UNIVERSITATEA DE MEDICINĂ ȘI FARMACIE
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ABSTRACT OF PhD THESIS

HORMONAL DYNAMICS IN ENDOCRINE AND MALIGN PATHOLOGIES

SCIENTIFIC COORDINATOR

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CONTENT OF PhD THESIS

Key words: leptin, adiponectin, FGF23, colorectal cancer

The content of PhD Thesis:

- theoretical part in 4 chapters (49 pages);
- personal researches in 4 chapters (99 pages);
- 132 tables and 57 figures (personal part);
- references (320 titluri).

Note: this PhD abstract contains tables and figures, respecting the numerotation and content of PhD Thesis.

INTRODUCTION

The hormonal secretion varies in disease conditions either in the context of an adaptive mechanism or as a consequence of impaired homeostasis. The evaluation of the hormonal profile can, therefore, be used for the prognosis of some systemic conditions.

Colorectal cancer is one of the most common types of cancer, with over 700,000 deaths annually (Hafner et al. 2016). The risk of occurrence, evolution and prognosis of the treatment of colorectal cancer have been correlated with some adipokine markers, considered to have a protective role (adiponectin, leptin) as well as with some agents with unfavorable role, with angiogenetic effects, with potential to stimulate the proliferation of cancer cells (visfatin, resistin) (Slomian et al. 2014). The incidence of colorectal cancer is growing in Eastern Europe, in parts of Asia and South America. The increased frequency of colorectal cancer in developed countries indicates the involvement of environmental factors in the etiology of this pathology.

Leptin is a hormone secreted in the adipocyte, with an essential role in regulating dietary intake and energy homeostasis, but also with immunomodulatory functions and metabolic and neuroendocrine regulation. The main role of leptin is to modulate energy homeostasis, metabolism and neuroendocrine functions, but some experimental studies have also shown proliferative and anti-apoptotic effects (Kelesidis et al., 2010). The role of leptin in the pathogenesis of colorectal cancer is not yet fully elucidated. However, leptin has been investigated for its role as a possible biomarker for the diagnosis and prognosis of certain types of cancer, including colorectal cancer (Al-Maghrabi et al. 2018). The results repported by resesarchers reveal conflicting data on both serum leptin levels and the role of the LepRb receptor as a prognostic factor in colorectal cancer. Leptin expression was also negatively associated with lymph node invasion in colorectal cancer patients (Koda et al., 2007).

Adiponectin is a serum adipokine secreted in the adipocyte, but also in hepatocytes, cardiomyocytes, skeletal muscle, osteoblasts, placenta, pituitary or digestive tract. Most of the adiponectin production occurs in adipocyte, as secreted adiponectin from other tissues does not appear to affect circulating adiponectin concentration; its autocrine / paracrine role was suggested at these levels (Min et al., 2012, Akingbemi, 2013, Sharma et al., 2016).

Adiponectin, considered an adipocyte hormone that stimulates insulin secretion, fatty acid oxidation and energy consumption, has been negatively correlated with visceral adiposity and insulin resistance, suggesting a link between adiponectin, insulin resistance and colorectal cancer (Hanley et al. 2007).

Increased insulin levels may be related to colorectal cancer initiation, without being involved in disease progression (Jiang et al. 2014). In this context, in recent years has increased the number of reearch groups that tried to explain the role of adiponectin in the development of colorectal cancer has increased.

Fibroblastic growth factors (FGFs) play an important role in both cell growth and differentiation as well as in the normal functioning of the cells throughout the body. FGF 23 is part of the FGF category, being a major regulator of phosphorus metabolism (Juppner et al. 2011). FGF23 secretion is elevated under conditions of hyperphosphatemia or increased vitamin D levels (Liu et al. 2006). FGF23 interferes with other phospho-calcium regulators (PTH and vitamin D). The rare disorders accompanied by an excess or deficiency of FGF23 can lead to severe phosphocalcial, metabolic and bone disorders. FGF23 variations have been studied in the context of chronic renal failure (CRI), but its role in primary hyperparathyroidism is poorly defined.

CHAPTER 5. STUDY REGARDING ROLE OF LEPTIN IN RECTAL CANCER

5.1. Objectives and reason of study

Colorectal cancer is the third most common neoplasm in the world, with an incidence of up to ten times higher in developed countries. The incidence of colorectal cancer has a growing trend in western and eastern Europe, including Romania (Trifan et al. 2006). Involvement of the environmental factors, dietary factors and lifestyle changes in the etiology of colorectal cancer is demonstrated by studies conducted on immigrant populations from regions with low incidence of colorectal cancer (Flood et al., 2000) as well as by the evolution of incidence in Asian countries. with rapid development in recent decades (Sung et al., 2005). Leptin is one of the markers proposed to be involved in detecting and monitoring the progression of rectal cancer (Koda et al., 2007a, Koda et al., 2007b, Paik et al., 2009, Kelesidis et al., 2010).

The aim of the study is to evaluate the changes in postoperative leptin levels in relation both to individual factors and different therapeutic approaches to patients with rectal cancer.

5.2. Materials and method.

The study was performed on a study group of 61 patients diagnosed with rectal cancer at the Regional Institute of Oncology Iasi. The patients were treated by surgery associated with or not with chemotherapy and / or radiotherapy. The study was conducted between August 2017 and August 2018. Informed consent was obtained from all included patients. Blood samples from the subjects included in the study were collected during the morning. Measurement of leptin levels was performed using the Human Adiponectin assay kit (R&D Systems, Minneapolis, MN, USA). Leptin levels were measured and compared in relation to a number individual parameters linked to the development of rectal cancer.

Subject distribution by gender: 38 men and 23 women. Distribution of subjects by age: 42 patients aged 50-69 years and 19 patients aged 70-89 years. Distribution of patients in relation to the location of rectal cancer: 17 patients with rectal cancer located in the upper third, 15 patients with rectal cancer located in the middle third, and 29 patients with rectal cancer located in the lower third. Subject distribution with respect to chemotherapy (absence of chemotherapy vs. 1-3 months post-operatory chemotherapy vs. 3-6 months post-operatory chemotherapy): 33 patients not included in chemotherapy, 18 patients included in chemotherapy 1-3 months, 10 patients who were included in chemotherapy 3-6 months. Distribution of subjects in relation to radiotherapy (absence of radiotherapy vs. 1-3 months post-operatory radiotherapy vs. 3-6 months post-operatory radiotherapy): 20 patients not included in radiotherapy, 31 patients included in radiotherapy 1-3 months, respectively 10 patients who were included in radiotherapy 3-6 months. Distribution of group of patients in relation to post-operatory changes of body weight (absence of weight loss vs. weight loss 0-9 kg vs. weight loss 10-20 kg: 33 patients who did not show weight loss, 20 patients who showed weight loss 0-9 kg , respectively 8 patients who had a weight loss of 10-20 kg.

We evaluated the influence of some parameters on the change of LEPTINA marker values in the patients affected by colorectal cancer. From the frequency distributions and from the normality tests, the necessity of using non-parametric tests emerged. We used the Mann-Whitney test to test the difference between independent groups for which the dependent variable is expressed in ordinal (rank) values or when it does not support a parametric test. The statistical analysis was performed, at an initial stage, at the level of the whole lot, by comparing the concentration changes of LEPTINE marker between different time intervals: preoperative - 24 hours postoperatively, preoperative - 72 hours postoperative, preoperative - 7 days

postoperative, 24 hours - 72 hours postoperatively, 24 hours - 7 days postoperatively, respectively 72 hours - 7 days postoperatively. In the second stage, the changes of the marker LEPTINA were analyzed in relation to the following variables:
1. SEX: Male (M) vs. Female (F);
2. AGE GROUP: 50-69 vs. 70-89;
3. CANCER LOCATION: Lower CR (inf) vs. CR medium (middle) vs. Upper CR (sup);
4. CHEMIOTHERAPY: Without chemotherapy vs. 1-3 months Chemotherapy Vs. 3-6 months Chemotherapy;
5. RADIOTHERAPY: Without chemotherapy vs. 1-3 months Radiotherapy vs. 3-6 months Radiotherapy;
6. WEIGHT LOSS: 0 (kg) vs. 1-9 (kg) vs. 10-20 (kg)

5.3. Results

Descriptive statistics are presented in Table 5.I and Figure 5.2. The mean value of leptin levels increases from 5,221 ng / mL preoperatively to 17,727 ng / mL at 24 hours, then decreases to 8,411 ng / mL at 72 hours, respectively at 8,622 ng / mL at 7 days.

Table 5.1. Descriptive statistics (Leptin- preoperatory, 24h, 72h, 7 days)

Statistics

		LeptinaPreop	Leptina24h	Leptina72h	Leptina7zile
N	Valid	61	61	61	61
	Missing	183	183	183	183
Mean		**5.221418**	**17.727793**	**8.411789**	**8.622749**
Std. Error of Mean		.7860109	2.3018165	1.4848352	1.3873839
Median		3.136000	13.213300	4.460200	5.641100
Std. Deviation		6.1389417	17.9777618	11.5969340	10.8358147
Skewness		2.182	1.635	2.315	2.830
Std. Error of Skewness		.306	.306	.306	.306
Kurtosis		6.263	3.527	5.290	10.841
Std. Error of Kurtosis		.604	.604	.604	.604

Figure 5.3.3 compares the evolution of Leptin levels in the group of patients with rectal cancer according to gender (male vs. female).

Figure 5.4 compares the evolution of Leptin levels in the group of patients with rectal cancer in relation to the age group (50-69 years vs. 70-89 years).

Figure 5.5. compares the evolution of Leptin levels in the group of patients with rectal cancer in relation to the localization of tumors (upper third vs. middle third vs. lower third).

Figure 5.6. shows the evolution of Leptin levels in patients with rectal cancer in relation to the therapeutic management by chemotherapy.

Figure 5.7. shows the evolution of Leptin levels in patients with rectal cancer in relation to the therapeutic management by radiotherapy.

Figure 5.8. compares the evolution of Leptin levels in colorectal cancer patients in relation to the degree of body weight loss.

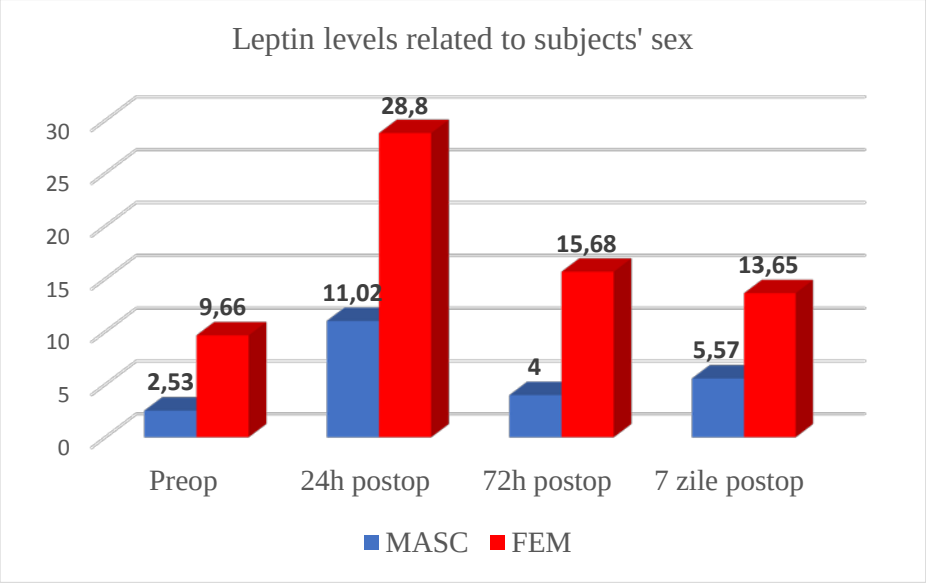


Figure 5.3. Leptin levels (ng/ml) for patients with rectal cancer (males vs. females)

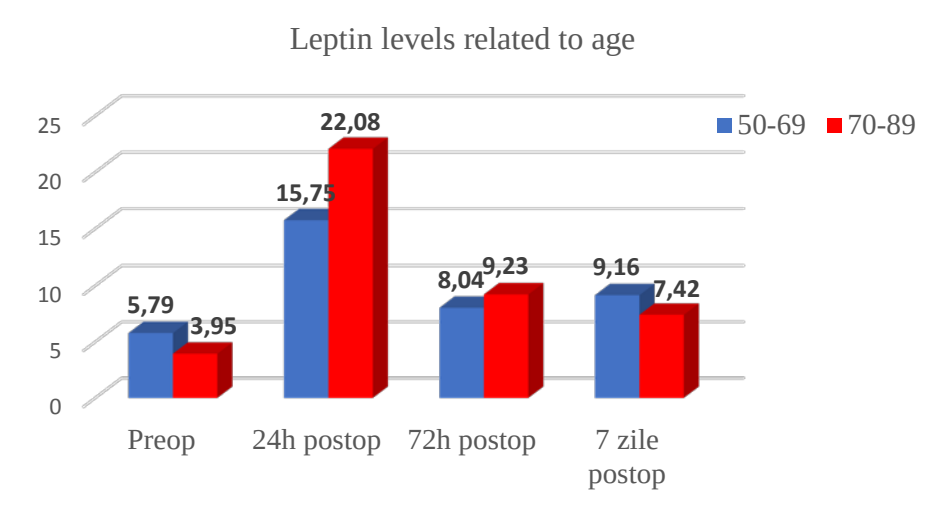


Figure 5.4. Leptin levels (ng/ml) for patients with rectal cancer (age 50-69 vs. age 70-89)

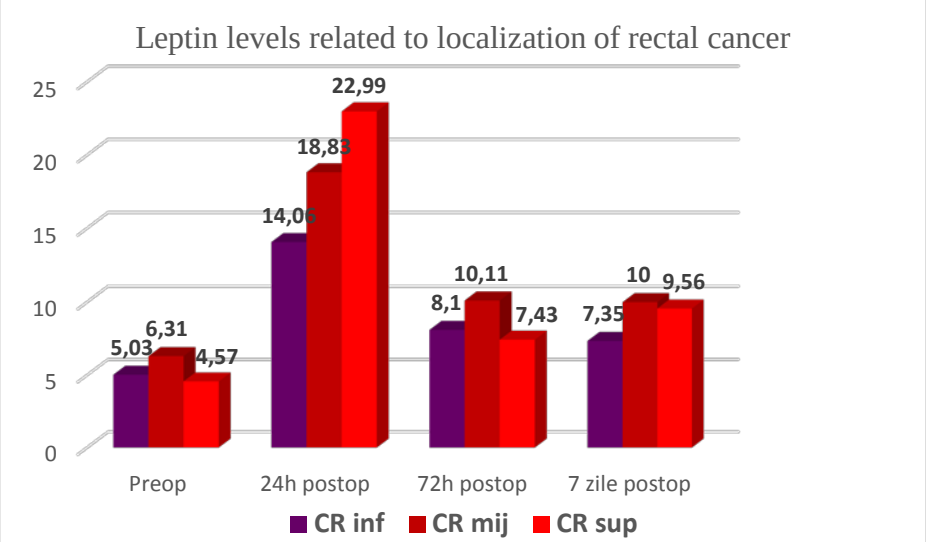


Figure 5.5. Leptin levels (ng/ml) for patients with rectal cancer (1/3 sup vs. 1/3 med vs. 1/3 inf)

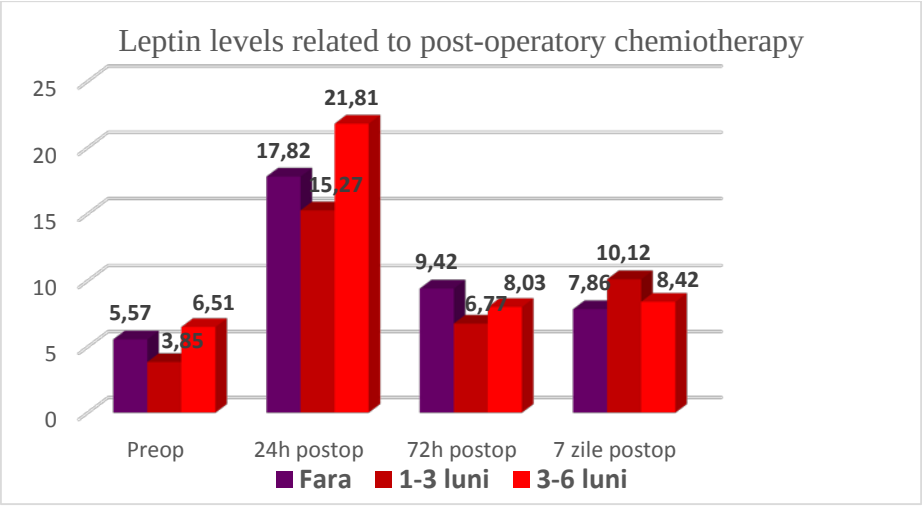


Figure 5.6. Leptin levels (ng/ml) for patients with rectal cancer (chemiotherapy: absent vs. 1-3 months vs. 3-6 months)

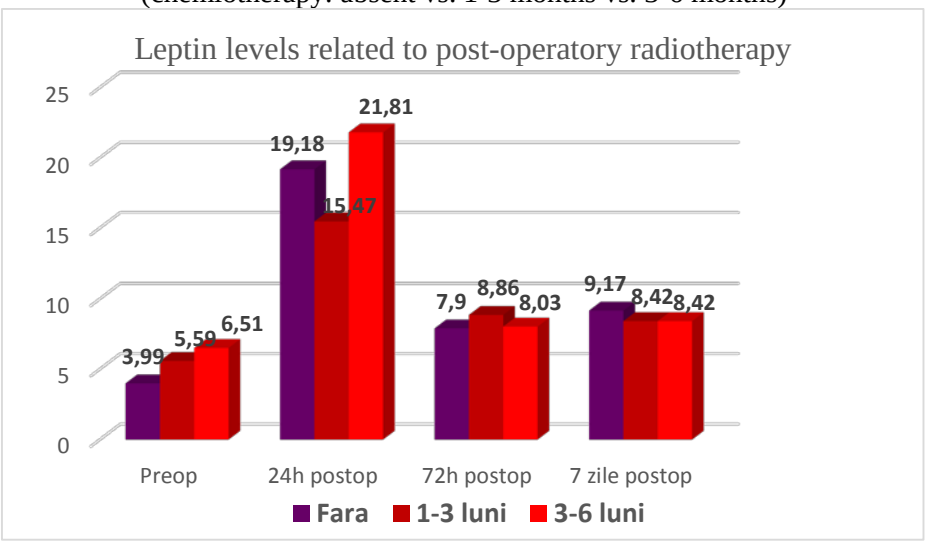


Figure 5.7. Leptin levels (ng/ml) for patients with rectal cancer (radiotherapy: absent vs. 1-3 months vs. 3-6 months)

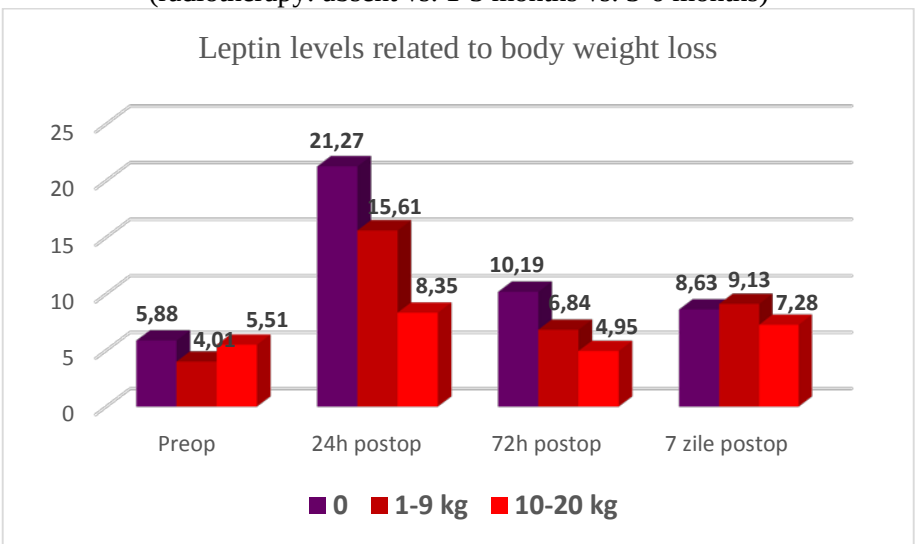


Figure 5.8. Leptin levels (ng/ml) for patients with rectal cancer (body weight loss: absent vs. 0-9 kg vs. 10-20 kg)

5.4. Discussions

Although leptin is known to be a modulator of energy homeostasis, metabolism and neuroendocrine functions, proliferative and anti-apoptotic effects involving leptin in cancer pathogenesis have been found (Kelesidis et al., 2010). Also, leptin expression was negatively associated with ganglionic invasion in patients with rectal cancer (Koda et al., 2007b, Paik et al., 2009).

In this context, our study demonstrated significant changes of leptin in the whole group in the postoperative period compared to the preoperative period, as follows: the average value of leptin levels increases from 5,221 ng / mL preoperatively to 17,727 ng / mL at 24 hours. , then decreases to 8,411 ng / mL at 72 hours, respectively at 8,622 ng / mL at 7 days. These data would suggest a role for leptin as a negative predictor of rectal cancer.

LepRb receptor expression is stronger in the early stages of colorectal cancer, with increased leptin receptor concentrations being associated with better histopathological differentiation and a better prognosis for colorectal cancer patients (Uddin et al. 2009, Aloulou et al. 2008). Low levels of leptin are considered markers of poor prognosis, similarly with an increased degree of tumor invasion, including at the ganglionic level (Paik et al., 2009).

In vitro studies on cancer cell lines have demonstrated the role of leptin as a stimulator for carcinomatous cell proliferation by activating pathways that mediate cell growth and survival (Chen et al, 2009). Synergistic action of leptin and VEGF accelerates angiogenesis and stimulates tumor invasion and metastasis of other tissues in colorectal cancer patients (Tutino et al., 2011, Martins et al., 2013). Leptin increases the expression of angiogenic factors, such as fibroblast growth factor (FGF), in cancer cells (Endo et al., 2011).

Koda et al. (2007) and Wang et al (2012) significantly correlated leptin expression in colorectal cancer with tumor histologic type, degree of evolution, but did not find an association between leptin expression and patient age.

Liu et al. (2011) determined the existence of a correlation between the expression of leptin and the degree of evolution of colorectal cancer or the presence of metastases, but did not determine the existence of an association between the level of expression of leptin and the location of colorectal cancer.

Chemotherapy, performed over a 3-month postoperative periode, led to stabilization in 40% of cases, partial response in 45% of cases, respectively cancer progression in 15% of cases, aspects associated with significantly higher leptin levels in patients with stabilization.

Studies that have investigated the effects of using short-term radiotherapy in the postoperative stage have shown that this therapy increases significantly the survival rate in colorectal cancer (Haffner et al., 2016; Ngan et al., 2012; Folkesson et al., 2016, De Caluwe et al. 2013, McCarthy et al. 2012).

5.5. Conclusions

1. The levels of leptin were higher in women than in men in patients with rectal cancer,
2. Leptin shows a particular dynamic in patients with rectal cancer of both sexes, having a significant increase at 24 hours postoperatively (by 340% compared to the preoperative value) and a significantly higher level up to 7 days postoperatively (by 60% compared with preoperative value). The use of leptin as a marker for postoperative evolution of rectal cancer may have prognostic applicability.
3. Dynamic tracking also showed the persistence of leptin levels differences in favor of women. There were no differences in the post-operative evolution of leptin by age group, localization of rectal cancer, use of radio and chemotherapy, or in relation to the change in body weight.
4. The correlation between leptin and body weight was maintained only in men, but not in women. This difference could be explained by the particularities of the hormonal profile, but also of the body composition and distribution of adipose tissue in women.

CHAPTER 6. STUDY REGARDING ROLE OF ADIPONECTIN IN RECTAL CANCER

6.1. Objectives and reason of study

Colorectal cancer is the third most common neoplasm in the world, with an incidence of up to ten times higher in developed countries. The incidence of colo-rectal cancer is increasing in western and eastern Europe, including Romania (Trifan et al., 2006).

Involvement of environmental factors, dietary factors and lifestyle changes in the etiology of rectal cancer is demonstrated by studies performed on immigrant populations from regions with low incidence of colo-rectal cancer (Flood et al., 2000) and by the evolution of incidence in Asian countries with a rapid development in recent decades (Sung et al., 2005). Adiponectin is one of the factors involved in the development of colorectal cancer (Saxena et al. 2012, Mutoh et al. 2011, Chen et al. 2009, Wei et al. 2005, Otake et al. 2010, Aleksandrova et al. 2012, Tae et al. 2014, Byeon et al. 2010).

The aim of the study is to evaluate the postoperative changes of adiponectin in patients with rectal cancer.

6.2. Materials and method.

The study was performed on a number of 61 patients diagnosed with rectal cancer at the Regional Institute of Oncology Iasi, who were treated by surgery associated with or not with chemotherapy and / or radiotherapy. The study was conducted between August 2017 and August 2018. Informed consent was obtained from the patients included in the study. Blood samples from the subjects included in the study were collected during the morning. Measurement of adiponectin levels was performed using the Human Adiponectin assay kit (R&D Systems, Minneapolis, MN, USA). Adiponectin levels were measured in relation to individual parameters, with a role in the development of rectal cancer.

Distribution of patients by gender (male vs. female): 38 men and 23 women. Distribution of group of patients by age group (50-69 years vs. 70-89 years): 42 patients aged 50-69 years and 19 patients aged 70-89 years. Distribution of patients in relation to the location of rectal cancer (upper third vs. middle third vs. lower third): 17 patients with rectal cancer located in the upper third, 15 patients with rectal cancer located in the middle third, and 29 patients with localized rectal cancer in the lower third. Distribution of patients in relation to chemotherapy (absence of post-operatory chemotherapy vs. 1-3 months post-operatory chemotherapy vs. 3-6 months post-operatory chemotherapy): 33 patients not included in chemotherapy, 18 patients included in chemotherapy 1-3 months, respectively 10 patients who were included in chemotherapy 3-6 months. Distribution of patients compared to radiotherapy (absence of post-operatory radiotherapy vs. 1-3 months post-operatory radiotherapy vs. 3-6 months post-operatory radiotherapy): 20 patients not included in radiotherapy, 31 patients included in radiotherapy 1-3 months, respectively 10 patients who were included in radiotherapy 3-6 months. Distribution of patients in relation to changes of body weight (absence of body weight loss vs. body weight loss 0-9 kg vs. body weight loss 10-20 kg): 33 patients who did not show body weight loss, 20 patients who showed body weight loss 0-9 kg, respectively 8 patients who showed body weight loss 10-20 kg.

It was assessed the influence of some parameters on the change of the values of ADIPONECTINA marker in the patients affected by rectal cancer. We used the Mann-Whitney test to test the difference between independent groups for which the dependent variable is expressed in ordinal (rank) values or when it does not support a parametric test. The statistical analysis was performed, at an initial stage, at the level of the whole lot, by comparing the concentration changes of ADIPONECTINA marker between different time intervals: preoperatively - 24 hours postoperatively, preoperatively - 72 hours postoperatively,

preoperatively - 7 days postoperatively, 24 hours - 72 hours postoperatively, 24 hours - 7 days postoperatively, respectively 72 hours - 7 days postoperatively. In the second stage, the changes of the marker ADIPONECTINA were analyzed in relation to the variables:
1. SEX: Male (M) vs. Female (F);
2. AGE GROUP: 50-69 vs. 70-89;
3. CANCER LOCATION: Lower CR (inf) vs. CR medium (middle) vs. Upper CR (sup);
4. CHEMIOTHERAPY: Without chemotherapy vs. 1-3 months Chemotherapy Vs. 3-6 months Chemotherapy;
5. RADIOTHERAPY: Without chemotherapy vs. 1-3 months Radiotherapy vs. 3-6 months Radiotherapy;
6. WEIGHT LOSS: 0 (kg) vs. 1-9 (kg) vs. 10-20 (kg).

6.3.Results

The descriptive statistics elements are presented in table 6.I and figure 6.2.a. The mean value of adiponectin levels decreases from 9,874 μg / mL preoperatively to 8,014 μg / mL at 24 hours, and then increases to 8,993 μg / mL at 72 hours, respectively at 9,794 μg / mL at 7 days.

Table 6.1. Descriptive statistics (Adiponectin- preoperatory, 24h, 72h, 7 days)

Statistics

		AdipoPreop	Adipo24h	Adipo72h	Adipo7zile
N	Valid	61	61	61	61
	Missing	183	183	183	183
Mean		**9.874923**	**8.014289**	**8.993635**	**9.794293**
Std. Error of Mean		.5698348	.4642159	.5146119	.5251004
Median		8.982800	7.578200	8.517600	8.965200
Std. Deviation		4.4505524	3.6256417	4.0192477	4.1011649
Skewness		.777	1.051	.730	.836
Std. Error of Skewness		.306	.306	.306	.306
Kurtosis		-.040	.832	-.212	.042
Std. Error of Kurtosis		.604	.604	.604	.604

Figure 6.3 shows the evolution of the levels of Adiponectin in the group of patients with rectal cancer in relation to gender (male vs. female).

Figure 6.4 compares the evolution of the levels of Adiponectin in the group of patients with rectal cancer in relation to age group (age 50-69 years vs. age 70-89 years).

Figure 6.5. compares the evolution of Adiponectin levels in the group of patients with rectal cancer compared to localization of the neoplasia (upper third vs. middle third vs. lower third).

Figure 6.6. compares the evolution of Adiponectin levels in the group of patients with rectal cancer compared to the therapeutic management by postoperative chemotherapy.

Figure 6.7. presents Adiponectin levels in relation to the therapeutic management by postoperative radiotherapy.

Figure 6.8. shows the evolution of Adiponectin levels in relation to body weight loss.

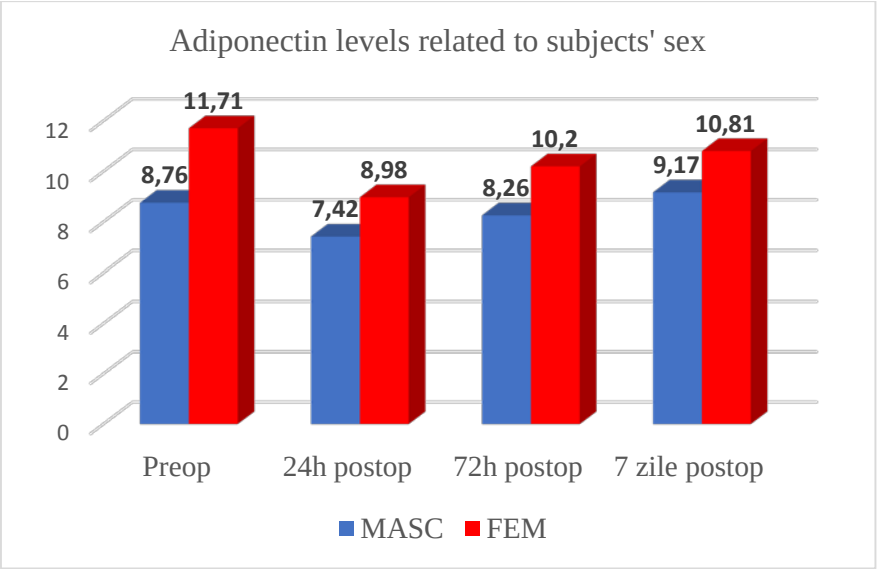


Figure 6.3. Adiponectin levels (µg/ml) for patients with rectal cancer (males vs. females)

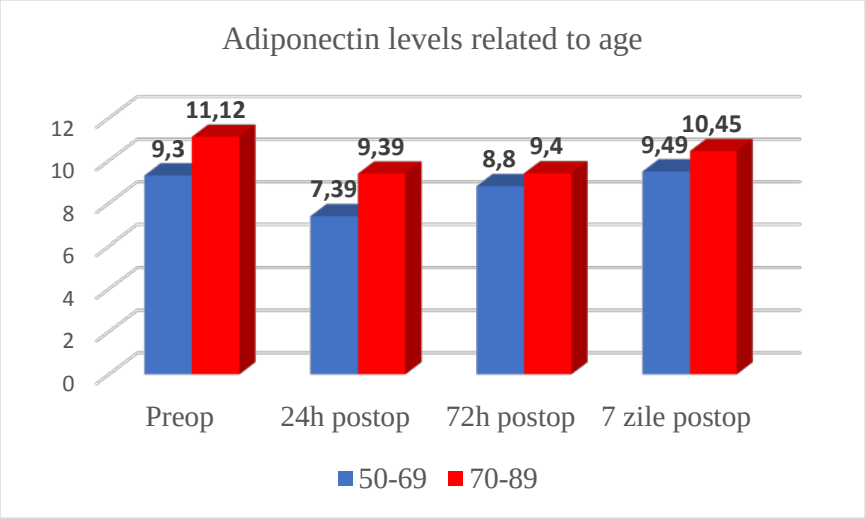


Figure 6.4. Adiponectin levels (µg/ml) for patients with rectal cancer (age 50-69 ani vs. age 70-89)

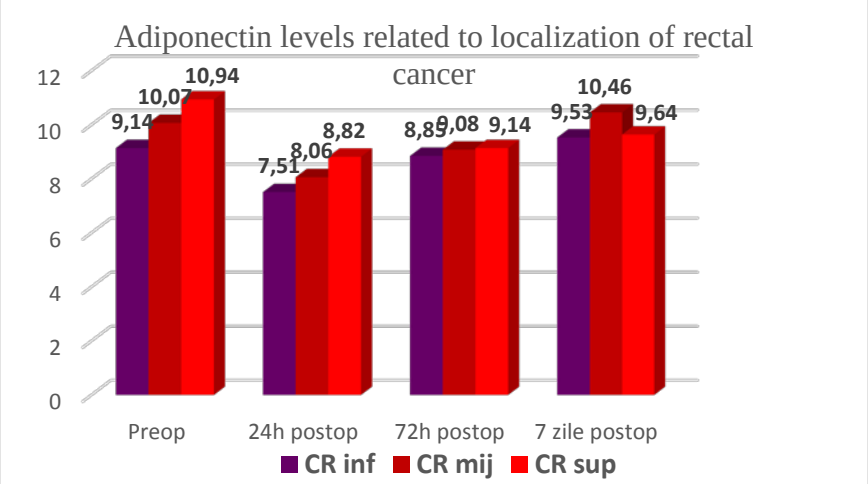


Figure 6.5. Adiponectin levels (µg/ml) for patients with rectal cancer (1/3 sup vs. 1/3 med vs. 1/3 inf)

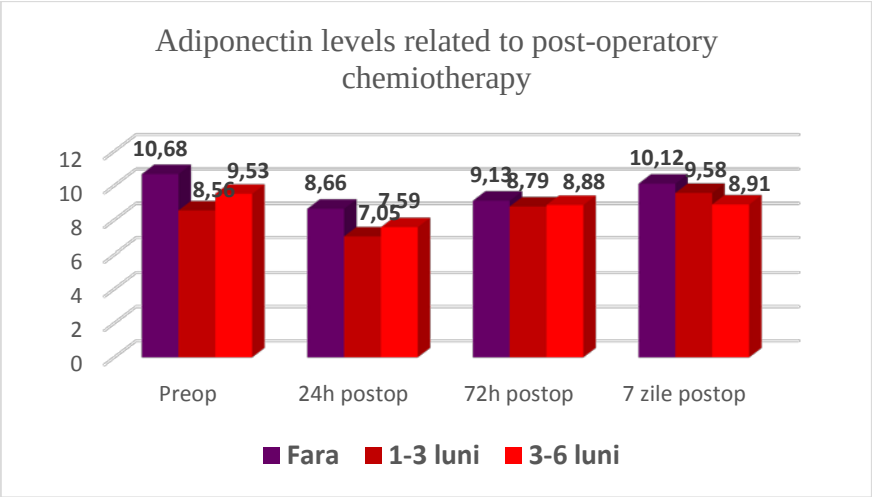


Figure 6.6. Adiponectin levels (µg/ml) for patients with rectal cancer (chemiotherapy: absent vs. 1-3 months vs. 3-6 months)

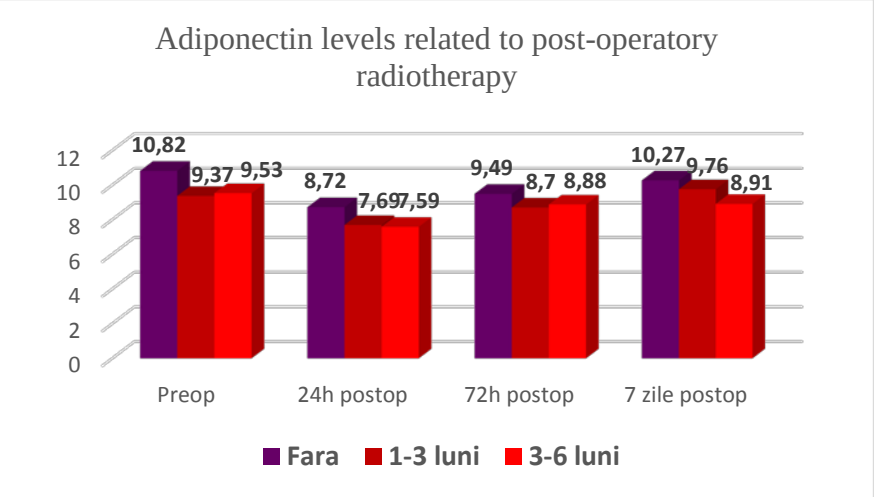


Figure 6.7. Adiponectin levels (µg/ml) for patients with rectal cancer (radiotherapy: absent vs. 1-3 months vs. 3-6 months)

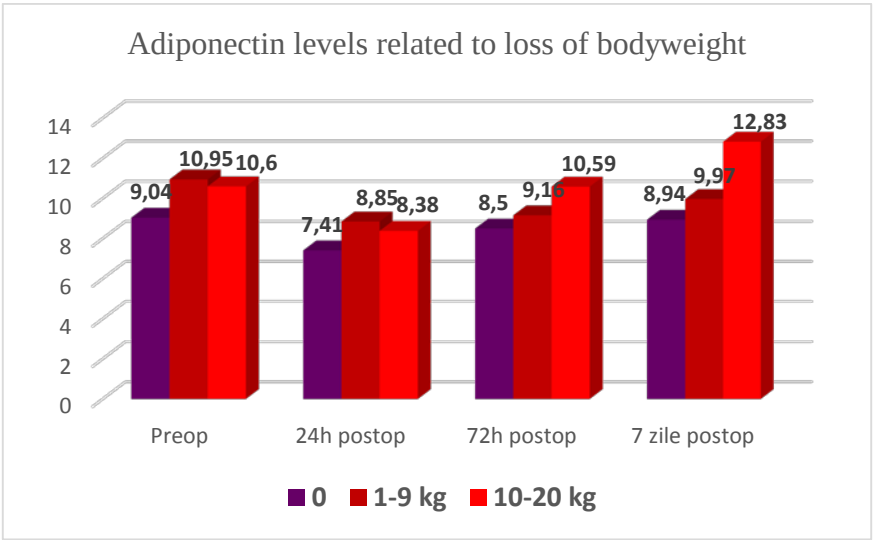


Figure 6.8. Adiponectin levels (µg/ml) for patients with rectal cancer (loss of body weight: absent vs. 0-9 kg vs. 10-20 kg)

6.4. Discussions

In our study, it was found that in the whole group of patients with rectal cancer the mean value of adiponectin decreases from 9.87 μg / mL preoperatively to 8.01 μg / mL at 24 hours, then increases to 8.99 μg / mL at 72 hours, respectively at 9.79 μg / mL at 7 days.

The results obtained support the data from the literature regarding the modification of adiponectin values after surgery in colorectal cancer. A study in a healthy female patient group reported adiponectin concentrations of 8.87 μg / mL (with a range of 2.77-25.03 μg / mL) for patients with normal weight, significantly higher compared to overweight patients (7.4 μ / mL, 2.76-22.38 μg / mL) (Nien et al. 2007).

Exogenous administration of adiponectin results in a significant reduction in the number and size of adenomatous polyps (Otani et al., 2010; Moon et al., 2013). Habeeb et al. (2011) repported the inhibitory role of adiponectin on tumor growth, as well as an increase in adiponectin receptor expression. Otake et al. (2010) have shown that low adiponectin levels are greater risk factor than triglyceride or BMI levels in patients with early-stage colorectal adenoma or carcinoma. Tae et al. (2014) demonstrated a lower expression of adiponectin receptors in colorectal cancer and advanced colorectal adenomas, compared to normal tissues.

Some studies have shown that low levels of leptin lead to an increased risk of colorectal cancer in men (Guadagni et al. 2009, Gialamas et al. 2011). The studies performed by Song et al. (2013), Yamaji et al (2010) demonstrated, after adjusting for multiple risk factors, the association of increased levels of adiponectin with reduced risk of colorectal cancer in male patients.

Gender significantly influences the clinical and pathological characteristics of colorectal cancer, including differences in incidence and mortality rates, age, complications, type of treatment, histopathological aspects, and survival rate (Koo et al. 2010).

Chemotherapy (6 sessions) leads to the increase of the adiponectin levels with 47%, respectively decrease of the levels of some agents that have proangiogenesis effects and stimulate cancer cells proliferation (visfatin, resistin) (Slomian et al, 2014).

Regarding the influence of the changes in body weight postoperatively, we could not compare the data obtained with those of other studies, because we did not identify similar studies performed in patients with colorectal cancer.

6.5. Conclusions

1. The basal values of adiponectin were higher in women than in men in colorectal cancer patients. This gender dichotomy has been described in the literature.
2. Adiponectin demonstrates a particular dynamic in patients with colorectal cancer operated, with significant decrease to 76.75% of preoperative values at 24 hours postoperatively and returning to baseline values in 7 days. The use of leptin as a marker for postoperative evolution of rectal cancer may have prognostic applicability.
3. Dynamic monitoring showed higher level of adiponectin in women.
4. There were no differences in postoperative evolution of adiponectin by age group, localization of rectal cancer or use of radio and chemotherapy.
5.Significant differences in adiponectin levels were observed in women, but not in men who suffered body weight loss compared to patients whose body weight remained constant. The correlation between adiponectin and body weight persisted postoperatively only in men, but not in women. This difference could be explained by the particularities of the hormonal profile, but also of the body composition and distribution of adipose tissue in women.

CHAPTER 7. STUDY REGARDING FGF23 ROLE IN PRIMARY HYPERPARATHYROIDISM

7.1. Objectives and reason of study

FGF23 is one of the factors involved in the modulation of phosphocalcic metabolism having possible interferences with the synthesis and secretion of the other two important regulators, PTH and vitamin D. The interference of these hormones is described in particular pathological situations, such as hyperparathyroidism secondary to renal failure, but FGF23 changes in primary hyperparathyroidism are insufficiently studied.

Primary hyperparathyroidism can cause symptomatology and complications that can affect patients' quality of life, starting from depression, anorexia, fatigue, polyuria, polydipsia and reaching renal lithiasis, impaired renal function, calcifications in soft tissues and blood vessels, or severe osteoporosis with pathological fractures (Spivacow et al. 2017).

The aim of the study is to compare the levels of FGF23 between patients with primary hyperparathyroidism and patients from a healthy control group, in relation to age group and menopausal status. At the same time, it was investigated the secretory dynamics of FGF23 in patients diagnosed with primary hyperparathyroidism.

7.2. Materials and method.

The study was performed on a number of 93 female patients from the general population (GEN group) and a number of 34 female patients diagnosed with primary hyperparathyroidism (PTH group), within the Department of Endocrinology of the "Sf.Spiridon" Hospital. Iasi (Figure 7.1.a). Informed consent was obtained from the patients included in the study.

FGF23 levels were measured and compared between study groups (general population, PTH group) with respect to some investigated parameters (age groups, premenopausal-postmenopausal) and between the group of patients selected from the general population and the group of patients with primary hyperparathyroidism.

In the group of patients in the general population, the distribution by age group was as follows: 41 patients under the age of 50, 22 patients aged between 50-60 years, 30 patients over 60 years. In the group of patients in the general population, the distribution in relation to the absence / presence of menopause was as follows: 38 patients in premenopause, respectively 55 patients in postmenopause. In the group of patients in the group with primary hyperparathyroidism (PTH), the distribution by age group was as follows: 9 patients under the age of 50, 12 patients aged between 50-60 years, 13 patients over 60 years. In the group of patients from the general population, the distribution in relation to the absence / presence of menopause was as follows: 12 patients in premenopause, respectively 22 patients in postmenopause.

It was used statistical tests to analyze the influence of some parameters on FGF23 marker values in patients in the general population and those affected by primary hyperparathyroidism. From the frequency distributions and from the normality tests, the necessity of using nonparametric tests has emerged. It was used the Mann-Whitney test to test the difference between independent groups for which the dependent variable is expressed in ordinal (rank) values or when it does not support a parametric test. Statistical analysis was performed in three stages.

In the first stage statistical analysis investigated the influence of the age group, respectively of the menopause, on FGF23 values, in the patients of the general population (GEN group):

- Age groups:
  <50 vs. 50-60;
  <50 vs. > 60;
  50-60 vs. > 60
- Menopause:

premenopausal vs. postmenopausal

In the second stage, it was investigated the influence of the age group, respectively of the menopause, on FGF23 values in the group of patients with primary hyperparathyroidism.

- Age groups:
  <50 vs. 50-60;
  <50 vs. > 60;
  50-60 vs. > 60
- Menopause:
  -premenopausal vs. postmenopausal

In the third stage, it was investigated the influence of the age group and the menopause on FGF23 values, comparing the patients in the general population (GEN group) and the group of patients with primary hyperparathyroidism (PTH group).

- Age groups (GEN vs. PTH):
  <50 (GEN) Vs. <50 (PTH)
  50-60 (GEN) vs. 50-60 (PTH)
  > 60 (GEN) vs. > 60 (PTH)
- Menopause (lot GEN vs. lot PTH):
  -postmenopausal (lot GEN) vs. postmenopausal (PTH group)
  -premenopause (lot GEN) vs. premenopausal (PTH group)

7.3.Results

Descriptive statistical elements are presented in table 7.1. The mean value of FGF23 levels is 77,65 ng/mL for patients in general population, respectively 77,76 ng/mL for patients with primary hyperparathyroidism (figure 7.2.c).

Table 7.1. Descriptive statistics (FGF- general population, primary hyperparathyroidism)

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Skewness		Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Std. Error
FGF23GEN	93	55.1532	120.2390	**77.649970**	16.3890078	.797	.374	-.293	.733
FGF23PTH	34	41.67	146.36	**77.7585**	24.11471	1.202	.403	1.761	.788
Valid N (listwise)	34								

Figure 7.5.a-b compares FGF23 values in the two groups (GEN vs. PTH group) for premenopausal patients and postmenopausal patients. The mean FGF23 values are 76.2 µg / ml in the group of patients from the general population in premenopause, respectively 65.81 µg / ml in the patients with hyperparathyroidism in the premenopause (figure 7.5.a). The mean FGF23 values are 76.64 µg / ml in the patients from the general population in premenopause, respectively 84.46 µg / ml in the patients with postmenopausal hyperparathyroidism (Figure 7.5.b).

In Figure 7.6.a-c. FGF23 values in the two groups (GEN vs. PTH) are compared with the age group. For patients under the age of 50, mean FGF23 values are 76.13 pg / ml in patients in the GEN group, respectively 74.42 pg / ml in patients in the PTH group (Figure 7.6.a). For patients aged 50-60 years, mean FGF23 values are 70.57 pg / ml in patients in the GEN group, respectively 73.22 pg / ml in patients in the PTH group (Figure 7.6.b). For patients over 60 years of age, mean FGF23 values are 81.23 µg / ml in patients in the GEN group, respectively 84.24 µg / ml in patients in the PTH group (Figure 7.6.c).

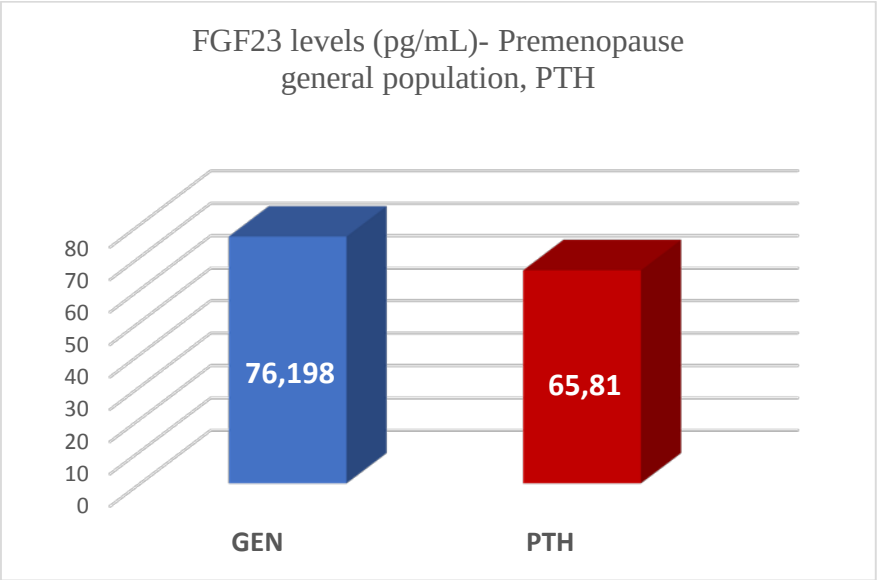


Figure 7.5.a. FGF23 levels (pg/ml) for patients from study groups GEN and PTH (premenopause)

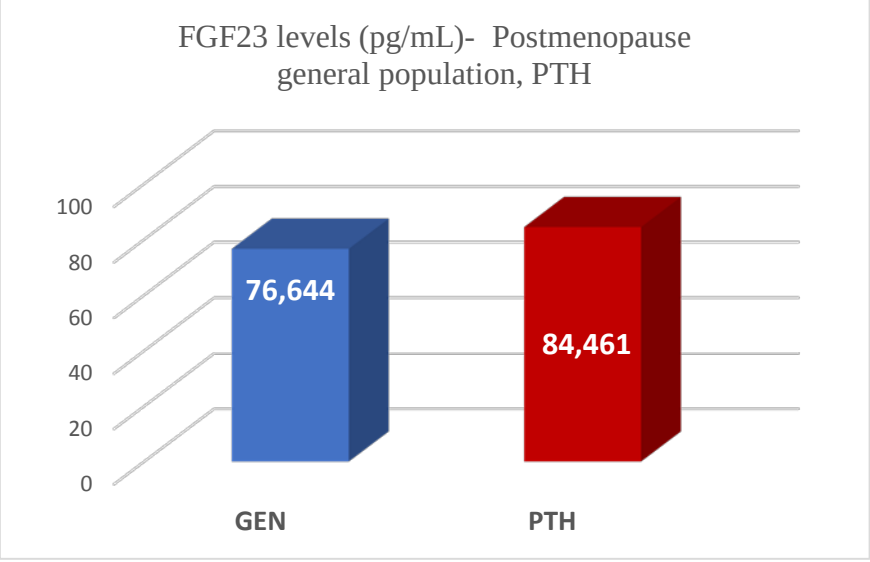


Figure 7.5.b. FGF23 levels (pg/ml) for patients from study groups GEN and PTH (postmenopause)

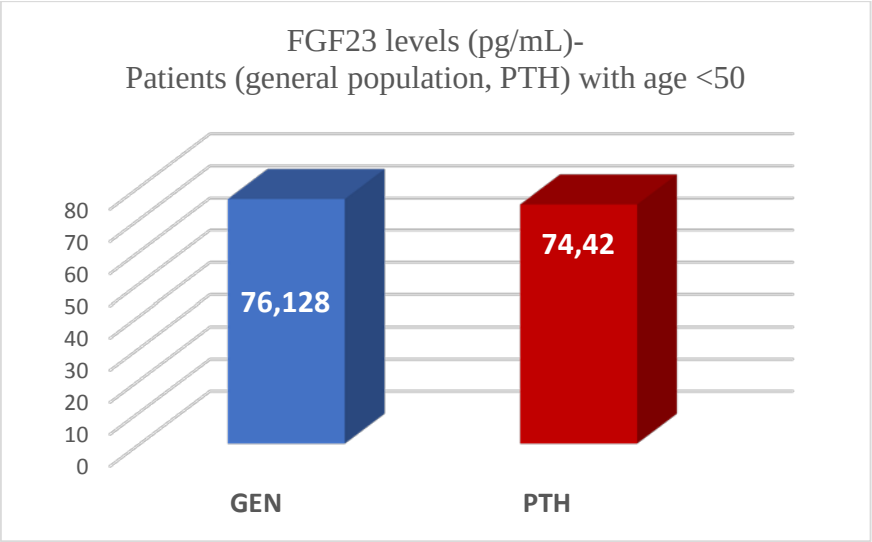


Figure 7.6.a. FGF23 levels (pg/ml) for patients with age < 50 ani (GEN vs. PTH)

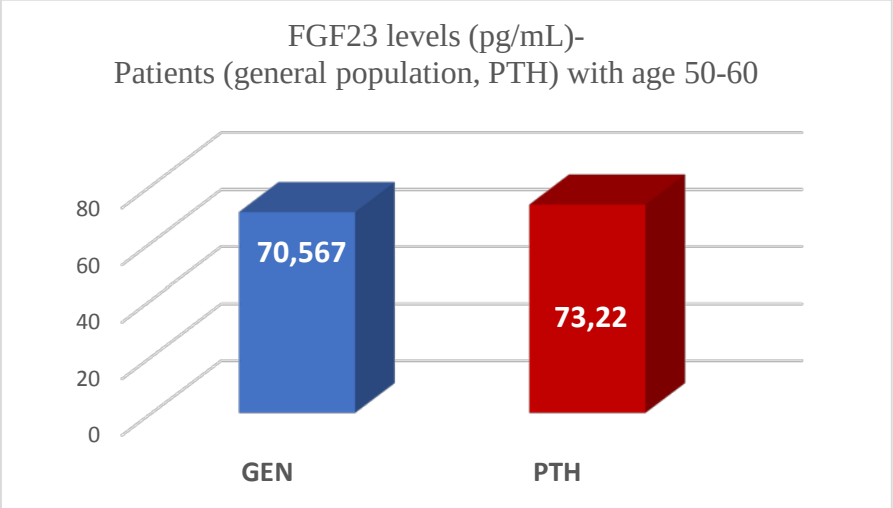


Figure 7.6.b. FGF23 levels (pg/ml) for patients with age 50-60 (GEN vs. PTH)

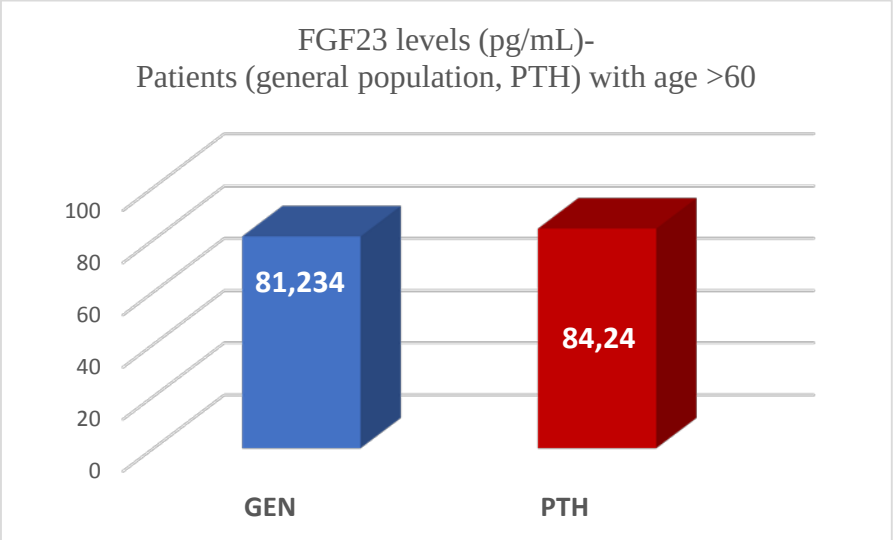


Figure 7.6.c. FGF23 levels (pg/ml) for patients with age >60 ani (GEN vs. PTH)

7.4. Discussions

It were investigated the age parameters and the premenopausal-postmenopausal status of the studied patients (general population, patients with primary hyperparathyroidism) under the conditions in which the age and estrogen deficiency characteristic of the postmenopausal period seem to play a role in the onset of secondary hyperparathyroidism (Khosla et al, 1997).Significant statistical differences were found between premenopausal and postmenopausal patients in the group of patients with hyperparathyroidism.It was recorded statistically significant differences between patients in the general population and patients with hyperparathyroidism in the group with pre-menopause periods (but not for post-menopause group). Significant statistical differences between FGF23 levels were found between patients in the general population and those affected by hyperparathyroidism both in the group under 50 years and in the groups 50-60 years, respectively over 60 years.FGF23 and parathyroid hormone (PTH) are key factors in the control of the phosphate ion homeostasis via the bone-parathyroid-renal tissue axis (Witteveen et al. 2012). There are studies showing that FGF23 is a direct or indirect inhibitor of PTH secretion, respectively, of mRNA expression and parathyroid cell proliferation under hypocalcemia (Canalejo et al. 2010)

The direct relationship between FGF23 and PTH levels was demonstrated by a study that shows that PTH administration in healthy mice leads to increased FGF23 levels, when parathyroidectomy prevents the increase of FGF23 levels or enhances FGF23 hypersecretion in renal failure mice. (Lavi-Moshayoff et al. 2010). Two studies have shown that administration of PTH conducts to decreased levels of FGF23 (Gutierrez et al., 2012; Saji et al., 2010). The diagnosis of primary hyperparathyroidism is essential in postmenopausal patients, when hyperparathyroidism is associated with accelerated bone resorption processes. The detection of the excess FGF23 is essential, as excess FGF23 causes hypophosphataemia, decreased renal reabsorption and inhibition of vitamin D. In these cases, aberrant vitamin D metabolism and bone pathology (osteomalacia, fractures) may occur.In our study, we investigated exclusively FGF23 changes in relation to age group, menopause and primary hyperparathyroidism. This is a limitation of the study, so further studies are needed to include analyzing the relationship of FGF23 with parameters such as calcemia, phosphatemia, calcitriol levels, or serum iron concentration. Also, in the modification of FGF23 levels local factors may be involved at the bone level (Martin et al. 2011), but the mechanism by which local factors act is less known.

The importance of FGF23 as a marker of health status is demonstrated by studies that draw attention to the relationship between increased phosphate levels in populations in industrialized areas (due to consumption of processed foods, with high phosphate content) and increased FGF23, under conditions where phosphate-rich diet (inorganic phosphate) will lead to compensatory increases of FGF23 to maintain serum phosphate levels within normal limits

7.5. Conclusions

1. Serum levels of FGF23 show no significant differences between patients in the general population (77.65 pg / mL) and patients in the hyperparathyroidism group (77.76 pg / mL).
2. Patients affected by primary hyperparathyroidism have higher values of FGF23 during the premenopausal period (84.46 vs. 65.81 pg / ml for postmenopausal patients with primary hyperparathyroidism). This difference is not observed in healthy patients (76.2vs76.64 pg / ml).
3. FGF23 values were not significantly altered by parathyroidectomy in our study. In our patients with primary hyperparathyroidism, FGF23 was inversely correlated with PTH.
4. The lowest FGF23 values are found in patients with premenopausal hyperparathyroidism (65.810pg / ml), followed by patients in the general population in premenopause (76.198pg / ml), patients in the general population in postmenopause (76.644pg / ml); the highest values of FGF23 are found in patients with postmenopausal hyperparathyroidism (84,461pg / ml).

CHAPTER 8. DYNAMICS OF LEPTIN AND ADIPONECTIN TO PATIENTS WITH RECTAL CANCER

8.1. Objectives and reason of study

There are numerous data in the literature that demonstrate a direct link between body weight gain and colorectal cancer risk. Adipose tissue plays an important endocrine role mediated by hormones (adipokines) secreted by adipocytes (Fasshauer & col, 2015). In this context, previous studies have focused on possible links between adipokines and colorectal cancer pathogenesis (Aleksandrova & al., 2016; Joshi & al., 2014; Riondino & al., 2014). There are numerous articles that associate adipokines with colorectal cancer, but there are few studies in groups of patients exclusively affected by rectal cancer.

The aim of the study is to evaluate leptin and adiponectin levels in patients with rectal cancer compared to healthy individuals and the dynamics of their postoperative evolution.

8.2. Materials and method

The study was performed on a study group of 21 female patients and 38 male patients diagnosed with rectal cancer (CR) in stages 2 and 3 within the Regional Institute of Oncology Iasi, between August 2017 and August 2018.

62 patients (40 - male; 22- female) entered the study, but the group of patients was reduced to 77 days postoperatively due to the dropout rate.

The exclusion criteria were the following:

- rectal cancer in stage 4;
- the presence of distant metastases;
- extreme obesity (BMI> 35 kg / m2);
- the presence of diabetes.

The group of patients diagnosed with rectal cancer was statistically compared with a group of healthy patients (control). 30 of these patients underwent chemotherapy for a limited period of two months preoperatively (pelvic irradiation with doses of 45Gy, capecitabine 1600-1650 mg / m2 / day). For this category the surgery was performed 60 days (+/- 10) after the completion of the chemotherapy.

From the point of view of the surgical technique, in the case of 42 patients the technique of resection at the inferior-anterior LAR level was applied, and in the case of 17 patients the technique of abdomen-perineal resection was applied. Preoperative adiponectin and leptin levels were measured at 24 hours, 72 hours, and 7 days postoperatively.

Serum levels of leptin and adiponectin were measured using the Luminex Screening Assay RD Systems device. The sensitivity limit was 10 pg / mL for leptin, and 148 pg / mL for adiponectin, respectively. The changes in body weight were recorded 1 year preoperatively.
Data were expressed as a mean +/- standard error of the mean.

Statistical analysis was performed with SPSS 20.0 software. Correlation analysis was used to investigate the relationships between leptin and adiponectin, respectively between body weight, leptin and adiponectin. Regression analysis was used to investigate the possibility that body weight parameter could be a confounding factor. Differences were considered statistically significant for $p < 0.05$.

8.3.Results

The results regarding adipokine levels in the two groups for male and female patients are presented in table 8.3. In the preoperative stage, in the group of healthy patients the leptin levels were significantly higher, and the adiponectin levels were significantly lower compared to the study group. The mean values of leptin were 32 +/- 4.34 ng / ml in the healthy patients. , compared with 9.51 +/- 1.73 ng / ml ($p = 0.00048$) in female patients with rectal cancer,

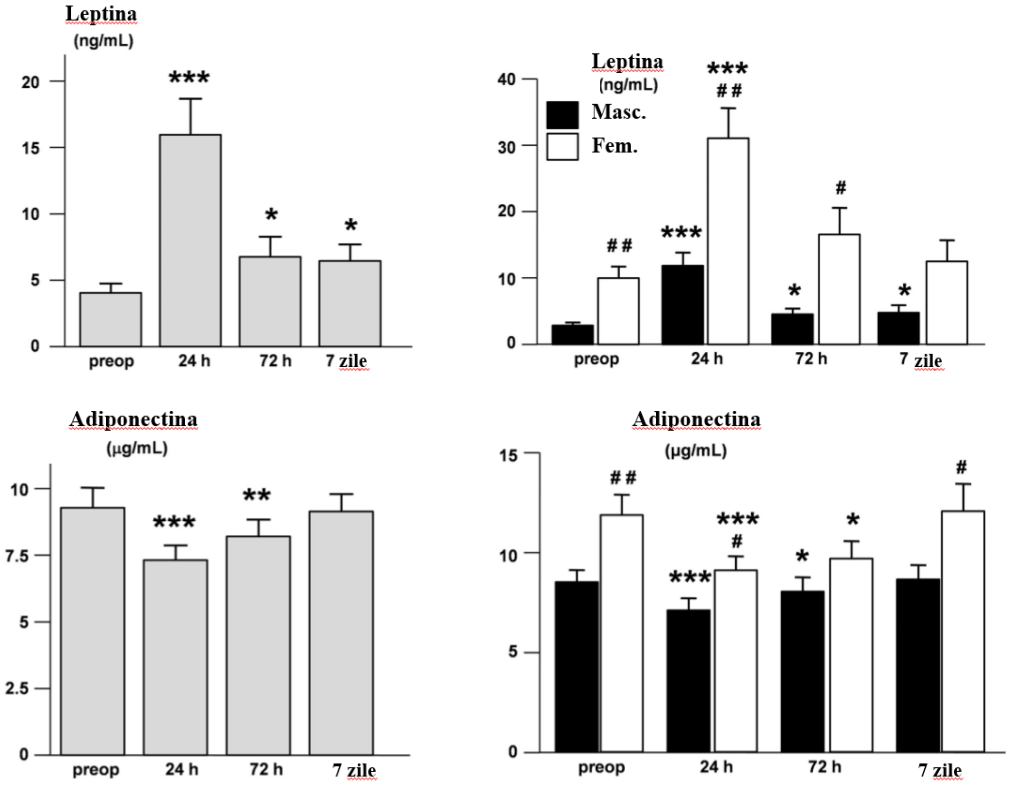
respectively 11 +/- 2.66 ng / ml. 2.54 +/- 0.39 ng / ml (p = 0.0032) in male patients, with statistically significant differences between the two groups, for both female and male patients. Mean adiponectin values were 9 +/- 0.64 ng / ml in healthy patients, compared with 11.85 +/- 1.02 µg / ml (p = 0.017) in female rectal cancer patients, respectively 7.39 +/- 0.51 ng / ml vs. 8.50 +/- 0.62 µg / ml (p = 0.019) in male patients, with statistically significant differences between the two groups, for both female and male patients.

Table 8.3. Adipokins levels in study group (CR) and control group (CTR) (preoperatory)

Adipokine	CTR (masculin)	CR (masculin)	p: CTR vs. CR (masculin)	CTR (feminin)	CR (feminin)	p: CTR vs. CR (feminin)
Leptina (ng/ml)	11+/-2.6***	2.5+/-0.4**	*p=0.0032*	32.2+/-4.3	9.5+/-11.7	*p=0.00049*
Adipokina (µg/ml)	7.44+/-0.5*	8.5+/-0.6**	*p=0.019*	9+/-0.6	11.99+/-1	*p=0.0171*

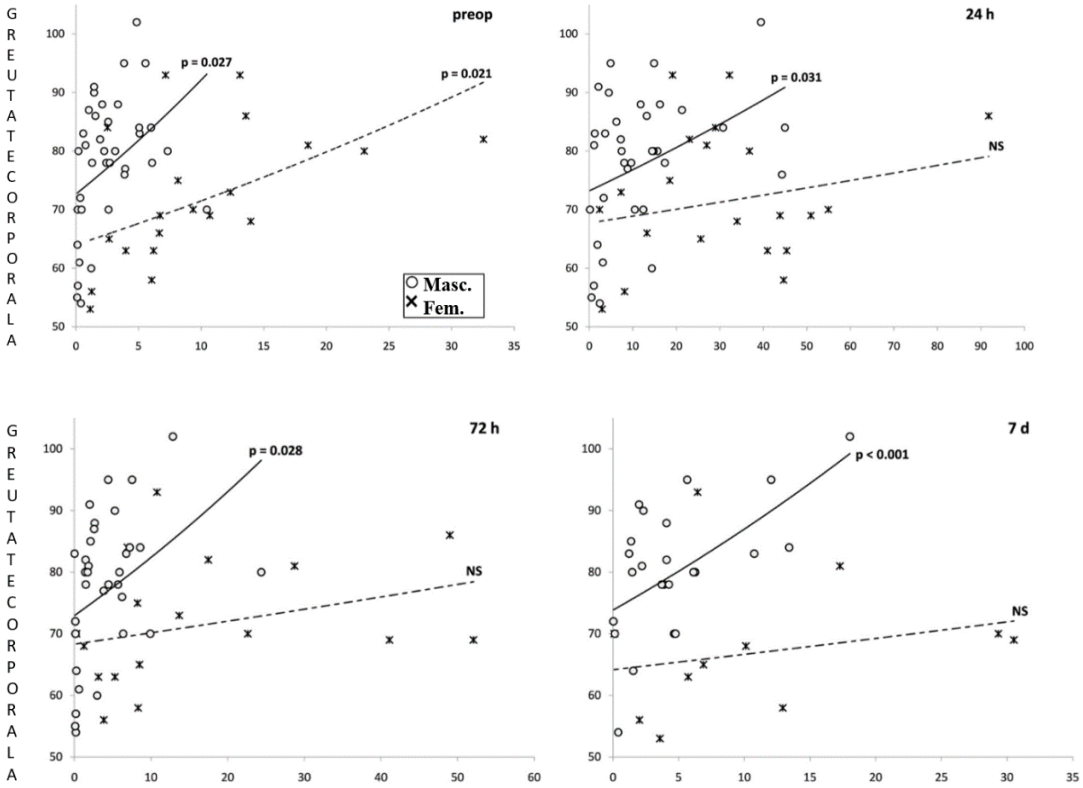
*p<0.005
**p<0.01
***p<0.001(comparison with female study group)

Surgical intervention in patients with rectal cancer increased the average leptin values to 18.7 +/- 2.42 µg / ml and to reduce the average values of adiponectin to 7.87 +/- 0.46 µg / ml. At 7 days postoperatively, the mean values of leptin and adiponectin returned to levels similar to those recorded preoperatively. The dynamics of the serum levels of leptin and adiponectin postoperatively at 24 hours, 72 hours, respectively 7 days compared with the serum levels recorded preoperatively is presented in figures 8.2.a-d.



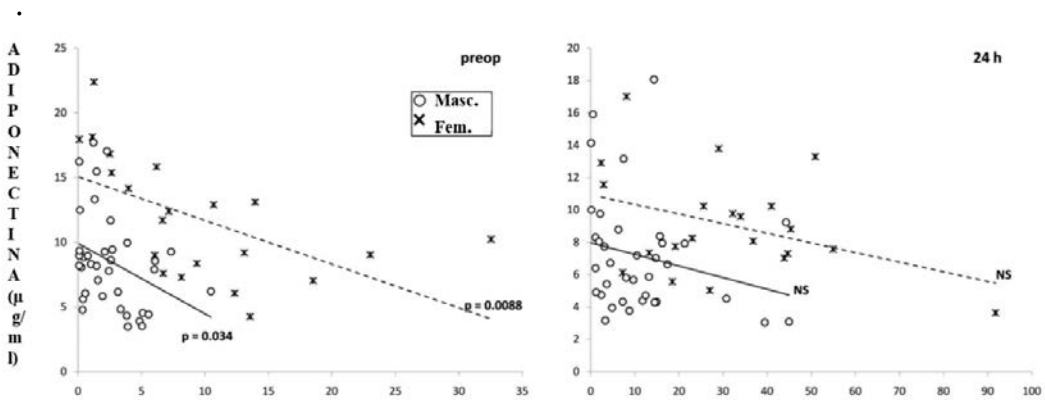
Figures 8.2.a-d. Postoperatory dynamics adipokins levels (***p<0.0001, ##p<0.01, #p<0.05)

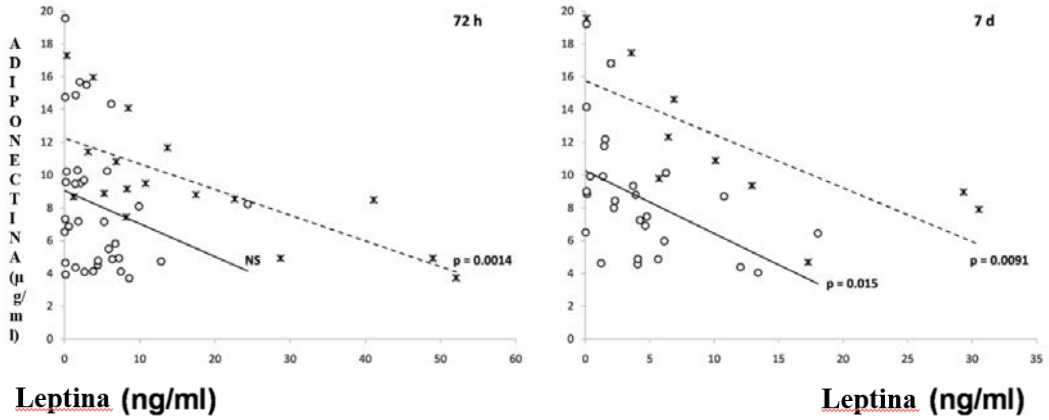
In Figures 8.3.-8.4. and Tables 8.4 and 8.5 are presented for regression analysis (correlation of adipokine levels with weight loss, correlation of leptin and adiponectin levels). Preoperatively, adiponectin levels were significantly correlated with body weight in both male and female patients. The significance of the correlation was maintained postoperatively in the male patients, but it disappeared in the female patients.



Figures 8.3.a-d. Correlations between body weight and leptin levels for patients with rectal cancer (preoperatory, at 24h, 72h, 7 days postoperatory) (significance at $p<0.05$; NS- non-significant)

Adipokine levels were not significantly correlated with tumor localization, type and duration of preoperative chemotherapy or surgical technique. Adipokine levels were significantly correlated with body weight during preoperative period in patients of both sexes. The surgery resulted in a decrease in the significance of the correlation of leptin and adiponectin levels with body weight exclusively in female patients (Table 8.4). Table 8.6 presents the results regarding the influence of some parameters on the profile of adipokines (tumor localization, type of surgery, chemotherapy, weight loss).





Figures 8.4.a-d.Correlations between leptin and adiponectin levels for patients with rectal cancer (CR) (preoperatory, at 24 h, 72 h, 7 days postoperatory) (significant at $p<0.05$; NS-non-significant)

Table 8.6. Preoperatory adipokins levels related to tumors localization (UR- superior rect, LR- inferior rect), surgical technique (LAR- low anterior resection, APR- abdominal-perineal resection), chemiotherapy (nCRT-, nCRT+), loss of body weight (W-, W+)

Sex		Leptina (ng/ml)				Adiponectina (µg/ml)			
		0	24h	72h	7 zile	0	24h	72h	7 zile
M	UR	2.6±0.5	13.5±3.4	4.4±1	5.3±1.7	8.4±0.9	7.2±0.9	8.4±1.2	8.8±2
	LR	3.1±0.6	11±2.6	4.9±1.4	4.3±1.1	8.7±1	7.1±0.8	8.2±1	8.5±0.9
	p	0.314	0.784	0.591	0.516	0.631	0.957	0.312	0.404
	LAR	2.5±0.4	13.2±2.5	4.2±0.8	5±1.2	8.8±0.8	7.3±0.8	8.5±1	8.8±1
	APR	2.8±0.9	8.4±3.2	4±1.8	2.7±0.8	8±0.9	6.9±0.9	8.2±0.9	8.2±0.9
	p	0.764	0.148	0.958	0.405	0.643	0.684	0.441	0.312
	nCRT-	2.1±0.4	8.8±2.3	2.8±0.6	3.5±1.1	9.4±1	7.9±0.4	8.1±1	8.1±0.9
	nCRT+	3.2±0.7	14.9±3.2	5.8±1.7	5.1±1.5	7.5±0.5	6.2±0.5	8.4±1.1	9.3±1.1
	p	0.099	0.215	0.137	0.689	0.136	0.218	0.679	0.081
	W-	2.2±2.4	12.1±2.9	3.5±1	4.7±2	8.7±1	7.4±1	8.5±1.3	9±1.4
	W =	2.3±0.6	11.1±2.8	4.6±1.2	4±0.9	8.3±0.8	7±0.7	8±0.9	8.5±0.9
	p	0.55	0.995	0.523	0.71	0.742	0.946	0.818	0.551
F	UR	10.6±3.2	27±5.5	11.6±2.9	12.3±4.1	12.6±1.7	9.8±1.2	10.8±1.1	11.6±1.9
	LR	9±2.4	26.4±5.8	27.5±11.3	13.7±8.5	11.4±1.4	8.9±0.9	8.4±2.1	13.3±2.8
	p	0.696	0.943	0.074	0.871	0.603	0.557	0.311	0.639
	LAR	9.5±1.9	32.3±5.3	15.5±4.2	10.1±3	12.2±1.1	9.3±0.8	9.9±1	11.9±1.5
	APR	9.8±4.7	25.5±9.2	24.7±16.4*	17.1±13.5*	10.5±2.6	8.1±1.3	8±0.5*	12.7±4.8*
	p	0.739	0.284	0.743*	0.947*	0.833	0.555	0.279*	0.902*
	nCRT-	10.9±2.8	30±7.1	17.9±6	10.4±3.5	11.9±1.5	9±1	9.8±1.4	12.4±1.8
	nCRT+	7.3±1.5	30.1±5.6	9.8±2.5	14±7.7	11.6±1.5	8.8±0.9	10.4±0.9	11.1±1.8
	p	0.151	0.909	0.436	0.777	0.944	0.793	0.425	0.982
	W-	7.7±3.9	21.7±6	12.5±6.2	9±7.2	14.6±1.8	10.9±1.2	11.7±1.6	15.8±1.3
	W =	10.6±1.6	36.8±6	18.8±5.3	12.7±3.2	10.2±1	8±0.7	8.6±1	9.7±1.1
	p	0.336	0.101	0.123	0.306	***0.028***	***0.043***	***0.004***	***0.021***

8.4. Discussions

In the context in which adipose tissue plays an important endocrine role, mediated by adipocyte-secreted hormones (adipokines) (Fasshauer & col, 2015), numerous studies have been performed to determine the possible links between adipokines and the pathogenesis of rectal cancer (Aleksandrova & col., 2016 ; Joshi & al., 2014; Riondino & al., 2014).

Leptin, one of the adipokines involved in nutritional behavior, is considered to be a pro-inflammatory factor that stimulates carcinogenesis in various types of neoplasms. (Riondino & col., 2014; Joshi & col., 2014).

Adiponectin plays a role in reducing adipose tissue mass, stimulates insulin secretion, it has anti-inflammatory effects, and inhibits angiogenesis, being considered an anti-carcinogenic protective factor (Riondino & al., 2014; Joshi & al., 2014). These properties have led researchers to consider adiponectin biomarkers of colorectal cancer, with potential for use in monitoring cancer progression and prognostic evaluation.

The results of the study are similar to those presented by Christen & col. (2018), Ho & col. (2012), Riondino & col (2014) and Alexandrova & col (2016), which report increased leptin levels in patients with rectal cancer.

The discrepancies between the data results from the literature can be explained by the variability of adipokine receptor distribution associated with tissue sensitivity (Erkasap & col., 2013; Nimptsch & col., 2017).

In this context, it is necessary to clarify the relationship (causality or adaptive phenomenon) between the modification of adipokine levels in patients with rectal cancer (Wang & col., 2016).

Another reason for the differences between the data in the literature may be related to the heterogeneity of the groups in terms of tumor localization, sex, changes in body weight, degree of tumor evolution, association or absence of chemotherapy.

Changes in adiponectin values after surgery to remove some tumors were presented by Chelazzi & col (2017) and Yamamoto & col (2016), which find significant reductions in postoperative leptin levels compared with preoperative ones.

8.5. Conclusions

1. It was noted a particular adipokinic profile of patients with rectal cancer compared to healthy control patients of the same age, body mass and same sex.
2. The levels of leptin were lower in patients with rectal cancer, and the levels of adiponectin were higher.
3. The stress induced by the surgery led to important changes in adipokine secretion, which returned to baseline values within a week.
4. Adipokine secretion in patients with rectal cancer differed by gender. Both leptin and adiponectin levels were higher in women than in men.
5. The correlation between adipokine level and body mass persisted in men but became insignificant one week after surgery in women.
6. The hierarchical regression analysis showed the presence of an important association between leptin and adiponectin only in women, this result being independent of body mass. The relationship independent of the body mass of the two parameters studied in the case of women could be explained by the way they interfere with other metabolisms.
7.The level of adiponectin was significantly higher in women, but not in men who lost weight, compared with same-sex patients who did not lose weight.
8. Differences in the response of adipokines in chronic and acute stress situations could find practical applicability in the prognosis of the evolution of neoplastic patients.

CHAPTER 9. GENERAL CONCLUSIONS

- Inflammatory processes, such as inflammation associated with malignancy, as well as stress significantly influence the secretion of these adipokines. In turn, adipokines may have a pro-inflammatory and oncogenic effect.
- Patients with rectal cancer have a significantly lower level of leptin and a higher level of adiponectin compared to healthy patients.
- Similar to the general population, female patients with rectal cancer have a higher basal adipokinic level than men with the same condition.
- Surgery for rectal cancer causes acute changes in leptin (growth) and adiponectin (decrease) 24 hours after surgery, after which the adipokine level returns to preoperative values 7 days after surgery.
- Adipokinic changes could play a prognostic role in postoperative evolution, with insufficiently explored applicability. There are gender differences in the postoperative evolution of adipokines. Their level continues to be higher in women at any time of postoperative evaluation. If in men a persistence of the correlation between adipokinic level and postoperative body weight was observed, this correlation disappeared in women up to one week postoperatively. Furthermore, in women it was observed a leptin - adiponectin correlation independent of body weight. This correlation could be mediated by the specific impact on insulin secretion and carbohydrate metabolism.
- Finally, we observed that the pre- and postoperative levels of adiponectin were significantly higher in women with rectal cancer who suffered a significant weight loss, but not in men.
- It were not observed influences of the anatomical localization of the tumor, of the chemo- or radiotherapy on the adipokine dynamics.
- Leptin and adiponectin are hormones secreted by the adipose cell, known as adipokines, which have pleiotropic effects. The two hormones are correlated with the mass of adipose tissue, thus with the body weight. If leptin is positively correlated, adiponectin is negatively correlated. Healthy women have higher levels of leptin and adiponectin than healthy men.
- FGF23 is an important regulator of phosphocalcic and bone metabolism. FGF23 modifications have been carefully studied in very rare pathological situations of bone metabolism, such as hypophosphatemic rickets, but also in commonly encountered changes, such as hyperparathyroidism secondary to chronic renal failure, but data on FGF23 variations in primary hyperparathyroidism are few or inconsistent.
- We found a significant difference in FGF23 levels in patients with primary hyperparathyroidism compared with patients in the general population. In contrast to healthy patients, in patients with primary hyperparathyroidism we found a significantly higher level in postmenopausal patients, suggesting a possible estrogenic interference with FGF23 secretion facilitated by excess PTH.
- FGF23 values were not significantly altered by parathyroidectomy in our study. In our patients with primary hyperparathyroidism, FGF23 was inversely correlated with PTH. The role of FGF23 appears to be attenuated by primary hyperparathyroidism, and its correlation with PTH is rather an epiphenomenon than the expression of direct interference.

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