Plasma Lipocalin Concentrations in Relation to Visceral Fat, Risk Factor for Endometrial Cancer

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Abstract

Objective The study aims to evaluate the presence of a correlation between visceral fat assessed by ultrasonography and the plasmatic level of lipocalin in patients diagnosed with endometrial cancer. *Material and Method* The study is a case-control analysis including 2 groups of patients: group I – 44 patients diagnosed with endometrial cancer, group II – 44 patients without gynecological pathology or inflammatory disorders. After the clinical examination and anthropometric measurements, these patients underwent ultrasonography (US) examination, in view of determining the visceral fat. At the patients included in this study, we also determined the plasmatic levels for lipocalin. *Results* At the patients diagnosed with endometrial cancer, the intraabdominal fat area evaluated by US and the plasmatic level of lipocalin is significantly larger (p<0.0001) compared to the control group. A correlation was also found between the intraperitoneal fat area evaluated by US and the plasmatic level of lipocalin. *Conclusions* The measurement of the intraperitoneal fat by US in correlation with the plasmatic level of lipocalin can be a screening method for endometrial cancer in obese patients.

Keywords: Endometrial cancer; lipocalin; ultrasonography; visceral fat

Introduction

The adipose cell has been considered for long an inert cell with no secretion, with the exclusive role of lipid storage. Recent studies confirm the capacity of the adipocyte to synthetize a whole range of substances with multiple functions, such as modulating immunity, adjusting insulin resistance, controling satiety, modulating inflammation, and influencing sexual and endothelial functions. There is a close interrelation between adipocytes and the other apparatuses and systems of the body, which is determined through adipocyte biomolecules, such as leptin, adiponectin, and lipocalin.

Obesity, usually the intraabdominal visceral adipose tissue, is associated with insulin resistance, hyperinsulinemia, and the increase of serum concentration of fatty acids. Although the detailed molecular processes which link obesity to its associated pathologies are not well understood, the accumulation of data suggests the fact that the inflammatory syndrome could be a strong mediator

[1,2]. As to the visceral distribution of the adipose tissue, intraabdominal obesity is considered a low level chronic proinflammatory state [3].

In the case of endometrial cancer, visceral obesity, as a risk factor, is associated to a chronic inflammatory process, confirmed by the increase of the inflammatory markers (PCR, IL-6, TNF-α) in the systemic circulation of obese patients [4]. The chronicity of the inflammatory process through a long-lasting stimulation of the innate immune cells creates a vicious circle which favours cancerogenesis.

If we refer to endometrial cancer, recent studies have shown the implication of lipocalin -2 (Lcn-2) in cancerogenesis and tumour progression [5,6]. Lcn-2 is released by tissues, such as the liver, the lung, or the kidney, but also by cells such as adipocytes and macrophages [7,8]. Certain inflammatory stimuli, such as lipopolysaccharides and IL-1β, can produce a significant form of lipocalin-2, as well as the increase of secretion of these cells [9]. Genes that codify lipocalin-2 have been selectively expressed in the liver and the adipose tissue of the obese/diabetic guinea pigs [10]. The Lcn-2 secretion is closely related to the cycle of cell proliferation and apoptosis during the reshaping of the endometrium[11]. The Lcn-2 expression is increased by the dexamethasone stimulation in the cell lines of the endometrial carcinoma (RL95-2) [12]. Lcn-2 appears to start the production of cytokines the cell lines of endometrial cancer [13].

Material and method

The study is a case-control analysis including 2 groups of patients: *group I* – 44 patients diagnosed with endometrial cancer, *group II* - 44 patients without gynecological pathology or inflammatory disorders (control group).

The diagnosis of endometrial cancer was determined through a histopathological examination where we analysed the tissue material obtained after an endometrial biopsy. The endometrial biopsy was made in the case of some significant metrorrhagias, climax mettrorhagias, as well as for certain aspects assessed by US (significantly thick and vascularised endometrium).

Group I
Inclusion criteria
- diagnosis of endometrial cancer following the histopathological examination
- aged 40-85
- will and capacity to take part in research procedures

Exclusion criteria
- other genital diseases except for endometrial cancer
- treatment with systemic corticosteroids
- metabolic or endocrine disorders
- autoimmune chronic diseases
- malignant tumours

Group II
Inclusion criteria
- aged 40-85
- will and capacity to take part in research procedures

Exclusion criteria
- other genital diseases except for endometrial cancer
- treatment with systemic corticosteroids
- metabolic or endocrine disorders
- autoimmune chronic diseases
- malignant tumours

After the clinical examination and the anthropometric measurements – body mass index (BMI) and abdominal/waist circumference (AC), we assessed the intraperitoneal fat of these patients by ultrasonography (US).

BMI was calculated according to the formula = weight (kg)/[height(m)]². AC (cm) was measured while standing, at the level of the umbilicus. The ultrasonography evaluation (Voluson 739) was done in dorsal decubitus position at the end of a normal expiration after a 12-hour digestive pause in order to assess the deposits of visceral fat. The visceral fat area determined by ultrasonography was calculated according to the formula 9,008+1,191×[the distance between the internal surface of the right abdominal muscle and the splenic vein (mm)] +0,987×[the distance between the internal surface of the right abdominal muscle and the posterior surface of the aorta (mm)] +3,644×[the fat thickness of the right kidney posterior surface (mm).

From every patient included in this study we collected a jeun 2 ml of blood by venipuncture in EDTA test tubes in order to make a complete blood count (CBC) and 2ml of blood in test tubes without anticoagulants in order to determine the plasmatic level of lipocalin. The serum obtain by centrifugation was separated and stored in freezing tubes of 600 μl each at a temperature of − 30⁰ C until samples were analysed in order to avoid repeated frost-defrost cycles. The minimum detected lipocalin value (Human, Lipocalin Immunoassay DLCN20 R&D Systems USA) was of 0.012.ng/ml. No significant crossed reactivity has been identified and no interference has been noticed.

We obtained the consent of all patients. The study has been approved by the Ethics Commission of the 'Iuliu Hatieganu' University of Medicine and Pharmacy from Cluj-Napoca.

All parameters were included in the study database and version 13 of the SPSS software and Microsoft Excel with Analysis Tool *Pack* were used for statistical analysis. The Kolmogorov Smirnov test was applied for the testing of normal distribution. The Student t test was used for the comparison of the means and the Mann-Whitney test for rank comparison in two independent samples. For multivariate models, the stepwise linear regression method was used.

Results

The characteristics of the patients included in this study are described in table 1.

**Table1. The characteristics of the patients included in this study**

Parameter	Group	m	stdev	SE	95% confidence interval		Min	Max	p
Age	Control	55.47	9.07	1.08	53.31	57.63	42.00	80.00	0.004
	Case	60.57	9.92	1.42	57.72	63.42	41.00	80.00	
Weight (kg)	Control	63.71	11.72	1.40	60.92	66.51	40.00	100.00	<0.0001
	Case	85.33	13.67	1.95	81.40	89.25	62.00	112.00	
BMI	Control	24.55	4.00	0.48	23.60	25.51	16.90	36.40	<0.0001
	Case	32.49	4.66	0.67	31.15	33.82	22.80	42.70	
CA	Control	76.37	8.58	1.03	74.33	78.42	64.00	119.00	<0.0001
	Case	97.57	11.78	1.68	94.19	100.96	71.00	123.00	
Intraperit. fat (mm)	Control	159.14	42.50	5.08	149.01	169.27	93.15	297.62	<0.0001
	Case	251.37	59.78	8.54	234.20	268.54	141.56	361.83	
Menarche	Control	12.01	0.88	0.10	11.81	12.22	11.00	14.00	0.63
	Case	12.15	1.96	0.29	11.57	12.72	9.00	16.00	
Menopause	Control	51.28	2.69	0.37	50.54	52.01	45.00	55.00	0.50
	Case	49.06	10.69	1.81	45.39	52.73	3.00	56.00	
Lipocalin ng/ml	Control	4.962	1.538	0.232	4.494	5.429	1.97	7.99	<0.001
	Case	9.673	2.350	0.354	8.959	10.388	3.431	15.421	

m = artithmetic means stdev = standard deviation; SE = standard error

In the case of the control group the visceral fat area had a mean value of 142.41±23.06 cm², while in the case of the group of patients with endometrial cancer, the visceral fat area had a mean

value of 232.72±57.14 cm². Thus, there was a statistically significant difference (p<0.0001) in intraperitoneal fat between the two groups.

The ROC curve for identifying the cut-off point for visceral fat is shown in Figure 1. The area under the curve was 0.95, p<0.0001. The identified cut-off point was 172. Intraperitoneal fat over 172 seems to be a cut-off value for the patients with endometrial cancer (Figure 1).
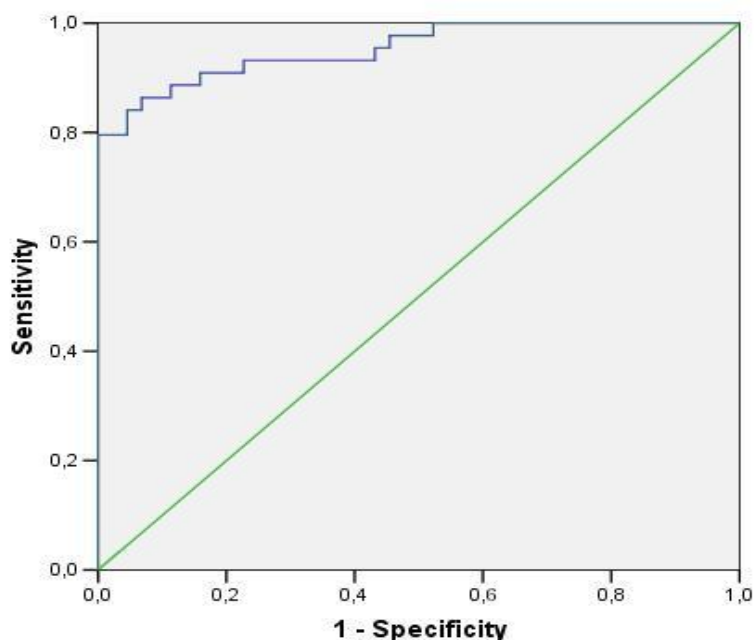
Figure 1. ROC curve for identifying the cut-off point for intraperitoneal fat

The plasmatic level of lipocalin in the case of the group with endometrial cancer is of 9.673±2.350 pg/ml , significantly higher (p<0.001) as compared to the control group, with a value of 4.962± 1.538pg/ml.

The cut-off for lipocalin between the sick group and the control group is 6.46 (AUC=0.96, p<0.001). If a patient's plasmatic level of lipocalin exceeds 6.46, she is exposed to the risk of endometrial cancer (Figure 2).

Intraperitoneal fat is in a positive linear correlation with the plasma lipocalin level (Figure 3). In order to establish the influence of the other variables on **lipocalin**, a *stepwise* multivariate linear regression analysis was performed. The following variables were entered in regression: age, BMI, CA, intraperitoneal fat, menarche, menopause. Of these variables, only the **intraperitoneal fat** had a significant influence on the lipocalin level.
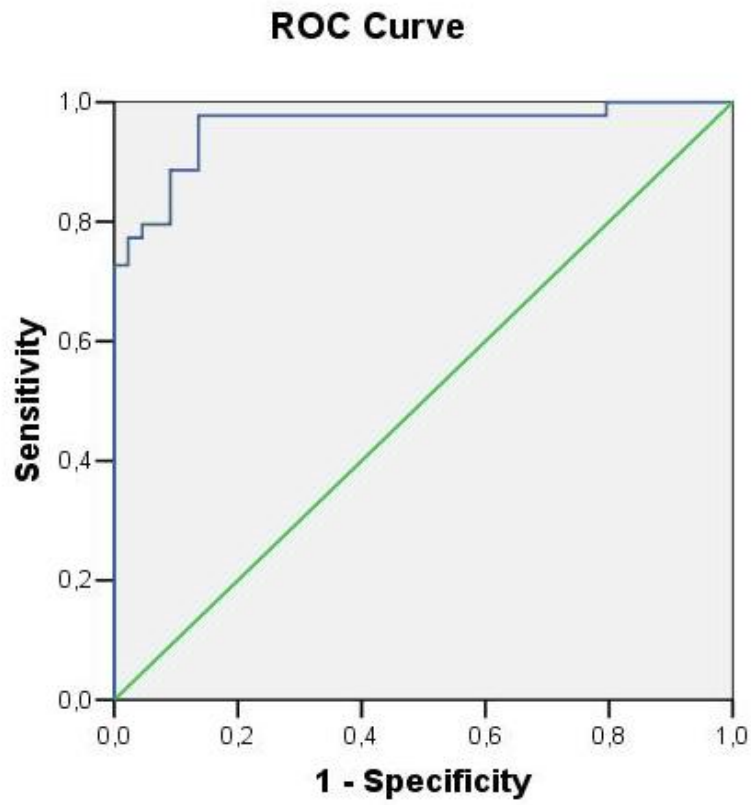
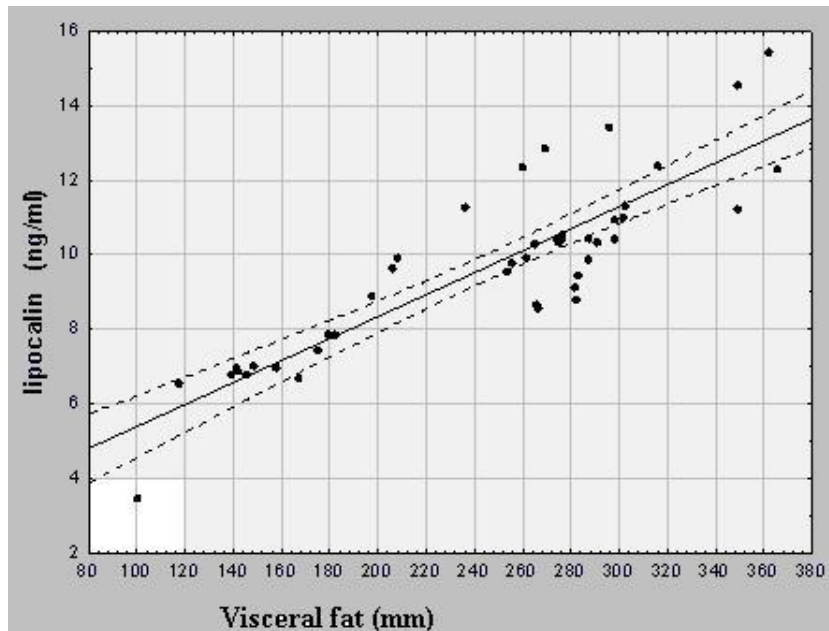**Figure 2.** ROC curve for identifying the cut-off point for lipocalin

Figure 3. Correlation intraperitoneal fat – lipocalin

Discussions

Endometrial cancer represents 2-3% of all malignant tumours, while its incidence is increasing especially in the societies with a high living standard [14]. Epidemiological studies have shown the influence of non-genetic environmental factors in endometrial cancer etiology, which are linked to a person's lifestyle: reduced physical activity and obesity. Obesity is an endemic disease of the XXIst century, which increases continually, especially in the case of young persons.

It is well known that the adipose tissue constitutes a risk factor for endometrial cancer, which is also sustained by this study. The patients from the group with endometrial cancer have increased values of anthropometric indices (BMI, AC), but also of visceral fat.

The traditional vision of the adipose tissue seen as a passive tank for energy storage is no longer valid. At present, we know that the adipose tissue releases and secretes a variety of bioactive peptides, known as adipokines, with a local and systemic role. Apart from these signals, the adipose tissue has numerous receptors which allow it to answer to these corresponding signals from traditional hormonal systems. Thus, the adipose tissue is totally involved in the coordination of diverse biological processes, including energetic metabolism, neuroendocrine functioning and the autoimmune function. The adipocyte is the central element and it integrates multiple metabolic and endocrine signals [14].

It is already known that abdominal obesity is a risk factor for cardiovascular diseases (coronary), metabolic (diabetes mellitus), as well as endometrial cancer. However, it has not yet been proved why the accumulation of adipose tissue in the abdominal region has an increased risk of complications as compared to other areas. This study sustains the existence of a link between the incidence of endometrial cancer and visceral fat. The study of the formation and secretion of adipokines in different body areas showed an increased leptin release and a decreased adiponectin release towards the visceral adipose tissue [15].

Our data show a highly positive correlation between obesity expressed through the BMI and AC, on the one hand, and the plasmatic level of Lcp-2, on the other hand. At the same time, our study underlines the idea that visceral obesity assessed by ultrasonography is related to the plasmatic level of Lcp-2.

Lcp-2 has recently been reported to be associated with obesity and insulin resistance in rats and men. Lcp-2 is intensely expressed by adipocytes in vivo and in vitro. Lately, is has been reported that the level of Lcp-2 was significantly higher in patients with coronary diseases [16]. Retinol Binding Protein-4(RBP4), another member of the lipocalin family, has recently been added on the list of adipokines which would link obesity to insulin resistance and type 2 diabetes mellitus.

During an ovulation cycle Lcn-2 is high in the uterus in the pre-ovulatory and ovulatory period and low during menstruation and after ovulation [17]. Lcn-2 plays an important role in maintaining the balance between cellular proliferation and the death of cells during the dynamic reshaping of the endometrium [18]. Lcn-2 induces IL-8 secretion through RL95-2 cells and it can be correlated with the suppression of cellular apoptosis.

Low chronic inflammation is considered a crucial mediator in the development of metabolic disorders associated with obesity [18]. The plasmatic level of the C-reactive protein (CRP) is linear and positively linked to the plasmatic level of Lcp-2. However, the Lcp-2 level is not significantly correlated with the level of IL-6 [19]. Thus, Lcp-2 could be more than a simple inflammation marker. There is probably an interconnection between the plasmatic level of Lcp-2 and the metabolic disorders induced by obesity and inflammation. The role played by Lcn-2 in the inflammation is suggested by the presence of Lcn-2 in the uterus during pre-implantation and parturition, and it is part of the local inflammatory answer associated with the birth [17,20].

Lcn-2 is secreted by the epithelial, macrophage, neutrophil, and tumour cells [21,22]. The increased level of Lcp-2 could be seen in the plasma, serum, and urine in different conditions, such as lung metastases and colorectal cancer, acute renal failure, and preeclampsia [23,24]. In tumour tissues, a high value of Lcn-2 was observed in lung, colorectal, ovarian, and pancreatic cancer [25,26]. Lcn-2 is involved in tumorigenesis and the metastasis of mammary tumours [27]; these proteins influence cell migration [28]. This study sustains the presence of a high plasmatic level of Lcn-2 in the patients with endometrial cancer.

At the same time, we noticed the increase of Lcn-2 expression in the atypical endometrial hyperplasia [29]. Lcn-2 is considered a multifunctional lipocalin in the pathophysiology of endometrial cell carcinoma. In the RL95-2 cells, the apoptosis is induced by Lcn-2 in 24 h from culture [12]. \The studies made on exogenous proteins, which were purified from the uterine fluid of rats, suggest that, in the presence of Lcn-2, the apoptosis can be suppressed; this leads to the improvement of cell survival.

Starting from the idea that visceral fat is positively correlated with the incidence of endometrial cancer, but at the same time it is the dominant parameter influencing the plasmatic level of Lcp-2, we can interpret that visceral fat constitutes a risk factor for endometrial cancer through the systemic inflammatory status [30].

Conclusions

1. An intraperitoneal fat area evaluated by US larger than 172 cm² is a risk factor for endometrial cancer.
2. A plasmatic level of lipocalin higher then 6.46 increases the risk of endometrial cancer.
3. The measurement of intraperitoneal fat by US in correlation with the plasmatic level of lipocalin can be a screening method for endometrial cancer in obese patients.

Acknowledgements

Conflict of Interest

None of the authors have a conflict of interest

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