

The prognostic role of inflammation and hormones in patients with metastatic cancer with cachexia

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Abstract We wanted to investigate the possible etiologic factors of cachexia. Forty-six patients diagnosed with cancer cachexia and 34 healthy controls were included in the study. Serum total testosterone, free testosterone, interleukin 1 (IL) alpha and beta, IL6, tumor necrosis factor (TNF) alpha, orexin, galanin, neuropeptide Y, tumor necrosis factor-like weak inducer of apoptosis and tumor necrosis factor receptor-associated factor 6, and C-reactive protein (CRP) levels were investigated. There were 36 male and 10 female patients in the cachexia group, and 24 male and 10 female patients in the control group. Median overall survival (OS) of the cachexia group after the diagnosis of cachexia was 8 (1–25) months. There were statistically significant relationships between OS and BMI, serum CRP, TRAF-6, albumin, and LDH levels in the cachexia group. In addition to cachexia, serum CRP, testosterone, and TNF alpha levels were statistically significantly correlated with OS in refractory cachexia. TRAF-6 levels was significantly correlated with type of cancer ($P = 0.02$). Although cachexia presents with a multifactorial ethio-pathogenesis, few of them affect the OS. Our novel results were that serum CRP, albumin, LDH, and TRAF-6 levels have a higher association with OS in

patients with cancer cachexia compared to the many other parameters. An ongoing cachexia also called refractory cachexia is a recent definition. This end-stage term of cancer duration may be predicted by decreasing serum testosterone and increasing serum TNF alpha levels, as well as serum CRP levels.

Keywords Cachexia · TRAF-6 · Inflammation · Hormones · Survival

Introduction

Cancer cachexia has been known to be a side effect of cancer for a long time. This condition, characterized by decreased physical activity, intolerance to the treatment of cancer, decreased overall survival (OS), and weight loss in this population, has been ignored as a clinical situation for several decades [1]. However, the etiology and treatment of cancer cachexia has been extensively researched in recent years. Following the decrease in muscle mass in cancer patients, cachexia emerges with an ongoing catabolism, a decline in performance status, unresponsiveness to the treatment, and deterioration of clinical performance [2]. Onset of cachexia prevents the patients from receiving the main cancer treatment and leads to patients receiving palliative care [3]. Thus, preventing this catabolic process is essential for palliative care as well as cancer therapy. In an ongoing process of catabolism, negative energy imbalance and protein metabolism are organized by three different but intertwined ways. These pathways are reduction in energy intake (anorexia, cancer-related reasons, etc.), systemic inflammation, and metabolic abnormalities. We concentrated on these three pathways in the etiology of cachexia

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and aimed to investigate the possible effects of these three pathways.

Materials and methods

Forty-six patients diagnosed with cancer who have become cachectic during the follow-up period were included in the study. Body mass index (BMI) was under 20 for all patients. The control group consisted of 34 healthy participants with any known chronic disease and weight loss. Patients with features such as previous gastrointestinal system operations, changes in eating habits, depressive mood, regular drug use, smoking, and excessive alcohol consumption were excluded from the control group. Our study performed during the period between May 2011 and February 2013 in Bulent Ecevit University Medical Oncology Clinic. Verbal and written informed consents were obtained from all patients. The study was performed after the ethical approval from Bulent Ecevit University, and this work was supported in part by Bulent Ecevit University under project BAP-2012-20-00-16.

BMI was calculated as body weight in kilograms divided by the square of body height in meters for all patients. BMI values of 20.0–24.9 are normal; BMI lower than 20 suggests cachexia, and a value of above 25 indicates that the person is overweight.

Patients who had a life expectancy lower than 3 months, are unresponsive to cancer treatment, and present with reduced performance status were criteria investigated for the diagnosis in patients with refractory cachexia.

Inclusion criteria were cancer diagnosis by pathologic examination, BMI with <20 , weight loss during cancer treatment, weight loss of more than 5 % prior to illness in the past 6 months, and continuing weight loss in the last few months.

Study exclusion criteria were BMI <20 at the time of diagnosis of cancer, hyperthyroidism, decompensating heart failure, weight loss after operation for cancer, steroid treatment except protocol for cancer chemotherapy, edema and/or ascites on physical examination at the time of diagnosis, symptoms of infection in medical history, and physical examination at the time of diagnosis.

Biochemical measurements

Serum IL1 β , IL1 α , IL6, and TNF α levels were measured by Boster (Fremont, CA, USA)-branded ELISA kits. Serum orexin-A, galanin TWEAK, and TRAF-6 levels were measured by Cusabio (Wuhan, China)-branded ELISA kits. Serum NPY levels were measured by Raybio (Norcross, USA)-branded ELISA kits. Study was

performed by ELx50 washing apparatus and ELx800 ELISA plate reader by Bio-Tek (Vermont, USA).

Weight and bio-impedance measurements

All parameters were measured by two models of commercially available foot-to-foot impedance device (Models TBF 300A; Tanita Inc., Tokyo, Japan). The measurements were performed in the morning fasting. Measured parameters by BIA were total weight, body fat % (BF %), fat mass (FM), fat-free mass (FFM), muscle mass, metabolic age, and basal metabolic rate (kcal). OS was defined as the time interval between the first assessment in the clinic and the patient's death date or the time of the last contact or news obtained while the patient was still alive.

Statistical analysis

The study's primary end point was OS with a planned enrollment of 46 patients to estimate the median OS. Descriptive and continuous variables were summarized as arithmetic means with standard deviation (SD) and medians with range, and categorical variables were summarized as relative frequency proportions and 95 % confidence intervals (95 % CI). OS is defined as the interval from the patient's date of diagnosis until date of death and was calculated using the Kaplan–Meier method, whereas Cox proportional hazards models were used to evaluate the multivariate prognostic effect. Hazard ratios (HR) were calculated along with their corresponding 95 % CI as a measurement of association. A *P* value of 0.05 or lower was considered significant in all cases. Analyses were done by SPSS v. 17 software package (SPSS, Inc., Chicago, IL).

Results

There were 36 male and 10 female patients in the cachexia group, and 24 male and 10 female patients in the control group. There was no statistical significance according to gender (*P* = 0.6). There was no significant difference for age, white blood cell, and liver functions between the groups. General characteristics of cachexia and control groups are presented in Table 1. BMI in the cachexia group was statistically lower than in the control group as expected (16.0 vs. 23.1, *P* = 0.0001). Liver function tests and renal function tests were similar in both groups but anemia, hypoalbuminemia, as well as high levels of alkaline phosphatase and LDH were prominent in the cachexia group.

Seven patients had hypertension, four patients had mild chronic obstructive pulmonary disease, and three patients had diabetes mellitus without any known macrovascular

Table 1 General features of cachexia and control groups

	Cachexia Mean (SD)	Control Mean (SD)	<i>P</i>
<i>N</i>	46	34	
Age (years)	60.9 (14)	57.9 (12)	0.2
M/F	36/10	24/10	0.6
BMI	16 (2.1)	23.1(1.2)	0.0001
WBC (uL)	7750 (4000)	10,000 (4500)	0.06
Hb (g/dL)	11.3 (1.8)	12.8 (0.9)	0.001
PLT (mm ³)	277,000 (12,000)	265,000 (11,000)	0.48
AST (U/L)	32 (21)	29 (23)	0.54
ALT (U/L)	24 (14)	18 (9)	0.19
Cre (mg/dL)	1.01 (0.3)	0.99 (0.33)	0.46
LDH (U/L)	249 (210)	113 (51)	0.002
Alb (g/dL)	3.0 (0.7)	3.86 (0.6)	0.0001
Protein (g/dL)	6.6 (0.9)	8.9 (1.1)	0.001
ALP (U/L)	174 (136)	30 (13)	0.0001
Glucose (mg/dL)	108 (38)	101 (11)	0.11

SD standard deviation, *N* number of patients, *M* male, *F* female, *BMI* body mass index, *WBC* white blood count, *Hb* hemoglobin, *AST* aspartate transaminase, *Cre* creatinine, *LDH* lactate dehydrogenase, *ALT* alanine transaminase, *Alb* albumin, *ALP* alkaline phosphatase

complications in the cachexia group. All of them were controlled with medication. In the control group, there were no chronic diseases present.

In this study, we examined serum testosterone, free testosterone, IL1 alpha and beta, IL6, TNF alpha, orexin-A, galanin, NPY, TRAF-6, TWEAK, and serum CRP as cytokine and hormonal parameters. Serum CRP, IL1 alpha, IL6, TNF alpha, and TRAF-6 levels were significantly higher in the cachexia group compared to the control group. Serum testosterone, TWEAK, orexin-A, and NPY levels were significantly lower in the control group compared to the cachexia group (as shown in Table 2). The mean total testosterone levels were significantly lower in cachectic women compared to control women (141 ng/dL vs. 312 ng/dL, *P* = 0.002). Cachectic males had lower total testosterone levels compared to control group (173 ng/dL vs. 515 ng/dL, *P* = 0.0001). According to the free testosterone level, there was no significant difference between the gender groups (*P* value for male = 0.6 and for female = 0.053).

The most common type of cancer in the cachexia group was gastroesophageal cancer (*n* = 24); the remaining seven patients had pancreatic cancer, seven had lung cancer, four had colorectal cancer, two had ovarian cancer, one had breast cancer, and one had laryngeal cancer.

All 46 patients had systemic metastasis at the time of study. The most common metastatic sites were lung and liver (shown in Fig. 1). Median survival of the cachexia

Table 2 Comparison of cytokine and hormonal parameters of both groups

	Cachexia Mean (SD)	Control Mean (SD)	<i>P</i>
<i>N</i>	46	34	
CRP (mg/L)	83 (25)	4 (2.6)	0.0001
IL1 alpha (pg/mL)	14 (9.9)	9.9 (7.5)	0.0337
IL1 beta (pg/mL)	3.68 (0.8)	3.59 (0.7)	0.6
IL6 (pg/mL)	16.5 (4)	5.7 (3.6)	0.0001
TNF alpha (pg/mL)	15.9 (7.1)	12 (5.2)	0.008
NPY (ng/mL)	0.175 (0.05)	0.21 (0.02)	0.0001
Orexin-A (ng/mL)	0.96 (0.2)	1.2 (0.3)	0.0001
Galanin (pg/mL)	16.2 (6.9)	15.9 (5.6)	0.8
TRAF-6 (ng/mL)	0.87 (0.4)	0.72 (0.12)	0.029
TWEAK (pg/mL)	726 (346)	823 (256)	0.015
Testosterone (ng/dL)	166 (155)	455 (281)	0.0001
Free testosterone (pg/mL)	4.95 (1.2)	7.39 (2.3)	0.14

SD standard deviation, *N* number of patients, *CRP* C-reactive protein, *IL* interleukin, *TNF* tumor necrosis factor, *NPY* neuropeptide Y, *TRAF* TNF receptor-associated factors, *TWEAK* TNF-like weak inducer of apoptosis

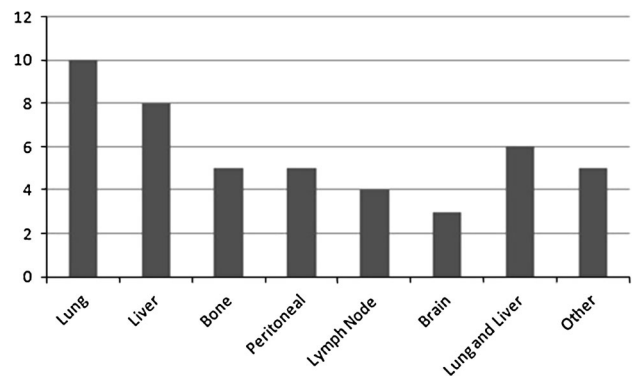


Fig. 1 Number of the metastatic site of the cachexia group

group after the diagnosis of cachexia was 8 (1–25) months. There were statistically significant relationships between OS and BMI, serum CRP, albumin, LDH, and TRAF-6 levels in the cachexia group (shown in Table 3). At the end of the study, all patients died.

OS-cachexia = 19.4-0.0177 CRP-3.16 TRAF-6 + 2.49 albumin + 0.00911 LDH + 1.42 BMI, and *R*² was 65.1 %. All parameters showed normal distribution and were useful for linear regression analysis.

BMI, TRAF-6, LDH, albumin, and CRP were the parameters that had statistically significant effects on OS. Only TRAF-6 was significantly correlated with type of cancer (*P* = 0.02). TRAF-6 levels had the lowest values in lung and stomach cancer (0.74–0.76 ng/ml, respectively),

whereas it was significantly higher in other cancer types including pancreatic and colorectal cancer (0.97–1.22 ng/ml, respectively). There was no significant relationship between the metastatic site of cancer, and BMI, TRAF-6, LDH, albumin, and CRP (P values were $P = 0.06$ for TRAF-6; $P = 0.8$ for ALP; $P = 0.75$ for Alb; $P = 0.6$ for LDH; $P = 0.09$ for CRP; and $P = 0.33$ for BMI).

Twenty-one patients were diagnosed with refractory cachexia, whereas the remaining 25 patients were in the cachexia group. All study parameters are compared and shown in Table 4. Serum CRP levels were significantly higher ($P = 0.001$), whereas serum testosterone and IL1 alpha levels were significantly lower in the refractory cachexia group compared to the cachexia group ($P = 0.0001$ and 0.05 , respectively).

Serum CRP, testosterone, and TNF alpha levels were statistically significantly correlated with OS in refractory cachexia ($P = 0.0001$, 0.0001 and 0.0017 , respectively).

Table 3 Parameters that have a significant impact on OS in multiple regression analysis

	Coef.	P
BMI	1.2222	0.009
CRP	-0.017423	0.0001
TRAF-6	-3.205	0.017
Alb (g/dL)	2.1488	0.001
LDH (U/L)	0.010579	0.001

BMI body mass index, *CRP* C-reactive protein, *TRAF* TNF receptor-associated factors, *Alb* albumin, *LDH* lactate dehydrogenase

Table 4 Comparison of cachexia patients with refractory cachexia patients

	Cachexia Mean (SD)	Refractory cachexia Mean (SD)	P
N	25	21	
Age (years)	58 (13)	64 (15)	0.18
BMI	16.5 (1)	15.6 (1)	0.017
LDH (U/L)	220 (119)	282 (180)	0.3
Alb (g/dL)	3.09 (0.7)	2.9 (0.77)	0.45
ALP (U/L)	190 (127)	156 (110)	0.4
CRP (mg/L)	34 (14)	130 (45)	0.001
IL1 alpha (pg/mL)	17 (11)	10 (5)	0.017
IL1 beta (pg/mL)	3.4 (0.4)	3.9 (0.9)	0.012
IL6 (pg/mL)	17.4 (8)	15.4 (5.4)	0.7
TNF alpha (pg/mL)	17 (7)	14 (7)	0.12
NPY (ng/mL)	0.17 (0.02)	0.17 (0.01)	0.9
Orexin-A (ng/mL)	0.94 (0.2)	0.99 (0.3)	0.5
Galanin (pg/mL)	14.8 (5)	17.9 (8)	0.13
TRAF-6 (ng/mL)	0.77 (0.12)	0.99 (0.5)	0.07
TWEAK (pg/mL)	731 (310)	719 (330)	0.9
Testosterone (ng/dL)	219 (170)	83 (45)	0.0001
Free testosterone (pg/mL)	3.4 (1.2)	6.8 (2.4)	0.06

SD standard deviation, *N* number of patients, *BMI* body mass index, *LDH* lactate dehydrogenase, *Alb* albumin, *CRP* C-reactive protein, *IL* interleukin, *TNF* tumor necrosis factor, *NPY* neuropeptide Y, *TRAF* TNF receptor-associated factors, *TWEAK* TNF-like weak inducer of apoptosis

Higher CRP levels, but lower TNF alpha and testosterone levels, were found as a risk factor for refractory cachexia. Multiple linear regression equation was “OS-refractory cachexia = 3.38-0.0154 CRP + 0.0260 Testosterone + 0.105 TNF alpha (pg/ml),” and R^2 was 76 %.

Discussion

In this study, the possible effects of inflammation and hormones on cancer cachexia, including levels of serum total testosterone, free testosterone, IL1 alpha and beta, IL6, TNF alpha, orexin, galanin, NPY, TRAF-6, TWEAK, and CRP levels, were investigated. Although liver and renal functions were similar in the control and cachectic groups, anemia, hypoalbuminemia, as well as higher serum levels of alkaline phosphates and LDH were more prominent in the cachectic group. Serum CRP, IL1 alpha, IL6, TNF alpha, and TRAF-6 levels were statistically significantly higher in the cachectic group compared to the control group. Inversely, serum testosterone, TWEAK, orexin-A, and NPY levels were significantly lower than those in the control group. Despite all these changes, a few parameters were correlated with OS in patients with cancer cachexia. These were BMI, serum CRP, albumin, LDH, and TRAF-6 levels. In addition, only serum testosterone, TNF alpha, and CRP levels were significantly correlated with OS in patients with refractory cachexia.

We can speculate that there are three different but interrelated mechanisms that may affect the ongoing

catabolism and protein energy pathway. First, one of the mechanisms was decreasing the energy intake and appetite; thus, serum orexin, galanin, and NPY levels were investigated. The second mechanism was inflammation; thus, serum levels of CRP, IL1 alpha and beta, serum IL6, and TNF alpha were observed. The last possible mechanism was systemic metabolic factors; thus, serum total and free testosterone levels, serum TWEAK, and TRAF-6 levels were investigated.

Production of pro-inflammatory cytokines in cancer patients triggers a systemic inflammation and causes an acute-phase reaction [4]. Also, increasing catabolism decreases serum albumin synthesis and atrophy in muscles [4]. An increased acute-phase reaction is associated with hypermetabolism and increased catabolism. Some acute-phase reactants, such as CRP, regulate the immune response and are accepted as a poor prognostic factor especially in patients with cancer [5]. Serum CRP levels have been observed in many studies involving cancer patients. It is well known that serum CRP level is a prognostic marker of systemic inflammation, anorexia, hypermetabolism, weight loss, and decreased survival in patients with cancer [6, 7]. Falconer et al. [7] investigated the relation of serum CRP levels and OS in 102 patients with unresectable pancreatic cancer. They found that patients with serum CRP levels >10 mg/L had significantly shorter survival (66 days vs. 222 days) compared to patients with serum CRP levels lower than 10 mg/L. A recent study showed that higher than 15 mg/L of serum CRP had 2.2 times mortality risk in 405 cancer patients with advanced stage [8]. In this study, many cancer types such as colorectal, pancreatic, gastric, and hepatobiliary cancers were included so we can advocate that serum CRP levels can be prognostic in many types of cancer. However, the median BMI of these 405 patients was 23 (± 3.6) so not all of the study population had cachexia. In our study, we found similar findings to previous studies according to serum CRP levels. In addition, we also found that serum CRP levels were more valuable than serum IL1, IL6, and TNF alpha levels even during follow-up of the cancer cachexia and an ongoing catabolism because only serum CRP levels were associated with OS in the inflammatory markers we investigated. Also, this is the first study which investigated the serum inflammatory markers in refractory cachexia, showing that the serum CRP levels were significantly higher in this group compared to the control group as well as the cachectic group. Thus, increasing serum CRP levels may predict the deterioration of cachexia.

Albumin levels were usually in the normal range in patients with early stage cancer but will drop in advanced stage, so this finding supports that albumin is a prognostic marker in the advanced stage of cancer [9, 10]. In a review of 59 studies involving nearly 16,000 patients with GI

malignancies, lung cancer, breast cancer, and ovarian cancer, a higher albumin level was a good prognostic marker and, conversely, a lower level of albumin was a poor prognostic marker in all of the above cancers [10]. In our study, we also found lower albumin levels in patients with cancer cachexia, but there was no significant difference between cachexia and refractory cachexia. This may support the finding that hypoalbuminemia's key role was more prominent in the early stage of cachexia rather than the late stage of this catabolic process.

TRAF protein is closely related with the synthesis of pro-inflammatory cytokines and pathways of the Tol/IL1 and TNF family. It is a cytoplasmic protein that plays a role in apoptosis and cell survival [11]. We do not know the exact mechanism of cancer cachexia, but atrophy in muscles may have a prognostic role in this process. The ubiquitin-proteasome system (UPS) is the major proteolytic machinery systematically activated in cachexia, and only proteasome inhibitors prevent increased proteolysis [12]. Sun et al. [13] investigated the TRAF-6 expression in patients with gastric cancer. They found that TRAF-6 was significantly up-regulated in the muscles involved in gastric cancer compared with the control muscles, and an overexpression of TRAF-6 in the muscles of gastric cancer was associated with TNM stage, level of serum albumin, and percent of weight loss. In addition, the authors concluded that this pathway may be a treatment target in cancer cachexia. Suppressed TRAF-6 synthesis prevented cancer cachexia in rats; thus, this novel finding was interpreted to be a new treatment target of cachexia [14]. To our knowledge, there are no studies measuring the TRAF-6 levels in patients with cancer cachexia. In this context, our study is the first investigation in the literature that showed elevated TRAF-6 levels in patients with cancer cachexia. Moreover, higher TRAF-6 levels were significantly associated with OS, with this result more prominent in pancreatic and colorectal cancer. Thus, we may speculate that TRAF-6-targeted therapies can be more effective in these cancers.

There are many animal studies in the literature which investigated NPY levels, but there have not been enough investigation in patients with cachectic cancer. In a study of 23 children diagnosed with cancer, NPY levels were significantly decreased during chemotherapy [15]. In another study including non-cachectic cancer patients, serum NPY levels did not significantly change after the cancer chemotherapy [16]. It is well known that there is a significant correlation with serum NPY levels and appetite, but we do not know the role of NPY in cancer cachexia. Our study showed that serum NPY levels were significantly decreased in cancer cachexia, but this drop was not significant in patients with refractory cachexia. Indeed, higher serum NPY levels were expected in cancer cachexia, but

we found an inverse relation; this may be a reason of previous cancer chemotherapies, advanced stage of cancer, or suppression of the other inflammatory cytokines such as TNF alpha, IL1, or IL6. Despite all these findings, serum NPY levels, as well as serum orexin and galanin levels, did not have a significant correlation with OS in our study.

TWEAK is a multifunctional protein and a member of the TNF family that functions with fibroblast growth factor-14. TWEAK has a role in cell survival, proliferation, angiogenesis, migration, and apoptosis [17]. Also, it has been shown that TWEAK and its receptor Fn14 were expressed in human malignant ovarian tumors, but not in normal ovarian tissues or in borderline/benign epithelial ovarian tumors [18]. To date, there has been no study investigating the serum TWEAK levels in cancer cachexia. In our study, serum TWEAK levels were significantly higher in cancer cachexia compared to the control group, but it is not an effect on OS.

Conclusion

Although cachexia presents with a multifactorial ethiopathogenesis, few affect OS. Our novel results were that serum CRP, albumin, LDH, and TRAF-6 levels have a higher association with OS in patients with cancer cachexia compared to the many other parameters. An ongoing cachexia also called refractory cachexia is a recent definition. This end-stage term of cancer duration may be predicted by decreasing serum testosterone and increasing serum TNF alpha levels, as well as serum CRP levels.

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Conflict of interest Cemil Bilir M.D., Huseyin Engin M.D., Murat Can M.D., Yasemin Bakkal Temi M.D., Derya Demirtas M.D. all of the authors declare no conflict of interest.

Ethical standard The authors of this manuscript certify that they comply with the ethical guidelines for authorship and publishing in the Journal of Cachexia, Sarcopenia, and Muscle.

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