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Apelin-12 levels in Obese Patients with Colon Cancer

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Abstract

Objective: The aim of the present study was to evaluate serum concentrations of apelin-12in relation with metabolic profile of patients with colon cancer.

Material and Methods: A total of 59 patients with colon cancer and 60colonoscopy-negative controls were eligible for the study. Clinicopathologic features and metabolic profile as well as apelin-12 levels were evaluated in each subject.

Results: Obese men with colon cancer exhibited higher serum concentrations of apelin-12compared with controls ($P=0.05$) and obese women ($P=0.004$). On the other hand, the obese women with colon cancer showed lower serum concentrations of apelin-12 than obese controls ($P=0.04$). Apelin levels were significantly correlated positively with body mass index ($P=0.03$) in non-obese patients. Regarding the colon cancer stages, the analysis showed an inverse correlation between serum apelin levels and the stages in obese males (r= -0.67, $P=0.05$).

Conclusion: The higher level of apelin-12 serum in obese male with colon cancer speculates that this adipocytokine may be a predictive or an important risk factor for colon cancer development.

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Introduction

Cancer is the third foremost cause of death worldwide [1,2]. There is no proper early detection and treatment method available relative to various cancers [4]. Colon cancer is an aggressive disease that continues to have a daunting impact on global health. It is the second cause of cancer death with 30% inheritance base [4]; in fact, half of all patients diagnosed with this invasive cancer would ultimately die [5,6]. To improve prognosis and reduce morbidity and mortality, a recent review has suggested a modification of diet,alteration of lifestyle and an early detection [7].

In recent years, several researchers have focused on obesity as a risk factor for colon cancer. Obesity and pathogenesis of cancer may share same metabolic and biological pathways. The relationship between body fat deposition and the pathogenesis of cancer has been the subject of many studies; however, no clear consensus has emerged linking these two biological processes. Although an excess of adipose tissue characterizes obesity, adipose tissue is as an endocrine organ, secretes various adipokines. Their existence was confirmed in 1994 through the identification of leptin [8]. Adipokines are characterized by a spectrum of local, peripheral, and central effects [9]. At the level of adipose tissue, adipokines contribute to the modulation of adipogenesis, immune cell migration into adipose tissue, adipocyte metabolism and function [10,11,12]. At the level of the whole body, adipokines modulate and regulate different biological processes in target organs including the brain, liver, muscle, vasculature, heart and pancreatic β-cells [10,12,13]. Adipose tissue is unique in generating a large number of adipokines. The secretion pattern of these adipokine reflects adipose tissue function. The list of molecules identified as adipokines has grown over recent years; one of the newly discovered adipokines is apelin.

Apelin is a peptide expressed in various tissues, including gastrointestinal tract, heart, lung, liver, and bone [14,15]. It has been reported in experimental and clinical studies that this bioactive protein stimulates proliferation and migration of retinal endothelial cells and is required to normal vascular development [14,16]. Apelin has been shown as a potentially important proangiogenic factor in cancers [14-17]. To date, several active apelin forms have identified, including apelin-36, -17, and -13 as well as the pyroglutamated isoform of apelin-13, which is produced by a post-translational modification of glutamine to pyroglutamine at the Nterminus [18].

The potential role of apelin-12 in colon cancer and their influence on cancer progression are not entirely explained. The aim of the present study was the investigation of (a) possible relationship between colon cancer and level of serum apelin-12 and (b) correlation of serum apelin-12 with clinical and blood parameters of cancer patients.

Material and Methods

Subjects

The present study included 59 Saudi colon cancer patients (29 females and 30 males) and 60 age and gender matched colonoscopy-negative controls (30 females and 30 males). The case group was selected from patients diagnosed with colon cancer via colonoscopy and confirmed by tissue biopsy at King Abdul Aziz University Hospital in Jeddah, KSA, between January 2010 and December 2012. Control group was selected from subjects visited the same hospital for routine screening and diagnosed as free from colon cancer and had normal blood pressure, blood sugar level, and were not under any treatment course. All participants gave their informed consent before enrollment in the study. The local ethical committee at KAU approved this study.

Anthropometric measurements

Standard methods were used to measure height, weight, waist circumferences (WC), and hip circumferences (HC). Body weight was measured with light clothing on, with up to 0.1kg precision. Height was measured up to 0.1cm precision. Body mass index (BMI) was calculated as weight (kg) divided by height in meters squared (m2). Waist-to-hip ratio (WHR) was also calculated as WC divided by HC.

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Biochemical measurements

Blood samples were collected in the morning after an overnight fast and after centrifugation (3500g for 15 min) serum samples were stored at -20°C until assayed. Biochemical parameters were determined in fasting status. In order to measure serum glucose, after collecting the samples in the morning after an overnight fast, the serum was separated and immediately analyzed. Serum glucose was determined by the glucose oxidase method. Serum concentrations of total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) were measured using routine enzymatic methods with commercial kits purchased from Human Diagnostics (Wiesbaden, Germany). Low-density lipoprotein cholesterol (LDL-C) levels were calculated using the Friedewald formula: (LDL-C= TC-HDL-C-TG/5 mg/dl). Serum apelin concentration was determined by using nonselective apelin-12 enzyme immunoassay kit (Phoenix Pharmaceuticals, Inc., USA). This test kit is effective in the range of 0.0 to 100 ng/mL. Duplicate measurements were performed in a single experiment.

Classification of the colon cancer patients

Patients with colon cancer were divided into four groups according to the size of tumors and status of lymph node metastasis. Histological grading of colon cancer was based on the Duke's classification (A, B, C, and D stages).

Statistical analysis

Data are presented as means ± S.D. Comparisons between two groups were made by the Mann-WhitneyU test. The correlation of serum apelin-12 level with anthropometric parameters and metabolic parameters was analyzed by Spearman's rank order correlation (non-parametric test). Differences with a P-value <0.05 were considered significant. All statistical analyses were carried out using the SPSS for Windows V16.0 (SPSS Inc., Chicago,Il., U.S.A).

Activation of both receptors occurs by binding of their respective ligand and results in activation of the same downstream signaling pathways, including beside others the Mitogen-Activated Protein Kinase (MAPK)-pathway and the Akt-pathway [23]. The therapeutic effect of both antibodies was determined by evaluation of the expression and phosphorylation level of selected key mediator proteins from MAPK-pathway (ERK 1/2) and Akt-pathway (Akt, mTOR and p70S6K) using the Meso Scale Discovery (MSD®) platform. The resulting phosphorylation status was considered as indicator for pathway activity. To gain a deeper view in treatment induced regulations of signaling protein isoform phosphorylation, we used the NanoPro™1000 technology in samples of three patients. In addition, tissue slices were analyzed by immunohistochemistry (IHC) assays to evaluate the EGF and IGF-1R receptor status, the phosphorylation pattern of Akt and ERK1/2 (MAPK) and to prove tissue viability by determination of the expression level of proliferation marker Ki67.

Results

Comparison between the patients and the control group

The physical and biochemical characteristics for the groups, including the serum concentration of apelin-12 are summarized in Table 1 and 2. When the patients and the controls were further divided into obese (BMI ≥ 25kg/m^2) and non-obese (BMI <25kg/m^2), Obese men with colon cancer exhibited higher serum concentrations of apelin-12 compared with both non-obese patients (P= 0.01, 0.85±0.76ng/ml vs. 0.29±0.24ng/ml, respectively)) and obese controls (P=0.05, 0.85±0.76ng/ml vs. 0.57±0.93ng/ml, respectively) (Figure 1). The results also showed that the obese men with colon cancer revealed higher level of apelin-12 than obese women with colon cancer (P=0.004, 0.85±0.76ng/ml vs. 0.26±0.24ng/ml, respectively). On the other hand, the obese women with colon cancer showed lower serum concentrations of apelin-12 than obese control women (P = 0.04, 0.26±0.24ng/ml vs. 0.72±0.85ng/ml, respectively) (Figure 2).

Variables	Patients							
	Female Mean ± SD			P^1	Male Mean ± SD			P^1
	N=10 BMI <25	N=19 BMI ≥ 25			N=18 BMI ≥ 25		N=12 BMI ≥ 25	
Age (years)	49.60± 15.28	54.53± 10.99		0.54	52.39± 11.61		58.25± 9.95	0.12
Height (cm)	158.60± 8.42	154.89± 7.89		0.40	165.11± 9.01		166.17± 81.77	0.88
Weight (kg)	51.50± 11.23	72.69± 8.71		0.0001**	59.89± 8.72		81.77± 7.66	0.0001**
BMI (kg/m^2)	20.32± 3.04	30.43± 4.35		0.0001**	21.88± 1.83		29.74± 3.79	0.0001**
Waist (cm)	43.25± 19.23	71.42± 28.33		0.008*	65± 23.89		86.37± 18.67	0.68
Hip (cm)	41.50± 15. 76	77.17± 31.67		0.001**	58.40± 23.64		85.75± 39.90	0.01*
WHR	1.09± 0.50	0.96± 0.36		0.54	1.2± 0.52		1.18± 0.57	0.57
Cholesterol (mg/dl)	215.80± 72.42	169.95± 41.42		0.05*	197.11± 69.99		214.17± 72.17	0.82
Triglyceride (mg/dl)	153.30± 56.07	165.95± 100.86		0.77	198.67± 112.50		231± 115.44	0.39
HDL (mg/dl)	53± 24.33	52.79± 10.62		0.73	55.05± 17.76		62.17± 28.43	0.57
LDL (mg/dl)	132.1± 74.15	83.96± 29.97		0.03*	103.32± 62.15		110.07± 49.51	0.52
Total lipid (mg/dl)	7.48± 0.53	7.06± 0.48		0.02*	7.24± 0.72		7.4± 0.57	0.75
Apelin (ng/ml)	0.35± 0.40	0.26± 0.24		0.76	0.29± 0.24		0.85± 0.76	0.01*
	0.02 Min	1.29 Max	0.002 Min 0.84 Max		0 . 0 1 Min	0.87 Max	0.18 Min	2.11 Max

Table 1: Characteristics of the non-obese and the obese female and male patients.
[1]Mann Whitney U Test,
* $P \leq 0.05$ Significant, ** $P \leq 0.001$ Highly significant.

Variables	Control							
	Female[a] Mean ± SD			Male[b] Mean ± SD				P[1]
	N=11 BMI <25		N=19 BMI ≥ 25		N=6 BMI <25		N=24 BMI ≥ 25	
Age (years)	44.82± 16.09		50.32± 13.80	0.33	48± 18.15		46.13± 12.70	0.74
Height(cm)	160.04± 10.61		156.92± 8.14	0.67	167.83± 4.44		169.94± 6.71	0.63
Weight(kg)	55.77± 6.04		82.29± 13.47	0.0001**	63.17± 7.33		88.14± 17.33	0.0001**
BMI(kg/ m^2)	21.94± 2.25		32.79± 4.89	0.0001**	21.38± 1.99		30.48± 5.67	0.0001**
Waist (cm)	57.64± 25.80		95.81± 2 4.51	0.001**	65.17± 28.81		75.29± 32.64	0.40
Hip(cm)	78.68± 28.49		102.55± 35.56	0.007*	67.67± 32.17		83.62± 37.50	0.27
WHR	0.83± 0.29		1.11±0.77	0.27	0.97± 0.10		0.93± 0.20	0.23
Cholesterol(mg/dl)	194.09± 44.40		206.79±52.35	0.58	169.33± 29.17		178.87± 41.36	0.53
Triglyceride(mg/dl)	77,82± 20.34		134.74± 70.54	0.003*	116.83± 62.57		205.08± 142.62	0.10
HDL(mg/dl)	57.91± 14.75		60.95±31.47	0.64	43.83± 5.88		43.08±8.67	0.67
LDL (mg/dl)	117.44± 37.87		119.53±29.10	0.87	102.13± 24.72		93.36±33.27	0.46
Total lipid (mg/dl)	7.31± 0.42		7.46±0.33	0.52	7.17± 0.38		7.18±0.53	0.67
Apelin (ng/ml)	0.72± 0.99		0.72±0.85	0.77	0.35± 0.085		0.57±0.93	0.59
	Min 0.068	Max 3.62	Min 058	Max 3.29	Min 0.253	Max 0.51	Min 0.016	Max 3.79

Table 2: Characteristics of the non-obese and the obese female and male controls.
[1]Mann Whitney U Test.
* $P \leq 0.05$ Significant , ** $P \leq 0.001$ Highly significant.

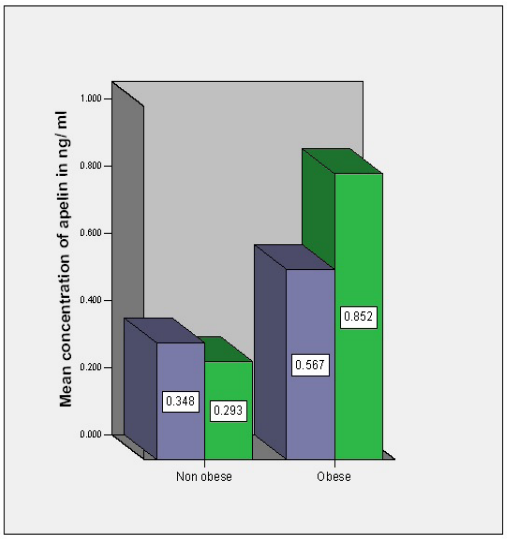

Figure 1: Mean apelin levels in male patients and controls according to the BMI weight state categories.

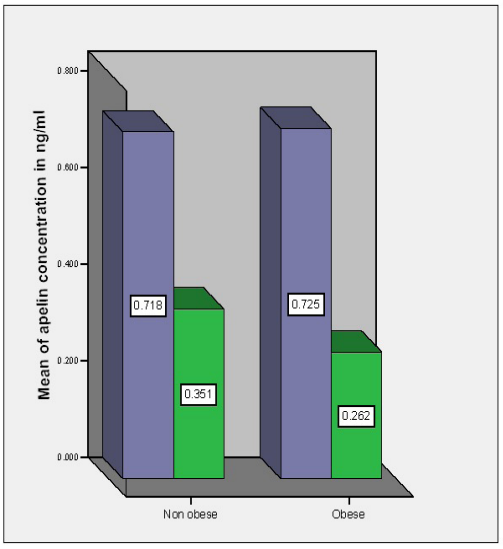

Figure 2: Mean apelin levels in female patients and controls according to the BMI weight state categories.

Serum apelin-12 levels in different clinical stages of colon cancer

The mean apelin-12 level for patients in A+B and C+D stages are summarized in Table 3. In comparing apelin-12 levels in the stages, obese males showed significant difference (P=0.04, 1.5±0.70ng/ml vs. 0.58±0.52ng/ml, respectively) in apelin-12 levels. Also the analysis showed an inverse correlation between serum apelin levels and the stages in obese males (r= -0.67, P=0.05).

Relationship between apelin-12 and other variables

The present study investigated whether serum apelin-12 level has

		Serum Apelin Mean± SD		P[1]
		Dukes' Stages A+B	Dukes' Stages C+D	
Females	Non- obese	0.38± 0.17	0.34± 0.49	0.38
	Obese	0.22± 0.13	0.31± 0.36	0.80
Males	Non- obese	0.29± 0.23	0.22± 0.18	0.64
	Obese	1.5± 0.70	0.58± 0.52	0.04*

Table 3: Serum apelin-12 levels in different clinical stages of colon cancer.
[1]Mann Whitney U Test.
- $P \leq 0.05$ Significant , ** $P \leq 0.001$ Highly significant.

any relationship with age, BMI, WHR, waist, hip, and lipid profiles. In non-obese female patients, serum apelin-12 level showed significant correlation with LDL ($P = 0.01$) and cholesterol (P=0.02). In non-obese male patients, serum apelin-12 level showed significant correlation with BMI (P=0.03), LDL (P=0.002), cholesterol (P=0.004) and total lipids (P=0.03). No significant correlations between apelin-12 and the other variables in the obese and the non-obese controls for both genders.

Discussion

The main function of adipose tissue is to store energy. In addition, as an endocrine organ, it secretes a variety of adipocytokines like apelin. In this study, we evaluated the association of serum apelin-12 level with the progression of colon cancer through a case-control study for Saudi females and males of newly diagnosed, untreated colon cancer. The observed result indicated that serum apelin-12 is not involved with tumor stage progression. The higher level of apelin-12 serum in obese male with colon cancer speculates that this adipocytokine may be a predictive or an important risk factor for colon cancer development in men. The present study is one of few reports to evaluate serum apelin-12 level in colon cancer patients and its involvement with tumor stage progression.

Our results are consistent with previous studies that suggested a relationship between apelin and colon carcinogenesis [19,20]. Apelinergic signalling has been implicated in the growth of colon cancer, with apelin expression upregulated in half of colon adenocarcinomas[20]. Moreover, both apelin and the apelin receptorare expressed in LoVo cells, a colorectal cancer cell line; in these cells, apelin-13 administration prevents apoptosis by inactivating a caspase pathway, while the aforementioned antagonistic apelin-13 mutant F13A significantly reduces cellular proliferation [20]. The serum apelin concentration was measured in patients with other cancers and adenomas. Apelin has been shown as a potentially important proangiogenic factor in cancers. Diakowska et al., observed that apelin levels increased significantly in tumor tissues and serum apelin was significantly higher in patients with gastroseophageal than in the controls [21]. Heo et al., have demonstrated a link between apelin expression and poor prognosis in human non-small cell lung cancer and a correlation between strong apelin expression and tumor recurrence and poor prognosis. They suggested that apelin directly affected oral cancer proliferation and migration through an autocrine mechanism and apelin receptor was expressed in oral cancer tissues and cell lines. Therefore, the multifaceted role of apelin in tumorigenesis may result from the differences in APJ expression in target cells [15]. Berta et al. concluded that apelin is an angiogenic factor in human non-small cell lung cancer. Moreover, it also provides the first evidence for a direct association of apelin expression with clinical outcome in a human cancer [16]. Kasai et al. observed that apelin signaling regulated pathologic retinal vascularization in cooperation with vascular endothelial growth factor or fibroblast growth factor [22]. Sorli et al.observed that apelin stimulates proliferation and is required to normal vascular development. They suggested that the apelin gene expression wasupregulated in a variety of human solid tumors [14].On the other hand, Yener et al. found no significant differences between the circulating apelin levels of patients with non-functioning adrenal adenomas and control cases [23]. This result shows similarity with our obese female patients.

Although apelin expression is physiologically regulated by insulin, growth hormone, TNF-a andhypoxia in several tissues [23], changes in apelin level in obese men with colon cancer could provide additional information in discriminating early stage cases. The result of our study suggests that apelin may be good biomarkers of colon malignant potential independently from anthropometric characteristics. Whether the change in this adipocytokine level is the result and/or effects of colon cancer development should be investigated further. Nonetheless, larger cohorts in different populations are warranted to replicate our findings and clarify the gender-associated differences.

In conclusion, we have investigated serum concentrations of apelin-12 in relation with metabolic profile of patients with colon cancer. Our finding speculates that the higher level of apelin-12 serum in obese male with colon cancer may be a predictive or an important risk factor for colon cancer development.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Both the authors substantially contributed to the study conception and design as well as the acquisition and interpretation of the data and drafting the manuscript.

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