Int J Clin Exp Med 2015;8(1):876-882
www.ijcem.com /ISSN:1940-5901/IJCEM0003279

Original Article

Alterations of ghrelin with weights and correlation among ghrelin, cytokine and survival in patients receiving chemoradiotherapy for gastrointestinal cancers

Feryal Karaca[1], Cigdem Usul Afsar[2], Meral Gunaldi[2], Erkut Erkurt[1], Vehbi Ercolak[2], Yasar Sertdemir[3], Semra Paydas[2]

Departments of [1]Radiation Oncology, [2]Medical Oncology, [3]Biostatistic, Faculty of Medicine, Cukurova University, Adana, Turkey

Received October 20, 2014; Accepted January 7, 2015; Epub January 15, 2015; Published January 30, 2015

Abstract: Aim: This study involved 30 patients (16 had gastric, 9 pancreatic and 5 gall bladder cancer) who had received concomitant chemoradiotherapy (CRT). Blood ghrelin and IL-6 values were compared before, in the last week of, and 3 months after CRT. Meanwhile, changes in body weight of patients were also investigated with changes in ghrelin and IL-6 levels before, in the last week of, and after radiotherapy (RT). Methods: Informed consent of the patients and the ethical committee approval from Cukurova University Medical Faculty were taken. Blood ghrelin and IL-6 levels were measured by using the ELISA method. Survival analysis was performed by the Kaplan Maier method, and data were evaluated by using the SPSS 19.0 package. Categorical measurements were calculated as numbers and percentages, whereas numerical data were summarized as mean and standard deviation. Results: The correlation between ghrelin and IL-6 values at the baseline of RT and overall survival rates at the end of the 30-month follow up was analyzed. Accordingly, ghrelin values were also changed in line with changes in patients' weights ($P < 0.001$). Patients with ghrelin values above 35 pg/ml before RT had longer survival rates at the end of the 30-month follow up ($P = 0.001$). Overall survival rates in patients with IL-6 value at or below 3.9 pg/ml before RT were longer than patients with IL-6 value above 3.9 pg/ml ($P = 0.021$). Conclusion: Therefore, the initiation of ghrelin analogue prophylactically in patients receiving chemoradiotherapy with gastrointestinal system malignancies can both prevent weight loss by increasing appetite and decrease severity of inflammation, thereby increasing survival.

Keywords: Ghrelin, weight, survival, radiotherapy, cancer

Introduction

Ghrelin is a hormone, which was identified in 1999 by a Japanese researcher Masayasuv Kojima, and it is endogenous ligand bound to the growth hormone secreting hormone receptor (GHS-R1a) [1]. Ghrelin is a polypeptide composed of 28 amino acids. Approximately 30% of circulating ghrelin originates from the gastrointestinal system [2]. Ghrelin has been shown to affect various systems in the organism. It has effects on eating, sleeping, cell proliferation, the cardiovascular system, carbohydrates and energy metabolism, as well as pancreatic exocrine and endocrine functions [1]. Eating and appetite mechanisms are controlled in the brain by arcuate nucleus in the hypothalamus. The effect of ghrelin on eating is independent of GH. In mammalians, ghrelin exerts its effects by both increasing appetite and GH secretion by binding to the GHS-R molecule through G protein and also acting directly on the hypophysis. Ghrelin is expressed from cardiac and aortic endothelial cells. It was shown that blood pressure was decreased; cardiac flow and index were increased in studies performed by administering intravenous ghrelin [3]. It has been suggested that ghrelin increases sleep. This effect is not definite.

The effect of the ghrelin hormone secreted primarily from the gastrointestinal system was

Table 1. General characteristics and treatment modalities of patients

Total number of patients	30
Gender	
Female	50%
Male	50%
Stage	
Stage 1-b	16.7%
Stage 2-a	30%
Stage 3	53.3%
Histopathology	
Adenocarcinoma	86.7%
Squamous cell carcinoma	13.3%
CT regimen	
5-FU	20%
FUFA (5-FU, Folinic Acid)	33.3%
Mac Donald	16.7%
DCF (Docetaxel, 5-FU, Cisplatin)	3.3%
Cisplatin	26.5%
Mean RT dose	46.5 Gy
Duration of follow-up	3 months

RT: Radiotherapy, CT: Chemotherapy, 5-FU: 5-Fluorouracil.

shown in appetite regulation. Intravenous ghrelin increased gastric peristalsis along with an increased secretion of gastric acid. This activation occurred via a parasympathetic effect that has been prevented by intravenous administration of atropine [4]. It has been shown that it exerts an antioxidative effect via inflammatory cytokine inhibition by acetylcholine secreted from the stomach. It exerts this effect in many organs like the stomach, heart, and pancreas.

IL-6 is a pro-inflammatory cytokine and it plays an important role in immunity. It causes development and differentiation of B cells. Similarly, growth cells play a role in malign plasma cells (plasmocytoma or myeloma), and self-growing plasmocytoma cells secrete IL-6 as the autocrine growth factor [5, 6]. It is differentiated into T cells and macrophages by secreting immunoglobulin. Although cytokines resemble hormones, they are not exactly hormones and they do not act as hormones [7].

Currently, gastric cancer is still one of the cancers with the highest mortality rate. Although this ratio is decreased by screening and preventative measures, it is still an important issue. Adjuvant 5-fluorourasil (5-FU) based concomitant chemoradiotherapy is still the contemporary treatment in patients with local or locally advanced gastric cancer [8].

Cisplatin-based regimens can only increase the survival for 10.6 months if they are given as adjuvant therapy in pancreatic cancer, one of the most fatal cancers with late-onset clinical symptoms along with being difficult to diagnosis and treat [9]. Median survival is only around 10 months in patients with unresectable pancreatic cancer, who have received curative 5-FU based chemoradiotherapy [10].

Gallbladder cancer is quite curable if it is diagnosed early. Five-year survival rates are 77% and 60% in stages I and II of the disease, respectively. However, the majority of patients are diagnosed coincidentally after cholecystectomy, thus many of them are encountered at locally advanced stages. Survival rates in stages III and IV are 29% and 3% respectively, after adjuvant 5-FU based chemoradiotherapy is performed following the curative resection in locally advanced gallbladder cancer cases.

Loss of appetite, fatigue, nausea, and weight loss are encountered in respect to the intensity of therapy in all patients, who have concomitant chemotherapy and radiotherapy. Even in some locally advanced diseases, complications of grade 3-4 toxicity can develop [11].

Materials and methods

Patient and treatment protocols

Informed consent from the patients and ethical committee approval from Cukurova University Medical Faculty were received. There were 30 patients enrolled in this study. They had been diagnosed with gastric or pancreatic or gallbladder cancer, and received concomitant chemoradiotherapy (**Table 1**). Weight monitoring of patients was performed each week during RT. Blood samples were taken and serum and plasma parts were separated before radiotherapy, in the fifth week of radiotherapy (acute effects of radiotherapy were observed), and 3 months after radiotherapy to investigate the chronic affects of radiotherapy. Blood ghrelin and IL-6 levels were measured by using the ELISA method. Adjuvant FUFA de Gramond and Mac Donald protocols were followed up in patients with gastric cancer during RT. Patients

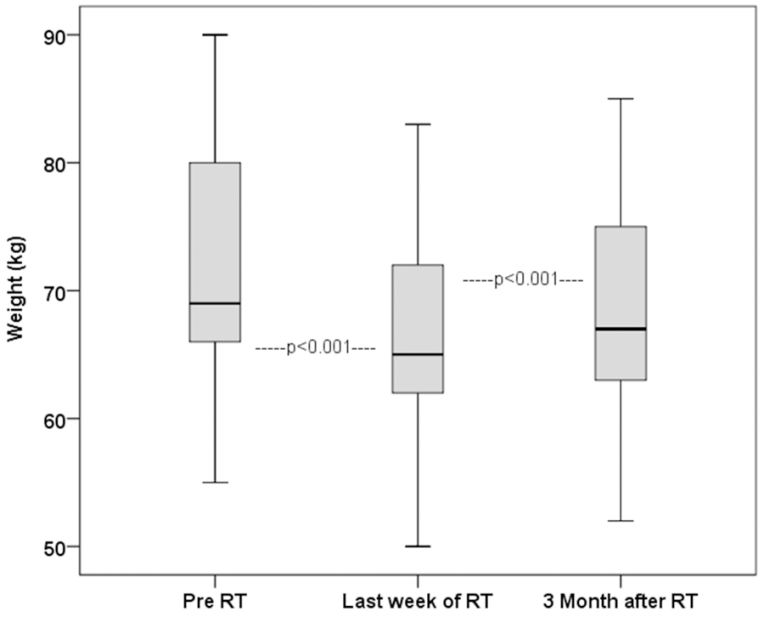

Figure 1. Weights decreased in the last week of RT, and increased in the 3rd month after RT.

Table 2. Changes in patients weights pre RT, in the last week of RT, and 3 months after RT

Weight	Mean	95% CI	P
Pre RT	73 ± 9.9	69-77	P < 0.001
In the last week of RT	67 ± 8.9	64-71	
3rd month after RT	69 ± 9.1	66-73	

RT: Radiotherapy.

with pancreatic carcinoma received 20-25 mg/m^2 cisplatin one day per week and 5-FU infusions five days per week during radiotherapy. Patients with gallbladder carcinoma received 5-FU infusions five days per week with radiotherapy. During concomitant chemoradiotherapy, using 6 MV and 18 MV X ray, 45 Gy (1.8 Gy/fx/day) a dose of curative radiotherapy was given to tumor, lymph nodes, and clips areas in Phase I; and a 5-10 Gy (1.8 Gy/fx/day) dose was given via boost in Phase II to selected risky patients. Patients were followed up for 30 months to compare the correlation between their standards of living and levels of ghrelin, and IL-6.

Laboratory analysis

Blood samples were taken (not less than 5 cc) in serum tubes to measure pro-inflammatory cytokine IL-6, and after the samples were centrifuged for 10 minutes at 3000 rpm, serums were separated. They were frozen and stored at -70°C until the day of study. For comparison of ghrelin hormone secretion levels, whole blood samples (again not less than 5 cc) were taken in tubes with etilendiamin tetraasetik asit (EDTA) before RT, in the last week of RT (to define acute effects of radiotherapy), and 3 months after radiotherapy to define chronic effects. Samples were centrifuged for 10 minutes at 3000 rpm, and plasma was separated. Plasma samples were frozen and stored at -70°C until the day of study. IL-6 and ghrelin levels were measured in pg/ml accordingly by using the ELISA method.

Statistical analysis

Data were evaluated by using SPSS 19.0 Statistical software. Categorical variables were calculated using counts and percentages, whereas continuous variables were summarized by mean and standard deviation (median and min-max when required). The Kaplan Meier method was applied to compare survival times between groups. Repeated measurement analysis was applied to detect changes of ghrelin and IL-6 in time. Statistical significance was accepted as $P < 0.05$ in all tests.

Results

This current study included 16 patients diagnosed with gastric cancer, and 14 patients diagnosed with pancreatic or gallbladder cancer, a total of 30 patients. Of these cases, 50% were females and the total follow up duration was 3 months after radiotherapy. Patients' stages were as follows; stage Ib (16.7%), stage IIa (30%) and stage III (53.3%). Adenocarcinoma histology was 86.7% and squamous was 13.3%. Chemotherapy regimens were 5-FU (20%), FUFA (33.3%), Mac Donald (16.7%), DCF (3.3%) and cisplatin (26.5%). The mean RT dose was 46.5 Gy.

Weights of patients before, in weeks 5-7, and 3 months after radiotherapy were 73 ± 9.9, 67 ± 8.9, and 69 ± 9.1 kg, respectively. The change

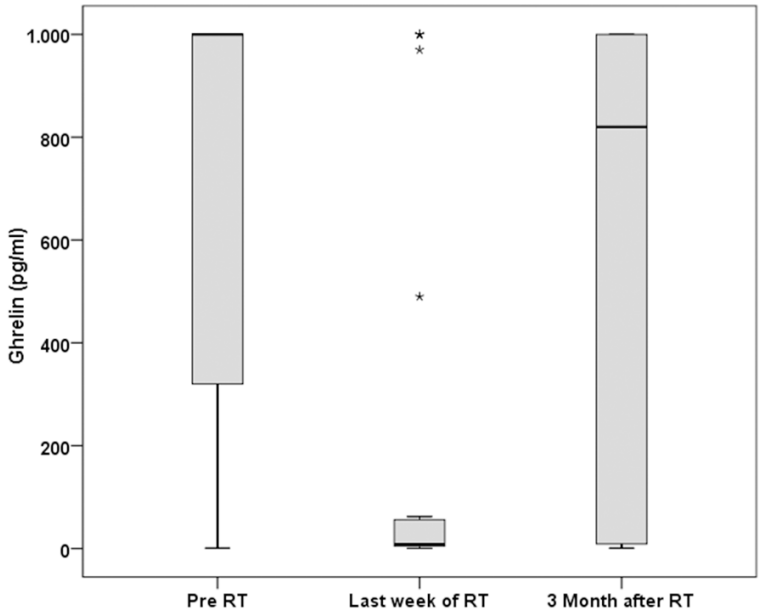

Figure 2. Ghrelin values are decreased in the last week of RT, and are increased in the 3rd month after RT.

Table 3. Changes in ghrelin values of patients pre RT, in the last week of RT, and in the 3rd month after RT

Ghrelin	Mean	95% CI	P
Pre RT	723 ± 427	561-885	
In the last week of RT	199 ± 379	55-344	P < 0.001
In 3rd month after RT	510 ± 476	329-691	

RT: Radiotherapy.

here was statistically significant over time in all patients (P < 0.001) (**Figure 1** and **Table 2**). When patients' weights measured during the initial acute side effects period were compared with the values before RT, median weights were observed as being lower. In measurements 3 months after RT had been completed, the median value had increased, but it was below the median value measured before RT. When ghrelin measurements of all patients were examined, the values were 732 ± 427 pg/mL, 199 ± 379, and 510.8 ± 476 before RT, in weeks 5-7 of, and in the 3rd month after radiotherapy, respectively. This change was statistically significant over time in all patients (P < 0.001) (**Figure 2** and **Table 3**). In the period when acute side effects of radiotherapy was initiated, the median ghrelin value was lower than the value measured before RT. The median value increased 3 months after RT, but it was lower than the median value measured before RT was begun.

When patients were divided into two groups according to their ghrelin level being below or above 35 pg/ml, the mean survival duration was 454 days in the former group, whereas it was 758 days in the latter (**Figure 3**; **Table 4**). Before RT was initiated patients were divided into two groups based on IL-6 levels being below or above 3.9 pg/ml. The mean survival durations were 750 days and 555 days, respectively. This change was statistically significant over time in all patients (**Figure 4**; **Table 4**).

Discussion

Cancer cachexia is commonly encountered in patients after long-term chemotherapy and radiotherapy. Cachexia not only increases the intensity of symptoms, but also decreases overall survival and muscle mass as a result of the chronic progressive disease. The most important factor related to cachexia is weight loss. Ghrelin is a hormone, which is composed of 28 amino acids. It increases appetite and is secreted mainly from the stomach. Ghrelin regulates appetite by binding to the GHS-R receptor, as well as prevents inflammation initiation by inhibiting pro-inflammatory cytokines [12-15]. Ghrelin suppresses sympathetic innervation by instigating the parasympathomimetic effect. Therefore, it prevents the initiation of inflammation by inhibiting the secretion of pro-inflammatory cytokines.

Garcia et al. performed a multi-center, placebo controlled, double blind study on 16 patients with cancer cachexia by giving oral ghrelin mimetic anamorelin and a placebo. They compared weight gain and appetite between the two groups. They gave anamorelin to the first group (n = 9), and a placebo to the second (n = 7). Both patient groups were similar to each other in demographic and disease characteristics. While baseline patient mean weight was 62.68 ± 11.33 kilograms (kg) in the group receiving anamorelin, weight after the treat-

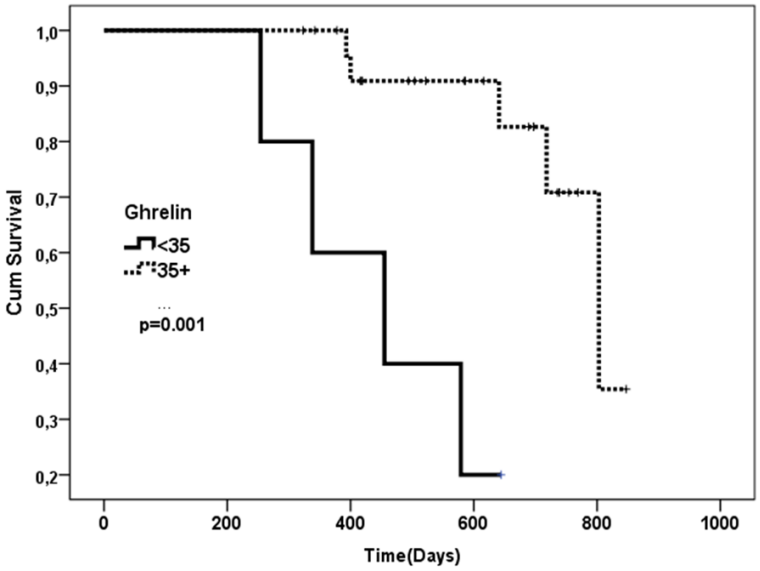

Figure 3. Survival according to ghrelin levels.

Table 4. Patients' survival durations when patients were grouped into two according to ghrelin and IL-6 levels before RT

	Mean	95% CI	Median	95% CI	P
Ghrelin < 35	454	327-581	455	204-706	0.001
Ghrelin > 35	758	695-821	803	681-925	0.001
IL-6 < 3.9	750	679-821	803	574-1032	0.021
IL-6 > 3.9	555	392-718	579	313-845	0.021

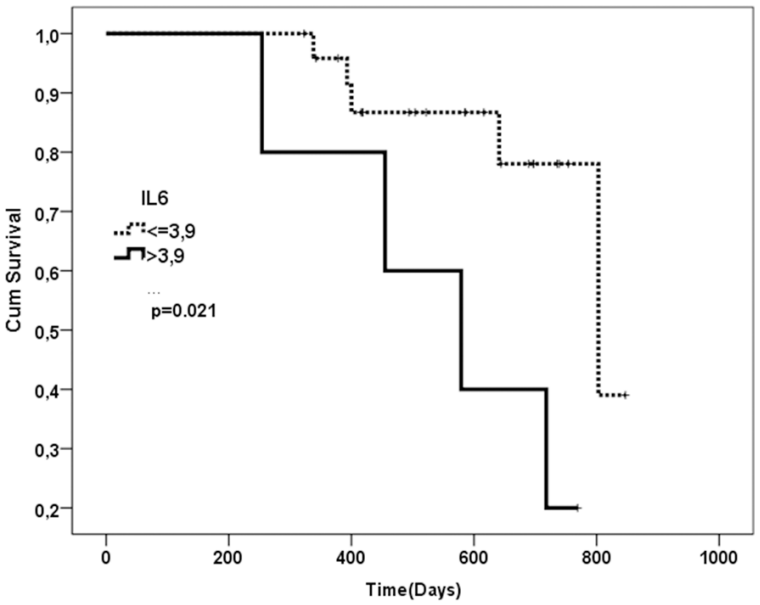

Figure 4. Survival according to IL-6 levels.

ment was 63.96 ± 11.45 kg. However, in the placebo group baseline weight was 63.06 ± 11.40 kg, and dropped to 62.73 ± 11.27 kg after treatment [16].

In our study, there was a significant difference between patient weights before RT and in the last week of RT. Although patient weights increased 3 months after RT, the values never reached the levels measured before RT. Weight change was significant over time ($P < 0.001$). Similarly, ghrelin levels before RT decreased in the last week of RT, and then increased 3 months after the completion of RT. However, they did not reach the values measured before RT commenced ($P < 0.001$). Loss of weight and appetite due to RT and toxicity of RT could be prevented by administering a ghrelin analogue.

Citrin et al. observed increased oral secretion of plasma cytokines in 11 patients with head and neck cancer after radiotherapy. Patients received IMRT (intensity modulated radiation therapy) treatment or curative radiotherapy by using the conformational radiotherapy technique. Saliva samples from buccal mucosa of patients, which were taken before, in the second week (while buccal mucosa was administered 10 Gy), in the third week (while buccal mucosa was administered 20 Gy), and in the fourth week of radiotherapy (while buccal mucosa was received 30 Gy), were investigated. When IL-6 levels in areas receiving high dose radiotherapy were compared with the areas of low dose radiation, IL-6 levels were higher in the former group ($P = 0.015$). Especially in cases with severe inflammation, IL-6 levels increased more than those of patients in other

groups [17]. Jacob et al. argued that it should be investigated whether ghrelin can be used safely in radiation damage caused by total body and half-body radiotherapy and in nuclear terrorism [18].

As the result of loss of appetite and weight, malnutrition and anorexia developed. It is known that ghrelin is a parasympathomimetic hormone. Plasma ghrelin inhibits cytokine secretion. Therefore, secretion of those cytokines, which cause oral mucositis, will also be inhibited. Administration of ghrelin in patients with head and neck cancers may prevent the side effects of radiotherapy if given before, concomitantly and after radiotherapy [19]. The weights of the patients will increase; they will gain weight, so their RT tolerance will be increased indirectly.

Arpin et al. performed a study with 96 patients who had non-small cell lung cancer between stages 1 and 3. They had received treatment with 3D conformal RT and thorax radiotherapy. They measured TNF alpha, IL-6 and IL-10 values before RT, every two weeks after RT was initiated, and 6 weeks and 8 weeks after the completion of RT. It was observed that radiation pneumonia increased in patients whose IL-6 levels were increased during radiotherapy (P = 0.047). In multivariate analysis, development of radiation pneumonia probability was defined as being high in patients with increasing IL-6 and IL-10 levels during the first week of radiotherapy (P = 0.011) [20]. According to the study, radiation pneumonia probability after RT was higher in patients with high baseline cytokine values.

In addition to the anabolic effects of ghrelin in the body, there may be many effects, which remain to be investigated. In our study, patients with high baseline ghrelin levels survived longer than the ones with low values during the 30-month follow up period (P = 0.001). In parallel with this finding, patients with a high baseline level of pro-inflammatory cytokine IL-6 had a lower survival rate than those with a low IL-6 (P = 0.021). Inflammation was not very severe in patients with high ghrelin levels, so weight gain and appetite were not affected much. Patients who did not have anorexia survived longer. Although our sample size was small, there have been few studies performed on the issue of patients with gastrointestinal cancers.

In future, patients who have gastrointestinal system malignancy and are having chemoradiotherapy may receive prophylactic ghrelin analogue during the treatment. This will both increase appetites, thus prevent weight loss of patients, and decrease the intensity of inflammation, thereby increasing overall survival rates. Therefore, further large scale studies are required to clarify this issue.

Acknowledgements

This study was funded by Cukurova University with the project number TF2011LTP27.

Disclosure of conflict of interest

None.

Abbrevations

GH, growth hormone; GHS-R1a, growth hormone secreting hormone receptor; 5-FU, 5-fluorouracile; RT, radiotherapy; IMRT, intensity modulated radiation therapy.

Address correspondence to: Dr. Cigdem Usul Afsar, Department of Medical Oncology, Faculty of Medicine, Cukurova University, Adana, Turkey. Tel: +903223386060 (3142); Fax: +903223386060; E-mail: cigdemusul@yahoo.com

References

[1] Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 1999; 402: 656-60.

[2] Ariyasu H, Takaya K, Tagami T, Ogawa Y, Hosoda K, Akamizu T, Suda M, Koh T, Natsui K, Toyooka S, Shirakami G, Usui T, Shimatsu A, Doi K, Hosoda H, Kojima M, Kangawa K, Nakao K. Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. J Clin Endocrinol Metab 2001; 86: 4753-8.

[3] Druce M, Bloom SR. Central regulators of food intake. Curr Opin Clin Nutr Metab Care 2003; 6: 361-7.

[4] Date Y, Nakazato M, Murakami N, Kojima M, Kangawa K, Matsukura S. Ghrelin acts in the central nervous system to stimulate gastric acid secretion. Biochem Biophys Res Commun 2001; 280: 904-7.

[5] Horii Y, Muraguchi A, Iwano M. Involvement of IL -6 in mesangial proliferative glomerulonephritis. J Immunol 1989; 143: 3949-55.

[6] Stahl RA. Chemoattractive cytokines (chemokines) and immune renal injury. Nephrol Dial Transplant 1995; 10: 307-9.

[7] Noronha IL, Niemir Z, Stein H, Waldherr R. Cytokines and growth factors in renal disease. Nephrol Dial Transplant 1995; 10: 775-86.
[8] Macdonald JS, Smalley SR, Benedetti J, et al. Postoperative combined radiation and chemotherapy improves disease-free survival (DFS) and overall survival (OS) in resected adenocarcinoma of the stomach and gastroesophageal junction: Update of the results of Intergroup Study INT-0116 (SWOG 9008). American Society of Clinical Oncology Gastrointestinal Cancers Symposium 2004; Abstract no. 6.
[9] Kalser MH, Ellenberg SS. Pancreatic cancer. Adjuvant combined radiation and chemotherapy following curative resection. Arch Surg 1985; 120: 899-903.
[10] Moertel CG, Frytak S, Hahn RG, O'Connell MJ, Reitemeier RJ, Rubin J, Schutt AJ, Weiland LH, Childs DS, Holbrook MA, Lavin PT, Livstone E, Spiro H, Knowlton A, Kalser M, Barkin J, Lessner H, Mann-Kaplan R, Ramming K, Douglas HO Jr, Thomas P, Nave H, Bateman J, Lokich J, Brooks J, Chaffey J, Corson JM, Zamcheck N, Novak JW. Therapy of locally unresectable pancreatic carcinoma: a randomized comparison of highdose (6000 rads) radiation alone, moderate dose radiation (4000 rads + 5-fluorouracil), and highdose radiation + 5-fluorouracil: The Gastrointestinal Tumor Study Group. Cancer 1981; 48: 1705-10.
[11] Suzuki H, Matsuzaki J, Hibi T. Ghrelin and oxidative stres in gastrointestinal tract. J Clin Biochem Nutr 2011; 48: 122-5.
[12] Deboer MD, Zhu X, Levasseur PR, Inui A, Hu Z, Han G, Mitch WE, Taylor JE, Halem HA, Dong JZ, Datta R, Culler MD, Marks DL. Ghrelin treatment of chronic kidney disease: improvements in lean body mass and cytokine profile. Endocrinology 2008; 149: 827-35.
[13] Dixit VD, Schaffer EM, Pyle RS, Collins GD, Sakthivel SK, Palaniappan R, Lillard JW Jr, Taub DD. Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T cells. J Clin Invest 2004; 114: 57-66.
[14] Granado M, Priego T, Martin AI, Villanua MA, Lopez-Calderon A. Anti-inflammatory effect of the ghrelin agonist growth hormone-releasing peptide-2 (GHRP-2) in arthritic rats. Am J Physiol Endocrinol Metab 2005; 288: E486-92.
[15] Li WG, Gavrila D, Liu X, Wang L, Gunnlaugsson S, Stoll LL, McCormick ML, Sigmund CD, Tang C, Weintraub NL. Ghrelin inhibits proinflammatory responses and nuclear factor-kappaB activation in human endothelial cells. Circulation 2004; 109: 2221-6.
[16] Garcia JM, Friend J, Allen S. Therapeutic potential of anamorelin, a novel, oral ghrelin mimetic, in patients with cancer-related cachexia: a multicenter, randomized, double-blind, crossover, pilot study. Support Care Cancer 2013; 21: 129-37.
[17] Citrin DE, Hitchcock YJ, Chung EJ, Frandsen J, Urick ME, Shield W, Gaffney D. Determination of cytokine protein levels in oral secretions in patients undergoing radiotherapy for head and neck malignancies. Radiat Oncol 2012; 7: 64.
[18] Jacob A, Shah KG, Wu R, Wang P. Ghrelin as a novel therapy for radiation combined injury. Mol Med 2010; 16: 137-43.
[19] Güney Y, Ozel Turkcu U, Hicsonmez A, Nalca Andrieu M, Kurtman C. Ghrelin may reduce radiation-induced mucositis and anorexia in head-neck cancer. Med Hypotheses 2007; 68: 538-40.
[20] Arpin D, Perol D, Blay JY, Falchero L, Claude L, Vuillermoz-Blas S, Martel-Lafay I, Ginestet C, Alberti L, Nosov D, Etienne-Mastroianni B, Cottin V, Perol M, Guerin JC, Cordier JF, Carrie C. Early variations of circulating interleukin-6 and interleukin-10 levels during thoracic radiotherapy are predictive for radiation pneumonitis. J Clin Oncol 2005; 23: 8748-56.