), LEP

(rs2167270) and ADIPO (rs822369) polymorphisms ($P>0.05$; Table V).

Discussion

The present study showed that leptin and non-HMW adiponectin levels were significantly higher in CRC patients compared with the controls. In addition, HMW adiponectin levels were significantly higher in patients who received chemotherapy compared with those who did not, whereas leptin levels were not significantly different between patients who received chemotherapy and those who did not. In addition, there was no association between LEPR (rs6588147), ADIPO (rs266729), LEP (rs2167270) and ADIPO (rs822369) polymorphisms and CRC.

The association between leptin levels and CRC remains controversial. In a prospective study, leptin concentration was found to be similar in CRC patients compared with the controls (32), whereas other studies reported a significant decrease in leptin serum levels in CRC patients (33,34). In agreement with the present study, studies have shown that leptin is significantly associated with CRC occurrence (35,36), and this may be due to the established tumorigenic, mitogenic and antiapoptotic effects of leptin (15,16). However, sex differences between control (5 females, 20 males) and CRC patients (13 females, 19 males) may influence leptin levels. Previous studies have shown females have 2-3 times higher levels of leptin than males regardless of BMI; higher fat mass may explain the higher levels of leptin in circulation in females compared with males (37-39). Therefore, in the present study, it is possible that sex variations may have contributed to the higher levels of leptin shown amongst CRC patients.

The results of the present study showed higher levels of non-HMW adiponectin in CRC patients compared with the control group, which may be closely associated with CRC occurrence. In contrast, a meta-analysis indicated that a low circulating non-HMW fraction may be considered as a risk factor for CRC (40). Increased levels of non-HMW adiponectin in CRC patients shown in the present study may be associated with the involvement of non-HMW adiponectin in the regulation of inflammatory processes, which is considered a key factor in cancer progression and metastasis in various types of cancer including CRC (23,41). However, the exact mechanism that may explain the higher levels of non-HMW adiponectin in CRC patients is unknown, and further studies are required to this end.

In the present study, CRC patients receiving chemotherapy had significantly higher HMW adiponectin levels compared with those who did not receive chemotherapy. Thus, it is likely that chemotherapy may have resulted in such an elevation. However, no significant difference in leptin levels was detected in patients who received chemotherapy compared with those who did not. A previous study has reported that adiponectin levels are increased after receiving chemotherapy in patients with CRC who exhibited a partial response (42) and in patients with lung cancer (43). These results may indicate that adiponectin levels are sensitive to chemotherapy (44). Therefore, further studies are required to evaluate the potential value of adiponectin levels as predictive or prognostic markers for the chemotherapy response (45); specifically, HMW adiponectin.

The results of the present study showed there was no association between LEPR (rs6588147), ADIPO (rs266729), LEP (rs2167270) and ADIPO (rs822369) polymorphisms and CRC. In agreement, Carvajal-Carmona *et al* (46) did not find an association between the ADIPO (rs266729) polymorphisms and CRC. However, previous studies have reported an association between these variants and CRC, particularly LEP (rs2167270) and ADIPO (rs266729) (47-49). The difference in the ethnic groups and the smaller number of samples may explain the differences in results.

The present study was performed on CRC patients from a large health center; however, it has some limitations. First, a small number of patients were enrolled. Secondly, due to the limited number of participants and less willingness of female participants to be involved in the study, we could not match for sex between control and patient group. Thirdly, due to sample size limitations, the results of this study are based only on univariate analyses. Finally, the effect of leptin on migration and invasion of CRC was not investigated. Thus, a future study that is more comprehensive and addressing whether leptin promotes migration and invasion in CRC patients is required.

In conclusion, the present study showed that higher plasma levels of leptin and non-HMW adiponectin may be associated with an increased risk of CRC, whereas HMW adiponectin levels may increase with chemotherapeutic regimens. The polymorphisms of LEPR (rs6588147), ADIPO (rs266729), LEP (rs2167270) and ADIPO (rs822369) were not associated with CRC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

NMM designed the study, assisted in collection of patient samples, contributed to the analysis and interpretation of the data, and assisted in manuscript writing. KHA was involved in the study conception and design, sample analysis and interpretation, and assisted in writing the manuscript. MAK participated in study design, collection of patient data, performed the molecular analysis of the samples, and assisted in manuscript writing. MNB was involved in the study design, subject recruitment and data interpretation. NH participated in study design, assisted in molecular analysis of the samples, and in manuscript writing. TMA was involved in study design, participated in collection of patient samples, interpretation of results and manuscript writing. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Institutional Review Board of Jordan University of Science and Technology (approval no. 80/2012). Written informed consent was obtained from all study participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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