УДК [61+615.1] (06)
ББК 5+52.81
А 43
ISBN 978-985-21-1865-1

Л. Чандракумар, С. Баранитаран

МОРФОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ В АТЕРОСКЛЕРОТИЧЕСКОЙ БЛЯШКЕ У БОЛЬНЫХ САХАРНЫМ ДИАБЕТОМ

Научный руководитель: д-р мед. наук, доц. В.В. Савош
Кафедра патологической анатомии и судебной медицины
с курсом повышения квалификации и переподготовки
Белорусский государственный медицинский университет, г. Минск

L. Chandrakumar, S. Baraneetharan

MORPHOLOGICAL CHANGES IN ARTHEROSCLEROTIC PLAQUE IN DIABETIC PATIENTS

Tutor: PhD, associate professor V.V. Savosh
Department of Pathological Anatomy and Forensic Medicine
with a Course of Advanced Training and Retraining
Belarusian State Medical University, Minsk

Резюме. В этом исследовании изучаются изменения коронарных бляшек у больных диабетом, подчеркиваются повышенные уровни ММП-1, ММП-9, a-SMA и CD68, которые коррелируют с нестабильностью бляшек и риском разрыва, а также подчеркивается роль металлопротеиназ в деградации бляшек и сердечно-сосудистом риске.

Ключевые слова: атеросклероз, MMP-1, MMP-9, a-SMA, CD68.

Resume. This study investigates coronary plaque changes in diabetics, highlighting increased MMP-1, MMP-9, a-SMA, and CD68 levels, which correlate with plaque instability and rupture risk, emphasizing the role of metalloproteinases in plaque degradation and cardiovascular risk

Keywords: atherosclerosis, MMP-1, MMP-9, a-SMA, CD68.

Actuality. In individuals with diabetes, atherosclerotic plaques within the arterial walls exhibit distinctive structural modifications and show an increased presence of specific biomarkers that are indicative of heightened inflammatory activity and instability of the plaques. These alterations are critical because they significantly influence the progression of cardiovascular disease and the risk of acute events such as heart attacks.

One of the key biomarkers identified is **alpha-smooth muscle actin (a-SMA)**, which is a protein expressed predominantly by smooth muscle cells (SMCs). These cells play a vital role in maintaining vascular integrity and in the formation of atherosclerotic plaques. In diabetic patients, the level of a-SMA is approximately 30% higher compared to non-diabetic individuals. This increase suggests an elevated activity or proliferation of smooth muscle cells within the plaques. Such hyperactivity can contribute to the thickening of the fibrous cap of the plaque, but paradoxically, it can also lead to instability if these cells produce matrix-degrading enzymes or if their proliferation results in a disorganized fibrous structure.

Another significant biomarker is **CD68**, a glycoprotein highly expressed by macrophages, which are immune cells involved in inflammation and tissue remodeling within atherosclerotic lesions. The increased presence of CD68 in diabetic plaques indicates a higher infiltration of macrophages. This heightened macrophage activity is associated with an inflammatory milieu within the plaque, promoting further tissue degradation, necrosis,

УДК [61+615.1] (06)
ББК 5+52.81
А 43
ISBN 978-985-21-1865-1

and destabilization. Macrophages release various enzymes and cytokines that can weaken the structural integrity of the plaque, making it more prone to rupture.

A particularly critical enzyme in this context is **Matrix Metalloproteinase type 9 (MMP-9)**. In diabetic individuals, the quantity of MMP-9 doubles compared to non-diabetics. MMP-9 is a member of the matrix metalloproteinase family, which is responsible for degrading components of the extracellular matrix (ECM). The ECM provides structural support to the vessel wall and the plaque itself. Elevated levels of MMP-9 reflect an increased breakdown of the ECM, especially the degradation of elastin and gelatins, which are essential for maintaining the elasticity and strength of the arterial wall. The overexpression of MMP-9 contributes to weakening the fibrous cap of the plaque, increasing the risk of rupture, which can lead to thrombus formation and subsequent cardiovascular events.

The enzymes **MMP-1 (collagenase-1)** and **MMP-9 (gelatinase-9)** are particularly crucial because they are directly involved in ECM remodelling, a process vital for tissue repair but also implicated in pathological tissue degradation when dysregulated. **MMP-1** primarily targets fibrillar collagen, the main structural protein in the fibrous cap of plaques. Its activity influences the stability of the plaque, as excessive collagen breakdown can weaken the cap and predispose it to rupture. **MMP-9**, on the other hand, degrades gelatins and elastin, which are key components of the vascular extracellular matrix that provide elasticity and resilience to the vessel wall. When these proteins are excessively broken down, the structural integrity of the plaque and the vessel wall is compromised, increasing the likelihood of destabilization. Aberrant expression of MMPs, particularly MMP-1 and MMP-9, is not only a hallmark of unstable plaques but also a contributing factor to various pathological conditions. Overactivity of these enzymes has been linked to diseases such as **cancer**, where ECM degradation facilitates tumour invasion and metastasis; **arthritis**, where joint destruction occurs due to breakdown of cartilage and synovial tissue; and **cardiovascular diseases**, where excessive ECM degradation leads to plaque rupture and arterial dissection.

In summary, the increased levels of smooth muscle actin-alpha, macrophage marker CD68, and matrix metalloproteinases (especially MMP-9 and MMP-1) in diabetic individuals' atherosclerotic plaques indicate a complex interplay of cellular proliferation, inflammation, and enzymatic tissue degradation. These processes collectively contribute to the destabilization of plaques, making them more susceptible to rupture, which is a primary event leading to acute coronary syndromes. Understanding these molecular and cellular mechanisms provides crucial insights into the heightened cardiovascular risk associated with diabetes and underscores the importance of targeted therapies aimed at modulating inflammation and ECM remodelling in diabetic patients

Aim: the main aim of the thesis is to analyse the distribution and variation of morphological and immunohistochemical changes in coronary vessels with atherosclerotic plaques in diabetic patients, focusing on structural alterations and biomarker expression related to plaque stability and instability.

Objectives:

1. Digital scanning of the received slides with Aperio Image scope for Immunohistopathological analysis.

УДК [61+615.1] (06)
ББК 5+52.81
А 43
ISBN 978-985-21-1865-1

2. Stained slides were analysed with the Positivity of the presence of the respective markers by digital annotations with the positive pixel count

3. The results were obtained for all the chosen markers and the positivity degree were compared with the statistical correlations.

Material and methods. Coronary artery atherosclerotic plaques from diabetic and non-diabetic patients were analysed using immunohistochemical staining, revealing the presence of CD68, MMP-1, MMP-9, and a-SMA. Patient data was collected from the cardiology departments of several hospitals in Minsk. The corresponding stained pathological slides, used for immunohistochemical analysis, were obtained from the Pathological Anatomical Bureau of Minsk. This allowed for the correlation of clinical information with pathological findings. Quantitative analysis of expression was performed using the programme Aperio Image Scope 12.4.6 and calculation of the ratio of positive markers to the total number of markers present, thereby determining the overall positivity for each marker.

Results and their discussion. The statistical analysis conducted in this study identified a noteworthy correlation between the presence of diabetes mellitus, whether type 1 or type 2, and the expression levels of MMP-1 within atherosclerotic plaques. This suggests that diabetic patients tend to exhibit higher levels of MMP-1, which is an enzyme involved in the breakdown of extracellular matrix components, possibly reflecting a heightened enzymatic activity associated with diabetes-related vascular changes. However, when the researchers compared the average expression levels of all the studied biomarkers—including MMP-1, MMP-9, alpha-SMA, and CD68—between groups of patients with and without diabetes, they did not observe statistically significant differences. Similarly, the degree of diabetes control or compensation, which indicates how well blood glucose levels are managed, did not significantly influence the expression levels of these markers. This indicates that while diabetes may be associated with increased MMP-1 activity, overall biomarker expression does not differ markedly across diabetic and non-diabetic groups, nor does it vary significantly with the level of glycaemic control.

Further analysis revealed that the expression levels of both MMP-1 and MMP-9 were strongly dependent on the stability of the atherosclerotic plaques. Specifically, in large, unstable plaques characterized by a lipid-rich core and a thin fibrous cap, the expression of these metalloproteinases was markedly higher. This heightened enzymatic activity is likely to contribute to the degradation of critical structural components of the plaque, such as collagen and elastin, weakening the fibrous cap and increasing the risk of rupture. The significance of this finding is underscored by the observation that MMP-1 levels were particularly elevated in plaques from patients who had succumbed to acute myocardial infarction, suggesting that increased MMP-1 activity may be directly involved in the processes leading to plaque rupture and subsequent cardiac events.

УДК [61+615.1] (06)
ББК 5+52.81
А 43
ISBN 978-985-21-1865-1

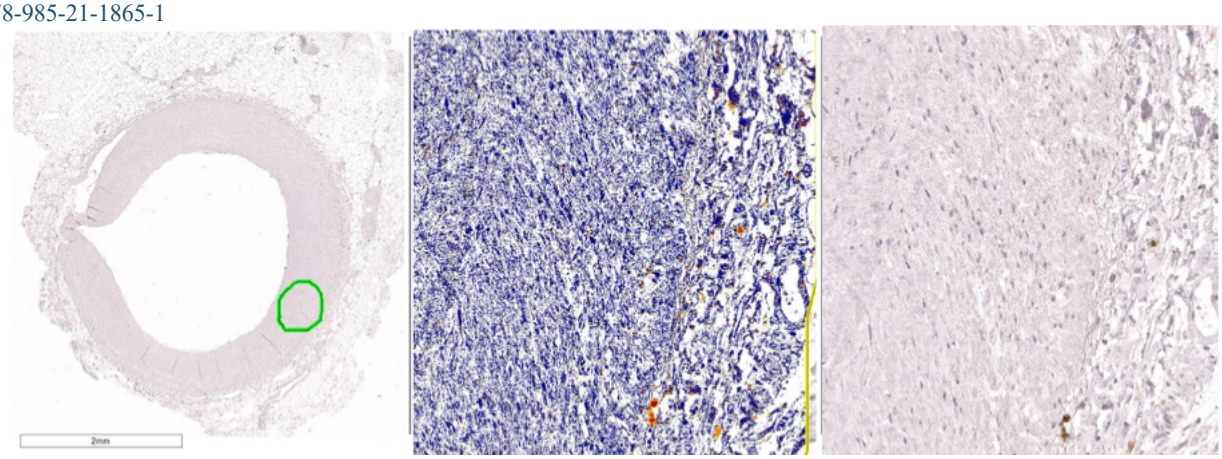

Fig. 1 – Representing the marked area of a coronary leumen with the scanned prelevance of MMP-1 Markers and the normal picture on the right corner before analyzed annotations

On the other hand, the study found no statistically significant correlation between the levels of alpha-SMA, a marker of smooth muscle cell activity, and CD68, a macrophage marker, with the severity or progression of atherosclerotic plaques in the diabetic patients examined. This means that the quantity or activity of smooth muscle cells and macrophages within the plaques did not consistently increase or decrease as the plaques became more advanced or unstable. In other words, the presence and levels of these cellular markers did not reliably reflect the degree of plaque deterioration or vulnerability. This finding suggests that cellular infiltration and smooth muscle cell activity, as measured by these markers, may not be sufficient indicators of plaque severity or instability in diabetic patients, and that other molecules or mechanisms might play more prominent roles in the process of plaque destabilization.

Overall, these findings highlight the complex and multifaceted nature of atherosclerosis in diabetic individuals. While certain enzymes like MMP-1 and MMP-9 are clearly associated with plaque instability and rupture, cellular markers such as alpha-SMA and CD68 do not show consistent relationships with plaque severity. This emphasizes the importance of focusing on enzymatic activity and extracellular matrix degradation pathways when assessing plaque vulnerability and developing targeted therapies aimed at preventing rupture and adverse cardiovascular events in diabetic patients.

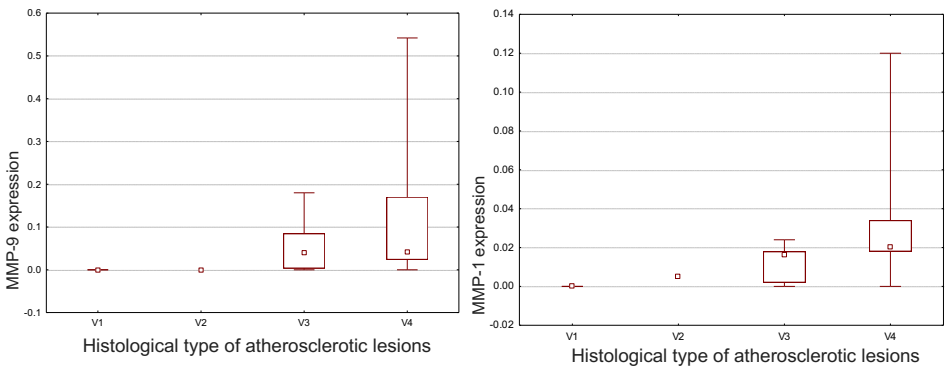

Fig. 2 – The degree of expression of both types of metalloproteinases depended on the degree of atherosclerotic plaque stability

УДК [61+615.1] (06)
ББК 5+52.81
А 43
ISBN 978-985-21-1865-1

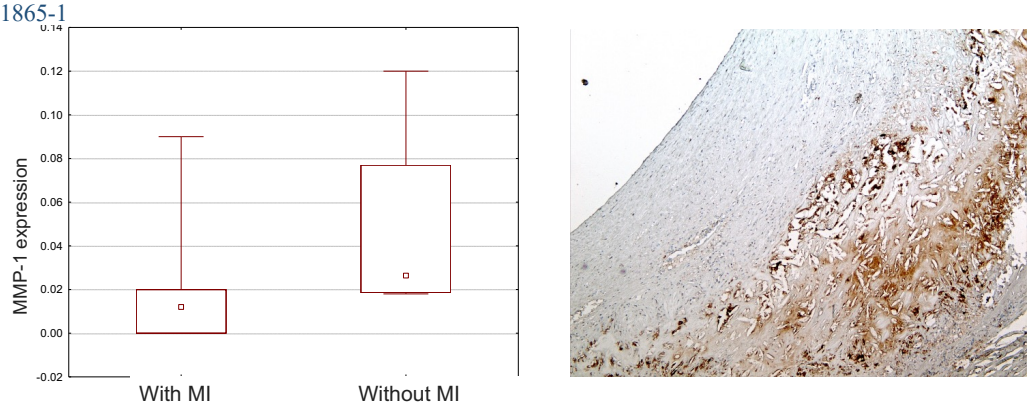

Fig. 3 – The level of MMP-1 expression was also higher in atherosclerotic plaques in patients who died of acute myocardial infraction

Conclusion: Significant expression of metalloproteinases (MMP-1 and MMP-9) results in more active degradation of extracellular matrix components, increases a risk of rupture of atherosclerotic plaques fibrous cover and makes them less stable.

Literature

1. Libby P. Inflammation in atherosclerosis. Nature. 2002 Dec 19–26;420(6917):868-74. doi: 10.1038/nature01323. PMID: 12490960.

2. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med. 2005 Apr 21;352(16):1685–95. doi: 10.1056/NEJMra043430. PMID: 15843671.

3. Falk E. Pathogenesis of atherosclerosis. J Am Coll Cardiol. 2006 Apr 18;47(8 Suppl):C7-12. doi: 10.1016/j.jacc.2005.09.068. PMID: 16631513.

4. Schaar JA, Muller JE, Falk E, Virmani R, Fuster V, Serruys PW, Colombo A, Stefanadis C, Ward Casscells S, Moreno PR, Maseri A, van der Steen AF. Terminology for high-risk and vulnerable coronary artery plaques. Report of a meeting on the vulnerable plaque, June 17 and 18, 2003, Santorini, Greece. Eur Heart J. 2004 Jun;25(12):1077-82. doi: 10.1016/j.ehj.2004.01.002. PMID: 15191780.

5. Brown BG, Zhao XQ, Sacco DE, Albers JJ. Lipid lowering and plaque regression. New insights into prevention of plaque disruption and clinical events in coronary disease. Circulation. 1993 Jun;87(6):1781-91. doi: 10.1161/01.cir.87.6.1781. PMID: 8504494.

6. Brown BG, Zhao XQ, Sacco DE, Albers JJ. Lipid lowering and plaque regression. New insights into prevention of plaque disruption and clinical events in coronary disease. Circulation. 1993 Jun;87(6):1781-91. doi: 10.1161/01.cir.87.6.1781. PMID: 8504494.