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Carnitine and adiponectin levels in breast cancer after radiotherapy

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Abstract: In this study, serum carnitine (CRNT) and adiponectin (APN) levels and the correlation of these parameters in patients with breast cancer before and after treatment with radiotherapy (RT) were determined.

Materials and methods: Serum adiponectin and carnitine levels were assessed in 58 patients with breast carcinoma and 30 control subjects. Serum carnitine and APN levels were determined using a specific enzyme-linked immunosorbent assay.

Results: While serum carnitine level was significantly lower in the patients with breast cancer after RT compared with the control group and before treatment (p=0.002 and p=0.019, respectively), serum APN level was significantly higher than in the control group and before treatment (p=0.003 and p=0.027, respectively). Carnitine level showed a negative correlation with APN level in the patients after RT (r= -0.626, p= 0.001). There was no correlation between carnitine and APN levels in subjects of control group and before treatment. Also, neither carnitine nor APN levels demonstrated correlation other parameters.

Conclusions: Results suggest that increased serum adiponectin and decreased carnitine levels in breast cancer after RT than control group. Carnitine level showed a negative correlation with APN level in the patient with breast cancer after RT. While carnitine, HDL-C and total cholesterol levels are decreased, trygliceride and LDL-C levels are increased in patients than control group. In addition, serum APN concentration was inversely correlated with serum carnitine levels. Furthermore, increased serum APN level in breast cancer after RT might be associated with hypocarnitinemia.

Keywords: Adiponectin; Carnitine; Breast cancer; Radiotherapy

1 Introduction

Many of the tumors can be removed surgically. However, surgical removal of the some tumors is impossible or surgery might be weaken. When surgical treatment is impossible in such circumstance radiotherapy (RT) is an important procedure for cancer treatment [1]. RT is a key procedure for different cancer type treatments. Nearly 90% of human cancers are carcinomas, the five most common being those of the lung, stomach, breast, colon/rectum, and uterine cervix. About 60% of all cancer patients receive RT for every year, either single or in along with chemotherapy or surgery [2,3]. Even the current treatment, the most common side effect of RT are include infertility, cardiovascular disease, nausea, hair loss, skin irritation, anemia, cognitive disability. Even the most side effect, RT is used as a primary or adjuvant procedure in along with surgery, chemotherapy, hormone therapy and immunotherapy. It is a fundamentally important treatment process that is required to kill cancer cells in the area of the body, but it also affects some of the normal cells nearby.

Carnitine is synthesized from lysine and ketoglutarate primarily in liver and kidney. There are high affinity uptake systems for carnitine in most tissue. Carnitine is necessary for the transport of long-chain fatty acids and the acyl coenzyme A derivatives across the inner mitochondrial membrane. Also it is required for muscle energy production and transfer of the products of peroxisomal beta-oxidation to the mitochondria and removal of short-chain and medium-chain fatty acids from these organelles, in

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which it is responsible for maintaining coenzyme A levels [4]. It has been widely shown that clinically significant oxidative stress occurs in patients with advanced cancer, as shown by increased levels of reactive oxygen species (ROS) such as superoxide and hydrogen peroxide [5,6]. Also, ROS are a source of chronic damage to tissue biomolecules. Two major products secreted by the adipose tissue, an active endocrine organ, are leptin and adiponectine. It regulates glucose and lipid metabolism in muscle and liver. Also, it is strongly suggested that APN downregulates the secretion of proinflammatory cytokines interleukines 6 and 8 and monocyte chemoattractant proteine-1. It has been showed that APN might be useful for preventing the development of atherosclerotic disorders [7]. Low levels of adiponectin are also associated with oxidative stress and endothelial dysfunction. Irradiation conditions such as radiotherapy causes damage to DNA, oxidative stress and other cellular events [8]. A relationship between increased oxidative stress and decreased circulating levels of APN in normal weight and metabolically obese humans has been estimated [9]. The mechanisms involved are as yet poorly understood but it appears that loss of expression of the glucose and lipid metabolism molecules have a key role in the ability of the tumor development.

Up to now, there are no published reports on serum carnitine and APN levels in breast cancer patients after RT. In the current study, we have examined serum carnitine and APN levels and the correlation of these parameters in breast cancer patients after RT.

2 Materials and methods

We studied 58 patients with breast cancer and 30 healthy women in eastern Turkish people. Staging was determined by the American Joint Committee on Cancer Guidelines. None of the subjects in this study had any lipid lowering drug. All of patients had undergone a mastectomy or lumpectomy. After mastectomy and lumpectomy all of patients had chemotherapy treatment. Per protocol, RT had to start within six weeks after completion of the adjuvant chemotherapy. In the mastectomy group, a dose of 50 Gy was delivered in 25 fractions over five weeks to the chest wall using tangential photon fields, and in cases of pN1 status, to the supraclavicular, infraclavicular and axillary nodes using an anterior field matched to the tangential fields. Breast-conserved patients received, in addition, a sequential boost of 10 Gy delivered in 5 fractions the initial tumor bed using a direct electron field. The study is restricted to women with invasive breast cancer; women with ductal carcinoma in situ are not included. The study has been carried out with the approval of the Ethics Committee of the Ataturk University Medical School (protocol number: B.30.2. ATA.0.01.00/93) and all participants have provided written informed consent. For blood sampling, 10 ml of blood was drawn by venipucture into glass tubes. Blood samples were centrifuged at 4000 rpm for 10 min and the serum samples obtained were stored at -80 ºC until assayed. Serum carnitine and APN levels were measured using a specific enzyme-linked immunosorbent assay (CUSABIA, Cat. No. CSB-E13242h- Wuhan China; and BioVendor, Cat. No: RD195023100-Germany; respectively). Serum lipid profiles were studied with autoanalyser systems (Beckman Coulter AU 5800, USA).

The data are given mean±standart deviation. The statistical analysis was done using SPSS version 19.0 (IBM-SPSS, Chicago, USA). Comparisons between groups were done using Mann-Whitney tests for continuous data. The relations between data were investigated by linear regression analysis. A p value lower than 0.05 was accepted as significant.

3 Results

Distributions of patients by demographic and clinical characteristics are presented in (Table 1). While serum carnitine level was significantly lower in the patients with breast cancer after RT compared with the control group and before treatment (p=0.002 and p=0.019, respectively), serum after treatment APN level was significantly higher than in the control group and before treatment (p=0.003 and p=0.027, respectively) (Table 2). Both control group and before treatment patients did not show statistically significant differences between carnitine and APN levels (p>0.05) (data not show). But carnitine level showed a negative correlation with APN level in the patients with breast cancer after RT (r= -0.626, p=0.001). However, neither carnitine nor APN levels demonstrated other correlation parameters. The study showed significant increase in serum triglycerides and LDL cholesterol levels in control groups compared with breast cancer patients after RT and before treatment (p = 0.045 and p=0.037 for TG; p=0.012 and p=0.017 for LDL-C, respectively). Significant decreased levels of total cholesterol and HDL cholesterol levels were observed in control group compared with breast cancer patients after RT and before treatment (p = 0.011 and p=0.023 for cholesterol; p = 0.009 and p=0.007 for HDL-C, respectively). There was no any correlation between TG, LDL-C and total cholesterol, HDL-C

Table 1: Clinical and demographic charecteristics of study subjects

Characteristic	Control subjects (n=30) No. (%)	Cancer subjects (n=58) No. (%)
Age (yr)*	42±5	47±4
BMI (kg/m2)	23.6±4	25.2±3
Duration of cancer (mo)*		8±4
Have at least one child	27 (90)	49 (84)
Stage diagnosis		
Stage II- B		5
Stage II- A		34
Stage III-B		13
Stage III-C		6
Education		
Up to secondary school	9 (30)	50 (86)
University	21 (70)	8 (14)
Married position		
Married	25 (83)	48 (82)
No married	5 (17)	10 (18)
Location of residence		
City area	22 (73)	51 (88)
Non-city area	8 (27)	7 (12)
Consumes alcohol		
Alcohol drinker	1 (3)	3 (5)
Non-alcohol drinker	29 (97)	55 (95)
Smoker		
Never or past smoker	27 (90)	50 (86)
Current smoker	3 (10)	8 (14)
No oral endocrine therapy	30 (100)	58 (100)
Type of surgery		
Mastectomy		47 (81)
Lumpectomy then mastectomy		11 (19)
Have health insurance		
Yes	30 (100)	58 (100)
No		

BMI=body mass index.
*Mean±SD.

levels. However, total cholesterol levels demonstrated a positive correlation with HDL-C levels in both before treatment and after treatment in patients with breast cancer (r=0.572, p=0.001 and r=0.633, p=0.001, respectively).

4 Discussion

Despite recent research, the link between breast cancer, one of the most common cancers, and mortality can be seen in worldwide. We have examined breast cancer after RT with a focus on serum carnitine and APN levels. It is fundamentally important changes in the homeosta-

Table 2: Levels of serum carnitine, adiponectin and lipids in control and patients groups

	Control subjects (n=30)	Before treatment subjects (n=58)	After treatment subjects (n=58)
Carnitine (nmol/mL)	56.34±6.97	50.71±5.42	33.12±3.94*+
Adiponectin (μg/mL)	1.24±0.21	1.32±0.26	1.98±0.37‡§
Triglyceride (mg/dL)	161.30±32.54ǁ‖	342.41±53.21	328.62±49.63
LDL-C (mg/dL)	117.11±19.21**++	182.32±23.98	186.20±44.51
Total cholesterol (mg/dL)	148.30±22.43‡‡§§	121.11±18.32	129.22±19.72
HDL-C (mg/dL)	53.58±7.55ǁǁ‖‖	20.13±3.81	23.66±4.39

Data are mean±SD. Comparisons are made between control subjects and before treatment patients with patients after radiotherapy.

*p=0.002 vs. control group and +p=0.019 vs. before treatment.
‡p=0.003 vs. control group and §p= 0.027 vs. before treatment.
ǁp=0.045 vs. after RT and ‖p=0.037 vs. before treatment.
**p=0.012 vs. after RT and ++p=0.017 vs. before treatment.
‡‡p=0.011 vs. after RT and §§p=0.023 vs. before treatment.
ǁǁp=0.009 vs. after RT and ‖‖p=0.007 vs. before treatment.

sis of fatty acid metabolism that are required to maintain the metabolic integrity and production of cellular energy levels. Inappropriate lipid metabolism can lead to degenerative conditions, such as obesity, cardiovascular disease, and cancer [10,11]. One of the major exogenous sources of DNA damage, cellular homeostasis, cell membrane dysfunction and oxidative stress is ionizing radiation [11,12]. The radiation produces a flux of hydroxyl radicals and organic radicals at the site of the tumor. The localized oxidative stress causes damage to all biomolecules in the tumor cell and it prevents tumor cell replication and inhibits tumor growth. In rats radiation-induced adverse effects have been prevented by administration of L-Carnitine. The radiation-induced dramatic morphological changes and germ cell apoptosis in the irradiated rat testis significantly reduced by L-Carnitine treatment. The precise molecular mechanisms of L-Carnitine supplementation are as yet unknown. However, one of the main effector functions of L-Carnitine supplementation during RT may be useful for spermatogenesis following testicular irradiation by decreasing germ cell apoptosis [13]. On the other hand, the relationships of L-Carnitine supplementation and reduced the degree of brain and retinal damage, decreased the malondialdehyde levels and increased the activity of antioxidant enzymes, superoxide dismutase, glutathione peroxidase and catalase are illustrated in the rat brain [14]. Supplemental L-Carnitine applied has been used for increase the activity of mitochondrial antioxidant system and decrease the free radical-induced oxidative stres, the development of secondary protective mechanism is considered the result of its endogeneous antioxidant and free radical scavenger characteristic [15-17]. In our study decreased serum carnitine level in breast cancer after RT may be influenced by caloric intake, metabolic requirements and pharmacological therapies [18]. Thus, blood L-Carnitine levels in the disease such as breast cancer plays a fundamental role in the energy and fatty acid oxidation by regulating the mitochondrial ratio of free coenzyme A to acyl-coenzyme A. while 25% carnitine is synthesized from lysine and ketoglutarate, primarily in liver and kidney, rest of its in the organism is synthesized from dietary source such as red meat and dairy rich amine products [18,19]. Fatty acids as the major source of energy use in both skeletal muscle and myocardium so that both tissues are carnitine-dependent. Since decreased caloric intake and increased metabolic requirements, cancer patients such as breast cancer are particularly at risk for carnitine deficiency. Additionally, since carnitine absorption, synthesis, and excretion effected by long term pharmacological therapy decreased blood levels of carnitine could be seen in breast cancer patients [20,21]. Some studies showed that serum carnitine levels are decreased in patients with chronic illness and cancer patients [22-25]. Our study showed that significant increase in serum triglycerides and LDL-C levels in breast cancer patients as compared to controls. On the other hand, significant decreased levels of total cholesterol and HDL cholesterol levels were observed in breast cancer patients compared

to control group. The study has been submitted altered pattern of lipid profile in breast cancer patients. The result for hypocholesterolaemia may be due to its increased utilisation by neoplastic cells for new cell biogenesis. Alteration in the amount of lipid profile is effected by the nutritional state of the individual, changed adipose tissue metabolism and RT side action; consequently, RT is main factor controlling the synthesis of blood carnitine and lipid profile levels in breast cancer patients.

It is clear that adipose tissue is hormonally an active endocrine organ. Biomolecules such as leptin, adiponectin, resistin, vascular endothelial growth factor, tumor necrosis factor alpha and interleukin 6 are all produced by adipocytes. In both in vivo and in vitro oxidative damage are prevented by high level adiponectin concentration [26,27]. All of cell and tissue are protected against radiation syndrome by cytokines and growth factors. Also, radiation-induced deaths are prevented by the administration of some cytokines before irradiation in the mice [28]. Cytokines such as tumor necrosis factor alpha and interleukin 6 and growth factors such as vascular endothelial growth factor play a main role in orchestrating the host's response to radiation and stimulate the congenital defense against ionizing radiation, which contributes protection [29]. Adipose tissue is an active endocrine organ. Its products are known as adipokines like leptin and adiponectin. Adiponectin increases insülin sensitivity. Its lack leads to insülin resistance. Also, adiponectin downregulates secretion of proinflammatory cytokines interleukins 6 and 8 and monocyte chemoattractant protein-1 [30-31]. APN is an important adipose tissue-derived antioxidant hormone in relation to insulin sensitivity, lipid metabolism and inflammation. Furthermore, by binding to various growth factors such as vascular endothelial growth factor associated with regulation of cell proliferation, oxidative stress, energy intake and expenditure. As discussed above, with regard to its known effects in regulating cell proliferation, oxidative stress, energy intake and expenditure, APN may effectively be associated with regulating tissue damage and repair following radiation therapy in breast cancer patients. In this study, increased serum APN levels in breast cancer patients after RT may be effected by decreased carnitine levels. We think that when serum carnitine level was decreased, lipid metabolism would have been dysfunctional so that oxidative stress and adipose tissue metabolism including adiponectin synthesis and amount. Then different oxidant molecules such as oxygen radicals, hydrogen peroxide and organic radicals might be increased in bloodstream. For decreases these oxidant molecules serum antioxidant concentration such as APN might be increased. This has the net effect of producing increasing amounts of oxidant agents, and it facilitates the synthesis of APN within the adipocyte. Because APN are not only mediators of inflammation, but are also involved in the regulation of oxidative balance, along with resolution of the clinical and metabolic features of breast cancer patients after RT. It has been shown that dysregulation of adipocytokines such as adiponectin, resistin and leptin may be associated with obesity, diabetes mellitus, dyslipidemia, and hypertension, and finally result in atherosclerotic vascular diseases [32,33]. Also, while serum carnitine levels decrease serum adiponectin levels are increased in patients with breast cancer after RT. Because APN might be useful for preventing the development of atherosclerotic changes and other metabolic dysfunctions in our patients. This study demonstrated that alterations in the amount of serum carnitine, adiponectin and lipid concentrations are effected by radiotherapic treatment in breast cancer patients. As we mentioned, all of data can be explain with metabolic integration.

Conflict of interest statement: Authors state no conflict of interest.

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