Adipocytokine levels in benign prostate hyperplasia and prostate cancer patients

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Abstract
The occurrence of prostate cancer in men is one of the most common types of cancer. Recent studies have found important links between cancer and adipocytokines. Adipocytokines are thought to be factors in the occurrence of a variety of diseases. In addition, adipocytokines studies in cancer patients have shown that these hormones may have an effect in the formation of cancer. The objective of this study was to evaluate the relationship between adiponectin, resistin, and leptin levels in BHP and prostate cancer patients. The study was conducted between September 2012 and April 2013 at the Department of Medical Biochemistry and the Department of Urology. Blood samples were collected from 20 people in the same age range who had been diagnosed by examination and biopsy as having BHP (benign prostatic patients) and prostate cancer patients who had not been operated on. Leptin, adiponectin, resistin, and human serum levels were measured using ELISA kit.

In the prostate cancer group, serum adiponectin and resistin levels were significantly lower than in the BHP group. However, serum leptin levels in the prostate cancer group were not significantly different from those in the BHP group. This information and our own findings show that adiponectin and resistin, which are from the adipocytokine family, may play an important role in the progression of prostate cancer, and thus it may be possible to use them as diagnostic markers. Therefore, similar studies should be considered with a greater number of patients at different stages.

Keywords: Benign prostatic hyperplasia, prostate cancer, adipocytokines, leptin, adiponectin, resistin

Introduction
Prostate carcinoma is the most commonly seen internal malignancy in men, and is the second commonest cause of cancer-related death. Hormonal factors are important in the development of this disease, which generally affects men over 50 years of age [1]. Adipocytokines are a group of adipose tissue derived hormones which were discovered at the beginning of the 1990s with the recognition of the first member of the family, leptin [2]. One classification of these hormones reflects their physiological roles. According to this classification, adipocytokines can be divided into two groups: resistin, “insulin resistance inducing factors” such as TNF-α and interleukin 6, and leptin, adiponectin and the recently recognized “insulin sensitive factors” such as visfatin [3].

Those which are most evident in the adipose tissue are leptin and adiponectin. Even though leptin is not a classical cytokine, various immune cells including polymorphonuclear leucocytes, monocytes, macrophages and lymphocytes carry leptin receptors, and their activity can be changed by leptin [4,5]. Leptin shows certain structural similarities to classic cytokines such as IL-6, GM-CSF or IL-12 [6]. Peripheral leptin has been recognized as having effects in many areas such as glucose and lipid metabolism, angiogenesis, blood pressure regulation and the formation of bone mass.

Adiponectin is the second best known of the adipocytokines secreted by the fat cells, but unlike leptin it has many useful and protective effects. Among these are anti-inflammatory, vasculoprotective and anti-diabetic effects [7]. It has been observed that the relationship between adiponectin and TNF-α are complementary [8]. Also, it is thought that adiponectin initiates its effect by inhibiting the NFKB pathway [8].

Resistin was given its name because of the resistance which it showed to insulin [9]. Resistin itself causes the secretion of TNF-α, IL-12 and many pro-inflammatory cytokines from macrophages and monocytes [10]. It shows its pro-inflammatory effect by inhibiting NF-kB [10].

One of the important effects on the human body of the hormones produced by the adipose tissue is in lipid and carbohydrate metabolism. One commonly held view is that they could be one of the factors in the causal relationship between endocrine dysfunction in the adipose tissue and insulin resistance/diabetes. Even though the mechanism has not been explained, it has been shown in epidemiological studies that obesity is a significant risk factor in the development of cancer. In many recent studies, hormones
produced by the adipose tissues have been shown to have a significant effect on tumor stroma and the development and proliferation of the malignant cells within it [11]. Most studies have shown that adiponectin potentializes the development of cancer cells in in vitro conditions, and it is thought that adiponectin may have a countering effect. There is a need for further research to decide whether adipose tissue has a direct effect on obesity-caused carcinogenesis. It has been known for a long time that there is a connection between obesity and some types of cancer [12], and therefore it is not surprising that researchers would investigate the likely role of adipocytokines in the regulation of carcinogenesis as another link between cancer and obesity [13].

In recent studies, significant links have been found between cancer and adipocytokines. It is thought that adipocytokines have an effect in the development of certain illnesses, and studies of adipocytokines in cancer patients have shown that these hormones may have an effect on cancer formation [14,15]. In this study we aimed to evaluate the relationship between adiponectin, resistin, and leptin levels in BHP and prostate cancer patients.

Materials and Methods

This consecutive prospective study was conducted at the Department of Medical Biochemistry and the Department of Urology. The study was approved by the hospital ethical committee (20478486-18, 16.01.2013).

Between September 2012 and April 2013, 20 males (age 63.55±8.10 years) from the patients referred to the outpatient clinics Department of Urology with PCa diagnosed by transrectal ultrasound-guided biopsy were enrolled in the study as the patient group. An additional 20 men (age 62.00±7.30 years) with clinically benign prostatic obstruction, normal prostate-specific antigen (PSA) levels (less than 2.5 ng/mL), and normal digital rectal examination findings, were enrolled in the study (Table 2).

All the patients and the volunteers involved in the study gave their informed consent. Trained physicians performed recruitment of subjects by convenience sampling at the outpatient Department of Urology. Malign and benign patients with diabetes mellitus, hypertension, and those who had been taking hormonal therapy such as thyroxin derivates, androgen replacement therapy, and medications for chronic diseases were excluded. Blood samples were taken from all patients.

**Group-1**—benign (before treatment) patient group (n=20)
**Group-2**—malign (before treatment) patient group (n=20).

**Assay**

Blood samples were kept at -80 °C until analysis. All samples from each patient were run in the same assay. This ELISA is a sandwich type enzyme-linked immunoassay consisting of primary (mouse anti-adipocytokine monoclonal) antibody-coated plate, secondary (rabbit anti-human adipocytokine polyclonal) antibody, detection (HRP-conjugated goat anti-rabbit IgG) antibody, substrate for HRP and an (recombinant human) standard. Adiponectin, resistin, and leptin levels were measured using Elisa kits (Millipore Corporation, Billerica, MA, USA) and the results were obtained in nanograms per milliliter.

**Statistics**

Nonparametric methods were performed in the cross-sectional analysis of biomedical data (Mann–Whitney U test). Two-tailed probability (p) values were calculated and statistical significance was defined as p<0.05. All analyses were performed using the statistical software SPSS 15.00.

**Results**

Age, gender (all of the subjects were male), weight, and height were not significantly different between the BHP patients and the prostate cancer group subjects (Table 2).

In the BHP group the mean adiponectin, resistin, and leptin levels were 45.66±8.06 nmol/mL, 5.68±0.54 ng/mL, and 7.31±1.1 ng/mL respectively. In the prostate cancer group, the mean adiponectin, resistin and leptin levels were 37.07±9.25, 4.71±0.36, and 7.11±2.8 respectively (Table 1).

**Table 1.** Adiponectin, Resistin, and Leptin levels in benign prostate hyperplasia (BHP) and prostate cancer patients

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Adiponectin (ng/mL)</th>
<th>Leptin(ng/mL)</th>
<th>Resistin (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate Cancer</td>
<td>20</td>
<td>37.07±7.06</td>
<td>7.11±1.25</td>
<td>4.71±0.39</td>
</tr>
<tr>
<td>BPH</td>
<td>20</td>
<td>45.66±9.75*</td>
<td>7.31±1.54</td>
<td>5.68±0.47*</td>
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<td>* p&lt;0.05</td>
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**Table 2.** Demographic characteristics of benign prostate hyperplasia (BHP) and prostate cancer patients

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<thead>
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<th>Groups</th>
<th>Age</th>
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<tbody>
<tr>
<td>Prostate Cancer</td>
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<tr>
<td>BPH</td>
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<td>20</td>
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In the prostate cancer group, serum adiponectin and resistin levels were significantly decreased but serum leptin was not significantly different from the benign prostate hyperplasia group (p<0.05).

**Discussion**

Prostate carcinoma, as well as being the commonest internal malignity seen in men, is also the second most common cause of cancer-related death. Hormonal factors are important in its development. It is generally a disease of men over the age of 50, and with advancing age symptomless microscopic incidental carcinoma foci can be seen at autopsy at a rate of up to 70% (1). Microscopically, a large proportion of prostate carcinomas are of the adenocarcinoma type, and may develop along a spectrum
Adipocytokines are cytokines produced from the mature adipocytes and pre-adipocytes of the white fatty tissue, and are formed from the polypeptide growth factor group. The tumor microenvironment was recognized as an important role in promoting tumor initiation, progression and metastasis. Adipose constitute major part of tumor microenvironment, and many exciting researchers reported adipose tissue adjacent to the tumor make significant contributions to cancer initiation and progression [18,19]. Adipose tissue around tumor, which is termed as cancer associated adipose, could secrete adipocytokines not only have a systemic effect through secretion in the serum and communication with distant site, but also influence tumor aggressive and metastasis by local paracrine [20].

Adipocytokines such as TNF-α, IL-6, hepatocyte growth factor, adiponectin, leptin, resistin, visfatin and apelin are cytokines secreted from the visceral adipose tissue, and their connection to metabolic syndromes is a very recent discovery. Many epidemiological studies have shown a positive correlation between an increase in the risk of cancer in various tissues (breast, prostate, endometrium and colorectum) and obesity. It has been suggested that adipocytokines play a part in tumor progression and the induction of carcinogenesis [21,23]. Finley et al. reported the result that adipocytokines in periprostate adipose tissue are much higher compared with serum level [24]. In a study by Fukumoto et al., it was observed that the levels of serum adiponectin in adenoma patients was slightly higher than in the healthy controls [25]. Obesity is a powerful risk factor in the progression of many cancers, and it increases the risk in such cancers as prostate, colon, and breast cancers. Adipocytokines are manufactured in the adipose tissue, and are being studied as new risk factors for cancer and metabolic syndromes [25].

Leptin is a protein of weight 16kDa which is produced in the white adipose tissue and is secreted into the blood both free and bound [26]. Apart from the adipose tissue, leptin is also found in the placenta, the gastric and colonic mucosa, the liver, and the epithelial cells of the breast [27,29]. Leptin is a multifunctional endocrine protein, active in the regulation of eating habits, bone formation, reproductive function, angiogenesis, etc. [30]. Although there have been many studies of leptin in relation to cancer cell invasion, migration and stimulation, the effect of the other adipocytokines on cancer remains unclear [31]. Mantzoros et al. [32] showed that associations were independent of the possible effects of major components of the IGF-1, leptin, BMI, and known risk factors for breast cancer. Therefore, the examination of each adipocytokine with independent factors for cancer cases may explain the mechanism in more detail. In addition, different plasma levels of adiponectin may directly or indirectly stimulate PCa’s growth, which is counterbalanced by its antiangiogenic activities [33,35]. A study by Kosova et al. showed that the leptin level in hypothyroid diabetic rats was lower than in the control group [36]. It was shown in a study by Aknc et al. [37] that leptin values in patients with thyroid cancer were clearly raised in comparison with the control group. In another study by Kosova et al., it was shown that there was no statistically significant difference in leptin values between a group of patients with benign colon disease and another with colon cancer [38]. Zhang et al, it shown that, IL-6 mRNA expression in periprostate adipose tissue was significantly higher than that in subcutaneous adipose, but Leptin and Adiponectin mRNA expression were similar in periprostate and subcutaneous adipose tissue. Leptin mRNA expression in prostate cancer tissue was significantly higher than that in subcutaneous adipose, and also higher than that in periprostate adipose, showing the leptin may be released by adipose tissue to the circulation and then combined with receptor in the surface of prostate cancer cell to influence the progression of prostate cancer [39]. In this study we did not find a statistically significant difference in leptin levels when we compared groups with BHP and prostate cancer. Adiponectin is a peptide hormone at a weight of 30 kDa which is synthesized only in the adipose tissue, and is seen to play a preventative role in the pathogenesis of atherosclerosis by the proliferation of endothelial cells and the inhibition of vascular smooth muscle [40,43]. Adiponectin increases the sensitivity of tissues to insulin and is connected with hypoadiponectemia insulin resistance [44]. Insulin has a mitogenic effect on both normal and neoplastic breast epithelial cells, and in addition to this, leptin has a direct mitogenic effect on human breast cancer cells [45]. Adiponectin is a protective against aggressiveness of prostate cancer. There might be intimate relationship between periprostate adipose and prostate cancer tissue [39]. In a study by Kosova et al., adiponectin levels were found to be clearly though non-significantly higher in a group with colon carcinoma than in patients with benign colon disease [38]. It has been shown that whatever the androgen level, adiponectin inhibits the proliferation of prostate cancer [46]. At the same time, men with low adiponectin levels have a much higher risk of BHP and prostate cancer [47]. In a study by Izadi et al., adiponectin levels were found to be lower in patients with prostate cancer [47]. Burton et al. maintained that there was a relationship between adiponectin levels and the degree of prostate cancer and said that adiponectin levels should be raised for treatment [46]. In the present study, we also compared BHP and prostate cancer groups, and found that the adipocytokine levels of the prostate cancer group were lower than those of the BHP group. Resistin is a newly discovered protein produced in the adipocytes, and has been shown to be strongly connected with insulin resistance and obesity [10,38]. Resistin is the most recently described adipocyte-derived peptide, and it was initially suggested that it plays a role in the

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development of insulin resistance and obesity [48]. Its receptor has not yet been identified and cloned, and little is known about the signalling of this adipokine [49]. In some breast cancers and solid carcinomas, there are receptors with direct paracrine and endocrine effects which increase adipocytokine invasion and metastasis capacity [38]. This may explain the high concentrations of resistin in colorectal cancer, which has a strong correlation with inflammation [10]. In addition, the previous studies revealed that resistin is expressed in adipose tissue, monocytes and macrophages, and correlated directly with C-reactive protein, tumour necrosis factor-α, and interleukin-6. The putative role of resistin as another marker of inflammation is the subject of growing interest [50].

The PI3K/Akt pathway is an important mediator of cell growth and survival in response to growth factors and other signals [51]. PI3K activates Akt serine/threonine kinase by generating specific inositol phospholipids, which recruit Akt to the cell membrane and enable its activation. Akt mediates cell survival and growth signals by phosphorylating and inactivating proapoptotic proteins [52]. Several studies have revealed that increased Akt activity is found in breast, colon and pancreatic cancers, as well as in prostate cancer. Recent studies demonstrate a role for Akt in aggressive phenotypes of prostate cancer, and its prognostic value in human prostate cancer [53,55]. In a previous study by Kosova et al., it was observed that resistin levels were higher to a statistically significant level in a group of patients with malignant colon cancer than in a group with benign colon disease [38]. In a study by Housa et al., it was reported that serum resistin levels were no different in patients with BHP and prostate cancer, but that serum resistin levels in advanced cancer patients showed a falling trend [38]. Chronic inflammation is known to be one of the causes of prostate the exact mechanism, the relationship of resistin to prostate cancer deserves additional study [49]. Shalev et al. [56] demonstrated that the mean serum resistin level in healthy control subjects was 30.7 ng/mL, and serum resistin levels were significantly elevated in patients with diabetes (49.7 ng/mL). In the present study also, a comparison between the groups with BHP and prostate cancer showed that resistin levels in the prostate cancer group were statistically lower than in the BHP group.

Radical prostatectomy is associated with acute perioperative changes in plasma levels of the neurohormonal stress adipocytokine leptin, as well as inflammatory cytokines, thereby affecting the activation of the immune system and the clinical course. Adipocytokine could serve as new marker of invasiveness in major urologic surgery [57].

In conclusion, it was clear from previous studies that adipocytokines have a significant place in carcinogenesis. This information and our own findings show that adiponectin and resistin from the adipocytokine family may play an important role in the progression of prostate cancer, and thus it may be possible to use them as diagnostic markers. It was seen when assessing the results of the study that these cytokines become more apparent with the severity and duration of the prostate cancer, and therefore they may be more valuable as markers in diagnosis. For this reason we feel that similar studies should be performed on a greater number of patients and at different stages.

References


