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Analysis of the levels of endotoxin and β-D-glucan in the synovial fluid of hemodialysis patients

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Abstract We analyzed the levels of endotoxin and β-D-glucan, which possibly induce cytokine production, in the synovial fluid of patients on long-term hemodialysis and compared the results to those in patients with osteoarthritis and rheumatoid arthritis. We studied 42 knees in 42 hemodialysis patients, 21 in 21 osteoarthritis patients, and 26 in 26 rheumatoid arthritis patients. The mean ages were 60.7, 63.2, and 59.7 years, respectively. The duration of hemodialysis in the long-term hemodialysis group averaged 14.0 years. The concentrations of endotoxin and β-D-glucan in the synovial fluid of these three groups were measured. The concentration of endotoxin was the same in the three groups. However, the concentration of β-D-glucan was significantly higher in long-term hemodialysis patients. This finding suggests that β-D-glucan may have some relation to the pathogenesis of the synovitis which exists in the hydrarthrosis of long-term hemodialysis patients.

Key words Endotoxin · β-D-glucan · Hemodialysis · Synovial fluid

Introduction

Amyloid arthropathy in the knee joint frequently occurs in long-term hemodialysis (HD) patients, and severe joint destruction occurs progressively (Fig. 1). This has also been reported in the hip joint.[1] Its pathogenesis remains to be elucidated and its therapeutic indication has not been yet established. Local accumulation of β-2-microglobulin has been suggested as the mechanism of pathogenesis,[2] but there are no exact correlations between the serum concentration of β-2-microglobulin and patient symptoms and frequency of complications. In addition, the variety of symptoms cannot be fully explained by the concentration of β-2-microglobulin. Therefore other factors should be considered. One of these is endotoxin in the dialysate, which might induce cytokine production and provoke various bone and joint complications.[3]

Recently, trials to quantify the components of the synovial fluid, to diagnose joint diseases and to understand their pathogeneses have become more interesting.[4–6] Based on analyses of synovial fluid components, specifically chondroitin sulfate isomers, we have previously reported, that synovitis, which is similar to rheumatoid arthritis (RA), may be present in the hydrarthrosis of long-term HD patients.[7] In the present study we analyzed the concentrations of endotoxin and β-D-glucan in the synovial fluid of patients on long-term HD and compared them with those of patients with osteoarthritis (OA) and RA. We also investigated the differences in long-term HD groups by clinical stage, roentgenological grade, and duration of HD.

Materials and methods

From December 1992 to August 1997, we obtained synovial fluid from 89 knees of 89 patients who had knee effusions with a gonalgia. The diagnoses were long-term HD (42 knees of 11 male and 31 female patients), OA (21 knees of 7 male and 14 female patients), and RA (26 knees of 3 male and 23 female patients). The mean ages were 60.7 (range 34–79), 63.2 (48–88), and 59.7 (37–76) years, respectively. All patients in the HD group underwent surgery after they developed carpal tunnel syndrome. They were diagnosed with HD-associated amyloidosis because amyloid deposi-

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Fig. 1. A 60-year-old woman who had been undergoing hemodialysis (HD) for chronic renal failure for the last 20 years. Relapsing hydroarthrosis with severe disability occurred 6 months earlier. Anteroposterior (**A**) and lateral (**B**) radiographs of the right knee show severe, progressive destruction of the joint, similar to a Charcot joint

tion was found during the pathological examination of specimens collected during surgery. Patients in the HD group all tested negative for the rheumatoid factor. Diagnoses of OA and RA were made according to the criteria recommended by the American Rheumatism Association.[8,9] The renal functions of all patients in the OA and RA groups were within the normal limits. All synovial fluid was examined microbiologically, and all results were found to be negative, including for fungal infection. We excluded patients who had had any trauma to the knee or had received a steroid injection into the joint within the last 3 months. The duration of HD in the HD group averaged 14.0 (4–24) years. The cellulosic membrane was used for all patients in the HD group.

The clinical stages were divided into three groups according to the evaluation system of the Japanese Orthopaedic Association,[10] which is a 100-point system. The ability to walk was given a maximum of 30 points, the ability to go up and down stairs 25 points, range of motion 35 points, and swelling 10 points. Patients who had a score of 80 points or more were categorized as in stage 1, 50–79 points in stage 2, and 0–49 points in stage 3. In the HD group, one patient was categorized in stage 1, 27 were in stage 2, and 14 were in stage 3. The roentgenologic grades were divided into four groups according to the extent of degeneration. Findings showing a small osteophyte only were categorized in grade 1, the clear formation of an osteophyte but with no signs of abnormality in the joint space was classified as grade 2, a narrowing of the joint space, or a varus or valgus deformity was assigned to grade 3, and irregularity of the joint surface and sclerotic findings of the subchondral bone which were more severe than grade 3 were placed in grade 4. In the HD group, 2 patients were classified as grade 1, 10 as grade 2, 22 as grade 3, and 8 as grade 4. The duration of HD in the HD group was divided into four groups spanning 6 years each. 3 patients with less than 6 years of HD were assigned to group 1, 14 patients with 7–12 years to group 2, 18 patients with 13–18 years to group 3, and 7 patients with 19–24 years to group 4.

Table 1. Analysis of glucan and endotoxin in the synovial fluid of patients on long-term hemodialysis (HD) and those diagnosed with rheumatoid arthritis (RA) and osteoarthritis (OA)

	Glucan (pg/ml)		Endotoxin (pg/ml)	
HD (42)	76.62 ± 126.84	*	5.13 ± 4.72	n.s.
RA (26)	17.70 ± 29.13	*	13.04 ± 12.26	n.s.
OA (21)	8.39 ± 4.58		8.07 ± 5.04	

Values are mean ± SD. Numbers of patients are in parentheses
* $P < 0.05$; n.s., not significant

The synovial fluid samples obtained were centrifuged at 10000 *g* for 15 min to remove cell and tissue debris. The supernatants were stored in aliquots at −20°C and analyzed within 6 weeks. The concentrations of endotoxin and β-D-glucan were determined by the colorimetric limulus test using chromogenic substrates.[11]

The differences in each parameter according to disease and clinical stage, roentgenologic grade, and duration of HD in the HD group were investigated. All the parameters were analyzed statistically using the Scheffe technique.

Results

The data obtained from the synovial fluid samples from patients with OA, RA, and HD are shown in Table 1.

The concentration of β-D-glucan in HD patients was significantly higher than in OA and RA patients, which showed a normal serum range (<20 pg/ml). Conversely, the concentrations of endotoxin were the same among the three groups. However, in the RA group, the concentration of endotoxin was higher than the normal serum level (<10 pg/ml).

In the HD group, the differences in clinical stage, roentgenologic grade, and duration of HD were investigated. The scatter for β-D-glucan levels within the HD group was found to be very large, and therefore statistically these results may

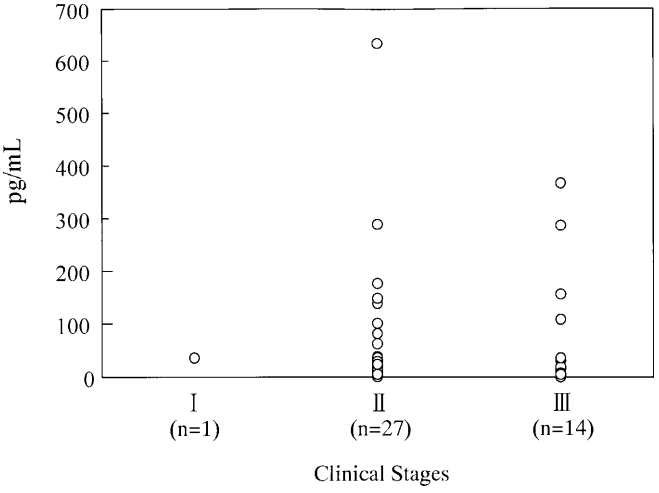

Fig. 2. Glucan in HD patients by clinical stage. There was no significant change in the proportion of glucan with clinical stage

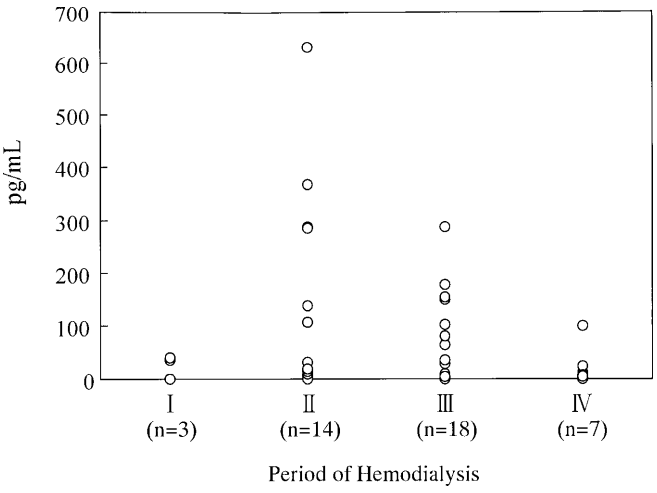

Fig. 4. Glucan in HD patients by duration of HD. There was no significant change in glucan with increasing duration of HD

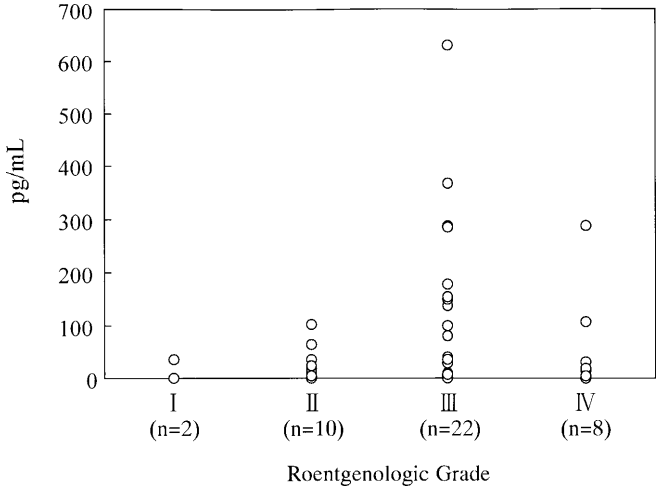

Fig. 3. Glucan in HD patients by roentgenologic grade. There was no significant change in glucan with changing roentgenologic grade

not be significant with regard to clinical stage (Fig. 2), roentgenologic stage (Fig. 3), and duration of HD (Fig. 4).

Discussion

Amyloid arthropathy is a common orthopedic complication, along with carpal tunnel syndrome and pathological fracture, in patients who undergo HD for more than ten years.[12] In the case of long-term HD patients, severe osteopenia due to renal osteodystrophy, and a vulnerability to infection due to immunodeficiency also exist. Therefore steroid injections into the joint or arthroplasty are not the ideal choice of treatment.[13] To date, the pathogenesis of amyloid arthropathy remains to be elucidated and the therapeutic indication has not yet been established.

We have already reported that the concentration of chondroitin sulfate isomers in the hydrarthrosis in long-term HD patients is similar to that in RA patients, and that synovitis may be present in the hydrarthrosis of long-term HD patients.[7] To our knowledge, there has been no previous report concerning the concentrations of endotoxin and β-D-glucan, which possibly induce cytokine production, in the synovial fluid of long-term HD patients. In the present study, β-D-glucan, but not endotoxin, was elevated in the synovial fluid of HD patients.

β-D-glucan is a major fungal cell-wall constituent. It has been reported that β-D-glucan enhances the hematopoietic response in mice[14] and induces cytokine synthesis in an in vitro human system.[15] Nemoto et al.[16] reported that cytokine gene expression by β-D-glucan was significantly affected by the structure of glucans. Obayashi et al.[17] pointed out that patients receiving HD with cellulosic membranes may have a high concentration of (1→3)-β-D-glucan in their blood, because these membranes inherently contain this polysaccharide.

Since it was confirmed by culture examinations that HD patients do not have fungal infections in the knee joints, the β-D-glucan detected in our study might be derived from a membrane component. Even if the β-D-glucan detected is not β-D-glucan itself, these structures are similar and therefore they may have similar effects.

Recently, several reports have suggested that cytokines TNF-α[18] and Il-1β[19,20] are produced during HD. Cytokines are essential mediators of inflammatory responses. The speculative "interleukin-1 hypothesis" of Shaldon et al.[21] suggested that repeated stimulation of these cytokines with each dialysis treatment might have a possible effect on the hydrarthrosis in long-term HD patients.

In the present study, there were no significant relationships between β-D-glucan levels and clinical stage, roentgenologic stage, or duration of HD. Only a small number of patients were included in this study, especially in stage 1, and levels of β-D-glucan were very widely scattered.

Therefore, reanalysis with a larger number of patients is necessary.

In conclusion, the present study suggests that β-D-glucan may have some relation to the pathogenesis of the synovitis existing in the hydrarthrosis of HD patients. Future issues that will be investigated are the relationships between β-D-glucan concentration and the conditions of the HD, the types of membranes and dialysates, and β-2-microglobulin concentrations.

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