Implication of visfatin levels in patients with colon cancer

Rowyda N. Al-Harithy

Department of Biochemistry, King Abdul-Aziz University, Jeddah, Saudi Arabia
Correspondence to: Dr. Rowyda N. Al-Harithy
P. O. Box 40288 Jeddah 21499, Kingdom of Saudi Arabia
Fax: 00966-2-6393640, E-mail: dr.alharithy@gmail.com

Abstract

Objective: Adipocytokines have been reported to contribute to the pathogenesis of colon cancer, yet few studies have evaluated these markers. Visfatin, an adipocytokine, is thought to play a role in the pathogenesis of metabolic-syndrome-related cancers. The aim of this study was to assess the association of visfatin level with the progression of colon cancer through a case-control study.

Methods: Serum visfatin levels were measured in 188 Saudi female subjects (90 colon cancer patients and 98 controls) by using enzyme-linked immunosorbent assay (ELISA) method. The patient and control group were matched on age, body mass index, waist-to-hip ratio, race, and gender. Colon cancer patients were categorized into four groups based on Duke’s classification (A, B, C, and D with N= 22, 20, 23, and 25; respectively). The levels of serum visfatin, anthropometric parameters and metabolic parameters were compared between patients and controls and between patients of different clinical stages of colon cancer.

Results: Mann-Whitney test was used for comparing patient and control group and showed that patients with colon cancer had significantly higher circulating visfatin levels than the control group (5.64±2.6 vs. 1.87±1.7 ng/ml, P< 0.0001). Kruskal Wallis test was used to compare the levels of visfatin in different clinical stages of colon cancer and the result showed no significant differences in the levels of visfatin between the colon cancer stages (P=0.95). Non-significant associations with anthropometric and metabolic parameters were observed for visfatin, except that visfatin level had a significant correlation with HDL (P=0.054).

Conclusions: The observed result indicates that visfatin is not involved with tumor stage progression. However, the increased levels of visfatin in patients with colon cancer strengthen the results of previous studies and speculated that this adipocytokine may be a predictive or an important risk factor for colon cancer development. Further studies are needed to elucidate the role of serum visfatin levels in colon cancer.

Key words: Visfatin, colon cancer, Saudi patients.

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Introduction

Colon cancer is a life-threatening disease. In Saudi Arabia, colon cancer occurrence ranks third among females (Al-Eid and Manalo, 2006). The incidence of colon cancer in the Kingdom of Saudi Arabia (KSA) has been on a constant rise over the past few years (Ibrahim et al., 2008). Research studies have indicated several risk factors that may increase a person's chance of developing colon cancer, but the disease still needs more investigation in its prevention and therapy. There are many known factors that increase or decrease the risk of colon cancer; some of these factors are modifiable and others are not. Abdominal obesity, that is related to the accumulation of visceral fat, has been associated with increased risk of some type of cancers (Schapira et al., 1990; Folsom et al., 1990), including colon cancer (Giovannucci et al., 1995; Murphy et al., 2000; Ceschi et al., 2007).

Metabolic changes resulting from imbalances in hormones such as adipocytokines that are derived from white adipose tissue are linked to colon cancer (Tulubas et al., 2013). Visfatin is an adipokine identified in 2004 and accordingly named for the suggestion of its high levels of expression in visceral fat cells (Fukuhara et al., 2005). Visfatin gene sequencing has been revealed to correspond to previously identified cytokine named Pre-B-cell colony-enhancing factor (PBEF), described in 1994 as a cytokine produced by lymphocytes (Samal et al., 1994). Visfatin was also known as the formerly described Nicotinamide phosphoribosyltransferase (Nampt), the limiting enzyme in nicotinamide adenine dinucleotide (NAD) biosynthesis. Thus, it is able to regulate cellular levels of NAD⁺, exerting an influence on cell energy metabolism and the activity of NAD⁺/NADH dependent enzymes (Manolescu et al., 2008).

Visfatin is not a fat-specific protein; it is released predominantly from macrophages rather than from adipocytes in visceral adipose tissue (Curat et al., 2006). In this regard, there is sufficient evidence to consider that visfatin is expressed by the macrophages infiltrating adipose tissue and is produced in response to inflammatory signals (Curat et al., 2006; Varma et al., 2007). Dendritic cells and colonic epithelial cells are also sources of visfatin (Moschen et al., 2007). In this regard, visfatin was considered as a proinflammatory adipocytokine (Alexander et al., 2006; Curat et al., 2006; Varma et al., 2007). This protein is also involved in pathophysiological angiogenesis including adipose tissue angiogenesis. Kim and his colleagues demonstrated that visfatin activates migration, invasion, and tube formation in human umbilical vein endothelial cells (HUVECs). They also showed that visfatin induces activation of the extracellular signal-regulated kinase 1/2 (ERK1/2) in endothelial cells, which is closely linked to angiogenesis. Their results revealed that visfatin promotes angiogenesis via activation of mitogen-activated protein kinase ERK-dependent pathway (Kim et al., 2007).

The discovery of this adipokine has the potential to enhance our understanding of metabolic-syndrome-related cancers. The biological role of visfatin is still illusive. Association between visfatin and several medical conditions such as metabolic syndrome, insulin-mimetic effect, obesity, and diabetes are still controversial (Zhong et al., 2008; Fukuhara et al., 2005; Revollo et al., 2007; Ye et al., 2005; Berndt et al., 2005; Haider et al., 2006A; Haider et al., 2006B; Dogru et al., 2007; Toruner et al., 2009; Hammarstedt et al., 2006; Takebayashi et al., 2007; Lopez et
The level of visfatin in visceral fat and subcutaneous fat is also controversial (Terra et al., 2012; Sandeep et al., 2007; Berndt et al., 2005; Araki et al., 2008). With the aforementioned, the association of visfatin level with the progression of colon cancer through a case-control study was the aim of the presented project.

Materials and Methods

Subjects
The present study included 188 Saudi female subjects (90 colon cancer patients and 98 age, body mass index (BMI), waist-to-hip ratio (WHR), race, and gender-matched healthy controls). 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 2009 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.

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. BMI is used to reflect the total body fat, while WC and WHR are indirect measurements of body fat centralization.

Biochemical measurements
Blood samples after a 12-hour overnight fasting were collected and then the serum was immediately separated and stored at −20°C to evaluate fasting blood glucose, triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol (TC), insulin, and visfatin. Serum visfatin was measured by a commercial visfatin C-terminal enzyme-linked immunosorbent assay kit (ALPCO Diagnostics, Salem, NH) using the microplate reader (sensitivity 30pg/ml). The intra- and inter-assay coefficient of variation (CV) was 5.04% and 6.67%, respectively. The test kit is effective in the range of 0.073 to 4.8ng/ml with observed value of 80-120%. Duplicate measurements were performed in a single experiment. Lipid profiles were measured by an automatic biochemistry analyzer (Hitachi HS 2003m, Japan). This research was approved by the local ethical committee.

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. Mann-Whitney test was used for comparing the patient and the control group. To compare visfatin levels between tumor stages, Kruskal Wallis test was performed. The correlation of serum visfatin with anthropometric parameters and metabolic parameters was analyzed by Spearman Rank order 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, Ill., U.S.A).

Results

Comparison between the patients and the control group
There was no significance difference in age, height, weight, BMI, WHR, cholesterol, HDL, and total lipid between the patient and the control group (Table 1). There was a statistically significant difference in waist ($P=0.01$), hip circumference ($P=0.03$), and triglycerides level ($P=0.009$) between the two groups. When serum visfatin levels were compared in cases and controls, the serum visfatin levels were significantly higher in the colon cancer group ($P=0.0001$) (Table 1). In the patient group, mean visfatin level was 5.64±2.6 and 1.87±1.7 in the control group.

Serum visfatin levels in different clinical stages of colon cancer
The result showed that the mean visfatin level for patients in A, B, C, and D stages were 4.9, 5.3, 4.7, and 4.9 ng/ml, respectively. In comparing visfatin levels in the stages, the result showed that there in no significant differences ($P=0.95$).

Relationship between visfatin and other variables
The present study investigated whether there was any relationship between the serum visfatin levels, age, BMI, WHR, waist, hip, and lipid profiles. No significant correlations with anthropometric and metabolic parameters were observed for serum visfatin level, except for HDL ($P = 0.054$).

Discussion
In the present study, the association of visfatin level with the progression of colon cancer through a case-control study for Saudi females was evaluated. The observed result indicates that visfatin is not involved with tumor stage progression. The higher level of visfatin serum in patients with colon cancer strengthen the results of previous studies and speculates that this adipocytokine may be a predictive or an important risk factor for colon cancer development. The present study is one of few reports to evaluate visfatin level in colon cancer patients and its involvement with tumor stage progression.

Adipocytokines produced by adipose tissue have been the subject of intense investigation as novel risk markers not only of metabolic syndrome but also for
cancer (Petridou et al., 2000; Hsing et al., 2001; Miyoshi et al., 2003; Petridou et al., 2003; Stattin et al., 2004; Goktas et al., 2005; Wei et al., 2005). Visfatin is an insulin mimetic adipocytokine, which directly interacts with the insulin receptor as insulin-like growth factor receptor, and can subsequently promote cancer cell proliferation (Fukuhara, et al., 2005; Tilg and Moschen, 2008). Visfatin contributes to the generation of nicotinamide adenine dinucleotide (NAD) biosynthesis and also affects cellular metabolism, cell life span and longevity (Bi and Che, 2010). Several studies have identified changes in the circulatory levels of visfatin in diseases. Notably among them are obesity, diabetes mellitus, kidney diseases and bone disorders.

Table 1: Comparison between the patient and the control group

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients Mean ± SD</th>
<th>Controls Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>52.0±11.7</td>
<td>53.9±13.8</td>
<td>0.52</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>158.1±9.1</td>
<td>155.4±7.9</td>
<td>0.25</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71.6±15.9</td>
<td>65.3±14.1</td>
<td>0.11</td>
</tr>
<tr>
<td>BMI (kg/cm²)</td>
<td>28.9±6.8</td>
<td>26.8±6.4</td>
<td>0.24</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>84.6±30.8</td>
<td>62.0±29.2</td>
<td>0.01*</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>93.8±34.7</td>
<td>70.3±33.4</td>
<td>0.03*</td>
</tr>
<tr>
<td>WHR</td>
<td>1.01±0.7</td>
<td>0.95±0.3</td>
<td>0.62</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>202.1±50.1</td>
<td>185.5±57.4</td>
<td>0.28</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>114.5±64.4</td>
<td>166.1±87.1</td>
<td>0.009**</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>60.1±26.4</td>
<td>53.7±16.4</td>
<td>0.29</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>119.7±34.2</td>
<td>98.5±54.9</td>
<td>0.12</td>
</tr>
<tr>
<td>Total lipid (mg/dl)</td>
<td>7.41±0.37</td>
<td>7.20±0.53</td>
<td>0.11</td>
</tr>
<tr>
<td>Visfatin (ng/ml)</td>
<td>5.64±2.6</td>
<td>1.87±1.7</td>
<td>0.0001**</td>
</tr>
</tbody>
</table>

*Significant, **highly significant

WHR: waist-to-hip ratio, BMI: body mass index, HDL: high-density lipoprotein, LDL: low-density lipoprotein.

Values were represented as the mean ± standard deviation.

The relationship between visfatin and colon cancer has been established, therefore Nakajima and his group suggested that it could be considered to be a new and promising biomarker of colorectal cancer (Nakajima et al., 2010). In the few published articles about visfatin serum level in colon cancer patients, there is no consensus result. A study reported that visfatin level in the malign group was not statistically significantly different from those in the benign group (Kosova et al., 2013), while others indicated that increased serum visfatin is associated with the incidence of colon cancer (Chen et al., 2013; Fazeli et al., 2013; Tuluba et al., 2013). The result of this study is in agreement with the studies that found increased levels of visfatin in patients with colon cancer than in the control group. This study confirms that patients with colon cancer are characterized with higher level of visfatin. The data postulate that higher visfatin concentrations in colon
cancer could probably serve as an early marker with prognostic value for the later development of the colon cancer. Chen et al. divided the patients into two subgroups early and advanced cancer and showed that increased level of visfatin was a strong risk factor for both early and advanced colorectal cancer in Chinese patients (Chen et al., 2013). Fazeli and his group suggested that visfatin may has a potential role in the development of colorectal cancer through mechanisms other than the direct mechanisms that are active in the association between obesity and colorectal cancer (Fazeli et al., 2013). Tuluba and his team implied that visfatin might play an important role in colon carcinogenesis (Tuluba et al., 2013). In addition, to explore the link between visfatin level and the progression of colon cancer, the levels of visfatin were compared between all the clinical stages and showed no significant differences. To our knowledge, there has not been any report comparing the level of visfatin between the clinical stages of colon cancer. This analysis may provide additional information in regard to the role of visfatin in colon cancer and the use of visfatin as a therapeutic target.

In the presented study, the analysis of visfatin levels documented no correlation with anthropometric parameters and metabolic parameters, except HDL among patients. Chen and his group observed that visfatin is not related to most anthropometric parameters and most parameters of metabolic syndrome, except HDL and LDL levels in female subjects (Chen et al., 2007). They suggested that visfatin might play a role in cholesterol homeostasis in women. Fukuhara et al. (2005) demonstrated that plasma visfatin levels correlated strongly with the visceral fat area and weakly with the subcutaneous fat area in male and female subjects. Because waist circumference and WHR are good surrogates of visceral fat, it is expected that visfatin level correlate with waist circumference and WHR. However, previous reports and this study have not found this correlation except a very recent project done be Chen et al. (2007) where they found a significant correlation with WHR among colorectal cancer patients and controls. The reason of this discrepancy is unclear. These findings may contribute to our current knowledge about visfatin in metabolic syndrome. Lipid profiles are also an important component of metabolic syndrome. The analysis showed that only HDL was associated with visfatin levels in female patients. This result and the result reported by Chen et al. (2007) in a Taiwanese population showed a correlation with HDL levels in female subjects and imply a role of visfatin in homeostasis of lipid in females. However, the mechanism is currently unknown.

These findings should be considered in light of a number of limitations due to the relatively small sample size of subjects that limit the statistical power. Moreover, the possibility of misclassifications of disease status cannot be excluded, since the controls were not thoroughly 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, BMI, WHR, and sex.

In conclusion, visfatin is not involved with tumor stage progression and did not correlate with most anthropometric and metabolic parameters. The result of this study confirms previous findings that patients with colon cancer are characterized with higher level of visfatin and consistent with other studies linking visfatin with cancer states. Since no conclusive evidence exists so far with regards to the role of
visfatin in colon cancer, more molecular, physiological and clinical studies are needed to determine the role of visfatin in the etiology and pathogenesis of colon cancer.

References


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