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The study of the protein complement in myocardial infarction

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ABSTRACT

Background: Activation of the complement system during myocardial ischemia and reperfusion results in its injury by multiple processes. The aim of this study was to evaluate contribution of innate, humoral mechanisms of nonspecific immune response in the myocardium subjected to infarction. Complement components and inhibitors were analyzed.
Materials and methods: Myocardial specimens from the archives of Chair and Department of Pathology, Medical University of Warsaw from 2010 to 2013, were used in the study. The examined proteins were evaluated using immunohistochemistry and immunofluorescence techniques. Tissues from 36 men and 14 women, mean age 65.02 ± 14.65, were used in the study. The control group comprised healthy myocardial tissue collected from 10 subjects.
Results: Statistical analysis of IHC reaction for proteins and inhibitors of the complement system and membrane attack complex demonstrated markedly higher immunoreactivity level in the myocardium with ischemic necrosis versus healthy myocardial tissue. A correlation analysis demonstrated statistically significant positive correlation between the examined proteins and inhibitors of the complement system and protectin and membrane attack complex. A significant correlation was not found between immunoreactivity of the examined proteins and clinical and morphological parameters of the analyzed cases.
Conclusions: Studies have shown that of the complement proteins presence on the surface of the myocardium subjected to ischemic destruction exacerbate.

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1. Introduction

Widespread cardiovascular diseases in the global population are an increasing health, psychosocial and economic problem. Cardiovascular disease, an ischemic heart disease in particular with its extreme form – myocardial infarction, is the cause of half of the deaths in Poland and globally and therefore constitutes one of the main problems of the current medicine [1–4]. Apart from increased premature mortality due to cardiovascular disease, important consequences of atherosclerosis-dependent diseases pandemics include marked loss of productive work force, worsening of quality of live and increasing costs of treatment of this vast group of patients. Despite the fact that multiple studies examined this disease, its pathomechanism remains incompletely understood. Currently this disease progression is thought to be modulated by multiple immunological, biochemical and biophysical factors, related to genetic and environmental mechanisms [5].

To better understand the problem raised in this paper we must understand elements of the examined structure and processes on its surface since this will allow us to move smoothly throughout this study.

Multiplicity of risk factors as well as their uneven contribution hamper attempts of their accurate classification. One of the simplest and oldest classifications, based on possibility of successful intervention, includes two groups: modifiable and non-modifiable factors. The first group includes, e.g. cigarette smoking, abnormal diet, poor physical activity, lipid abnormalities, including increased LDL cholesterol and reduced HDL cholesterol fraction, as well as preexisting diseases, e.g. hypertension, hyperglycemia/diabetes mellitus, obesity, vascular diseases caused by atherosclerosis. Non-modifiable factors include: age (men > 45 years, women > 55 years), male sex, family history of premature cardiovascular diseases caused by atherosclerosis (in men < 55 years, in women < 65 years) as well as genetic predisposition [6]. This classification, with small modifications, is included in European guidelines on cardiovascular diseases prevention in clinical practice [7].

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Myocardial infarction is a clinical entity that is diagnosed basing on clinical events in combination with laboratory tests. Fulfillment of these criteria results in a diagnosis of myocardial infarction. Definition of myocardial infarction by physicians and researchers in daily practice and in research studies in a different way resulted in confusion. To clarify these issues, European Society of Cardiology, American College of Cardiology, American Heart Association and World Health Federation in 2000 prepared and published a joint report that included an international and commonly accepted definition of myocardial infarction. Progress of medical science and numerous reports provided new data directly related to aspects of this definition. Therefore, an international expert group was appointed that in 2007 published a new definition of myocardial infarction that still remains valid [8–11].

The complement system is composed of approximately 40 proteins that are present in plasma as well as in other body fluids along with their numerous receptors and regulatory proteins. The complement plays an important role in innate, humoral mechanisms of nonspecific immunity, but is also closely associated with some mechanisms of specific immunity [12]. The complement activation involves series of enzymatic and nonenzymatic cascade reactions. The complement activation results in formation of two important enzymes: C3 and C5 convertase that very potently enhance its activity. Irrespective of the method of its activation, the end stages of all these reactions are identical and result in formation of a membrane attack complex – MAC that is composed of C5b, C6, C7, C8 and polymeric C9 [13,14]. The complement system requires regulation which is emphasized by examples of pathologies caused by its excessive activation. For this purpose a system of complement regulatory (controlling) proteins is present in the plasma and on the surface of cellular membranes. These factors play an inactivating role and their activity usually involves shortening of already short half life of C3 and C5 convertase. One of the plasma regulatory factors is factor H that binds C3b and facilitates inhibition of C3 convertase by factor I. Factors that are present on cells are responsible for scavenging the complement include: membrane protein cofactor (CD46) – binding C3b and C4b that is present essentially on all nucleated cells in the body; decay accelerating factor (CD55) – dramatically shortens the half like of convertases; homologous restriction factor (CD59) – binding C8 and C9, inhibits MAC formation [12,15,16].

2. Materials and methods

Ethical Review board from the Medical University of Warsaw approved our project (approval no. AKBE/51/14).

Tissue specimens (left ventricular) used in the study were fixed in 4% formalin solution and embedded in paraffin according to routine procedure. Paraffin blocks were taken from the archives of Chair and Department of Pathology, Medical University of Warsaw from 2010 to 2013 and were material to diagnostic tests. The complement proteins and inhibitors were studies in tissue specimens from 50 subjects who underwent autopsy (done within 2 days of their death). Eligibility criteria of the study group included: pathological and histopathological diagnosis of acute myocardial infarction, no malignant changes in histopathology examination, no old myocardial infarction or diseases that could make the qualification of material from this group impossible. Control group is the myocardium without significant pathology, obtained from 10 subjects aged 20–28 years who died as a result of accidents.

Paraffin specimens underwent immunohistochemistry and immunofluorescence staining. The following antibodies were used to detect the complement components and inhibitors in the studied tissue material: Mouse Anti Human CD46 (AbD Serotec, UK); Mouse Anti Human CD55 (AbD Serotec, UK); Mouse Anti Human CD59 (AbD Serotec, UK); Monoclonal Antibody to Human Factor H (Quidel, USA); Anti Human C4d Antibody (Oxford Biosystems, UK); Human Complement Component C9 (Novocastra, UK); Polyclonal Rabbit Anti-Human C1q complement (Dako, Dania); Anti C5b-9 Antibody (Abcam, UK).

The immunohistochemistry reaction was performed in the following way: routinely deparaffined specimens were treated with 3% hydrogen peroxide to block an endogenous peroxidase and 5% donkey plasma (Jackson Immunoresearch, USA) and subsequently applied to solutions of primary antibodies, incubated in a humid chamber over night at +4 °C. A kit of primary antibodies conjugated with peroxidase ImmPress Reagent Kit Anti-Mouse/Rabbit Ig (Vector Laboratories, USA) was used to detect the primary antibodies. 3–3′ diaminobenzidine (Dako, Dania) was used as a chromogen. Subsequently the specimens were stained with hematoxillin, dehydrated and closed.

For immunofluorescence examinations secondary antibodies conjugated with fluorochromes were used: Donkey Anti-Goat Alexa 555, Donkey Anti-Rabbit Alexa 488, Donkey Anti-Mouse Alexa 555 (Invitrogen, USA). After completion of staining, the specimens were closed in a medium Vectashield (Vector Laboratories, USA). Results of the reaction were analyzed in a confocal microscope Leica TCSSP5 (Leica Microsystems, Germany) and a fluorescence microscope Nikon Eclipse 80i (Nikon, Japan).

Immunohistochemistry reactions were evaluated using a semi-quantitative scale IRS (Immunoreactive Score) according to Remmele and Stagner that included both percent of positive cells (PP – percent of positive cells; 0 – no cells with positive reaction; 1 – up to 10% cells with positive reaction; 2 – 11% to 50% cells with positive reaction; 3 – 51% to 80% cells with positive reaction; 4 – >80% cells with positive reaction) and staining intensity (SI – staining intensity, 0 – no color reaction; 1 – poor color reaction; 2 – moderate color reaction; 3 – intensive color reaction) in at least five visual fields of the light microscope at 200× magnification. The final results are a product of scores for evaluated parameters and can range from 0 to 12 (0–2 poor reaction; 3–5 moderate reaction; 6–12 intensive reaction) [17].

Test results were expressed as means ± standard deviation and were tested using Shapiro–Wilk test for normal distribution. Nonparametric *U* Mann–Whitney test for independent variables and Spearman rank correlation test were used to compare the obtained data. $P < 0.05$ was treated as a statistically significant difference. The statistical analysis was performed using a statistical software Statistica 10 (StatSoft Inc., USA).

3. Results

Table 1 summarizes the most important clinical and morphological features of the study group and control group.

Statistical analysis of IHC reaction for complement proteins (C1q, C4d, C9), inhibitors (CD45, CD55, CD59, fH) and membrane attack complex demonstrated a significantly higher immunoreactivity level in the myocardium with ischemic necrosis versus healthy myocardial tissue (*U* Mann–Whitney test; $p = 0.000001$). Spearman rank test demonstrated a statistically significant correlation between the tested complement proteins (C1q, C4d, C9) and inhibitors (CD46, CD55, CD59, fH) ($p = 0.0001$). The same test demonstrated a positive, statistically significant correlation between CD59 and C5b-9 immunoreactivity ($p = 0.001$). No significant correlation was found between immunoreactivity of the tested proteins and clinical or morphological parameters of the analyzed cases. Results of immunohistochemistry reactions are presented below (Table 2).

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