Autopsy Case Reports

Original Article

Immunohistochemical expression of plasminogen activator inhibitor-1 in subcutaneous versus omental adipose tissue in patients after elective abdominal surgery

Mehmet Yildiz[a], Emine Bozkurtlar[b], Abdulmunir Azizy[c], Mehmet Agirbasli[d]

How to cite: Yildiz M, Bozkurtlar E, Azizy A, Agirbasli M. Immunohistochemical expression of plasminogen activator inhibitor-1 in subcutaneous versus omental adipose tissue in patients after elective abdominal surgery. Autops Case Rep [Internet]. 2019 Oct-Dec;9(4):e2019121. https://doi.org/10.4322/acr.2019.121

ABSTRACT

Plasminogen activator inhibitor-1 (PAI-1) is a biomarker of thrombosis. Adipose and vascular tissues are among the major sources of PAI-1 production. Previous studies indicated that fat deposits mediate increased cardiovascular risk among obese individuals. We investigated the immunohistochemical (IHC) expression of PAI-1 in adipose and vascular tissues from the omentum and the subcutaneous tissue. The pathology samples were selected from 37 random patients who underwent elective abdominal surgery between 2008-2009. PAI-1 expression was semi-quantitatively scored and compared between the groups. Significant differences were noted in the IHC expression of PAI-1 between the omental and the subcutaneous adipose tissues (1.1 ± 0.8 versus 0.8 ± 0.6, respectively (p=0.05)). Adipose tissue displayed higher IHC expression of PAI-1 compared to vascular wall tissue in both omentum and subcutaneous sections (1.1 ± 0.8 versus 0.5 ± 0.9 (p=0.004), and 0.8 ± 0.6 versus 0.4 ± 0.6 (p=0.003), respectively). In conclusion, our study compared PAI-1 expression in the omentum versus the subcutaneous tissue and adipose versus vascular tissues. IHC expression of PAI-1 level was significantly higher in the omental adipose tissue compared to the subcutaneous adipose tissue. Adipose tissue displayed significantly higher PAI-1 expression than vascular tissue. The study elucidates the biological differences of adipose and vascular tissue from subcutaneous versus omental sections.

Keywords
Adipose Tissue; Immunohistochemistry; Plasminogen Activator Inhibitor-1

INTRODUCTION

Plasminogen activator inhibitor (PAI-1) is a member of the superfamily of serine-protease inhibitors and plays a crucial role in the inhibition of both tissue and urinary type of plasminogen activators.[1] PAI-1 is produced in various cells as the liver, brain, muscle, and adipocytes. PAI-1 production is stimulated by various cytokines such as IL-6, TNF-α, and TGF-β.[2,3] Basic and clinical science studies confirmed that obesity and metabolic syndrome are associated with alterations in the fibrinolytic system and supported the hypothesis

[a] Cleveland Clinic Fairview Hospital, Department of Internal Medicine. Cleveland, OH, USA.
[b] Marmara University Medical School, Department of Pathology. Istanbul, Turkey.
[c] Marmara University, Medical School, Department of Medicine. Istanbul, Turkey.
[d] Medeniyet University Medical School, Department of Cardiology. Istanbul, Turkey.

that an increased PAI-1 expression by the adipose and vascular tissues might contribute to cardiovascular disease progress.[1,4,5]

The volume of adipose tissue, but also the types of adipose tissue is associated with the development of cardiovascular risk.[6,7] Adipocytes display biological differences based on their origin. The visceral adipose tissues were associated with increased risk of major cardiovascular events compared with the adipose tissue of the subcutaneous.[6,8] In this study, we examined immunohistochemical (IHC) expression of PAI-1 in surgical pathology samples from patients who underwent elective abdominal surgery.

METHODS

Surgical Pathology Samples

The study was approved by the Marmara University Medical School Institutional Review Board. The pathological samples were selected retrospectively from the archives of Marmara University Pathology Department. Randomly 37 patients were selected who underwent elective abdominal surgery for various clinical indications between 2008 and 2009 (Table 1).

The types and indications for abdominal surgery included splenectomy for thalassemia and immune thrombocytopenia, lymph node resection for Non-Hodgkin's lymphoma, total pancreatectomy and gastrectomy for cancer, and colon resection for ulcerative colitis. Nearly 20% of patients had the history of diabetes mellitus. Since the study samples were selected retrospectively, no informed consent was required.

Immunohistochemical Expression

IHC expression of PAI-1 staining (Rabbit anti-human PAI-1 antiserum, Molecular Innovations, Inc) was performed. Three to five fields were evaluated per section for PAI-1 expression. Adipose tissue from the omentum and the subcutaneous tissue was removed immediately and cut in 2 mm thick slices. These were fixed in 10% formalin and embedded in paraffin followed by the IHC reaction. IHC expression of PAI-1 semi-quantitatively scored from the adipose and vascular wall tissues from the omentum and subcutaneous. Blinded pathologists selected at least three pathological slides per block at 10X (magnification) and analyzed on the cellularity and density of PAI-1 expression. Then, BX51 Olympus^{R} microscopy was used to count cells that expressed PAI-1. A visual grading scale (0 to 3) was used for the assessment of the intensity IHC expression of PAI-1: Score "0" represented no expression, score "1" represented mild expression, score "2" represented moderate expression, and score "3" or more represented the most extensive PAI-1 expression.[9] The average score was calculated for each pathological samples. Comparison between groups and semi-quantitative scores were performed for statistical evaluation. A value of $p<0.05$ was considered statistically significant.

Table 1. Characteristics of the study sample as median age and gender

	Age (years)	Females (%)
Mean ± SD	53 ± 14	43
Median (IR)	55 (26)	

IR = interquartile range; SD = standard deviation.

RESULTS

Significant differences were noted in the IHC expression of PAI-1 between the omental and the subcutaneous adipose tissues. Mean ± standard deviation scores of IHC expression of PAI-1 for the omental versus the subcutaneous adipose tissue were 1.1 ± 0.8 versus 0.8 ± 0.6 ($p=0.05$). The omental adipose tissue also displayed higher IHC expression of PAI-1 compared to omental vascular wall (1.1 ± 0.8 versus 0.5 ± 0.9 ($p=0.004$)). Similarly, the subcutaneous adipose tissue displayed higher IHC expression of PAI-1 compared to subcutaneous vascular wall (0.8 ± 0.6 versus 0.4 ± 0.6 ($p=0.003$)), while the IHC expression of PAI-1 semi-quantitative scores were similar between the omental versus the subcutaneous vascular wall tissues (0.5 ± 0.9 versus 0.4 ± 0.6 ($p=0.32$)) (Figures 1-3).

DISCUSSION

Adipose tissue has a crucial role in the development of cardiovascular diseases. Adipose tissues secrete adipokines. Considerable interest remains in understanding the endocrine role of

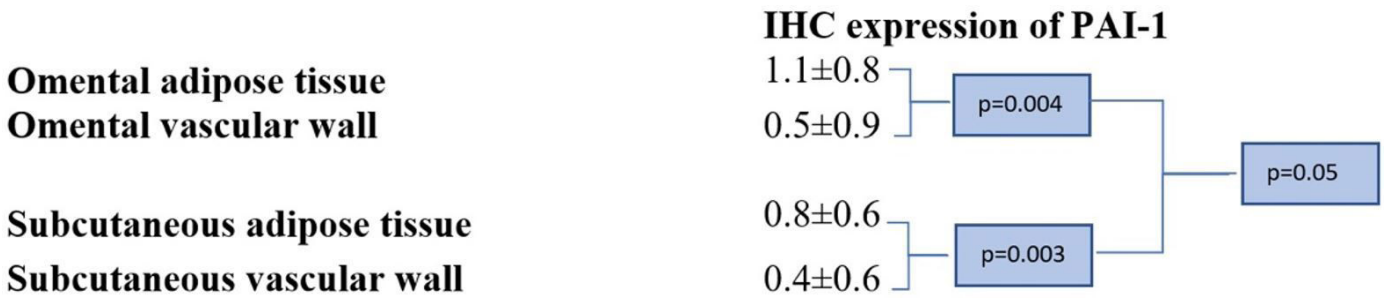

Figure 1. Comparison of IHC PAI-1 expression between omental and subcutaneous tissues. IHC = immunohistochemical; PAI-1 = plasminogen activator inhibitor-1.

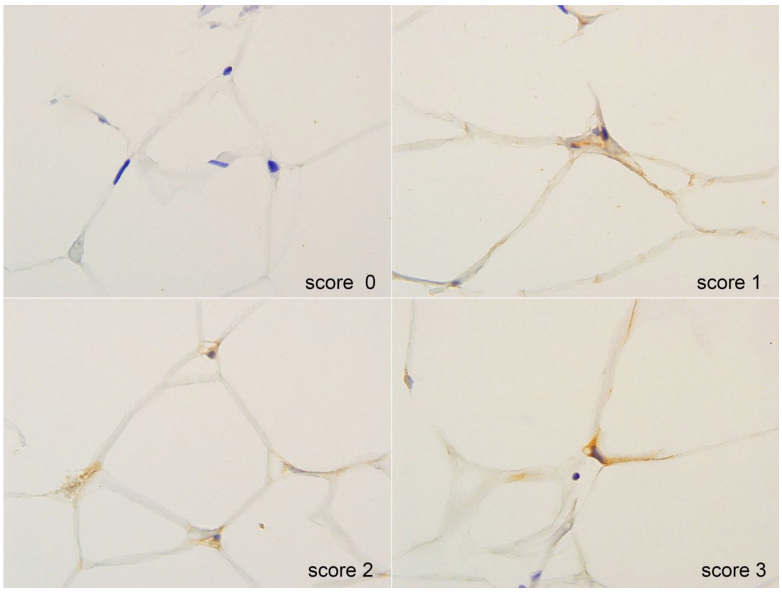

Figure 2. Scoring of IHC PAI-1 expression in adipose tissue.

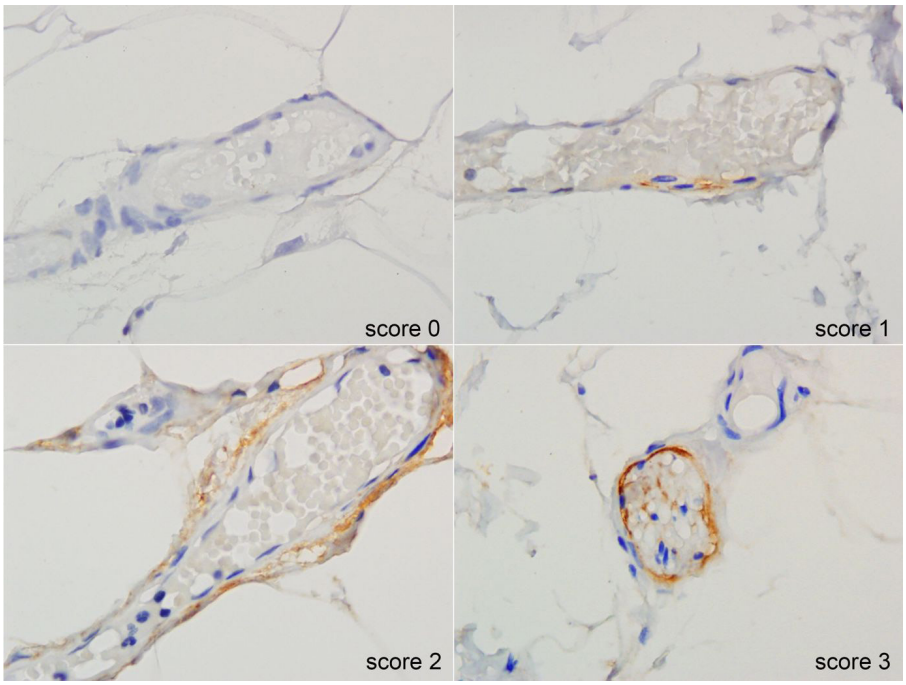

Figure 3. Scoring of IHC PAI-1 expression in vascular tissue.

adipokines. Adipocyte-derived PAI-1 can serve as a link between the increased body fat of the obese and the cardiovascular risk. The biological importance of the PAI-1 molecule is not limited to the coagulation-fibrinolysis cascade but is also demonstrated in many physiological and pathological processes, including obesity, inflammation, insulin resistance, and malignancy.[4,10] PAI-1 is produced and released by adipose tissues; however, the PAI-1 production varies not only with the adipose tissue burden but also with the type of the adipose tissue.[11,12] In this context; the biological differences of adipose tissues, subcutaneous versus omentum, have been demonstrated in favor of different gene expression, secretion of various inflammatory markers, growth factors, and fibrinolysis agents.[13-16]

Several ex-vivo human studies showed an increased production of PAI-1 by the visceral adipose tissues compared with the subcutaneous adipose tissues.[17-19] However, conflicting data exist in the literature about the biological differences between the omental versus the subcutaneous adipose tissues. Van Harmelen et al.[20] reported higher PAI-1 mRNA expression and PAI-1 antigen secretion by the subcutaneous adipose tissue compared with the visceral adipose tissue. He et al.[21] reported that no differences exist in PAI-1 expression between the omental and the subcutaneous adipose tissue; however, yet after incubation, a higher expression of PAI-1 was observed in the omental than the subcutaneous adipose tissue. Our study extends the evidence by using IHC and demonstrates the relation of increased PAI-1 production by the visceral origin adipose tissues (omentum). Our observations might be supportive for the prior findings by several other groups. Hardy et al.[22] showed that inflammation in the omental adipose tissue is associated with insulin resistance. O'Connel et al.[23] indicated that omental adipocyte size was an indicator of liver fibrosis, metabolic disease, and possibly progression from hepatic steatosis to fibrosis. Yang et al.[24] reported that mesentery adipose depot played a pivotal role in obesity-related diabetes. Harman-Boehm et al.[25] reported that preferential macrophage infiltration into the omental adipose tissue compared to the subcutaneous adipose tissue, among the obese population, might be the link between with increased risk of coronary artery disease and central obesity

Accumulated fat and enhanced adipose tissue-derived PAI-1 might induce fibrosis and chronic inflammation. As a potential explanation, TGF-β is a strong inducer of PAI-1 expression.[26] A previous study reported that the molecular mechanism involves tissue-specific TGF-β response to Ang II infusion; hence the PAI-1 response to TGF-β can display tissue-specific characteristics.[27]

Our study has several limitations. It was a retrospective surgical pathology sample analysis of PAI-1 expression in the tissues of patients who underwent elective intra-abdominal surgery. There was no blinding in the pathology assessment of IHC expression of PAI-1. Therefore, the PAI-1 expression in different tissues can vary based on patients' baseline vascular risk characteristics. We did not measure the plasma PAI-1 antigen and activity levels; therefore, we cannot assess the overall fibrinolytic activity in these patients. The sample size was relatively small, and this could be related to the restrictive eligibility criteria and availability of the tissue samples to assess PAI-1 expression. However, our study directly compares the IHC expression of PAI-1 in different tissues. We did find significant differences in IHC expression of PAI-1 in different tissue segments. We believe our data can provide clinicians with a rationale for a detailed assessment of the patients with cardiovascular risk.

In conclusion, IHC staining scores displayed higher levels of PAI-1 expression in the adipose tissues than the vascular wall and higher levels of PAI-1 expression in the omental adipose tissues than the subcutaneous adipose tissues. Future studies in large cohorts involving patients' characteristics, plasma PAI-1 levels, and follow-up data would be more explanatory for the role of PAI-1 in cardiovascular and metabolic diseases.

REFERENCES

1. Cesari M, Pahor M, Incalzi RA. Plasminogen activator inhibitor-1 (PAI-1): a key factor linking fibrinolysis and age-related subclinical and clinical conditions. Cardiovasc Ther. 2010;28(5):e72-91. http://dx.doi.org/10.1111/j.1755-5922.2010.00171.x. PMid:20626406.

2. Tsantes AE, Nikolopoulos GK, Bagos PG, Bonovas S, Kopterides P, Vaiopoulos G. The effect of the plasminogen activator inhibitor-1 4G/5G polymorphism on the thrombotic risk. Thromb Res. 2008;122(6):736-42. http://dx.doi.org/10.1016/j.thromres.2007.09.005. PMid:17949795.

3. Kolev K, Machovich R. Molecular and cellular modulation of fibrinolysis. Thromb Haemost. 2003;89(4):610-21. http://dx.doi.org/10.1055/s-0037-1613567. PMid:12669114.

4. Agirbasli M. Pivotal role of plasminogen-activator inhibitor 1 in vascular disease. Int J Clin Pract. 2005;59(1):102-6. http://dx.doi.org/10.1111/j.1742-1241.2005.00379.x. PMid:15707473.

5. Barnard SA, Pieters M, De Lange Z. The contribution of different adipose tissue depots to plasma plasminogen activator inhibitor-1 (PAI-1) levels. Blood Rev. 2016;30(6):421-9. http://dx.doi.org/10.1016/j.blre.2016.05.002. PMid:27233154.

6. Abraham TM, Pedley A, Massaro JM, Hoffmann U, Fox CS. Association between visceral and subcutaneous adipose depots and incident cardiovascular disease risk factors. Circulation. 2015;132(17):1639-47. http://dx.doi.org/10.1161/CIRCULATIONAHA.114.015000. PMid:26294660.

7. Poirier P, Després J-P. Waist circumference, visceral obesity, and cardiovascular risk. J Cardiopulm Rehabil. 2003;23(3):161-9. http://dx.doi.org/10.1097/00008483-200305000-00001. PMid:12782898.

8. Fox CS, Massaro JM, Hoffmann U, et al. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. Circulation. 2007;116(1):39-48. http://dx.doi.org/10.1161/CIRCULATIONAHA.106.675355. PMid:17576866.

9. Sobel BE, Woodcock-Mitchell J, Schneider DJ, Holt RE, Marutsuka K, Gold H. Increased plasminogen activator inhibitor type 1 in coronary artery atherectomy specimens from type 2 diabetic compared with nondiabetic patients: a potential factor predisposing to thrombosis and its persistence. Circulation. 1998;97(22):2213-21. http://dx.doi.org/10.1161/01.CIR.97.22.2213. PMid:9631870.

10. Yamamoto K, Takeshita K, Kojima T, Takamatsu J, Saito H. Aging and plasminogen activator inhibitor-1 (PAI-1) regulation: implication in the pathogenesis of thrombotic disorders in the elderly. Cardiovasc Res. 2005;66(2):276-85. http://dx.doi.org/10.1016/j.cardiores.2004.11.013. PMid:15820196.

11. Landin K, Stigendal L, Eriksson E, et al. Abdominal obesity is associated with an impaired fibrinolytic activity and elevated plasminogen activator inhibitor-1. Metabolism. 1990;39(10):1044-8. http://dx.doi.org/10.1016/0026-0495(90)90164-8. PMid:2215252.

12. Giltay EJ, Elbers JM, Gooren LJ, et al. Visceral fat accumulation is an important determinant of PAI-1 levels in young, nonobese men and women: modulation by cross-sex hormone administration. Arterioscler Thromb Vasc Biol. 1998;18(11):1716-22. http://dx.doi.org/10.1161/01.ATV.18.11.1716. PMid:9812909.

13. Cigolini M, Targher G, Andreis IAB, Tonoli M, Agostino G, De Sandre G. Visceral fat accumulation and its relation to plasma hemostatic factors in healthy men. Arterioscler Thromb Vasc Biol. 1996;16(3):368-74. http://dx.doi.org/10.1161/01.ATV.16.3.368. PMid:8630661.

14. Mertens I, Van Gaal LF. Visceral fat as a determinant of fibrinolysis and hemostasis. Semin Vasc Med. 2005;5(1):48-55. http://dx.doi.org/10.1055/s-2005-871741. PMid:15968580.

15. Atzmon G, Yang XM, Muzumdar R, Ma XH, Gabriely I, Barzilai N. Differential gene expression between visceral and subcutaneous fat depots. Horm Metab Res. 2002;34(11-12):622-8. http://dx.doi.org/10.1055/s-2002-38250. PMid:12660871.

16. Miyazawa-Hoshimoto S, Takahashi K, Bujo H, Hashimoto N, Saito Y. Elevated serum vascular endothelial growth factor is associated with visceral fat accumulation in human obese subjects. Diabetologia. 2003;46(11):1483-8. http://dx.doi.org/10.1007/s00125-003-1221-6. PMid:14534780.

17. Cigolini M, Tonoli M, Borgato L, et al. Expression of plasminogen activator inhibitor-1 in human adipose tissue: a role for TNF-alpha? Atherosclerosis. 1999;143(1):81-90. http://dx.doi.org/10.1016/S0021-9150(98)00281-0. PMid:10208482.

18. Gottschling-Zeller H, Birgel M, Röhrig K, Hauner H. Effect of tumor necrosis factor alpha and transforming growth factor beta 1 on plasminogen activator inhibitor-1 secretion from subcutaneous and omental human fat cells in suspension culture. Metabolism. 2000;49(5):666-71. http://dx.doi.org/10.1016/S0026-0495(00)80046-3. PMid:10831181.

19. Bastelica D, Morange P, Berthet B, et al. Stromal cells are the main plasminogen activator inhibitor-1-producing cells in human fat: evidence of differences between visceral and subcutaneous deposits. Arterioscler Thromb Vasc Biol. 2002;22(1):173-8. http://dx.doi.org/10.1161/hq0102.101552. PMid:11788479.

20. Van Harmelen V, Hoffstedt J, Lundquist P, et al. Regional variation in plasminogen activator inhibitor-1 expression in adipose tissue from obese individuals. Thromb Haemost. 2000;83(4):545-8. http://dx.doi.org/10.1055/s-0037-1613860. PMid:10780314.

21. He G, Pedersen SB, Bruun JM, Lihn AS, Jensen PF, Richelsen B. Differences in plasminogen activator inhibitor 1 in subcutaneous versus omental adipose tissue in non-obese and obese subjects. Horm Metab Res. 2003;35(3):178-82. http://dx.doi.org/10.1055/s-2003-39078. PMid:12734779.

22. Hardy OT, Perugini RA, Nicoloro SM, et al. Body mass index-independent inflammation in omental adipose tissue associated with insulin resistance in morbid obesity. Surg Obes Relat Dis. 2011;7(1):60-7. http://dx.doi.org/10.1016/j.soard.2010.05.013. PMid:20678967.

23. O'Connell J, Lynch L, Cawood TJ, et al. The relationship of omental and subcutaneous adipocyte size to metabolic disease in severe obesity. PLoS One. 2010;5(4):e9997. http://dx.doi.org/10.1371/journal.pone.0009997. PMid:20376319.

24. Yang Y-K, Chen M, Clements RH, Abrams GA, Aprahamian CJ, Harmon CM. Human mesenteric adipose tissue plays unique role versus subcutaneous and omental fat in obesity related diabetes. Cell Physiol Biochem. 2008;22(5-6):531-8. http://dx.doi.org/10.1159/000185527. PMid:19088435.

25. Harman-Boehm I, Blüher M, Redel H, et al. Macrophage infiltration into omental versus subcutaneous fat across different populations: effect of regional adiposity and the comorbidities of obesity. J Clin Endocrinol Metab. 2007;92(6):2240-7. http://dx.doi.org/10.1210/jc.2006-1811. PMid:17374712.

26. Vaughan DE. PAI-1 and TGF-beta: unmasking the real driver of TGF-beta-induced vascular pathology. Arterioscler Thromb Vasc Biol. 2006;26(4):679-80. http://dx.doi.org/10.1161/01.ATV.0000209949.86606.c2. PMid:16556860.

27. Sato O, Ashizawa N, Ohtsuru A, et al. Fibrotic response to angiotensin II is blunted in the kidney, but not in the heart, in insulin-sensitive long-lived transgenic dwarf rats. Int J Mol Med. 2007;19(1):23-7. http://dx.doi.org/10.3892/ijmm.19.1.23. PMid:17143544.

Author contributions: Yildiz M collected the samples and analyzed the data. Azizy A collected the patients data. Bozkurtlar E was in charge of the immunohistochemical analysis. Agirbasli M designed the paper and analyzed the data. All authors collectively proofread the final version of the manuscript and approved for publication.

The study was approved by the Marmara University Medical School Institutional Review Board

Conflict of interest: None

Financial support: The project was funded by the Scientific Research and Projects Commission of Marmara University (BAPKO SAG-B-060510-0112).

Submitted on: February 23rd, 2019
Accepted on: August 20th, 2019

Correspondence
Mehmet Agirbasli
Department of Cardiology - Medeniyet University Medical School
Yesilbahar Sok. 68/14 Goztepe – Istanbul – Turkey
34726
Phone: +90 532-7468840
magirbasli@gmail.com