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Activity of metalloproteinases and adiponectin in obese patients—a possible factor of incisional hernias after bariatric procedures

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Abstract: Purpose: Metalloproteinases are a key component of the pathogenesis of abdominal hernias. Obesity is considered a risk factor in herniogenesis and hernia recurrence. The aim of this study was to evaluate the serum concentrations of metalloproteinase-2 (MMP-2), MMP-9, MMP-13, and adiponectin in morbidly obese and non-overweight controls. Materials and methods: The participants were recruited from among patients undergoing bariatric and non-bariatric surgery and divided into two groups: I (body mass index (BMI)≥35 kg/m^2, n=40) and II (BMI<25 kg/m^2, n=30). Serum concentrations of MMP-2, MMP-9, MMP-13, and adiponectin were measured using enzyme-linked immunosorbent assay (ELISA). Results: A statistically significant difference between groups was observed for MMP-2 concentration. The median MMP-9 concentration was higher in the obese group, but the difference was not statistically significant. Median MMP-13 concentrations did not differ between groups. Serum adiponectin concentration was insignificantly higher in the non-obese group. Conclusions: The elevated serum MMP-2 and MMP-9 concentrations in obese individuals may be related to the higher incidence of incisional hernias in this population.

Key words: Metalloproteinase; Adiponectin; Obesity; Incisional hernia
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1 Introduction

The exact cause of incisional hernias is not completely clear. Several factors have been proposed, such as incorrect surgical technique, increased intra-abdominal pressure, and abnormal connective tissue metabolism, reflected by a disrupted synthesis and breakdown of its fibrous elements; in consequence, one in every five laparotomies results in an incisional hernia (Mohebali et al., 2009).

Obesity is considered a risk factor in herniogenesis and hernia recurrence, and bariatric surgery has proved to be the most efficient treatment for obesity (Sugerman et al., 1996; Frühbeck, 2015). The role of obesity as an etiological factor of hernia is quite intriguing. On the one hand, everyday practice indicates that a large proportion of morbidly obese patients retain body wall integrity for many years. On the other, even the smallest injury to the fascia in one out of 3–5 cases leads to fascial disintegration, resulting in an incisional hernia.

Many substances are synthesized in the intra-abdominal adipose tissue including adipokines, which have proinflammatory or atherogenic properties (Jung and Choi, 2014). They contribute to insulin resistance and influence the secretion of metalloproteinases (MMPs). After bariatric procedures the plasma levels of proinflammatory agents decrease significantly (Catalán et al., 2007; Gumbau et al., 2014; Tham et al., 2014).

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MMP-2, MMP-9, and MMP-13 are believed to break down the extracellular matrix of the connective tissue and to have an influence on herniation. Adiponectin influences a number of metabolic processes, particularly the metabolism of glucose and fatty acids, thus increasing insulin sensitivity. It also displays anti-inflammatory and anti-atherosclerotic properties, which is why these substances were chosen for evaluation of a possible role in the development of incisional hernias.

The aim of this study was to evaluate the concentrations of selected MMPs (MMP-2, MMP-9, and MMP-13) and adiponectin in the serum of morbidly obese and non-overweight controls.

2 Materials and methods

2.1 Study design and conduct

The study group consisted of 70 patients (42 females and 28 males) aged 19–65 years recruited from the Department of Hepatobiliary and General Surgery of the No. 1 Dr. A. Jurasz University Hospital in Bydgoszcz (Poland). The study group was divided into two subgroups: Group I included patients scheduled for elective bariatric surgery (body mass index (BMI)≥ 35 kg/m^2, n=40); Group II consisted of patients scheduled for elective operations for non-inflammatory conditions (BMI<25 kg/m^2, n=30). The morbidly obese patients in Group I included 22 females and 18 males aged 22–58 years and the non-obese patients in Group II included 20 females and 10 males aged 19–65 years. In Group I, 20 patients had been diagnosed with type 2 diabetes mellitus and 21 with arterial hypertension. However, in Group II only 6 patients had arterial hypertension. For the purpose of this study, diabetes mellitus was defined as constant uptake of insulin or oral hypoglycemic drugs. Hypertension was diagnosed if systolic blood pressure exceeded 140 mmHg (1 mmHg=133.3 Pa) and/or diastolic blood pressure was above 90 mmHg. Baseline characteristics of the study participants are presented in Table 1. Only two patients in the Group I and six in Group II were smokers. Therefore, smoking was disregarded in our analysis.

2.2 Blood sampling and laboratory analyses

Venous blood samples were collected using a "Vacutainer" system (Becton Dickinson, Franklin Lakes, New Jersey, USA) without anticoagulant. The samples were harvested in the morning, after 8 h of fasting prior to the scheduled surgery. The serum obtained by spinning the blood for 15 min at 3000g was kept at −20 °C until the measurements could be performed.

Human MMP-2 was determined by a sandwich enzyme-linked immunosorbent assay (ELISA) method (R&D Systems, Wiesbaden, Germany). The limit of detection was 0.16 ng/ml (intra-assay coefficient of variation (CV) 3.4%–5.7%, inter-assay CV 7.4%–8.2%) and the reference range was from 117 to 410 ng/ml. Human MMP-9 was assayed by a quantitative sandwich enzyme immunoassay technique (R&D Systems, Wiesbaden, Germany). The limit of detection was 0.156 ng/ml and the reference range was from 169 to 705 ng/ml (intra-assay CV 1.9%–2.9%, inter-assay CV 6.9%–7.9%). Human MMP-13 was determined using an ELISA test kit (Bender MedSystems GmbH,

Table 1 Baseline characteristics of the study participants

Parameter	Morbidly obese patients (BMI≥35, n=40)	Non-obese patients (BMI<25, n=30)	P-value
Age (year)	40.5 (37.5–45.5)	42 (32–47)	ns
Females, n (%)	22 (55)	20 (67)	ns
Males, n (%)	18 (45)	10 (33)	ns
BMI (kg/m^2)	48.7 (44.3–54.8)	25.4 (23.2–26.2)	0.00001
Diabetes mellitus, n (%)	20 (50)	0	0.00001
Hypertension, n (%)	21 (52)	6 (20)	0.00001
MMP-2 (ng/ml)	178.32 (164.34–205.40)	161.43 (142.53–198.31)	0.04
MMP-9 (ng/ml)	532.70 (389.00–760.90)	324.60 (280.50–753.10)	0.09
MMP-13 (ng/ml)	0.37 (0.36–0.38)	0.37 (0.37–0.39)	ns
Adiponectin (μg/ml)	3.39 (2.62–5.17)	3.54 (2.62–4.86)	ns

Values are presented as median (25th–75th quartile) or number (percent). BMI: body mass index; MMP: metalloproteinase; ns: not significant

Vienna, Austria). The limit of detection of MMP-13 was less than 0.18 ng/ml and the cutoff value was 9.7 ng/ml. Total human adiponectin was determined using an ELISA test kit (R&D Systems, Wiesbaden, Germany). The limit of detection was 0.0025 μg/ml and the reference range was from 0.865 to 21.424 μg/ml (intra-assay CV 2.5%–4.7%, inter-assay CV 5.8%–6.9%).

2.3 Statistical analysis

The Kolmogorov-Smirnov test was used to assess the normality of distribution of investigated parameters. All data are presented as the median and the 25th and 75th percentiles. Comparisons between the groups were performed using the Mann-Whitney *U*-test and the Kruskal-Wallis test for non-normally distributed variables. Pearson correlation was used to analyze associations between variables. *P* values of <0.05 were considered statistically significant. The clinical utility of laboratory parameters was determined by analysis of receiver operating characteristic (ROC) curves (Table 2). Statistical analysis was performed using Statistica 10.0 for Windows (StatSoft, Tulsa, OK, USA).

3 Results

3.1 Baseline characteristics and results of basic statistics

Baseline characteristics of the morbidly obese and non-obese groups, including age, sex, BMI, clinical parameters, and MMP-2, MMP-9, MMP-13 and adiponectin concentrations are shown in Table 1. Patients with morbid obesity presented higher values of MMP-2 and MMP-9, but lower levels of adiponectin in comparison with the non-obese group. However, the median values for MMP-13 did not differ between the study groups.

3.2 Diagnostic utility of investigated markers

Finally, we evaluated the ROC curves to assess the diagnostic accuracies of the investigated variables for the prediction of obesity occurrence. The highest level of discrimination of obesity was found for MMP-9 (area under the curve (AUC)=0.66). However, this was not significantly different from the diagnostic accuracy of MMP-2 (AUC=0.63). The diagnostic accuracies of all variables are shown in Table 2. For all measured parameters, sensitivity and specificity were calculated. Among them, the most valuable results were those obtained for MMP-9 and MMP-2.

4 Discussion

In research on the origin of hernia throughout recent decades, particular attention has been given to connective tissue metabolism, especially the synthesis and breakdown of the extracellular matrix (including the role of MMPs). Many authors have reported a genetically determined decrease in the collagen I to III ratio both within the hernial orifice and in remote tissues (Klinge et al., 2001).

MMPs have an important role in many physiological and pathological processes, including aortic aneurysm, wound healing (anastomotic leaks, venous ulcers), malignant metastases and local expansion of malignancies, and tissue remodeling (Henriksen et al., 2013). The influence of MMPs on the development and recurrence of hernia has been the subject of investigation for over a decade. Many reports have shown increased MMP concentrations both in the blood and the connective tissue of patients with inguinal and postoperative hernias, both primary and recurrent. Simultaneously, a decreased expression of the inhibitors of these enzymes was noted (Bellón

Table 2 Diagnostic usefulness of the assayed parameters for the occurrence of obesity

Variable	MMP-2 (ng/ml)	MMP-9 (ng/ml)	MMP-13 (ng/ml)	Adiponectin (μg/ml)
AUC	0.635	0.666	0.392	0.524
SE	0.070	0.100	0.064	0.082
95% CI	0.499–0.772	0.470–0.862	0.267–0.517	0.363–0.686
Cut-off value	149.800	405.500	0.344	1.931
Sensitivity	0.940	0.730	1.000	1.000
Specificity	0.420	0.730	0.100	0.180

AUC: area under the curve; SE: standard error; CI: confidence interval; MMP: metalloproteinase

et al., 2001). Henriksen et al. (2013) found no statistically significant difference in MMP-9 or tissue inhibitor of metalloproteinase 1 (TIMP-1) level when comparing patients with and without incisional hernias. Elevated pro-MMP-9 levels in patients with recurrent incisional hernias might suggest increased collagen degradation in these cases, while technical factors may play a more important role in primary incisional hernia formation (Henriksen, 2016).

Belón et al. (2001) observed a strong immunohistochemical reaction to MMP-2 in the transversalis fascia of patients with direct inguinal hernia (Sørensen, 2006). In their later studies, they confirmed the activity of this enzyme in cultures of fibroblasts harvested from the fascia and skin specimens of hernia patients (Pascual et al., 2010). The activity of MMP-2 in the serum of patients with inguinal hernias is significantly higher than that in healthy controls (Smigielski et al., 2009). Smigielski et al. (2011) discovered an overexpression of MMP-2 and TIMP-2 in recurrent hernias (TIMP-2 overexpression as a reaction to increased MMP activity). Salameh et al. (2007) found increased MMP-2 activity in biopsy specimens harvested from surgical scars in patients with incisional hernias. The results of Aren et al. (2011) indicated the roles of MMP-1, MMP-2, and MMP-9 in the etiology of inguinal hernia.

The results of this study have shown MMP-2 activity to be significantly higher in the obese than in the non-obese patients. Similar results have been reported by Andrade et al. (2012) in a group of obese females. Such results may indicate an increase of MMP-2 activity during the healing of surgical wounds in obese individuals, possibly resulting in an increased breakdown of type I collagen. This would lead to an increased proportion of type III collagen, resulting in the synthesis of a mechanically weak tissue. According to Klinge et al. (2001) such a ratio of collagen types is characteristic of incisional hernias. Bouloumié et al. (2001) have observed that adipocytes may be a source of MMP-2 and MMP-9. This would explain the elevated activity of these enzymes seen in extremely obese patients. However, Gummesson et al. (2009) found no positive correlation between MMP-9 mRNA activity and BMI.

Derosa et al. (2008) and Catalán et al. (2009) observed a positive correlation between BMI and the activity of MMP-2 and MMP-9. MMP-9 has a pivotal role in the process of wound healing. It is produced by neutrophils and macrophages, and its physiological role is to break down types IV and V collagen as well as other components of the extracellular matrix, such as gelatin or elastin. Increased MMP-9 and MMP-2 levels have been reported in a number of clinical and experimental trials on chronic problem wounds. In these cases, the concentration of TIMPs was reduced. Increased MMP-9 level in the wound exudate was a predictor of wound healing quality in diabetic foot ulcers (Liu et al., 2009). Similarly, in post-herniotomy wounds with increased MMP-9 activity, hydroxyproline accumulation was lower. The influence of inflammation cannot, however, be ruled out (Agren et al., 1998). In our own material, MMP-9 concentrations were higher in the obese group. Similar results have been reported by Głowińska-Olszewska and Urban (2007), in a population of obese children and teenagers. However, it is unclear how these alterations influence incisional hernia formation in obese populations.

In this study, the two groups displayed significant differences in the incidence of diabetes mellitus and hypertension. Arterial hypertension is most likely not a standalone factor in MMP secretion (Giannakos et al., 2016). The role of diabetes is significant and also affects other aspects of wound healing (Tsioufis et al., 2012). The high prevalence of diabetes in the obese group must have influenced our results. In the future, we will strive for better comparability of the study groups.

The data concerning the influences of MMP-13 on wound healing and herniation are contradictory. Stumpf et al. (2002) investigated the activity of this enzyme in colorectal anastomoses in humans. They found evidence of MMP-13 activity in 90% of anastomoses that failed. The enzyme was active in 43% of cases in which the anastomoses healed uneventfully. Rosch et al. (2002) could not confirm the activity of MMP-13 mRNA in skin fibroblasts of patients with inguinal hernias or the expression of this enzyme in these cells. MMP-13 may have a role in inguinal hernia recurrence (Antoniou et al., 2009). In our material, MMP-13 levels were nearly identical in both groups.

The role of adiponectin in wound healing is unclear. Its physiological role is to decrease insulin resistance and act as an anti-inflammatory and antiatherogenic agent. Its serum concentration is inversely proportional to the mass of the adipose tissue

and lepin concentration (Bienertová-Vašků et al., 2014). A marked increase in serum level of this hormone has been observed after bariatric procedures (Trakhtenbroit et al., 2009). In our study, we found slightly higher adiponectin concentration in the non-obese group. This is a surprising finding and one for which we have no ready explanation. A possible answer might be the phenomenon described by Gómez-Ambrosi et al. (2011) in which some individuals with a non-obese BMI may exhibit high total body and visceral adiposity together with insulin resistance.

In recent years, new proinflammatory cytokines and interleukins have been discovered (interleukin 32 (IL-32), aquaglyceroporins, or the L-α-lysophosphatidylinositol/GPR55 system) in the obese. These compounds have a potential role in wound healing and hernia formation, pending further study of their metabolism and properties (Rodríguez et al., 2011; Moreno-Navarrete et al., 2012; Catalán et al., 2016).

5 Conclusions

The concentrations of the investigated MMPs secreted by the adipose tissue differed between obese and non-obese individuals. The heightened activity of MMP-2 and MMP-9 in the obese group may adversely influence wound healing, as shown in other pathologies. The elevated serum MMP-2 and MMP-9 concentrations in obese individuals may be related to the higher incidence of incisional hernias in this population.

Compliance with ethics guidelines

Wojciech SZCZĘSNY, Magdalena KULIGOWSKA-PRUSIŃSKA, Stanisław DĄBROWIECKI, Jakub SZMYTKOWSKI, Adrian REŚLIŃSKI, and Maciej SŁUPSKI declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from all patients for being included in the study.

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中文概要

题　目：肥胖患者体内金属蛋白酶和脂联素的活性——肥胖手术后造成切口疝的可能因素

目　的：主要研究病态肥胖患者与正常人血清中金属蛋白酶 2（MMP-2）、MMP-9、MMP-13 和脂联素的浓度。

创新点：建立血清中 MMP-2、MMP-9、MMP-13 和脂联素的浓度与肥胖和切口疝的关系。

方　法：参与实验的人员为进行肥胖手术的患者和不进行肥胖手术的患者，并将他们分为两组：I（体重指数（BMI）≥35 kg/m^2，n=40）和 II（BMI<25 kg/m^2，n=30），并使用酶联免疫吸附实验测定受试人员体内血清中 MMP-2、MMP-9、MMP-13 和脂联素的浓度。

结　论：MMP-2 的浓度在肥胖组中更高，且在两组血清中有显著性差异。虽然 MMP-9 的浓度在肥胖组中更高，但是两组之间没有显著性差异。MMP-13 在两组间没有差异。血清中脂联素的浓度在非肥胖组更高，但无显著性差异。因此，血清中 MMP-2 和 MMP-9 的浓度在肥胖人群中与更高的切口疝发病率有关。

关键词：金属蛋白酶；脂联素；肥胖症；切口疝