Adiponectin mRNA and Metabolic Profile in Malignant Obesesity

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The aim of our study was to assess mRNA levels in visceral fat tissue and to correlate them with clinical-biological parameters in morbidly obese patients. 38 morbidly obese patients were selected and 8 cases of non obese subjects. The expression levels of adiponectin mRNA were determined through RT-PCR. Also we assessed biochemical parameters like seric glucose level, total cholesterol and triglicerides. The expression levels of adiponectin mRNA were correlated with demographic parameters (age, sex, BMI) and with clinical parameters (HTA, hypothyroidism and type 2 diabetes). This study showed significant correlations between adiponectin mRNA levels with obesity, age, triglicerids, total cholesterol and hypothyroidism. In conclusion, the expression level of adiponectin could be used as a molecular marker in the management of obesity.

Keywords: adiponectin mRNA, obesity, clinical-bilogical parametres

Obesity is now recognized as the illness of the last half century. World Health Organization reported in 2014 that 13% of the adults of the planet are obese (IMCee30) and 39% of adults over 18 years of age are overweight (IMCee25)[1]. In Romania the prevalence of obesity was 21.3% in 2014 and of overweight adults 31.1% in the same year [2].

Adiponectin, also known as GBP-28, apM-1, AdipoQsi Acrp30, is an adipocytokine involved in regulating lipid and glucose metabolism. The AdipoQ gene is placed on chromosome 3 in position 3q27 and it extends over 16 kb including 3 exons and 2 introns (fig.1) [3]. Recently, on the 3q27 site were described susceptibility locuses for type 2 diabetes and for the metabolic syndrome which could show that mutations of the AdipoQ gene might be involved in metabolic disorders. The product of this gebne is a 30kd protein composed of 244 aminoacids with a C-terminal globular domain and a collagen-like N-terminal domain [4]. In plasma, adiponectin is present under three multimeric forms: low molecular weight (LMW) trimers, middle molecular weight (MMW) hexamers, high molecular weight (HMW) oligomeric structures and globular adiponectin (gC1q domain) [5] (fig.2). The protein is expressed and secreted exclusively by adipocytes, in high concentrations for the visceral fatty tissue and in lower concentrations in the subcutaneous fat [6]. The level of expression is consistent with the degree of differentiation, the secreted quantity increasing with the differentiation degree of the adipocyte. Studies suggest that adiponectin has antihyperglycaemic, antiatherogenic and anti-inflammatory properties [7]. Decreased adiponectin plasma levels are associated with obesity and related cardiovascular risk factors, type 2 diabetes, endothelial dysfunction and dyslipidemia [8].

Increased levels of seric adiponectin are associated with high insulin sensitivity and glucose tolerance [9]. Studies on animal models show that the ablation of the adiponectin

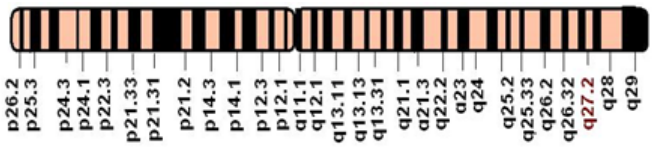

Fig. 1. AdipoQ gene localization on chromosome 3

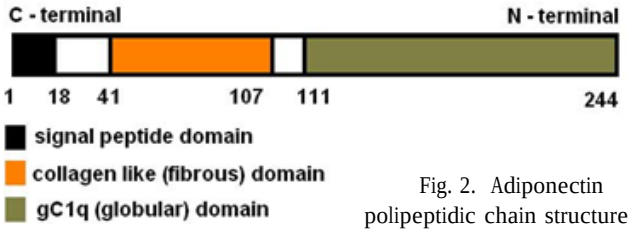

Fig. 2. Adiponectin polipeptidic chain structure

gene causes the development of severe insulin-resistance and pronounced lipid accumulation in the skeletal muscle cell [10].

The aim of our study was to determine the expression levels of adiponectin in visceral adipose tissue in patients with malignant obesity and to evaluate the correlations between these expression levels and the clinical and biological pannel of the subjects.

Experimental part

Materials and methods

The biological material was obtained through biopsies from subjects hospitalised for bariatric surgery (n=38). Morbidly obese patients were selected (BMI ≥ 40). The control group (n=8) was represented by adipose visceral tissue samples from non-obese patients, obtained during abdominal surgical interventions. Clinical and biological parameters were acquired from the medical documents of patients. All patients were admitted in Department 2 of Surgery at The Emergency County Hospital Timisoara. All patients provided written informed consent before the study.

PureLink® RNA Mini Kit (Invitrogen, 2183018A, Carlsbad,USA) was used to extract total RNA from adipose tissue. Total quality and quantity of purified RNA was assessed using a NanoDrop spectrophotometer. Total RNA was reverse-transcribed and amplified using Applied Biosystems® TaqMan® RNA-to-Ct™ 1-Step Kit (4392938), TaqMan specific assay and the 7900 HT Fast Real-Time PCR System. The results were normalized to Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression values. The relative amounts of the gene were expressed as ΔCT.

Data are presented as means ± standard deviation (SD) or as medians and interquartile range (IQR) for variables with skewed distribution. Differences between groups were

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analyzed with Student t test or Mann-Whitney U test as appropriate. Univariable regression analyses were carried out to evaluate the significance of the relation between continuous variables. P-values for all hypothesis tests were two-sided, and statistical significance was set at $P<0.05$. All analyses were conducted with Stata 9.2 (Statacorp, Texas, USA).

Results and discussions

Thirty-eight morbidly obese patients were analised, 73,69% of which were women and 26.31% men. We assessed the correlation between adiponectin gene expression from visceral adipose tissue and demographic characteristics (age, body mass index) and clinical-paraclinical profile (blood sugar, cholesterol, triglycerides, hypertension, type II diabetes, hypothyroidism)(table 1).

The adiponectin mRNA levels were assayed by RT-PCR using relative quantification to the GAPDH housekeeping gene. Adiponectin mRNA trascription level was lower in obese patients (-2.3130±0.2362) compared with control group (-0.802±0.6600) ($p<0.0147$) (fig.3). We compared adiponectin expression levels and the gender of obese patients. Our study did not show statistically significant differences between men and women (fig. 4). According to our study no correlations could be found between adiponectin levels and body weight, BMI, glycaemia. Using univariable linear regression models, we found a negative statistically significant correlation with cholesterol and triglycerides and a positive statistically significant correlation with age of patients (table 2).

Also adiponectin levels were compared with some associated pathologies of the metabolic syndrome: hypertension, type II diabetes and hypothyroidism. Statistical analysis showed a significant correlation only with hypothyroidism and not with the other considered pathologies (table 3).

It is recognised today that fat accumulation in obesity is the result of altered expression of some hormones, growth factors and adipokines. Adiponectin acts as a protection factor: endocrine/paracrine/autocrine for the prevention and/or progression of lethal diseases induced by obesity [11]. Studies in literature report levels of adiponectin inversely proportional to body fat mass in obese subjects [10,12,13]. Our study showed that adiponectin mRNA were lower in obese patients compared to the control group with signifiacant correlation ($p<0.0147$) but no significant negative correlations could be foud within the obese subjects ($p=0.9647$). The reverse balance between adipocytokines (increase of leptin and the decrease of adiponectin) and inflammatory cytokines (IL-6 and TNF-α) contributes to the inflamatory status in obesity [14]. Research on normal and obese human subjects showed that adiponectin mRNA levels were significantly reduced in visceral fat tissue of healthy patients and insignificantly reduced in obese subjets. The results support the theory that adiponectin gene expression is much lower in visceral adipocytes than in the subcutaneous ones sugesting subcutaneus adipose tissue to be more important for circulating adiponectin levels [15]. Some studies on

Parameter	mean±SD or median (IQR)
Age (years)	40.74±11.17
Adiponectin	-2.31±1.46
BMI	48.84±10.54
Body weight (kg)	124 (116-168)
Glycaemia (fasting) (mg/dl)	93 (89-105)
Cholesterol (mg/dl)	193.5 (162-203)
Triglycerides (mg/dl)	126.95±43.53

Table 1
CLINICAL-BIOLOGICAL DATA OF THE STUDIED PATIENTS (n=38)

Parameter	Variable	Coef	Std. Err.	P
Adiponectin	Age	0.0814	0.0169	<u>≤ 0.001</u>
	Glycaemia	0.0113	0.0069	0.112
	Cholesterol	-0.0135	0.0064	<u>0.043</u>
	Triglycerides	-0.0129	0.0051	<u>0.016</u>
	BMI	-0.0073	0.0230	0.752
	Body weight	0.0003	0.0069	0.965

Table 2
UNIVARIABLE REGRESSION ANALYSIS FOR ADIPONECTIN

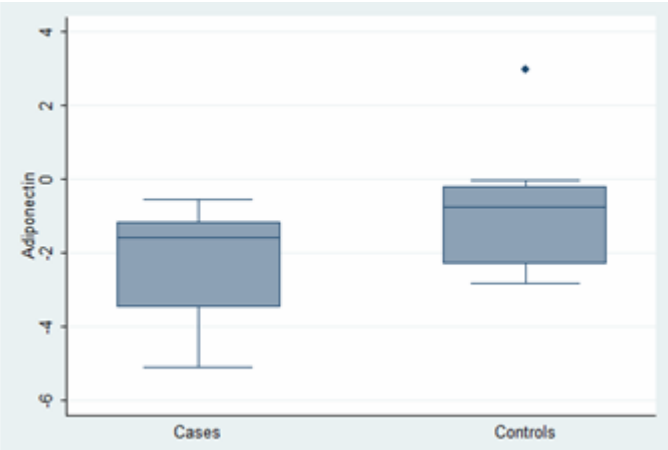

Fig.3 Adiponectin mRNA levels in visceral adipocytes for the study and control group (relative quantification normalized to GAPDH gene)

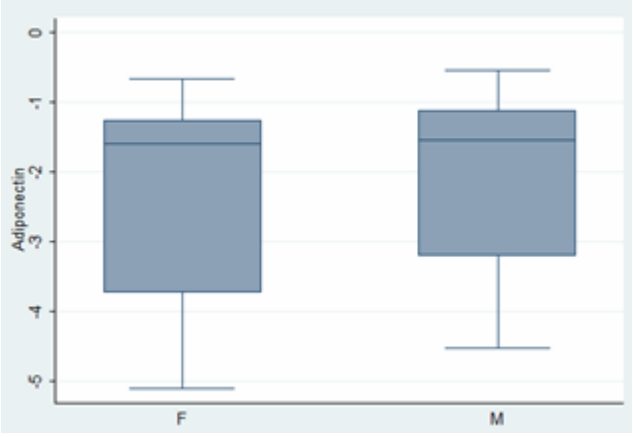

Fig.4. Adiponectin mRNA levels in visceral adipocytes by gender (relative quantification normalized to GAPDH gene)

Gene / Pathology	No. of patients (n)	Pahtology present (+) absent (-)	Adiponectin	P
Hypertension	n=16	-	-2.4696±1.4231	0.5788
	n=22	+	-2.1990±1.5029	
Type 2 diabetes	n=29	-	-2.4670±1.5421	0.2472
	n=9	+	-1.8166±1.0609	
Hypothyroidism	n=30	-	-1.9078±1.1543	0.0004
	n=8	+	-3.8325±1.5335	

Table 3
ADIPONECTIN EXPRESSION AND ASSOCIATED PATHOLOGY (DATA IS PRESENTED AS MEAN VALUES AND STANDARD DEVIATION)

experimental models support that adiponectin administration decreases body weight in laboratory animals [16]. On the contrary, Ahima *et al.* show that adiponectin transgenic mice crossed with ob/ob show a partial improvement in some of the co-morbidities of obesity (insulin resistance and type 2 diabetes), but not in obesity [7]. This suggests that the increase of adiponectin concentration has a direct effect on insulin resistance, independent of the amount of fatty tissue of the subject. Another survey reports that adiponectin administration in mice increases thermogenesys and decreases body mass, lipids and glycemia [17]. Many authors appreciate that adiponectin is a marker of obesity-related illnesses, and therefore, stimulating its synthesis and the synthesis of its receptors may be a therapeutic target of the future against obesity and its co-morbidities [7,11].

BMI is used in the evaluation diagnostics of body mass. Thus BMI ≥ 25 defines owervright, BMI ≥ 30 defines obesity and BMI ≥ 40 falls within the category of malignant obesity. In our study all patients belonged to this last category, we could not find statistically significant adiponectin levels compared to BMI. The literature is unanimous in appreciating the inverse correlation between levels of adiponectin and BMI [18-22]. In a survey on healthy adults, Kuo *et al.* don't support the hypothesis that obesity per se affects adiponectin levels and that obesity / serum adiponectin levels reported by other studies may be a consequence of metabolic syndrome associated with obesity [23]. The BMI / adiponectin / obesity comorbidity relationship is also revealed by other studies: Pilz *et al.* state that hypoadiponectinemia associated with increased BMI may be an early diagnostic factor of atherosclerosis in young obese patients [24], and Yatagai *et al.* show that hypoadiponectinemia contributes to the accumulation of visceral fat in men with type 2 diabetes [21]. Kirstop *et al.* appreciate that the decrease of adiponectin levels are associated with BMI in obese patients. For healthy subjects, decreased levels of of this hormone can be considered dangerous, a predictor of mortality, independent of the risk factors for cardiovascular disease. BMI is associated with cardiovascular disease, but part of this relationship is mediated by adiponectin [20]. *In vitro* research on human omental adipocytes shows that, visceral adiponectin synthesis was greater than that achieved by adipocytes harvested from subcutaneous adipose tissue, but maintained a strong negative correlation with the BMI of the subjects from which the harvest was made. In contrast, the synthesis of adiponectin in subcutaneous fat tissue did not correlate with BMI [18]. Adiponectin / BMI inverse correlation is also supported by studies conducted on patients before and after bariatric surgery, that have shown that BMI reduction by bariatric surgery increases adiponectin plasma levels. The physiopathological explanation would be that the increase in adiponectin plasma levels will act through a feed-back mechanism on the single tissue that produces them and thus obesity will be inhibited [25].

Specialty literature appreciates that adiponectin synthesis is closely associated with sexual hormones, women having a higher body fat mass than men at the same BMI [26,27]. It is estimated that testosterone has a regulatory role on adiponectin secretion, which is correlated with the observation that hypogonadal men have elevated levels of adiponectin that are resolved with testosterone treatment [28]. Studies that sought to highlight the adipokine / sex hormone relationship report that leptin and adiponectin are more elevated in women than men, but in contrast with leptin, adiponectin is decreased in obesity and it increases as a response to massive weight lost [22,29]. A number of studies support the correlation between adiponectin, gender and co-morbidities. Thus it was shown that adiponectin has weak antiatherosclerotic protective effects in men, and in women, the levels of serum adiponectin are inversely associated with coronary events [30]. In contrast, Nikizawa *et al.* show that androgen induced hipoadiponectinemia increases the risk of atherosclerosis in men. They also show that plasma adiponectin concentration is significantly lower in males than in females, but does not differ in pre- and post-menopausal women. The same study states that testosterone reduces adiponectin secretion even in adicpocyte cultures [31]. Another study reports positive correlation of adiponectine with sex hormone binding globulin (SHBG) and a reverse correlation with free-androgen index and estradiol. The authors speculate that SHBG is regulated by adiponectin, and that testosterone would have an inhibitory effect on the adiponectin gene [19]. Finally, Cohen *et al.* report a strong correlation between levels of adiponectin, age, HDL cholesterol and HTA in women regardless of race [32]. According to our results pacient gender did not correlate with the adiponectin levels.

It is unanimously accepted that visceral fat obesity is closely correlated with type 2 diabetes, hyperlipidemia, hypertension and atherosclerosis [3]. In our research, similar data were found; the total cholesterol and triglicerides were significantly correlated with adiponectin measurements in obese subjects ($p=0.043$, $p=0.016$ respectively). Adiponectin favors oxidation of fatty acids and decreases glucose synthesis in the liver; circulating levels of adiponectin have a pleiotropic effect upon cholesterol and triglicerides [33,34]. Adiponectin also lowers the accumulation of lipids in macrophages and thereby provides endothelial protection in cardiovascular diseases [3,35]. Some studies argue that plasma levels of adiponectin positively correlate with cholesterol and triglycerides, regardless of gender, age, and BMI [36]. Moreover, they correlate significantly with triglycerides and HDL and also remain significantly correlated after BMI

adjustment [19]. Other studies have found that low adiponectin is associated with low serum HDL cholesterol, LDL and triglyceride levels in young healthy men [8]. Opposite results are also repported. Thus, Tschritter *et al.* find a strong positive correlation between adiponectin and HDL cholesterol in women, and a reverse correlation between adiponectin and triglycerides. They assess that increased levels of adiponectin associated with elevated HDL cholesterol levels and the decrase in triglycerides demonstrate the antiatherogenic role of adiponectin. Moreover, the study states that elevated levels of circulating adiponectin predict the increase in insulin sensitivity, the relationship being independent of the decrease in body fat [37]. Cnop *et al.* show the same positive correlation adiponectin - HDL cholesterol and reversed for adiponectin-triglyceride, but warns that the increase in BMI due to visceral fat is associated with insulin resistance and the atherogenic lipoprotein profile [22]. Another study reports low levels of adiponectin correlated with total unmodified cholesterol and elevated serum triglyceride levels in patients with obesity and co-morbidities related to metabolic syndrome [28]. A study focusing on the evaluation of adiponectin mRNA reports a negative correlation with triglycerides; also it appreciates that BMI is inversely correlated with serum and intracellular gene expression of adiponectin [38].

Data is scarce regarding glycemic levels of obese subjects without type 2 diabetes. In our study no significant correlation could be found between adiponectin levels and glycaemia. Studies report the association between serum adiponectin levels with decreased glucose synthesis in the liver [33] and the inverse correlation between low levels of circulating adiponectin with elevated plasma glucose levels in patients with metabolic syndrome [28]. Yamauki *et al.* assess that blocking the ADIPOR2 receptor in hepatocytes stops adiponectin binding and activity, which induces insulin resistance and glucose intolerance [9].

In healthy individuals, low levels of plasma adiponectin are associated with an increased risk of cardiovascular disease. Patients with border-line hypertension show insulin resistance and dyslipidemia in higher proportion than subjects with normal blood pressure [13]. Several studies show significant negative associations between low levels of adiponectin and hypertension in young hypertensive men [8], in patients with essential hypertension [39] and in obese subjects [3]. Studies on obese children report a reverse correlation between adiponectin and blood pressure (systolic and diastolic) [40]. Opposite, studies focused on adiponectin-cardiovascular disease relation appreciate that adiponectin levels do not correlate with ischemia and hypertension, that high adiponectin levels represent an independent factor for HTA and that they appear as a response to HTA medication with Angiotensin II [8,20,28, 50]. Our study also confirmed these findings.

Literature associates obesity with the development of type 2 diabetes; increased adiponectin levels decreasing the risk of diabetes in different populations [27,37]. Our research did not reveal a significant correlation of type 2 diabetes with adiponectin expression within the obese group. Some clinical studies report strong correlations between decreased adiponectin levels and obesity, insulin resistance, diabetes, vascular inflamation and atherosclerosis [7,8,13,41]. Halleuxa *et al.* assess that the apM1 gene is negatively regulated by glucocortioids an positive by insulin wich contributes to the decrease of apM1 mRNA in patients with insulin resistance, diabetes and matabolic syndrome [42]. Some authors propose adiponectin as a biomarker for the early identification of diabetes [43,44].

The adipose tissue is an endocrine organ wich plays a critical role in the energy homeostasis not only by depositing triglycerides, but also through the secretion of adipokines wich controle appetite, thermogenesis, immunity and neuroendocrine functions [7]. Data obtained in the present study showed significant statistical correlations between adiponectin mRNA values and hypothyroidian subjects ($p=0.0004$). Recent studies report increased levels of leptin and decreased levels of adiponectin correlated with TSH, cholesterole and triglicerides [28,45,46]. Other sudies report decreased levels of circulating adiponectin in hypothyroidism and euthyroidism [47,48]. In their study on hypothyroidian rats, Seifi *et al.* reported low levels of adiponectin mRNA in parallel with the plasmatic decrease of T_3 and T_4 [49].

Conclusions

The results of our study sugests that visceral adipose tissue is involved in the metabolism of obesitydirectly regulating the levels of risk factors like total cholesterol and triglicerides. The significant correlation between obesity and hypothyroidism shows the involvment of thyroid dysfunction in the development and evolution of malignant obesity. The assessment of the molecular characterization of visceral and subcutaneous fatty tissue can help elucidate the causal relationship between changes in hormone levels, including adipokines, and the pathogenesis of the disease.

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