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THE ROLE OF ANTIBODIES TO XANTHINE OXIDASE IN THE DEVELOPMENT OF IMMUNOPATHOLOGICAL REACTIONS IN RHEUMATOID ARTHRITIS AND SYSTEMIC LUPUS ERYTHEMATOSUS

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Introduction/aim

The aim of the research was to develop the additional criteria for clinical laboratory diagnostics reumatic diseases (RD) by using immobilized antigen nanosystems (ANS) based on xanthine oxidase (XOD).

Material and methods

The serum of 30 donors, 142 patients with rheumatoid arthritis (RA) and 68 patients with systemic lupus erythematosus (SLE) was analyzed. Commercial preparation of xanthine oxidase was used as an antigen. ANS were the double polyacrylamide microgranules in size of 10–100 microns with the immobilized antigen in their structure. The traditional variant enzyme immunoassay (ELISA) as well as the option we have developed ELISA using the ANS was used to investigate the amount of autoantibodies to XOD in the blood serum of patients. The activity of XOD and Xanthine dehydrogenase (XDG) in serum was determined by standard methods. The XOD/XDG coefficient was used in the calculations.

Results/discussion

Preliminary studies have shown that the 20% polyacrylamide gel (PAAG) is the best carrier for use as antigen array of biopolymers with a molecular weight of not less than 30 Kd (XOD-300 Kd). ANS antigen array was formed by using the aqueous solutions with the optimal antigen concentration of XOD-50 µg/ml. The use of ANS based XOD in proposed ELISA option, would increase the sensitivity of this method is 4 times as compared to the traditional version, due to: 1) increase the concentration of antigen in 20–40 times and 2) frequency of the immobilized antigens with relevant immunoglobulins with help of a magnetic stirrer. Antibodies to XOD (anti-XOD) were found in 51,4% of RA patients and 47,1% of SLE patients. The severity of autoimmunisation to XOD in patients with RA depends on activity (DAS28 index) disease ($p=0,038$) and the presence of extra-articular manifestations ($p=0,008$); increased anti-XOD levels were associated with lesions of the organs of the reticuloendothelial system and with severe cytopenia ($p=0,018$). With the increase in the activity of the pathological process, an increase in the coefficient of XOD/XDG ($p=0,022$) was observed due to the growth of XOD and reduction of XDG in serum. We compared the performance of RA with minimal and moderate activity. The positive correlation of anti-XOD level with values SLEDAI index ($r=0,406$) and a negative correlation with the biochemical activity of XOD in the blood serum ($r=-0,316$) were observed in patients with SLE. The highest levels of anti-XOD were observed in SLE patients with liver disease ($p=0,017$), gastrointestinal tract ($p=0,048$) and in patients with severe cytopenia ($p=0,035$). In patients with SLE an evidential increase in the XOD/XDG ratio ($p=0,04$) was found due to a significant decrease in XDG activity in comparison with healthy individuals. Changes in functional activity XOD in patients with inflammatory RD may be associated with autoimmune disorders, because of the impact of specific anti-

bodies the transformation processes D-form of the enzyme (XDG) in the O-form (XOD) is amplified, which ultimately leads to a significant increase in the generation of superoxide radicals that have a damaging effect on lipids, proteins and other cellular components, whereby they acquire the properties of self-antigens and stimulate the production of antibodies having secondary damaging effects.

Conclusions

Consequently, the research of clinical correlation of autoantibodies to XOD can improve the diagnostic approaches and detail the role of the given antibodies in damaged organs and systems in RA and SLE.

THE ROLE OF ANGIOPOETIN-SIMILAR PROTEINS 3 AND 4 TYPES AS ACTIVATORS OF ANGIOGENESIS IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Introduction/aim

The family of angiopoietin-like proteins actively participates in both physiological and pathophysiological, especially inflammatory processes. The angiopoietin-like 3 protein (ANGPTL3) and angiopoietin-like 4 protein (ANGPTL4) are of interest due to their contribution to the processes of angiogenesis and lipid metabolism. It is known that in inflammatory rheumatic diseases, such as rheumatoid arthritis (RA), the intensification of angiogenesis processes is the basis of synovial proliferation in the joints. The purpose of research – to study the influence ANGPTL3 and ANGPTL4 and the characteristics of angiogenesis in RA.

Material and methods

The study included 18 RA patients (aged from 33 to 64 years, the average duration of the disease was $8,42 \pm 5,2$ years, women – 77,8%, DAS28 > 3,2). A control group (12 people) comprised healthy individuals aged 28 to 52 years (women – 75%). Levels of ANGPTL3 and ANGPTL4 in serum were determined by the enzyme immunoassay using the commercial test systems Human Angiopoietin-like Protein 3 ELISA and Human Angiopoietin-like Protein 4 ELISA from Bio Vendor (Czech Republic). Ultrasound examination of the wrist joints was carried out according to the standard procedure using a linear sensor with a frequency of 5–12 MHz on the ultrasonic diagnostic system Accuvix V10 (Samsung Medison, South Korea). The features of the blood flow were studied by color and energy dopplerography (ED) (the number of color loci was visually assessed). Statistical analysis of the data was carried out using the software «STATISTICA 10.0 for Windows» package, including conventional methods of parametric and non-parametric analysis.

Results/discussion

The search for new serological markers is an important stage in clinical trials in RA. These markers can be used as objective indicators of various pathological processes, such as neovascularization, developing in inflammatory joint diseases. The level of ANGPTL3 and ANGPTL4 was significantly higher in patients with RA ($p=0,043$ and $p=0,038$, respectively), than in the control group. The level of ANGPTL4 in patients with RA correlated ($r=0,38$, $p=0,002$) with indicators of ED hypervascularization significantly.