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Serum Leptin Hormone as an Indicator of Bad Prognosis in Colon Cancer Patients

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Abstract

Background: Leptin has been linked to the pathology of several types of cancers related to obesity, particularly colon cancer. This could be related to leptin' s influence on the equilibrium of specific intracellular mechanisms that control cellular growth, differentiation, apoptosis, neovascularization and invasiveness thus participating in the pathophysiology of colon cancer growth and metastasis. Additionally, ghrelin is a gut peptide secreted from the fundus of gastric mucosa and adiponectin is an adipocytokine released from adipose tissue and their low levels in obese subjects have been linked to an increased risk of development of colon cancer.

Subjects and methods: Forty (40) patients were enrolled from Cairo University hospitals and included in this study beside the control group which comprised 20 age and sex-matched healthy subjects. Patients were divided into two groups: Group 1: Included 20 patients suffering from colon cancer (stage II-A) without lymph node involvement or distant metastasis. Group 2: Included 20 patients suffering from colon cancer (stage III-C) with lymph node involvement but no distant metastasis. Serum Leptin, ghrelin and adiponectin were measured in all patients using a radioimmunoassy technique.

Results: Serum leptin levels were significantly higher in colon cancer patients compared to that of control subjects ($p<0.001$). Serum leptin levels were also significantly higher in stage II-A patients as compared to stage III-C ($p<0.001$). Serum ghrelin and adiponectin levels were found to be significantly lower in colon cancer patients compared to the control subjects ($p<0.001$). Moreover, serum ghrelin and adiponectin levels were found to be significantly lower in patients belonging to stage III-C compared to stage II-A ($p<0.001$). A negative correlation was noted between seum leptin levels and both serum ghrelin and adiponectin levels in colon cancer patients enrolled. **Conclution:** Serum leptin levels could serve as a good prognostic marker in colon cancer patients in addition to serum ghrelin and adiponectin levels to predict the severity and the development of colon cancer metastasis.

Keywords : Serum leptin, serum ghrelin, serum adiponectin, colon cancer, lymph node, matastasis.

Introduction

During the past ten years, adipose tissue has been considered not only as a tissue responsible for calorie storage, but as one of the vital endocrine tissues in the body as well [(1)]. Many cytokines called adipocytokines are produced and secreted by adipose tissue. Leptin is the most available adipocytokine . It is a 16-kDa hormone that plays a key role in regulating energy intake and expenditure, including appetite and hunger thus playing a major part in regulating appetite and satiety and modifying food consumption, energy storage and body mass index [(2)]. Leptin also makes a significant contribution to lipid and glucose metabolism, reproductive, adrenal, and thyroid functions, cardiovascular system, immunity and brain functionality [(3)].

Leptin has been incriminated in the pathology of several types of cancers linked to obesity, particularly colon cancer [(4)]. This could be related to leptin's influence on the equilibrium of specific intracellular mechanisms that control cellular growth, differentiation, apoptosis and neovascularization, thus participating in the pathophysiology of cancer [(5)].

Leptin promotes the production and secretion of several pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin-1 β (IL-1β) and chemokine (C-X-C motif) ligand 1 (CXCL1) in humans, which have been linked to colon carcinogenesis [(6)]. Leptin (mediated by the effect of CXCL1) promotes vascular endothelial growth factor (VEGF) activity by epithelial cells, and thus provides a tool for tumor-associated angiogenesis, nurturing

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tumor existence and proliferation [7]. Therefore, by enhancing angiogenesis, leptin accelerates tumor growth and metastasis of adjacent organs [8].

Leptin affects many cellular signal transduction pathways, and can act as an important gene expression modulator. The genes *AKT1, STAT3* and *MCL1* are up-regulated at the early stage of colon cancer, and on the other hand the gene *STAT5B* is quelled [9].

Additionally, differences in the gene expression profile between early and late stages of colon cancer suggest that leptin plays a role in the dynamic and variable structure of the malignant tumor. Leptin may consequently be partly accountable for tumor progression by means of transcription activation, repression or silencing [8].

Ghrelin is a growth-hormone-releasing acylated peptide. It is a 28 amino acid hormone produced by P/D_1 cells lining the fundus of the human stomach. It is known to have a variety of metabolic functions that range from stimulation of gastric acid and regulation of digestive tract motility to regulation of energy storage and modulation of appetite [10]. In some respects, it can be considered as the opposite of the hormone leptin; leptin is known to suppress the appetite. In rats and mice, systemic or central application of ghrelin increases food intake and increases fat mass as a result of its action at the hypothalamus [11]. In contrast to leptin, the satiety hormone, ghrelin plays a role at the beginning of a meal with ghrelin blood levels rising before and falling after food consumption [10].

The physiological role of ghrelin is understood to be greater than simply its effect on metabolism; studies advocate that ghrelin is expressed in human T lymphocytes and monocytes and acts through the growth hormone secretagogue receptor (type 1a) to inhibit the expression of the pro-inflammatory cytokines interleukin 1β, interluekin 6, and tumor necrosis factor-α [12] thus having the opposite effect to leptin on the risk of development of gastrointestinal tumors such as colon cancer and subsequent metastasis to distant organs.

Colon cancer has been proven to be an obesity-related disease, and adiponectin is one of the important adipocytokines that is also important to be considered in colon cancer patients. Adiponectin is secreted by adipocytes and consists of 244 amino acids that represent a full 30 kDa-long protein hormone. The gene for Adiponectin is located at chromosomal band 3q27 [13] . There is little information in literature about adiponectin and all of it has been obtained in the past ten years. A study carried out in 2005 suggested that a decrease in plasma adiponectin concentration was associated with the development of colon adenoma in Japanese patients [14]. Later in a prospective case control study it was suggested that the risk of colorectal cancer is higher in patients with low adiponectin plasma levels [15]. A third study on a mouse model, showed that adiponectin depressed colorectal carcinogenesis and leads the way for further investigations [16].

Aim of the work

The aim of the present study is to determine if measuring serum leptin could be of any prognostic value in patients with stage II-A colon cancer (a model of non-metastatic colon cancer) and patients with stage III-C colon cancer (a model of colon cancer spreading to adjacent lymph nodes just before the occurrence of distant metastasis). The present study also aims at investigating the inter-relationship between serum leptin in colon cancer patients enlisted and other adipocytokines suspected of having an influence on colon cancer development and metastasis such as serum gherlin and adiponectin.

Subjects and Methods

Subjects:

Forty (40) colon cancer patients were enrolled from Cairo University Hospitals and included in this study beside the control group which comprised 20 age and sex-matched healthy subjects not complaining from any disease related to the present study. Mean patient age was 55.6 ± 14 years. The patient group comprised 20 males and 20 females. Patients were divided into two groups:

Group 1 **:**Included 20 patients suffering from colon cancer (stage II-A) without lymph node involvement or distant metastasis selected according to the TNM staging system from the American Joint Committee on Cancer (AJCC) [17].

Group 2**:** Included 20 patients suffering from colon cancer (stage III-C) with metastasis to 4 or more regional lymph nodes but with no

distant metastasis selected according to the TNM staging system from the AJCC.

Methods:

All patients and controls were subjected to the following:

Clinical Evaluation: full history recording included age, sex, any medications taken including the standard chemotherapeutic regimens followed in the patient groups. Pulse monitoring and blood pressure measurement were carried out as well as a meticulous clinical examination for each subject. Weight and height were measured using the standard technique. BMI was calculated as body weight (kg) divided by height in meters squared (Kg/m^2). The patients enrolled (20 colon cancer patients in each group) were all obese with a mean BMI of 33.5 ± 3.2.

Staging of colon cancer was done using the TNM (for tumours/nodes/metastases) system, from the AJCC. The TNM system (T= tumour, N= lymph node, M= distant metastasis) assigns a number based on three categories.

T categories for colorectal cancer:

Tx: `No description of the tumour's extent is possible because of incomplete information.

Tis: The cancer is in the earliest stage (in situ). It involves only the mucosa. It has not grown beyond the muscularis mucosa (inner muscle layer).

T1: The cancer has grown through the muscularis mucosa and extends into the submucosa.

T2: The cancer has grown through the submucosa and extends into the muscularispropria (thick outer muscle layer).

T3: The cancer has grown through the muscularis propria and into the outermost layers of the colon or rectum but not through them. It has not reached any nearby organs or tissues.

T4a: The cancer has grown through the serosa (also known as the visceral peritoneum), the outermost lining of the intestines.

T4b: The cancer has grown through the wall of the colon or rectum and is attached to or invades into nearby tissues or organs.

N categories for colorectal cancer

N categories indicate whether or not the cancer has spread to nearby lymph nodes and, if so, how many lymph nodes are involved. To get an accurate idea about lymph node involvement, most doctors recommend that at least 12 lymph nodes be removed during surgery and looked at under a microscope.

Nx: No description of lymph node involvement is possible due to incomplete information.

N0: No cancer in nearby lymph nodes.

N1: Cancer cells are found in or near 1 to 3 nearby lymph nodes.

- **N1a:** Cancer cells are found in 1 nearby lymph node.
- **N1b:** Cancer cells are found in 2 to 3 nearby lymph nodes.
- **N1c:** Small deposits of cancer cells are found in areas of fat near lymph nodes, but not in the lymph nodes themselves.

N2: Cancer cells are found in 4 or more nearby lymph nodes.

- **N2a:** Cancer cells are found in 4 to 6 nearby lymph nodes.
- **N2b:** Cancer cells are found in 7 or more nearby lymph nodes.

M categories for colorectal cancer

M categories indicate whether or not the cancer has spread (metastasized) to distant organs, such as the liver, lungs, or distant lymph nodes.

M0: No distant spread is seen.

M1a: The cancer has spread to 1 distant organ or set of distant lymph nodes.

M1b: The cancer has spread to more than 1 distant organ or set of distant lymph nodes, or it has spread to distant parts of the peritoneum (the lining of the abdominal cavity).

Stage grouping

Once a person's T, N, and M categories have been determined, usually after surgery, this information is combined in a process called *stage grouping*. The stage is expressed in Roman numerals from stage I (the least advanced) to stage IV (the most advanced). Some stages are subdivided with letters.

Stage 0

Tis, N0, M0: The cancer is in the earliest stage. It has not grown beyond the inner layer (mucosa) of the colon or rectum. This stage is also known as *carcinoma in situ* or *intramucosal carcinoma*.

Stage I

T1-T2, N0, M0: The cancer has grown through the muscularis mucosa into the submucosa (T1) or it may also have grown into the muscularis propria (T2). It has not spread to nearby lymph nodes or distant sites.

Stage II-A

T3, N0, M0: The cancer has grown into the outermost layers of the colon or rectum but has not gone through them (T3). It has not reached nearby organs. It has not yet spread to the nearby lymph nodes or distant sites.

Stage II-B

T4a, N0, M0: The cancer has grown through the wall of the colon or rectum but has not grown into other nearby tissues or organs (T4a). It has not yet spread to the nearby lymph nodes or distant sites.

Stage II-C

T4b, N0, M0: The cancer has grown through the wall of the colon or rectum and is attached to or has grown into other nearby tissues or organs (T4b). It has not yet spread to the nearby lymph nodes or distant sites.

Stage III-A

One of the following applies.

T1-T2, N1, M0: The cancer has grown through the mucosa into the submucosa (T1) and it may also have grown into the muscularis propria (T2). It has spread to 1 to 3 nearby lymph nodes (N1a/N1b) or into areas of fat near the lymph nodes but not the nodes themselves (N1c). It has not spread to distant sites.

T1, N2a, M0: The cancer has grown through the mucosa into the submucosa (T1). It has spread to 4 to 6 nearby lymph nodes (N2a). It has not spread to distant sites.

Stage III-B

One of the following applies:

T3-T4a, N1, M0: The cancer has grown into the outermost layers of the colon or rectum (T3) or through the visceral peritoneum (T4a) but has not reached nearby organs. It has spread to 1 to 3 nearby lymph nodes (N1a/N1b) or into areas of fat near the lymph nodes but not the nodes themselves (N1c). It has not spread to distant sites.

T2-T3, N2a, M0: The cancer has grown into the muscularis propria (T2) or into the outermost layers of the colon or rectum (T3). It has spread to 4 to 6 nearby lymph nodes (N2a). It has not spread to distant sites.

T1-T2, N2b, M0: The cancer has grown through the mucosa into the submucosa (T1) or it may also have grown into the muscularis propria (T2). It has spread to 7 or more nearby lymph nodes (N2b). It has not spread to distant sites.

Stage III-C

One of the following applies:

T4a, N2a, M0: The cancer has grown through the wall of the colon or rectum (including the visceral peritoneum) but has not reached nearby organs (T4a). It has spread to 4 to 6 nearby lymph nodes (N2a). It has not spread to distant sites.

T3-T4a, N2b, M0: The cancer has grown into the outermost layers of the colon or rectum (T3) or through the visceral peritoneum (T4a) but has not reached nearby organs. It has spread to 7 or more nearby lymph nodes (N2b). It has not spread to distant sites.

T4b, N1-N2, M0: The cancer has grown through the wall of the colon or rectum and is attached to or has grown into other nearby tissues or organs (T4b). It has spread to at least one nearby lymph node or into areas of fat near the lymph nodes (N1 or N2). It has not spread to distant sites.

Stage IV-A

Any T, Any N, M1a: The cancer may or may not have grown through the wall of the colon or rectum, and it may or may not have spread to nearby lymph nodes. It has spread to 1 distant organ (such as the liver or lung) or set of lymph nodes (M1a).

Stage IV-B

Any T, Any N, M1b: The cancer may or may not have grown through the wall of the colon or rectum, and it may or may not have spread to nearby lymph nodes. It has spread to more than 1 distant organ (such as the liver or lung) or set of lymph nodes, or it has spread to distant parts of the peritoneum (the lining of the abdominal cavity) (M1b).

All patients involved in the study were staged after a resection-anastomosis surgery of the colon including a safety margin and biopsy followed by histopathology of the resected portion of the colon affected by the tumour as well as lymph nodes removed during the operation.

Laboratory assessment: Venous blood sampling was performed after a 12h fasting then patients were subjected to the following:

1. Routine complete blood picture**:** was carried out using an electronic Coulter counter (Cobas, Roch Inc. Switzerland) according to the standards specified by Cheng *et al* [18].
2. Routine complete liver and kidney function tests were also undertaken for each participant in this work.
3. **Serum Leptin assessment:** The level of serum leptin was analysed in all subjects

enrolled by radio- immune assessment using recombinant human leptin (Leptin- Human Ria-CT) according to the method described by Feldkamp and Smith [19].

4- Serum Gherlin assessment:

The level of serum gherlin was evaluated in all subjects joining in the study by using serum immune-reactive ghrelin that was measured in duplicates by a radioimmunoassay involving ^{125} I-labelled Ghrelin and a Ghrelin antiserum to determine the level of active Ghrelin in serum by the double antibody/PEG technique. This was done according to the method described by Feldkamp and Smith [19].The lower and upper limits of detection were 24 and 740 pmol per liter.

5-Serum adiponectin assessment:

The level of serum adiponectin was estimated in all subjects joining in the study by using serum immune-reactive adiponectin that was measured in duplicates by a radioimmunoassay that utilizes ^{125}I-labeled murine adiponectin and a multispecies adiponectin rabbit antiserum to determine the level of adiponectin in serum by the double antibody/PEG technique. The adiponectin standards were prepared using recombinant human adiponectin and were used to determine the circulating levels of adiponectin in human serum samples. This was done according to the method described by Feldkamp and Smith[19].

Statistical analysis

Statistical analysis was performed using Statistica *v.* 10 software. Quantitative results were expressed as mean ± SD. T-test or the Mann-Whitney U test was used to compare quantitative variables in the two qualitative groups and ANOVA or the Kruskal Wallis test was used in more than two groups. Pearson test and Spearman's rho test were used to assess the correlation of two quantitative variables. *P* value <0.05 was considered as statistically significant. *P* value <0.01 was considered as statistically highly significant. All statistical analysis were performed according to Snedcor and Cochran [20].

Results

A very highly significant difference ($p<0.01$) in serum leptin levels was found between colon cancer patients enlisted in this study and healthy control subjects. Also a highly significant difference ($p<0.01$) was found between colon cancer patients without metastasis (stage II-A) and those with metastasis (stage III-C). These results are illustrated in table 1 as well as figure 1.

Table (1): Mean ±SD and P values of serum leptin, gherlin and adiponectin levels in control subjects, colon cancer patients without lymph node metastasis (stage II-A) and colon cancer patients with lymph node metastasis (stage III-C)

	Controls (n=20)	Colon Cancer without metastasis (n=20)	Colon Cancer with metastasis (n=20)
Mean Serum Leptin Level (ng/ml) ± SD	8.6±0.8	25.2 ± 2.8	30.1 ± 4.3
P value	<0.001**		
Mean Serum Gherlin Level (pmol/l) ± SD	324.1± 14.2	229.05± 12.4	156± 11.5
P value	<0.001**		
Mean Serum Adiponectin Level (pmol/l) ± SD	8.1± 0.9	6.2± 0.7	2.5± 0.4
P value	<0.001**		

SD: standard deviation, P: probability, p< 0.05 = *Significant, p<0.01 = highly significant.**

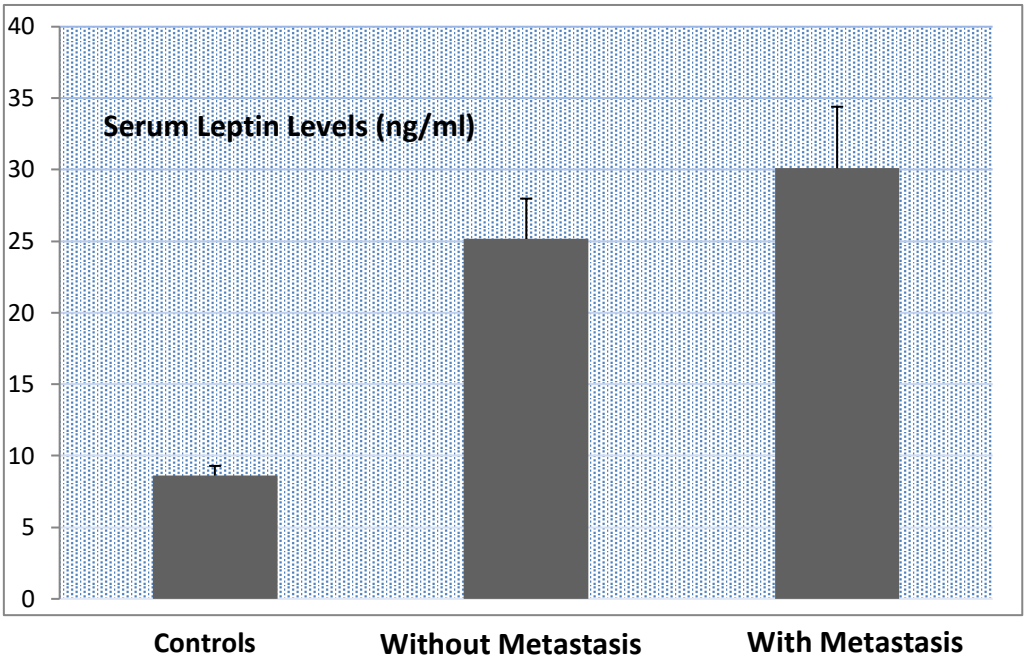

Figure 1: Mean ±SD serum leptin levels (ng/ml) in controls, colon cancer patients without lymph node metastasis (stage II-A) and those with lymph node metastasis (stage III-C).

In addition, a very highly significant difference ($p<0.01$) in serum gherlin levels was observed between colon cancer patients enrolled in this study and healthy control subjects. A highly significant difference ($p<0.01$) was also found between colon cancer patients without metastasis (stage II-A) and those with metastasis (stage III-C). These results are illustrated in table 1 as well as figure 2.

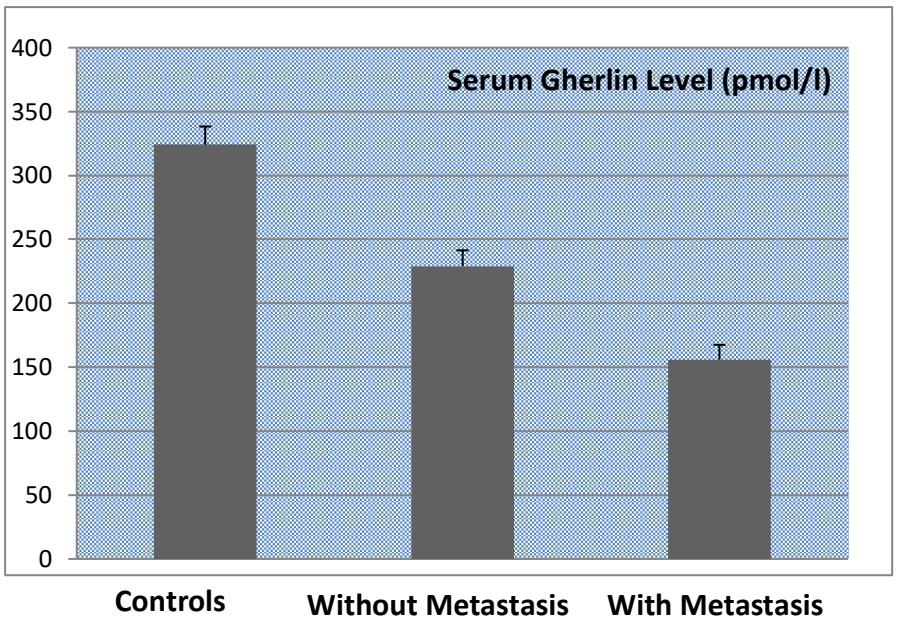

Figure 2 : Mean ±SD serum gherlin levels (pmol/l) in controls, colon cancer patients without lymph node metastasis (stage II-A) and those with metastasis (stage III-C) respectively.

A negative correlation was observed between seum leptin and serum gherlin levels in the two groups of colon cancer patients enrolled in the study. ($r = - 0.50$, $r^2 = 0.25$). This correlation is shown in figure 3.

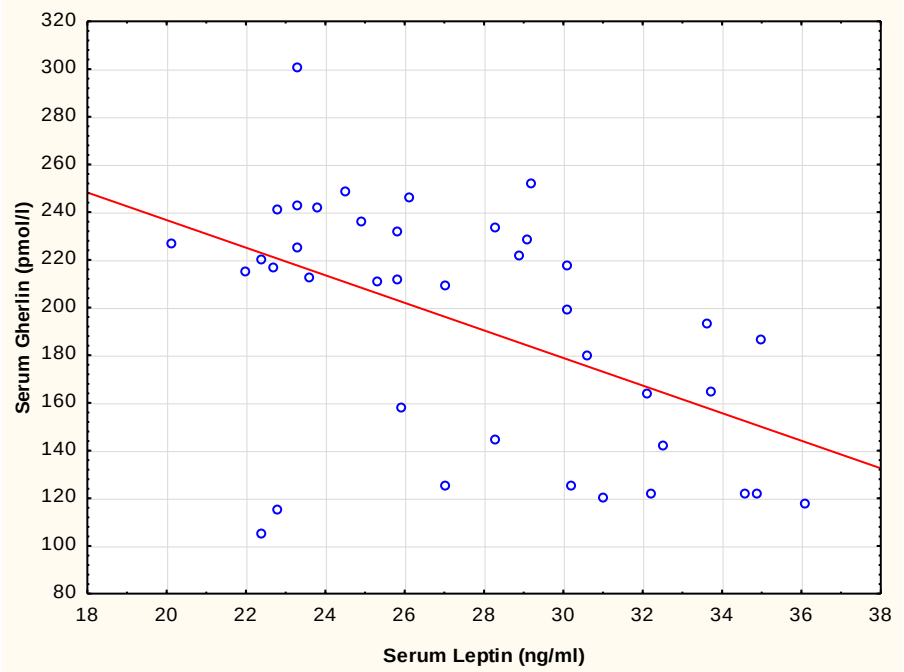

Figure 3: Correlation between serum leptin (ng/ml) and ghrelin levels (pmol/l) in colon cancer patients.

A very highly significant difference ($p<0.01$) in serum adiponectin levels was found between colon cancer patients enrolled in this study and control subjects. Also a highly significant difference ($p<0.01$) was found between colon cancer patients without metastasis (stage II-A) and those with metastasis (stage III-C). These results are illustrated in table 1 as well as figure 4.

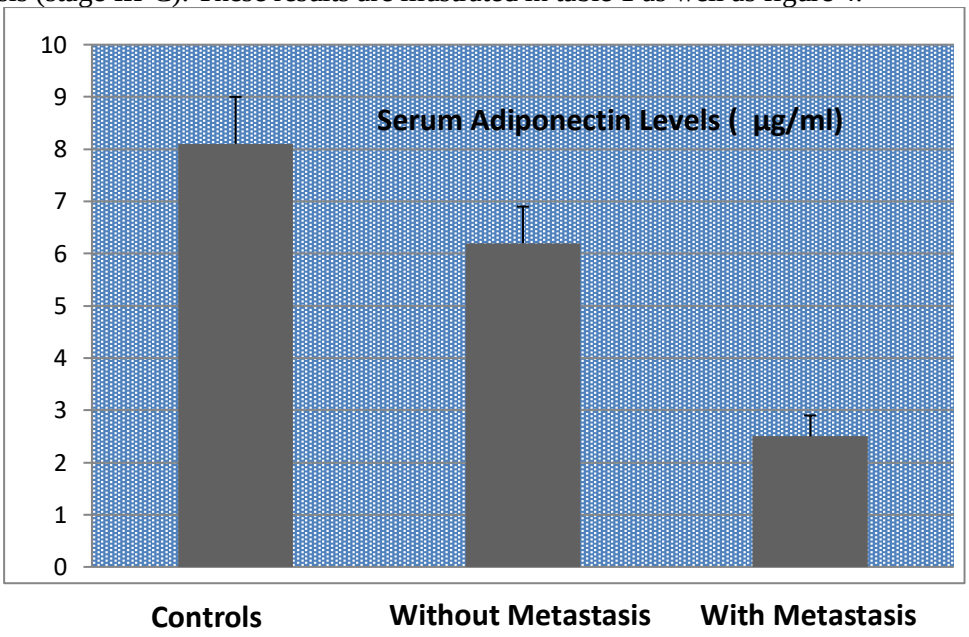

Figure 4 : Mean ± SD serum adiponectin levels (µg/ml) in controls, colon cancer patients without lymph node metastasis (stage II-A) and those with lymph node metastasis (stage III-C).

A negative correlation was observed between serum leptin and serum adiponectin levels in the two groups of colon cancer patients enrolled in the study. ($r = - 0.51$, $r^2 = 0.26$). This correlation is shown in figure 5.

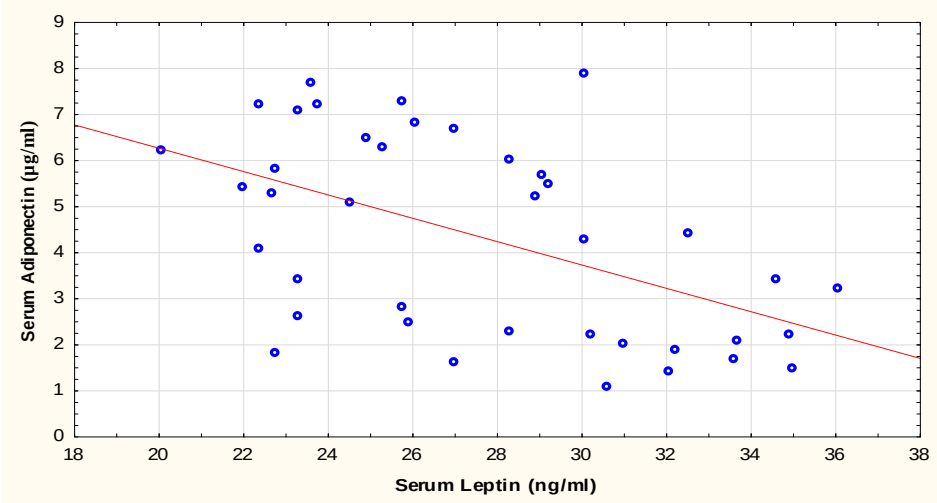

Figure 5: Correlation between serum leptin (ng/ml) and adiponectin levels (µg/ml) in colon cancer patients.

Discussion

Colorectal cancer is considered the second leading cause of cancer-related deaths in many countries worldwide including the United States. Early diagnosis, nevertheless, could in many cases lead to a better prognosis. Almost all colon cancers start in glands in the lining of the colon and rectum. However; colon cancer is usually diagnosed at advanced stages, when it is usually fatal [21].

Excess body weight, as defined by the body mass index (BMI), has been linked to several diseases and includes subjects who are overweight (BMI≥25-29.9 kg/m^2) or obese (BMI≥30 kg/m^2). Around 11% of colorectal cancer (CRC) cases have been attributed to overweight and obesity in Europe. Epidemiological data suggest that obesity is associated with a 30-70% increased risk of colon cancer in men, whereas the association is less consistent in women. The relative risk of colorectal cancer of obese patients is about 1.5 times higher than in normal-weight individuals and obesity is also associated with premalignant colorectal adenoma. Visceral fat, or abdominal obesity, seems to be of greater concern than subcutaneous fat obesity, and any1 kg/m^2 increase in BMI confers additional risk. Obesity might be associated with worse cancer outcomes, such as recurrence of the primary cancer or mortality. Several factors, including reduced sensitivity to anti-angiogenic-therapeutic regimens, might explain these differences [22]. Comstock *et al* [23] documented that obesity is a key risk factor for the development of colon cancer in white obese males.

In the present study, the patients belonging to the two groups selected (20 colon cancer patients each) were all obese with a mean BMI of 33.5 ± 3.2.

Aleksandrova *et al* [24] estimated the extent to which biomarkers with inflammatory and metabolic actions mediate the association of adiposity measures, waist circumference (WC) and body mass index (BMI), with colon cancer in men and women.

It is hypothesized that obesity is a chronic low grade inflammatory process that results in increased secretion of products from adipose tissue that include leptin, interleukin-6, interleukin-17, tumor necrosis factor-alpha, and associated decreased blood levels of ghrelin and adiponectin that appear to have a shielding effect against the development of several types of cancer, including colon cancer. These products induce malignancy-related metabolic alterations in colon cancer cells leading to metabolic syndrome, insulin resistance and modifications in levels of adipocytokines that seem to be of great importance [25].

The leptin hormone can modulate several important functions of the gastrointestinal tract. It interacts with the vagus nerve and cholecystokinin to delay gastric emptying and has a complex effect on motility of the small bowel. Leptin modulates absorption of macronutrients in the gastrointestinal tract differentially in physiologic and pathologic states. In physiologic states, exogenous leptin has been shown to decrease carbohydrate absorption

and to increase the absorption of small peptides by the PepT1 di-/tripeptide transporter. In certain pathologic states, leptin has been shown to increase absorption of carbohydrates, proteins, and fat. The hormone has been shown to be upregulated in the colonic mucosa in patients with inflammatory bowel disease. Leptin stimulates gut mucosal cell proliferation and inhibits apoptosis. These functions have led to speculation about the role of leptin in tumorigenesis in the gastrointestinal tract, which is complicated by the multiple immunoregulatory effects of this hormone [26].

Kemik *et al* [27] investigated the inter-relationship between leptin, associated cytokines and the development of colon cancer to elaborate these suspected links. The study found significantly higher serum C- reactive protein (CRP), interleukin 1α (IL-1α), IL-1β, IL-6, IL-8, IL-10, tumour necrosis factor α (TNF-α), midkine, vascular endothelial growth factor-A (VEGF-A), VEGF-C, VEGF receptor 1 (VEGFR1) and leptin in colon cancer patients. These factors are known to promote chronic inflammatory processes and enhanced cell growth and proliferation together with increased angiogenesis culminating into enhanced carcinogenesis.

Miyoshi *et al* [28] also hypothesized that leptin plays a pivotal role in the pathogenesis of colorectal cancer (CRC).

The present study showed a very highly significant statistical increase in serum leptin levels in colon cancer patients compared to normal healthy controls ($p< 0.001$). These results were very similar to those of Guadagni *et al* [29] and Chia *et al* [30] who noted that leptin levels were significantly higher in colon cancer patients compared to healthy control subjects. Yet, these results were contradictory to those of Arpaci *et al* [31] who found significantly lower serum levels of leptin in patients with colorectal cancer. They explained this discrepancy with other studies by suggesting that the increased weight of colon cancer patients in the earlier stages of the disease was not the sole factor in determining their serum leptin levels and that other factors could be involved. Similarly, Kosovva *et al* [32] also speculated that leptin levels in colon cancer patients were not statistically significantly different from those in the benign group.

Elevated serum leptin levels have been incriminated in colon cancer growth and development of metastasis to lymph nodes or distant metastasis [33]. Tutino *et al* [34] documented that high circulating levels of leptin receptor occur in patients with advanced stage of colon cancer, suggesting a role of leptin in cancer progression and aggressiveness. The results of a study done by Erkasap *et al* [35] suggested a role of leptin on the progression of colon carcinoma to metastatic disease without weight loss.

The present study included two groups of patients. Group 1 included 20 patients who were diagnosed as stage II-A colon cancer (without lymph node involvement or distant metastasis) while group 2 included 20 patients with stage III-C colon cancer with 4 or more lymph nodes involved. The selection of these patients was based on the study's aim to compare colon cancer patients who did not have lymph node involvement and those who did as regards serum leptin levels in order to predict the increased risk of lymph node metastasis and eventual distant metastasis using this hormone as a prognostic marker. The results showed that serum leptin levels were significantly higher in patients with stage III-C colon cancer compared to stage II-A colon cancer patients ($p<0.001$). These results are in agreement with those of Wang *et al* [36] who found significantly higher serum leptin levels in stage III colon cancer patients compared to those belonging to stage II.

Ghrelin is a metabolism-regulating hormone recently investigated for its role in cancer survival and progression. Moreover, low ghrelin levels observed in obese people may be implicated in cancer development and progression. Limited data are currently available on the effects exerted by ghrelin on intracellular proteolytic pathways in cancer. Both the lysosomal and the proteasomal systems are fundamental in cellular proliferation and apoptosis regulation. Preliminary *in vitro* fluorimetric assays have evidenced for the first time a direct inhibition of 20S proteasomes by ghrelin, particularly evident for the trypsin-like activity [37].

Many studies have pointed out a possible role of gut peptides, such as ghrelin, in the pathogenesis of gastrointestinal malignancies including colon cancer which is one of the most common death causes in the western world [38].

The present study showed that there was a statistically significant difference in serum ghrelin levels of the patients *vs.* the controls, with the patients having much lower levels ($p<0.001$). These results are confirmed Legakis *et al.*[39] who found that colon cancer patients had significantly lower circulating levels of ghrelin than healthy controls.

The role of low serum levels of ghrelin in enhancing cell proliferation and apoptosis has been illustrated, however; studies also implicate that low levels of ghrelin may influence the cancer cells motility or ability to metastasize. D'Onghia *et al* [38] found that ghrelin plays a role in protecting against colon cancer metastasis.

The results of the present study showed that serum ghrelin levels were significantly lower in patients with stage III-C colon cancer compared to stage II-A colon cancer patients ($p<0.001$) showing a possible protective role for ghrelin against colon cancer lymph node affection and metastasis.

A negative correlation was observed between serum leptin levels and serum gherlin levels in the patients enrolled in this study. A similar study done by Kemik *et al* [40] also established a negative correlation between serum leptin and ghrelin in colon cancer patients. On the other hand, such a negative correlation could not be observed in another study carried out by Wolf *et al.*[41] who investigated serum leptin and ghrelin levels in colon cancer patients. This discrepancy may be attributed to the fact that the patients enrolled in the latter study were belonging to a more advanced stage of colon cancer and were suffering from cachexia with disturbed leptin/ghrelin balance.

Another adipocytokine investigated in this study was adiponectin. This hormone has some known effects on the metabolic process such as gluconeogenesis, glucose uptake, lipid β-oxidation, triglyceride clearance, protection from endothelial dysfunction, insulin sensitivity and weight loss [42].

Recent studies have indicated a significant correlation between the reduced plasma adiponectin levels (associated with obesity) and the increased risk of various cancers. Additionally, these studies have also demonstrated some of the antiangiogenic and antitumoral effects of adiponectin [43]. Endometrial, breast, prostate, colon, pancreatic cancer, and more recently non-small cell lung cancer and esophageal cancer have been found to be correlated with low serum adiponectin levels.

Adiponectin exerts its effects via 5′ adenosine monophosphate-activated protein kinase (AMPK). Increased concentrations of adenosine monophosphate (AMP), calcium-dependent kinases and Ser/Thr liver kinase B1 (LKB1) contribute to AMPK activation which in turn interferes with cellular growth signaling through mammalian target of rapamycin (mTOR) thus inhibiting the promotion of carcinogenesis. AMPK also promotes growth arrest and apoptosis via increased p53 and p21 expression, respectively [44]. In contrast, the findings of a study done by Song *et al* (2013) [45] supported a positive role for adiponectin in colorectal carcinogenesis in men. They documented an association of plasma adiponectin and soluble leptin receptor (sOB-R) with colorectal cancer .

The present study showed that there was a highly significant statistical difference in serum adiponectin levels of the colon cancer patients *vs.* the controls with the patients having much lower levels ($p<0.001$). These results are in accordance with other studies by Gulcelik *et al* [46] and Guadagni *et al* [9] that demonstrated significantly lower levels of adiponectin in colon cancer patients compared to control subjects.

In addition, adiponectin via AMPK pathway causes inhibition of tumor cell adhesion and migration in general thus inhibiting the metastasis of many types of tumours [47]. The results of the present study showed that serum adiponectin levels were significantly lower in patients with stage III-C colon cancer compared to stage II-A colon cancer patients ($p<0.001$) showing a possible sheilding role for adiponectin against colon cancer lymph node affection or metastasis. These results are in agreement with another study by Gialamas *et al* [48] that demonstrated that serum adiponectin levels and tissue expression of adiponectin receptors are associated with risk, stage, and grade of colorectal cancer.

Data of the present work speculated a negative correlation between serum leptin levels and serum adiponectin levels in the patients joining in this study. This result is in accordance with that of Gauadagni *et al* [7] who

demonstrated that leptin inversely correlated with adiponectin in colon cancer patients.

The results of the present study highlighted important alterations occurring in serum adipocytokines, (leptin and adiponectin) in addition to the gut peptide ghrelin in colon cancer patients. It could be concluded from the striking features of this study that serum leptin levels could serve as a good prognostic marker to predict the clinical outcome of colon cancer and subsequent occurrence of metastasis in patients especially as it is the most readily available adipocytokine secreted by adipocytes. In addition to this, measuring serum levels of this hormone could also help clinicians decide whether or not to start aggressive therapy at an earlier stage before the occurrence of lymph node involvement or metastasis depending on the predictive value of leptin hormone. Serum ghrelin and adiponectin hormones are the counterparts of leptin and their determination in serum could also be used in conjunction with serum leptin level determination to predict clinical outcome and prognosis in these patients. However; further investigations are required to add more information on the relationship between serum leptin levels and colon cancer staging.

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