Resistin in human colon cancer

Increased expression independently of resistin promoter C–180G genotype

Rowyda N. Al-Harithy, *MSc, PhD,* ***Ayat B. Al-Ghafari,*** *MSc.*

ABSTRACT

الأهداف: دراسة العلاقة بين مستوى الرزيستين (RETN) والنيوكليوتيد المنفرد المتعدد الأشكال C-180G في المرضى السعوديون المصابين بسرطان القولون.

الطريقة: أجريت دراسة الحالة في قسم الكيمياء الحيوية – جامعة الملك عبدالعزيز – جدة – المملكة العربية السعودية – خلال الفترة من أبريل حتى ديسمبر 2009م. تم قياس مستوى جين الرزيستين في مصل الدم بواسطة تقنية المقايسة المناعية الإنزيمية (ELISA) في 60 مريض مصاب بسرطان القولون و 60 شخص من الأصحاء. ثم دراسة التغيرات الجينية لنيوكليوتيد المنفرد المتعدد الأشكال SNP C-180G بواسطة التفاعل المبلمر المتسلسل (PCR) و تقنيات اختلاف أشكال أطوال الشدف المتقطعة (RFLP).

النتائج: أظهرت النتائج في هذا البحث أن نسبة جين الرزيستين لدى مجموعة المرضى المصابين بسرطان القولون أعلى من الأصحاء (19.44±8.46 مقابل 5.45±2.73 ng/ml) بمعدل إحصائي p=0.0001، ظهر ارتفاع لدى النساء في كلا الفئتين عن الرجال (p=0.03). كذلك أظهر الطرازان الجينيان (CG) و (GG) ارتفاع مستوى جين الرزيستين لدى المرضى (p=0.08) مقارنة بالطراز الجيني CC ولم يلاحظ هذا التغير خلال طراز نيوكليوتيد المنفرد المتعدد الأشكال SNP C-180G لدى الأصحاء (p=0.78).

خاتمة: أظهرت نتائجنا أنه لا توجد علاقة بين التغير الجيني لدى النيوكليوتيد المنفرد المتعدد الأشكال في جين الرزيستين C-180G SNP وبين مستوى جين الرزيستين في مصل الدم، أوضح البحث أيضاً أن ارتفاع مستوى جين الرزيستين في المرضى المصابين بسرطان القولون يلعب دوراً مهماً في زيادة معدل خطورة سرطان القولون.

Objectives: To determine the relationship between resistin gene (RETN) C-180G variant and circulating resistin concentration in Saudi colon cancer patients.

Methods: This case-control study was conducted in the Biochemistry Department, King Abdul-Aziz University, Jeddah, Kingdom of Saudi Arabia from April 2009 to December 2009. The serum concentration had been measured with enzyme-linked immunosorbent assay in 60 colon cancer patients and in 60 controls matched in gender and age. The single nucleotide polymorphism (SNP) C-180G was genotyped using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques.

Results: We observed a significantly higher serum resistin level in colon cancer group compared with control group (19.44±8.46 versus 5.45±2.73 ng/ml; p=0.0001), with significant (p=0.03) higher levels showed in women than in men in patients and controls. In patients, the heterozygous (CG) and homozygous (GG) genotype carriers showed higher (p=0.08) levels of serum resistin compared to CC homozygous. This difference was not observed (p=0.78) among SNP C-180G genotypes in control group.

Conclusion: Our result showed no association between the C-180G SNP and the serum resistin concentrations and suggests that the high resistin level in colon cancer patients may play an important role in colon cancer development.

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From the Department of Biochemistry, King Abdul-Aziz University, Jeddah, Kingdom of Saudi Arabia.

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Address correspondence and reprint request to: Dr. Rowyda N. Al-Harithy, Biochemistry Department, King Abdul- Aziz University, PO Box 40288, Jeddah 21499, Kingdom of Saudi Arabia. Tel. +966 505444238. Fax. +966 (2) 6393640. E-mail: dr.alharithy@gmail.com

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Accumulating data supports a role for obesity in the etiology of colon cancer. Although the exact mechanism remains to be determined, the hormonal changes associated with obesity are considered to be responsible for this relationship, with particular emphasis being placed recently on the increased production of adipocytokines or adipokines.[1] A number of adipokines have been identified in the last 20 years and shown to communicate with other tissues and organs such as the skeletal muscle, adrenal cortex, brain, and sympathetic nervous system.[2-4] Changed levels of adipokines may alter appetite, glucose tolerance, fatty acid oxidation, and angiogenesis.[5,6] The adipocytokines may be classified into 3 different sgroups according to their source. The first group includes the hormones that are produced predominantly or exclusively by adipocytes of white adipose tissue such as leptin and adiponectin.[1,2] The second group consists of the hormones that are produced primarily in other tissues (immune cells) with simultaneous adipose tissue production such as tumor necrosis factor-α (TNF-α).[7] The third group comprises the hormones that are produced mainly in the white adipose tissue such as resistin.[8] Resistin is a special addition to the family of adipokines, and is poorly understood in humans. The human resistin gene, designated as resistin gene (RETN), is located on chromosome 19p13.3, comprises 4 exons with 3 introns, and spans approximately 1,750 base pair. The processed ribonucleic acid (RNA), product of RETN, is 478 nucleotides in length.[9,10] The protein translation starts from exon II and ends in the middle of exon IV, leading to a product consisting of 108 amino acids.[10] It is, however, associated with inflammation and insulin resistance at increased concentrations and is secreted more at higher body mass index (BMI).[11,12] In evaluating the influence of gender, Nogueiras et al[13] showed that resistin mRNA expression in adipose tissue is higher in male than in female rats, and these levels are highest during puberty, but then decrease with aging in both gender. They also showed that resistin mRNA expression was undetectable in 15-day-old male and female rats, and then they markedly increased during puberty, reaching the highest levels at 45 days in both gender, and declined thereafter.[13] Similar research was applied to human subjects by Yannakoulia et al,[14] whose study reported that resistin concentrations were significantly higher in females compared with males. These results were confirmed by 2 other studies, suggesting a significant gender difference.[15,16] However, a subsequent study examining both age and gender in relation to the serum resistin indicated a lack of association in healthy subjects, as well as patients with type i and type 2 diabetes mellitus.[17] Several SNPs in the RETN have been described in the literature, with most of them in the non-coding regions.[18-23] One of which, C-180G (rs1862513), is located in the promoter region and known as C-420G. Although C-180G SNP is an uncommonly studied variant, it has been suggested that SNPs including C-180G in the promoter of resistin gene is a determinant of resistin mRNA expression.[23,24] Other study has shown that polymorphism C-180G in RETN gene did not influence serum RETN concentration.[25] Based on this knowledge, this study was designed to investigate the relationship between resistin polymorphism at C-180G and circulating resistin concentration in Saudi colon cancer patients.

Methods. Sixty colon cancer patients (31 males and 29 females) were randomly selected from King Abdul-Aziz Hospital and Oncology Center in Jeddah; Kingdom of Saudi Arbia. Among subjects who visited the same hospital for annual health examinations, we selected 60 unrelated volunteers, as age, gender, and BMI matched controls (30 males and 30 females). All study subjects were of Saudi origin without any known ancestors of other ethnic origins. All subjects underwent complete physical examinations and routine biochemical analysis of blood. They all gave their written informed consent of their participation in the study. The study was a master degree project approved by the Biochemistry Department, King Abdulaziz University, Riyadh, Kingdom of Saudi Arabis and did not require ethical approval. In this study the Duke's system was used to characterize the disease.[26]

Anthropometric measurement. Body weight was measured with light clothing on with up to 0.1 kg precision. Height was measured up to 0.1 cm. The BMI was calculated by dividing the body weight (in kilograms) by the square of the height (in meters). The waist circumference was taken as the minimum standing horizontal circumference between rib cage and iliac crest; the hip circumference was taken as the maximum standing horizontal circumference of the buttocks. The waist-hip ratio was calculated.

Biochemical measurement. Blood samples were collected after overnight fasting for more than 12 hours. Serum resistin concentration was measured using human resistin enzyme-linked immunosorbent assay kit enzyme (ALPCO Diagnostics, Inc. San Francisco, USA), with a measurement range of 0-50 ng/ml, a minimum detectable concentration of 100 pg/ml.

Single nucleotide polymorphism (SNP) detection and genotyping. Genomic DNA was extracted from peripheral blood leukocytes using a commercial kit (QIAamp DNA blood Mini kit; Hilden, Germany). Deoxyribonucleic acid samples were genotyped using polymerase chain reaction (PCR) assays combined with restriction fragment length polymorphism (RFLP). The C-180G

SNP of the RETN gene was screened by restriction fragment length polymorphism (RFLP) analysis after digestion with BbsI. Two pairs of primers (5.0 nmol) from TIB (TIB-MOLBIOL Inc., Berlin, Germany) were used. For exon-2 amplification, the forward primer (5'-GAAGTAGACTCTGCTGAGATGG-3') and the reverse primer (5'-TATCAGTGTAGGAGGTCTGTGATG-3') were used. The PCR reactions were carried out by a Mastercycler gradient thermocycler (Eppendorf, Hamburg, Germany). All of the PCR reactions were carried out by a Mastercycler gradient thermocycler (Eppendorf, Hamburg, Germany). Each 25µl of the PCR reactions contained 2µl genomic DNA (0.2µg), 12.5µl HotStarTaq Master Mix, 10.1µl RNase free water, and 0.2µl of each primer (0.1µM) were used. After the first denaturing for 5 minutes at 96˚C, PCR was carried out for 40 cycles with denaturing at 96˚C for 35s, annealing at 51˚C for 35 seconds and extension at 72˚C for 45 seconds, with a final extension for 4 minutes. The amplified PCR products were electrophoresed on a 2% agarose gel.

Statistical analysis. All statistical analyses were performed with the SPSS (Version 16) for Windows software. Continuous variables were expressed as means and SD. Genotype distributions, allele frequencies, odds ratio, and risk ratio between the study groups were computed by 2x2 contingency table analysis. The Hardy-Weinberg equilibrium was tested for the genotypes. Results were considered statistically significant at P-value <0.05.

Results.

Clinical characteristics. The clinical characteristics of the patient and control groups, including the serum concentration of resistin, are presented in **Table 1**. The subjects were divided into 2 sub-groups according to gender. In patients, the number of females was 29 and males were 31. In controls, the number of females was 30 and males were 30. In comparing the colon cancer patients to the controls, there was no significant difference in age (p=0.09) and height (p=0.12). The results also showed a highly significant increase in the control group regarding the waist circumference (p=0.01), BMI (p=0.004), weight (p=0.0001), and hip circumference (p=0.0001).

Genotype and allele frequencies of C-180G variant in patients and controls. The genotype and allele frequencies of C-180G variant were examined (**Table 2**). The genotypic frequencies of the patients were 27% normal (CC), 55% heterozygous (CG), and 18% homozygous (GG). In controls, the results showed 40% CC, 33% CG, and 27%GG. In patient subjects, the frequencies of the C alleles was 55% and G alleles was 45%. In controls, the frequencies of the C and G alleles were 56.5% and 43.5%. Genotype distribution for colon cancer patients is in Hardy-Weinberg equilibrium (goodness of fit x^2=0.67, df = 1, p=0.41). For the controls the genotype distribution is out of Hardy-Weinberg equilibrium (goodness of fit x^2=6.18, df =1, p=0.013).

Serum resistin level analysis. In the colon cancer patients, the mean resistin level was 21.88±9.89 ng/ml in females and 17.16±6.18 ng/ml in males. In comparison, the mean resistin level in the controls was 6.22 ± 3.0 ng/ml in females and 4.68±2.23 ng/ml in males. The result showed that the females had significantly (p=0.03) higher resistin level than males (**Table 1**). When pooling the females and males and comparing patients to controls, patients (19.44+8.46 ng/ml) showed a significantly (p=0.0001) higher resistin concentration than controls (5.45+2.73 ng/ml).

Association between C-180G SNP and circulating resistin concentration. The result of association between C-180G SNP and circulating resistin concentration is

Table 1 - Physical and biochemical comparison between patient and control groups.

Variables	Patients (mean±SD)	Controls (mean±SD)	*P*-value
Age (years)			0.09
All	54.28 ± 12.32	50.47 ± 12.26	
Female	53.48 ± 13.64	51.96 ± 11.94	
Male	55.03 ± 11.12	48.96 ± 12.57	
Height (cm)			0.12
All	160.79 ± 9.14	163.50 ± 9.70	
Female	155.79 ± 8.05	158.07 ± 9.07	
Male	165.47 ± 7.55	168.93 ± 6.93	
Weight (Kg)			0.0001*
All	66.23 ± 13.93	77.42 ± 18.17	
Female	64.61 ± 14.27	71.58 ± 15.86	
Male	67.75 ± 13.66	83.25 ± 18.68	
Waist (cm)			0.01*
All	66.98 ± 24.75	80.17 ± 31.31	
Female	61.02 ± 28.05	84.63 ± 30.78	
Male	72.55 ± 20.11	75.69 ± 31.7	
Hip (cm)			0.0001*
All	66.04 ± 30.18	88.56 ± 35.66	
Female	66.81 ± 32.11	93.80 ± 34.66	
Male	65.32 ± 28.77	83.32 ± 36.44	
Waist hip ratio			0.15
All	1.09 ± 0.43	0.98 ± 0.47	
Female	0.95 ± 0.32	1.01 ± 0.65	
Male	1.23 ± 0.47	0.95 ± 0.19	
Body mass index			0.004*
All	25.73 ± 5.64	28.96 ± 6.35	
Female	26.77 ± 6.36	28.82 ± 6.7	
Male	24.75 ± 4.77	29.10 ± 6.08	
Resistin (ng/ml)			0.0001*
All	19.44 ± 8.46	5.45 ± 2.73	
Female	21.88 ± 9.89	6.22 ± 3.0	
Male	17.16 ± 6.18	4.68 ± 2.23	

*highly significant (<0.01). P values were obtained using student t tset.

Table 2 - Genotypes and allele frequencies of RETN gene C-180G for patients and controls.

RETN polymorphism	Frequencies %		*P*-value*	Odds ratio (95% confidence interval)	Risk ratio (95% confidence interval)
	Patients (n=60)	Controls (n=60)			
Genotypes					
CC	16 (27)	24 (40)		1.00 (Reference)	1.00 (Reference)
CG	33 (55)	20 (33)	0.03	2.48 (1.07 - 5.74)	1.48 (1.02 - 2.16)
GG	11 (18)	16 (27)	0.95	1.03 (0.38 - 2.79)	1.02 (0.56 - 1.84)
CG + GG	44 (73)	36 (60)	0.12	1.83 (0.85 - 3.96)	1.22 (0.95 - 1.58)
Alleles					
C	33 (55)	34 (56.5)		1.00 (Reference)	1.00 (Reference)
G	27 (45)	26 (43.5)	0.69	1.11 (0.67 - 1.84)	1.06 (0.79 - 1.4)

*Two sided x^2 test. RETN - resistin gene, CG - heterozygous, GG - homozygous, C - normal

Table 3 - The mean resistin level for the genotypes in patients and controls.

Genotypes	Patients (n=60) Mean ±SD	*P* value	Controls (n=60) Mean ±SD	*P* value
CC	16.28 ± 6.55 ng/ml	Reference	5.57 ± 2.93 ng/ml	Reference
CG	19.68 ± 8.74 ng/ml	0.17	5.45 ± 3.13 ng/ml	0.89
GG	23.31 ± 8.95 ng/ml	0.03*	5.27 ± 1.93 ng/ml	0.72
CG+GG	20.59 ± 8.84 ng/ml	0.08	5.37 ± 2.63 ng/ml	0.78

*Significant. P values were obtained using one way analysis of variance. CG - heterozygous, GG - homozygous, C - normal

summarized in **Table 3**. In subjects with colon cancer, a significant difference (p=0.03) in serum resistin level was observed between the GG genotype and the CC genotype carriers. Both the GG and the CG genotype carriers showed higher levels of serum resistin compared to the CC genotype carriers. When the effect of the C-180G SNP on serum resistin level was analyzed in controls, the resistin level did not show any differences between CG (5.45 ± 3.13 ng/ml), GG (5.27 ± 1.93 ng/ml), and CC (5.57 ± 2.93 ng/ml) genotype carriers.

Association between serum resistin level and clinical stages of colon cancer. Patients were divided into groups according to the clinical staging based on the Duke's system (**Table 3**). Duke's A (n=4 cases, representing 6.7%), Duke's B (n=14 cases, representing 23.3%), Duke's C (n=27 cases, representing 45%) and Duke's D (n=12 cases, representing 20%). There was unclear information for 3 cases (representing 5%). In comparing the level of resistin in different stages, the results showed that there was a significantly (p=0.04) higher resistin level in Dukes' A/B (23.93 ± 10.31 ng/ml) compared with Dukes' C/D (17.99 ± 6.62 ng/ml) colon cancer patients.

Discussion. The present study showed that serum resistin levels were significantly elevated in colon cancer patients, and the increase in the resistin concentrations has no association with the RETN C-180G polymorphism. Circulating resistin, like other adipokines, has been implicated in the pathogenesis of several obesity-related diseases. It is rational that resistin, mainly released from adipose tissue, may exert some potential impact on the colon cancer disease. Our results showed that serum resistin levels were significantly (p=0.0001) elevated in the colon cancer patients when compared with controls. Our result is agreeing with a research carried out on colorectal carcinoma and adenoma in Polish patients.[27] Moreover, 2 other research studies on breast cancer in Chinese and Korean populations found that resistin concentration is higher in females suffering from breast cancer than controls.[28,29] In contrast, Wagsater et al[30] found that no significant difference was seen in the levels of resistin in plasma of colon cancer patients in comparison with controls, but there was a significant difference in the levels of resistin lysate protein in cancer tissue in comparison with paired normal tissue.[30] In our study,

although the mean BMI, hip, and waist circumference are significantly less in patients than in controls, the result demonstrated that the increase in serum resistin level was independent of all these parameters. We think that the independent relationship strengthens the statistical power of this analysis. Our result suggests that serum resistin concentration might be a factor that contributes to increase colon cancer risk. To clarify the biology of this relation, serum resistin was determined in different clinical stages of the disease. We found out that serum concentration differ in patients depending on the clinical stage of the disease. In comparing the clinical stages, the result showed a significant higher (p=0.04) circulating resistin in Dukes'A/B compared with both C/D stages. Existing data showed that resistin regulate the matrix metalloproteinases (MMPs) and vascular endothelial growth factor (VEGF), which is considered of importance for promoting tumor invasion and angiogenesis in colorectal cancer.[31-33] In our study, we found that resistin concentration in the C/D stages was lower than that in the A/B stages, suggesting that resistin has no correlation with generation and metastasis of colon carcinoma. This result should be taken with concern due to the low number of cases in the present study. Further investigation in human is needed.

To explore the molecular mechanism underlying resistin role in colon cancer, genetic factors might help in the explanation. It has been reported that genetic polymorphisms in the promoter region of the resistin gene may be independent predictors of circulating resistin concentrations in humans.[34,35] However, our result confirms a previous study that showed polymorphism C-180G in RETN gene did not influence serum RETN concentration[25] and shows that it is unlikely C-180G polymorphism in RETN gene may be responsible for resistin levels in colon cancer. Accordingly, it could be speculated that the RETN gene C-180G polymorphism with another polymorphism determines higher resistin gene expression, causing in turn an increased risk to develop colon cancer.

In this study, we analyzed the RETN C-180G SNP in colon cancer patients. To our knowledge, the present study is the first to report the association between RETN C-180G SNP and colon cancer risk in Saudi patients. Genotype distributions for the SNP C-180G in patients and controls differ significantly, suggesting that the polymorphism may modify the risk for colon cancer. The data of the present study showed that carriers of the heterozygous (CG) genotype had more than 2-fold (OR=2.48, 95%CI 1.07-5.74, p=0.03) higher risk of colon cancer than carriers of the normal (CC) genotype. Our finding suggests a potential role for the SNP C-180G in the genetic predisposition to colon cancer disease among Saudi. These findings should be considered in light of a number of limitations because of the small number of subjects that limit the statistical power. Moreover, we cannot exclude the possibility of misclassifications of disease status, since the controls were not rigorously examined for the absence of colon cancer. Despite these limitations, the study design was relatively strong because the controls were recruited from the same cohort as the colon cancer patients. Also, the cases and controls have been matched by age and gender.

In conclusion, our result showed no association between the C-180G SNP and the serum resistin concentrations and suggests that the high resistin level in colon cancer patients may play an important role in colon cancer development.

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