THE VARIATION OF PLASOMATIC CONCENTRATION OF HAEMOGLOBIN AND THE EVALUATION OF HEAT SHOCK PROTEINS IN RHEUMATIC PATIENTS

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Abstract

Rheumatoid arthritis is a systemic inflammatory disease with still unknown aetiology. The purpose of our study was to comparatively investigate the level of haemoglobin, as well as of the heat shock proteins HSP60 and HSP70 as well as their specific antibodies serum levels in rheumatic patients, in order to evaluate their potential role as an aid in the diagnosis of this chronic pathology. This study was performed on patients with rheumatoid polyarthritis. The haemoglobin plasmatic concentration was assessed by a quantitative determination using the automatic analyzer Beckman Coulter Act5diff, while the thermal shock proteins HSP 60, HSP 70 as well as their respective serum antibodies were determined by Western blot method and ELISA assay, respectively. All the patients involved in this study exhibited low values of haemoglobin, known to be an important marker of haematological disorders. These results maintain the idea that anaemia, heart disease, osteoporosis are the most frequent complication for rheumatoid arthritis. The concept of overexpression of endogenous HSPs is central to hypotheses in which HSPs are implicated in the pathogenesis of autoimmune rheumatic disease. The quantification of HSPs levels in the serum of the rheumatic patients showed that both Hsp60 and Hsp70 levels are higher, especially in those patients who had as a secondary disease, like cardiac insufficiency and obesity. The HSP60 and HSP70 antibodies were also highly expressed in our patients. These lesions could be partially due to the fact that despite the ubiquitous and high homology of heat shock proteins among different species, they also represent important antigenic targets of the cellular and humoral immune response. Besides the low level of haemoglobin, the presence of a high level of heat shock proteins and of their corresponding antibodies may be considered as useful markers which could be correlated with the evolution and the severity of a long chain of pathological processes, including the rheumatic diseases.

Keywords

Rheumatic patients, haemoglobin, anemia, heat shock proteins (HSP), anti-HSP

Introduction

Rheumatoid arthritis is a systemic inflammatory disease with still unknown aetiology [1], having as major characteristics synovitis and serosity [2,3,4]. This disease is more frequent in women, the sex ration being approximately 3:1 [5]. Till present, there is no clear elucidation regarding the causes which predispose women to this disease, but some X-linked genetic factors seem to be involved, as well as hormonal profiles [6]. The existing data relates account for a less probability that a pathogenic agent could cause the disease [7]. However, the bacterial antigens with autoimmune potential can be transported into the articulations and initiate or amplify an abnormal immune response [8,9,10]. Stress or heat shock proteins (HSPs) were first discovered in 1962 and since then it has been shown that HSPs are constituting one of the most conserved superfamily of chaperone proteins throughout different phylogenetic groups [11], from prokaryotes, yeast, and plants, to higher
eukaryotic organisms and were reported as an important factor in different diseases [12,13].

Experiment Details

This study was performed on a group of patients with rheumatoid polyarthritis, admitted for investigations at the Rheumatology Department of Ploiești Municipal Hospital. The respective patients have been also diagnosed with a secondary diagnosis, such as: obesity, osteoporosis and cardiac insufficiency. From the enrolled patients who signed an informed consent to participate in the present study, there were taken biological samples, in total accordance with the bioethics convention.

The haemoglobin plasmatic concentration was assessed by a quantitative determination using the automatic analyzer Beckman Coulter Act5diff.

Quantification of HSP60 and HSP70 protein serum levels. Serum Hsp level was determined using Western dot blot. Briefly, 5 μL of serum was loaded by pipetting on a PDVF membrane and left at 37°C in order to be absorbed. The membrane containing transferred proteins was blocked and further treated according to WesternBreeze® Chromogenic Kit (Invitrogen). Briefly, the membrane was incubated with first antibody [rabbit anti-GroES-10kD (Sigma-G8909); rabbit anti-GroEL-60kD (Sigma-G6532); mouse monoclonal anti-Heat Shock protein 70 (Sigma-H5147)] over night at 40°C. Each membrane slice was then washed and treated with alkaline phosphatase labeled goat anti-rabbit immunoglobulin G (for HSP10 and HSP60) or goat anti-mouse immunoglobulin G (for HSP70) and finally reacted with BCIP/ NBT. The levels of HSPs were quantified by integrated optical density using Java-ImageJ 1.33u (Wayne Rasband National Institutes of Health, USA, http://rsb.info.nih.gov).

Quantification of HSPs antibodies serum levels. ELISA was performed to measure the amount of the human anti-HSP60-IgG, A, M and anti-HSP70-IgG, A, M antibody patients sera using anti-HSP60-IgG, A, M and anti-HSP70-IgG, A, M ELISA kit (Enzo Life Sciences BVBA, Belgium) according with producer instructions.

Results and Discussions

Most chronic inflammatory rheumatic diseases are complicated by hematologic abnormalities, including anemia, disorders of leukocytes, platelets, and the coagulation system, and hematologic malignancies [14]. Patients with rheumatoid arthritis (RA) may suffer from a variety of hematologic disorders, the prevalence of anemia ranging from 30 to 70 percent in various studies [15,16]. The mechanism of anemia in rheumatic patients is not completely understood, but it seems that it could be explained by trapping of iron in macrophages, making it relatively unavailable for new hemoglobin synthesis and by the inability of the morphologically normal marrow to increase erythropoiesis in response to the anemia [17]. The anemia is aggravated by the pro-inflammatory mediators over-produced in such pathological states (tumor necrosis factor-alpha, interleukin-1, interleukin-6, interleukin-10, and interferon gamma). All the patients involved in this study exhibited low values of haemoglobin, an important marker of haematological disorders. These results maintain the idea that anaemia, heart disease, osteoporosis are the most frequent complication for rheumatoid arthritis. Anaemia, generally correlate with the disease evolution and frequently starts asthenia. The cause of normochrome and normocyte anaemia is an un-efficient erythropoiesis. The concept of overexpression of endogenous heat shock proteins (hsps) is central to hypotheses in which HSPs are implicated in the pathogenesis of autoimmune rheumatic disease [11]. In different kinds of environmental stresses (hyperthermia, heavy metals, thiol-reactive chemicals, UV radiation, viruses, oxyradicals, cytokines), both prokaryotic and eukaryotic cells activate mechanisms which allow them to synthesize a group of highly conserved proteins--the so-called heat shock proteins.
(HSP). Some of these proteins are now known to facilitate the recovery of normal RNA processing and protein synthesis after exposure to hyperthermia and to protect the cell against further damage. They also play an important role in the synthesis and transport of normal proteins in unstressed cells, and perhaps also the export from cells of abnormal proteins of host or viral origin [12]. Because many of the factors which stimulate stress protein induction occur during inflammatory and immune responses, there has been increasing interest in the possible role of stress proteins in inflammatory disease such as arthritis [13]. In order to evaluate the potential role of specific antibodies to HSP60 and HSP70 in different infectious diseases, we have investigated the levels of these proteins and of their specific antibodies in the bloodstream of rheumatic patients. The quantification of heat shock proteins levels in the serum of our rheumatic patients showed that both Hsp60 and Hsp70 levels are higher, especially in those patients who had as a secondary disease, like cardiac insufficiency and obesity. The potential of synovial inflammation to induce cartilage destructions and bone erosions, followed by changes in joint integrity, may trigger the synthesis of heat shock proteins and could explain their high level. The HSP60 and HSP70 antibodies were also highly expressed in our patients. It is well known the fact that the most precocious damages in rheumatoidal synovitis are: microvascular lesions, high number of synovial corpuscle and the perivascular infiltration with mononuclear corpuscles [18]. These lesions could be partially due to the fact that despite the ubiquitous and high homology of heat shock proteins among different species, they also represent important antigenic targets of the cellular and humoral immune response.

Conclusions

Besides the low level of haemoglobin, the presence of a high level of heat shock proteins and of their corresponding antibodies may be considered as useful markers which could be correlated with the evolution and the severity of a long chain of pathological processes, including the rheumatic diseases.

References


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