

IGF-I and IGF-binding proteins in synovial fluid of patients with Lyme arthritis

Bączyk J.^{1A,B,C,D*}, Guszczyn T.^{2B,D}, Romanowicz L.^{1F}, Pancewicz Sł.^{3F}, Moniuszko A.^{3E}, Popko J.^{2F}

1. Department of Medical Biochemistry, Medical University of Białystok, Białystok, Poland
2. Department of Paediatric Orthopaedics and Traumatology, Medical University of Białystok, Białystok, Poland
3. Department of Infectious Diseases and Neuroinfections, Medical University of Białystok, Białystok, Poland

A - Conception and study design, B - Data collection, C –Data analysis, D - Writing the paper,
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ABSTRACT

Purpose: Multiple cellular functions are stimulated by a Insulin-like Growth Factor-I (IGF-I). The biological activity of IGF-I is modulated by IGF-binding proteins (IGF-BPs) and at the same time, the availability of IGF-BPs may be regulated by the proteolytic activity of some metalloproteinases (MMPs). The aim of the present study was to compare the amounts of IGF-I and IGF-BPs in relation to the activity of MMP-9 in serum and knee synovial fluid from patients with Lyme arthritis (LA) and post-traumatic damage (PTD).

Material and methods: Serum and synovial fluids were taken from knee joints of patients with PTD and LA. ELISA (for IGF-I assay), polyacrylamide gel electrophoresis following Western immunoblotting (for IGF-I and IGF-BPs expression), and zymography (for metalloproteinases detection), were used.

Results: The concentration of IGF-I in serum and

synovial fluid from LA patients were significantly lowered in comparison to PTD patients. Interesting, the synovial fluid /serum ratio of IGF-I concentrations was also lower in LA patients. Low expression IGF-BP3 and high activity of MMP-9 were detected in the LA synovial fluid.

Conclusions: The high proteolytic activity of MMP-9 results in a cleavage of both IGF-I and IGF-BP3 causing a decrease in content of these substances in LA synovial fluid. In addition, the reduction in IGF and IGF-BP amounts may affect the repair processes in joint tissues of LA patients. The low concentration of IGF-I and IGF-BP3 slows down the repair processes in the joint tissues of LA patients.

Key words: Insulin-like Growth Factor-I, Insulin-like Growth Factor-Binding Protein, metalloproteinase, synovial fluid, Lyme arthritis, post-traumatic damage

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***Corresponding author:**

Justyna Bączyk

Department of Medical Biochemistry, Mickiewicza 2C
15-089 Białystok, Poland

Tel/Fax.: 48 857485578; e-mail: justynarzeczycka@wp.pl

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INTRODUCTION

Most typically, Lyme arthritis (LA) evoked by *Borrelia burgdorferi* is an oligoarticular process affecting knees of Lyme disease patients. In septic arthritis caused by other bacteria, a combination of bacterial proteases and host proteases from neutrophils has been postulated to lead to rapid destruction of the articular cartilage. In contrast, Lyme arthritis results from a very slowly occurring process, with synovial fluid studies, typically revealing only moderate elevations in the number of inflammatory cells. It has been previously shown that *Borrelia burgdorferi* does not secrete proteases capable of digesting extracellular matrix proteins [1,2]. Instead, it has been suggested that *B. burgdorferi* utilizes proteases from its mammalian hosts to degrade these proteins [3,4]. Left untreated, *B. burgdorferi* infection can eventually lead to the development of an erosive arthritis with histopathological similarities to rheumatoid arthritis [5]. It has been reported that an imbalance in the production of matrix metalloproteinases (MMPs) is likely to be responsible for degradation of articular cartilage proteins in patients with rheumatoid arthritis [6]. The main enzymes involved in cartilage destruction in rheumatoid arthritis and LA are collagenases (MMP-1, MMP-13) gelatinases (MMP-2, MMP-9) and stromelysins (MMP-3, MMP-10) [6,7]. It has also been reported that synovial fluid from patients with LA contains at least 4 MMPs: MMP-1, MMP-2, MMP-3 and MMP-9 [4,8]. Previous studies have shown that MMP-9 is not required for the dissemination of the *Spirochete* to distant sites [9]. However, it does play an important role in the regulation of inflammation in Lyme arthritis, potentially through the cleavage of collagen type I. Furthermore, collagen type I degradation products after MMP-9 action result in increased monocyte chemoattraction [9]. Recently we reported on the high proteolytic activity of MMP-9 in juvenile idiopathic arthritis patients with low amount of IGF-binding protein 3 (IGF-BP3), possibly by impairing the reparation processes stimulated by Insulin-like Growth Factor-I (IGF-I) [10]. It is well known that increased activity of metalloproteinases is responsible for the intensified degradation that occurs, whereas the lack of anabolic action of growth factors would be responsible, at least in part, for the decreased reconstruction of extracellular matrix.

Peptide growth factors, especially IGF-I and IGF-BPs, are known to play an important role in regulation of cell division and in maintaining equilibrium between synthesis and degradation of extracellular matrix components [11].

IGF-I is an important metabolic and mitogenic factor involved in cell growth and differentiation. It is also recognised as a stimulator of collagen and sulphated glycosaminoglycans

biosynthesis [12,13]. IGF-I forms complexes with binding proteins which are important regulators of its biological activity in plasma and other tissues. At least six different high-affinity IGF-BPs have been identified in humans and other species [11]. Under normal conditions most IGF-I circulates in high molecular weight complex (150 kDa), containing IGF-BP3 and acid labile subunit [14, 15]. It has been suggested that such a complex prolongs the half-life of IGF-I and increases cell responsiveness to IGF-I stimulation. Bioavailability of IGF-I regulated by IGF-BPs may contribute to the remodelling of extracellular matrix in articular tissues. They may also be involved in reparation of inflammation-induced damage of synovial tissue [16, 17].

Therefore, the characterisation of IGF-I, IGF-BPs and IGF-BP-cleaving proteases in synovial fluid is important for understanding the modulation of synovial fluid metabolism in inflammatory processes affecting the knee joints.

The aim of the present study was to measure the concentration of IGF-I, expression of IGFBP-1 and IGFBP-3, and to evaluate gelatinolytic activity in both articular exudates and serum of patients with Lyme arthritis (studied group) and compared with those of patients with post-traumatic damage (control group).

MATERIAL AND METHODS

All the protocols in this study that involved human patients were conducted in accordance with the Declaration of Helsinki and were accepted by the Committee for Ethics and Supervision of Human and Animal Research of the Medical University of Białystok, Poland.

Patients and sample collection

Serum was collected from patients of the Medical University of Białystok. Shortly, after the first admission of the patients to the hospital, synovial fluid was collected during routine diagnostic or therapeutic arthrocentesis. A total of 30 patients were included in this study, 15 (9 males and 6 females, between 9-48 years old) with post-traumatic damage (PTD) of the knee joints were considered as a control group and those, diagnosed with Lyme arthritis, (8 females and 7 males, between 58-68 years old) were the studied group. The diagnostic of Lyme arthritis (LA) was based on clinical findings (e.g. inflammation of knee joint with exudate) and immunoblot testing on serum samples. All LA patients had tick-bite history and/or erythema migrans. The Lyme arthritis patients were treated with the regular antibiotic regime indicated in these cases, tetracycline and ampicillin. The PTD patients received non-steroid anti-inflammatory drugs. None of the patients included in the study received intra-articular injection of corticosteroids.

Samples processing

The samples of blood were collected into glass test tubes and allowed to clot at room temperature. The retracted clot was removed by centrifugation. The serum was collected and stored at -70°C until further use.

The samples of synovial fluid were taken from the knee joints of both groups of patients during the arthrocentesis performed for diagnostic or therapeutic purposes. They were centrifuged, and the supernatants were collected and stored at -70°C prior to further use.

Sodium dodecyl sulphate / polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to the Laemmli method [18] using molecular weight standards (Bio-Rad).

Gelatin zymography

To detect metalloproteinase activity, gelatin zymography was performed by the method described by Unemori and Werb [19]. The serum and synovial fluid were mixed with a Laemmli sample buffer [18] containing 3.0% SDS (without reducing agent). Equal amounts (10 µg) of protein were electrophoresed under no reducing conditions on 7.5 % polyacrylamide gel containing gelatin in a concentration of 1 mg/ml.

After electrophoresis, the gels were submitted to extraction of SDS with 0.25% Triton X-100 (2 x 15 min) at room temperature and then transferred into 0.05 M Tris/HCl, pH 7.5, containing 10 mmol/l CaCl₂, 1 µmol/l ZnCl₂, 0.02% NaN₃ and 1% Triton X-100. The gel was then incubated in buffer for 16 hours at 37°C.

After incubation, the gels were stained with 1% Coomassie Brilliant Blue R-250 for 14 hours and destained with 10% acetic acid plus 25% isopropanol. The position of gelatin-degrading enzymes present in the serum and synovial fluid became visible as clear zones on a blue background. A representative zymogram was presented.

IGF-I assay

The content of IGF-I in the studied materials was determined with quantitative assay, Human IGF-I Quantikine ELISA Kit provided by R&D Systems according to the manufacturer's instruction.

Western blot analysis

After SDS-PAGE, the gels were allowed to equilibrate for 5 min in 25 mmol/l Tris, 0.2 mol/l glycine in 20% (v/v) methanol for 5 min. The protein was transferred to 0.2 µm pore-sized nitrocellulose membranes at 100 mA for 1 hour using Bio-Rad Mini-Protean 3. The membranes were incubated with specific primary monoclonal antibodies against investigated proteins diluted 1:500 in 5% dried milk in TBS-T (20 mmol/l Tris-HCl buffer, pH 7.4,

containing 150 mmol/l NaCl and 0.05% Tween 20) for 1 hour, followed by alkaline phosphatase-conjugated secondary antibody against mouse Fc IgG, added at a concentration 1:2 000 in TBS-T. Incubation was continued for 30 minutes with slow shaking. Then the nitrocellulose was washed with TBS-T (5 times for 5 minutes) and submitted to the action of Sigma - Fast BCIP/NBT reagent for band visualization.

Detection of IGF-I, IGF-BP1 and IGF-BP3

IGF-I, IGF-BP1 and IGF-BP3 were detected by Western immunoblot analysis with the use of respective specific primary monoclonal antibody (Sigma-Aldrich). Representative blots were presented.

Determination of protein concentration

Protein concentration in serum and synovial fluid samples was measured by Bradford method [20].

Statistical analysis

The mean values for IGF-I concentration ± standard deviations (S.D.) were calculated. The results were submitted to statistical analysis with Student's "t" test, accepting p < 0.05, as significant.

RESULTS

Zymography technique with gelatin as a substrate indicated that both the serum and the synovial fluid of all investigated patients contain matrix metalloproteinase 2 and 9 (Fig. 1). Serum of control (PTD - lane 1) and studied group (LA – lane 3) demonstrated two light bands corresponding to active form of MMP-9 (82 kDa) and latent form of MMP-2 (72 kDa). No active form of MMP-2 and latent form of MMP-9 was detected for both investigated groups of patients. Those bands were more intensive for serum of LA patients (lane 3).

It is of interest that the synovial fluid of PTD patients demonstrated only a trace amount of active form of both MMPs (lane 2). In contrast, an intensive band corresponding to latent MMP-2 was visible (lane 2). No latent form of MMP-9 was observed for PTD patients (lane 2). Conversely, the synovial fluid of LA patients demonstrated the presence of intensive bands corresponding to an active form of MMP-9, a latent form of MMP-2, and a week band corresponding to active form of MMP-2 (Fig. 1, lane 4).

The concentrations of IGF-I in the serum and synovial fluid of both groups of patients measured by ELISA technique was showed on Figure 2a and ratio of IGF-I concentration in synovial fluid and serum was presented on Figure 2b. Large amount of investigated peptide growth factor was found in serum of PTD patients (about 320 ng/ml). Distinctly

lower concentration of IGF-I was determined in serum of LA patients (about 240 ng/ml).

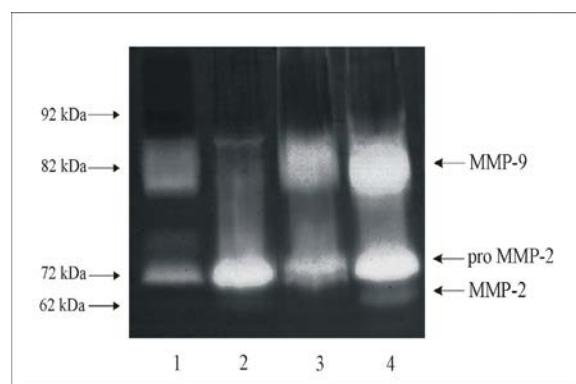


Figure 1. Gelatinolytic activity of synovial fluid and serum of PTD (control) and LA patients. Lanes: 1 – serum of PTD patient, 2 – synovial fluid of PTD patient, 3 – serum of LA patient, 4 – synovial fluid of LA patient. The same amount of protein (10 µg) was applied on each lane

The synovial fluid taken from PTD patients contained about 170 ng /ml IGF-I. Whereas the

material taken from LA patients contained distinctly less of that growth factor, about 90 ng/ml. Interestingly, both the serum and synovial fluid of the LA patients contained distinctly less IGF-I than respective samples of the PTD patients (Fig. 2a).

As could be seen from Figure 2b, not only values of IGF-I concentrations was distinctly lower in LA compared to that of PTD patients, but also synovial fluid/serum ratio of IGF-I concentrations of LA patients was significant lower (Fig. 2b).

Western Immunoblot with the use of specific anti-IGF-I antibody showed on Figure 3 indicated that the synovial fluid and serum of both PTD and LA patients contained investigated growth factor.

During the electrophoresis in reducing conditions two bands appeared. One of them (the less intensive band) of the molecular weight around 7.5 kDa, corresponded to free IGF-I. The other one (the distinctly more intensive band) of molecular weight about 40 kDa, indicated that IGF-I was in complex with other protein. No differences between PTD-serum (lane 1) and LA-serum (lane 3), PTD-synovial fluid (lane 2) and LA-synovial fluid (lane 4) were found (Fig. 3).

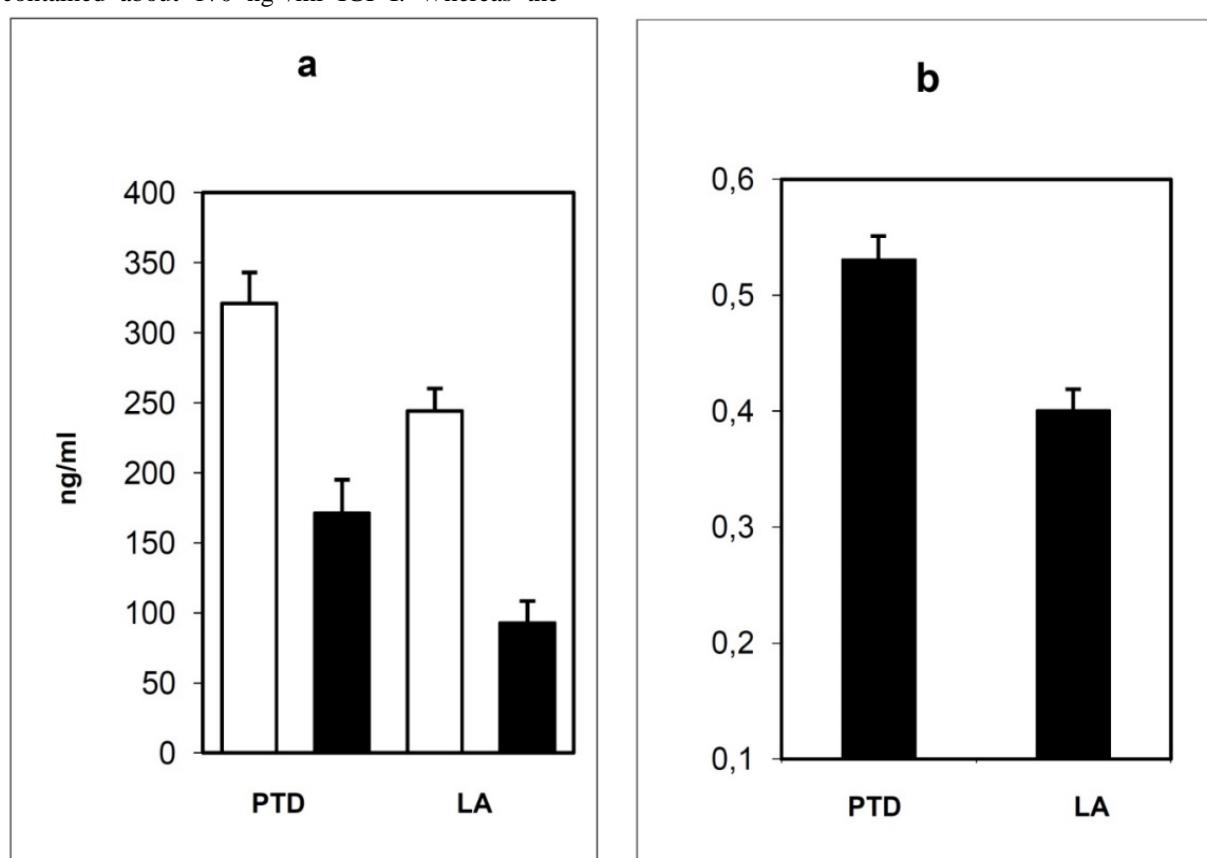


Figure 2. a - Concentration of IGF-I in serum [□] and synovial fluid [■] and **b** – synovial fluid / serum ratio of IGF-I concentrations of those patients ($p<0.05$)]

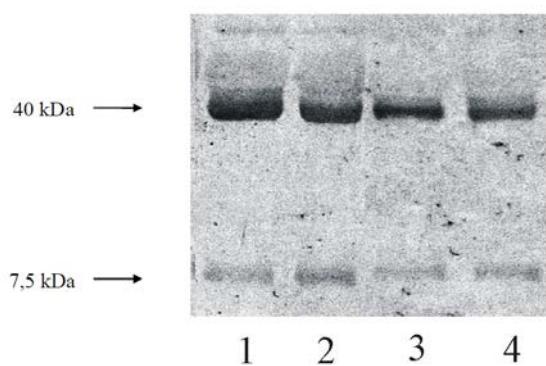


Figure 3. Western immunoblot analysis for IGF-I in reducing conditions. Lanes: 1 – serum of PTD patient, 2 – synovial fluid of PTD patient, 3 – serum of LA patient, 4 – synovial fluid of LA patient. The same amount of protein (10 µg) was applied on each lane

Figure 4 showed IGF-BP3 presence in both serum and synovial fluid of PDT and LA patients. A homogenous, high molecular weight complex (about 170 kDa) that interacted with anti-IGF-BP3 antibody was detected for all investigated samples without reduction of disulfide bonds (Fig. 4a, lanes 1-4). Notably, the intensity of 170 kDa band for synovial fluid of LA patients was weaker (Fig. 4a, lane 4) than of that for PDT patients (Fig. 4a, lane 2).

Under the reducing action of β -mercaptoethanol the homogenous 170 kDa band disappeared and few narrow bands with lower molecular weight were visible. Weak band of 35 kDa, corresponding to a free form of IGF-BP3, was observed for all investigated materials (Fig. 4b, lanes 1, 2, 3, 4). As in the case of high molecular complex (170 kDa), the intensity of bands corresponding to complexes with lower molecular mass was weaker for LA patients (lane 4) in comparison to those of PDT patients (Fig. 4b, lane 2).

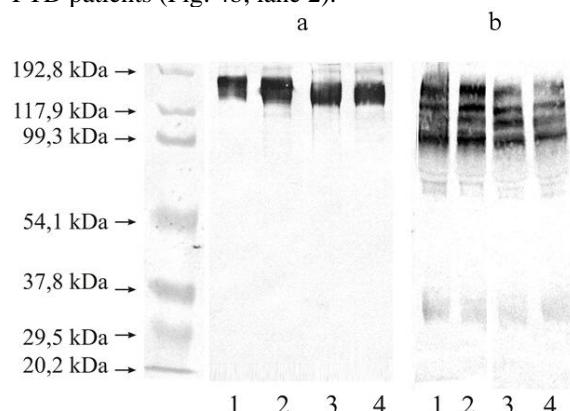


Figure 4. Western immunoblot analysis for IGF-BP3 in non-reducing (a) and reducing (b) conditions. Lanes: 1 – serum of PTD patient, 2 – synovial fluid of PTD patient, 3 – serum of LA patient, 4 – synovial fluid of LA patient. The same amount of protein (10 µg) was applied on each lane

It was evident from the immunoblot presented on Figure 5 that both the serum and the synovial fluid from PTD and LA patients contained a protein which interacted with anti-IGF-BP1 antibody. After electrophoresis in non-reducing conditions there were visible one band of the molecular weight about 170 kDa with similar intensity for all investigated samples (Fig. 5a, lane 1 - 4).

It could be seen from Fig. 5b that the action of β -mercaptoethanol resulted in a dissociation of high molecular weight complex into components of lower molecular weight. Only trace amounts of free form of IGF-BP1 (29 kDa) were detected. No differences between compared samples was found. Some bands of higher molecular weight (in the range of 65 -120 kDa) were visible. No differences between PTD and LA serum as well between PTD and LA synovial fluid were found (Fig. 5b).

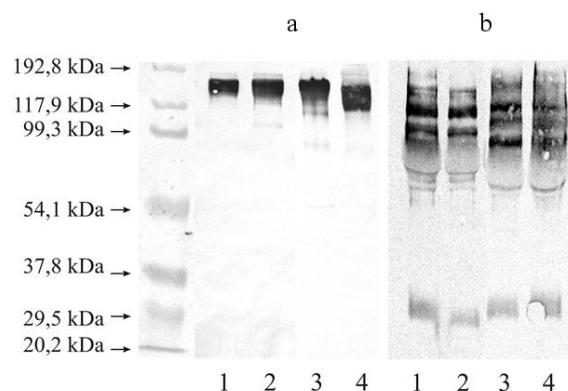


Figure 5. Western immunoblot analysis for IGF-BP1 in non-reducing (a) and reducing (b) conditions. Lanes: 1 – serum of PTD patient, 2 – synovial fluid of PTD patient, 3 – serum of LA patient, 4 – synovial fluid of LA patient. The same amount of protein (10 µg) was applied on each lane

DISCUSSION

Metabolism of fluid in joint cavity is directly dependent on the pathological processes taking place within the joint. This allows us to suggest that both trauma and inflammatory processes induce a local increase in IGF-I production by damaged tissues and its secretion into synovial fluid. Higher amounts of IGF-I may promote the repair process in the injured knee joint by stimulation of cell divisions and anabolic processes. By formal and ethical reasons, acquisition of classic control group - synovial fluid from healthy individuals is not possible. Collection of control material of synovial fluids from dead people would not create a good comparison group, because of the changes resulting from intensified proteolysis after death [21]. Because of that we decided to use synovial fluid of patients with post-traumatic disease (PTD) as a control material. Additionally, PTD seems to be a

good choice because it has different etiology than Lyme disease.

The arthritis evoked by *B. burgdorferi* infection leads to the development of histopathological changes similar to rheumatoid arthritis [5]. Studies on patients with rheumatoid arthritis have shown that an imbalance in the production of proteolytic enzymes, such as MMPs and peptide growth factors including IGF-I is likely to be responsible for degradation of the articular cartilage, thereby impairing the reparation processes [6]. Although the pathogenesis of rheumatoid arthritis and LA is different, the final pathway of joint destruction in each of them seems to be similar. A disruption of homoeostasis occurs between the synthesis and the degradation favouring the catabolic processes. Consequently, this leads to a loss of cartilage.

It is generally known that the activity of metalloproteinases is modulated, at least in part, by the peptide growth factors, including IGF-I [11, 22-24].

According to some authors [3,4,8,9] LA patients demonstrate elevated pro-MMP content in their serum and synovial fluid. In contrast to findings of those authors [3,4], we show that synovial fluid obtained from LA patients demonstrates distinctly higher amounts of active forms of both MMP-2 and MMP-9 in comparison to control materials. Higher activity of those matrix metalloproteinases may promote degradation of IGF-I and its binding proteins and may lower their content.

Little is known about the involvement of the IGF-I in pathomechanism of LA. We found that the knee synovial fluid taken from patients with LA contained lower amounts of IGF-I compared to PTD synovial fluid. No data concerning IGF-I content in the synovial fluid of patients with LA have been published. Matsumoto et al. [23] demonstrated that synovial fluid of healthy adult subjects collected after death, contains about 20 ng of IGF-I in 1 ml, corresponding to about 10% of the concentration of this factor in plasma [10]. It can be calculated that the synovial fluid/serum ratio of IFG-I contents equals about 0.1. From our studies, it appears that the corresponding ratios in PTD and LA patients are distinctly higher (from 0.53 in PTD patients to 0.4 in LA patients). Higher values of synovial fluid/serum IGF-I content ratio come off that Matsumoto and colleagues [23] have used synovial fluid samples after death.

The decrease in synovial fluid/serum ratio of IGF-I concentrations seems to be specific for LA patients. We have reported previously that the corresponding ratio in juvenile idiopathic arthritis was distinctly higher, about 0.45 [10]. We therefore suggest that the destruction process in LA patients is more intensive than that causing juvenile idiopathic arthritis, and that lower amounts of IGF-I in synovial

fluid of LA patients may stimulate the reparation processes in joint tissues in lesser degree.

The main part of this factor is bound to high molecular weight complexes that interact with anti-IGF-BPs antibodies. In our study, we have found both IGF-BP1 and IGF-BP3. The bands corresponding to IGF-BP3 in LA synovial fluid are weaker in comparison to those observed in PTD synovial fluid. The expression of IGF-BP1 is similar in both groups of patients.

We have found that in both synovial fluid and serum IGF-I is complexed with IGF-binding proteins. Such a phenomenon is important for IGF-I stability. The half-life of free IGF-I equals 10 minutes, whereas that of IGF-I/IGF-BP3 complex is 10 to 15 hours [25].

The lower amounts of IGF-I and IGF-BP3 in LA synovial fluid co-exist with a high activity of MMP-9 and also MMP-2. We acknowledge that several metalloproteinases digest IGF-binding proteins, including IGF-BP3 [11,23,24]. It is very likely that the proteolytic action of MMP-2 and MMP-9 results in a cleavage of both IGF-I and IGF-BP3, causing a decrease of these substances content in LA synovial fluid.

It seems that the high activity of both metalloproteinases in LA synovial fluid causes a destruction of cartilage and synovial tissues [26, 27]. Low concentration of IGF-I and IGF-BP3 slow down the repair processes in the joint tissues of LA patients.

CONCLUSIONS

The high proteolytic activity of MMP-9 results in a cleavage of both IGF-I and IGF-BP3 causing a decrease in content of these substances in LA synovial fluid. In addition, the reduction in IGF and IGF-BP amounts may affect the repair processes in joint tissues of LA patients. The low concentration of IGF-I and IGF-BP3 slows down the repair processes in the joint tissues of LA patients.

Conflicts of interest

There is not any reason for a conflict of interest connected with publication of this paper.

REFERENCES

1. Coleman JL, Sellati TJ, Testa JE, Kew RR, Furie MB, Benach JL. *Borrelia burgdorferi* binds plasminogen, resulting in enhanced penetration of endothelial monolayers. *Infect Immun.* 1995 Jul; 63(7):2478-84
2. Klempner MS, Noring R, Epstein MP, McCloud B, Hu R, Limentani SA, Rogers RA. Binding of human plasminogen and urokinase-type plasminogen activator to the Lyme disease

- spirochete, *Borrelia burgdorferi*. J Infect Dis. 1995 May;171(5):1258-65.
3. Gebbia JA, Coleman JL, Benach JL. Borrelia spirochetes upregulate release and activation of matrix metalloproteinase gelatinase B (MMP-9) and collagenase 1 (MMP-1) in human cells. Infect Immun. 2001 Jan;69(1):456-62.
 4. Hu LT, Eskildsen MA, Masgala C, Steere AC, Arner EC, Pratta MA, Grodzinsky AJ, Loening A, Perides G. Host metalloproteinases in Lyme arthritis. Arthritis Rheum. 2001 Jun, 44(6):1401-10
 5. Steere AC, Duray PH, Butcher EC. Spirochetal antigens and lymphoid cell surface markers in Lyme synovitis: comparison with rheumatoid synovium and tonsillar lymphoid tissue. Arthritis Rheum 1988 Apr;31(4):487-95.
 6. Abeles AM, Pillinger MH. The role of the synovial fibroblast in rheumatoid arthritis: cartilage destruction and the regulation of matrix metalloproteinases. Bull NYU Hosp Jt Dis. 2006, 64(1-2):20-4.
 7. Fiedorczyk M, Klimiuk PA, Sierakowski S, Kita K. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in the pathogenesis of rheumatoid arthritis. Pol Merkur Lekarski. 2006 Feb; 20(116):228-31.
 8. Lin B, Kidder JM, Noring R, Steere AC, Klempner MS, Hu LT. Differences in synovial fluid levels of matrix metalloproteinases suggest separate mechanisms of pathogenesis in Lyme arthritis before and after antibiotic treatment. J Infect Dis. 2001 Jul; 5;184(2):174-80.
 9. Heilpern AJ, Wertheim W, He J, Perides G, Bronson RT, Hu LT. Matrix metalloproteinase 9 plays a key role in lyme arthritis but not in dissemination of. Infect Immun. 2009 Jul; 77(7):2643-9 *Borrelia burgdorferi*
 10. Guszczyn T, Rzeczycka J, Popko J. IGF-I and IGF-binding proteins in articular exudates of children with post-traumatic knee damage and juvenile idiopathic arthritis. Pathobiology. 2009, 76(5):260-6.
 11. Lejbach A, Muzes G, Feher J: The insulin-like growth factor system: IGFs, IGF-binding proteins and IGFBP-proteases. Acta Physiol Hung. 2005; 92:97-107.
 12. Guenther HL, Guenther HE, Froesch ER: Effect of insulin-like growth factor on collagen and glycosaminoglycan synthesis by rabbit articular chondrocytes in culture. Fleisch H.Experimentia 1982 Aug; 38(8):979-81.
 13. McQuillan DJ, Handley CJ, Campbell MA, Bolis S, Milway VE, Herington AC. Stimulation of proteoglycan biosynthesis by serum and insulin-like growth factor-I in cultured bovine articular cartilage. Biochem J. 1986 Dec;240(2):423-341.
 14. Collett-Solberg PF, Cohen P. Genetics, chemistry, and function of the IGF/IGFBP system. Endocrine. 2000 Apr;12(2):121-36.
 15. Martin JL, Baxter RC. Insulin-like growth factor binding protein-3: biochemistry and physiology. Growth Regul. 1992 Jun;2(2):88-99.
 16. Balcom NT, Berg-Johansen B, Dills KJ, Van Donk JR, Williams GM, Chen AC, Hazelwood SJ, Sah RL, Klisch SM. In vitro articular cartilage growth with sequential application of IGF-1 and TGF- β 1 enhances volumetric growth and maintains compressive properties. J Biomech Eng. 2012 Mar;134(3):031001-8.
 17. Hunziker EB, Kapfinger E, Martin J, Buckwalter J, Morales TI. Insulin-like growth factor (IGF)-binding protein-3 (IGFBP-3) is closely associated with the chondrocyte nucleus in human articular cartilage. Osteoarthritis Cartilage. 2008 Feb;16(2): 85-94.
 18. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 1970 Aug;227:680-85.
 19. Unemori EN, Werb Z. Reorganization of polymerized actin: a possible trigger for induction of procollagenase in fibroblasts cultured in and on collagen gels. J Cell Biol 1986 Sep, 103(3):1021-31.
 20. Bradford MM. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976 May;72:248-54.
 21. Arikere SM, Laveesh MR, Priyadarshini AVN, Suja P, Farsana UK, Michael N, Raveendran N. Determination of time since death by estimating sodium and potassium levels in synovial fluid. Int J Pharm Res. 2013;4(4):194-6.
 22. Li S, Zhang D, Yang L, Burnier JV, Wang N, Lin R, Lee ER, Glazer RI, Brodt P. The IGF-I receptor can alter the matrix metalloproteinase repertoire of tumor cells through transcriptional regulation of PKC-{alpha}. Mol Endocrinol. 2009 Dec;23(12):2013-25.
 23. Matsumoto T, Gargosky SE, Iwasaki K, Rosenfeld RG. Identification and characterization of insulin-like growth factors (IGFs), IGF-binding proteins (IGFBPs), and IGFBP proteases in human synovial fluid. J Clin Endocrinol Metab. 1996 Jan;81(1):150-5.
 24. Fowlkes JL, Threlkill KM, Serra DM, Suzuki K, Nagase H. Matrix metalloproteinases as insulin-like growth factor binding protein-degrading proteinases. Prog Growth Factor Res. 1995;6(2-4):255-63.
 25. Guler HP, Zapf J, Schmid C, Froesch ER. Insulin-like growth factors I and II in healthy man. Estimations of half-lives and production rates. Acta Endocrinol (Copenh). 1989 Dec;121(6):753-8.
 26. Kim KS, Choi HM, Lee YA, Choi IA, Lee SH, Hong SJ, Yang HI, Yoo MC. Expression levels and association of gelatinases MMP-2 and MMP-9 and collagenases MMP-1 and MMP-13

- with VEGF in synovial fluid of patients with arthritis. *Rheumatol Int.* 2011 Apr;31(4):543-7.
27. Itoh T, Matsuda H, Tanioka M, Kuwabara K, Itohara S, Suzuki R. The role of matrix metalloproteinase-2 and matrix metalloproteinase-9 in antibody-induced arthritis. *J Immunol.* 2002 Sep; 169(5):2643-7.