EXPRESSION OF METALLOPROTEINASES IN ATHEROSCLEROTIC PLAQUES IN DIABETIC AND NON-DIABETIC PATIENTS

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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.

Relevance. 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, 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

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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.

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

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correlations.

Materials and methods. Coronary artery atherosclerotic plaque samples from 20 deceased diabetic and non-diabetic patients were analyzed 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 programmer 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 study found a correlation between diabetes and increased MMP-1 expression in atherosclerotic plaques, indicating heightened enzymatic activity. However, overall biomarker levels did not significantly differ between diabetic and non-diabetic patients. Notably, MMP-1 and MMP-9 were higher in unstable plaques, linked to increased rupture risk, while alpha-SMA and CD68 levels did not reliably indicate plaque severity. This study examined marker levels (α-SMA, CD68, MMP-1, MMP-9) in atherosclerotic plaques from 20 patients (62-97 years, median 77). Type 2 diabetics (n=17) showed α-SMA (0.24-0.83, mean 0.53±0.15), CD68 (0.21-0.60, mean 0.43±0.12), MMP-1 (0.41-0.65, mean 0.52±0.09), and MMP-9 (0.54-0.83, mean 0.66±0.10). The single Type 1 diabetic had intermediate levels. Non-diabetics (n=2) had higher marker levels. Patients with myocardial infarction (MI, n=5) had higher α-SMA (0.32-0.88, mean 0.60±0.22) and MMP-9 (p=0.03). Although α-SMA and CD68 showed overlap, elevated MMP-9 strongly correlated with MI, suggesting it's a better indicator of plaque vulnerability than α-SMA or CD68 alone.

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

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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.

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.

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ЭКСПРЕССИЯ МЕТАЛЛОПРОТЕИНАЗ В АТЕРОСКЛЕРОТИЧЕСКИХ БЛЯШКАХ У ПАЦИЕНТОВ С ДИАБЕТОМ И БЕЗ ДИАБЕТА

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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.

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