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Prognostic value of the synovial fluid peroxidation indicators to determine the risk of implant instability in patients with gonarthrosis accompanied by articular surface defects

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Introduction The number of knee arthroplasties continues to increase in the recent years. Poor quality of implants, technical mistakes, excessive load, injury and osteoporosis are among the causes of their instability that have been most cited in the literature. Mechanical causes of instability may be associated with the changes in the peroxydation processes. **Purpose** To identify peroxidation products and study the activity of catalase, the main antioxidant enzyme, in the synovial fluid of the knee joint harvested prior to arthroplasty and investigate them as possible prognostic criteria of implant instability in primary arthroplasty **Materials and methods** Samples of the synovial fluid from 270 patients with gonarthrosis accompanied by defects of the articular surface (stage 3) during the operation of primary knee arthroplasty were harvested. Material of this prospective study was synovial fluid components of 70 patients from this cohort assigned progressively to two groups according to similar age, implant used and stability. Group 1 were patients (n = 35, mean age 67.4 ± 3.1 years) with implant instability developed within 3 years postoperatively. Group 2 patients (n = 35, mean age 69.4 ± 2.8 years) had stable implants. Control group 3 (cadaver material) was without any articular pathology that was recorded by an expert (n = 30, age of 68.4 ± 1.92 years). For a predictive value of the research, biochemical tests of the synovial fluid components were studied. **Results** Changes in the synovial fluid components in groups 1 and 2 that diverged in different directions were in the products of lipid peroxidation (malondialdehyde) and the activity of catalase. In patients of group 1, catalase activity was increased almost 2-fold, and in patients of group 2 it was reduced by 30% relative the control group. The content of malonic dialdehyde was increased only in group 2. **Conclusions** The parameters of lipid peroxidation products and the activity of the antioxidant enzyme system in the synovial fluid seem to be possible criteria for predicting instability after knee arthroplasty.
Keywords: synovial fluid, lipid peroxidation, oxidative modification of proteins, implant instability

INTRODUCTION

The number of knee arthroplasties continues to increase in the recent years [1, 2]. Alongside, the number of complications [3, 4, 5, 6] and revision knee arthroplasties due to implant component instability has been increasing [7, 8].

Poor quality of implants, technical mistakes, excessive load, injury and osteoporosis are among the causes of instability that have been most cited in the literature [6, 9, 10]. Mechanical causes of instability may be associated with changes in the peroxidation processes.

Peroxidation products are lipid peroxidation indicators (malonic dialdehyde and diene conjugates) and oxidative modification of proteins (aldehydes and ketones). Peroxidation products in the synovial fluid reflect the state of endogenous intoxication while the activity of catalase is the state of the antioxidant system. Changes in the peroxidation processes might be one of the pathogenetic causes of osteoarthritis (OA). One can hypothetically suggest that changes in the peroxidation processes may also be one of the reasons of implant instability [11, 12, 13]. This was shown by determination of the lipid peroxidation products in the blood serum of patients with instable hip implants.

It is assumed that the determination of peroxidation products in the synovial fluid of the knee joint by primary arthroplasty might have an important prognostic significance and reveal probable risk criteria of implant instability [14].

The purpose of this study was to determine the products of peroxidation and the activity of the main antioxidant enzyme, catalase, in the synovial fluid of the knee joint immediately before arthroplasty as possible prognostic criteria in the development of instability following primary arthroplasty.

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MATERIAL AND METHODS

Synovial fluid (SF) samples were harvested from 270 patients with gonarthrosis that was accompanied by defects of the articular surfaces (OA stage 3) during the operation of primary knee arthroplasty. The material of this prospective study was patients of two groups from this cohort that were formed at follow-ups according to the following inclusion criteria: similar age, implant used and its stability/instability. Group 1 was SF samples of 35 patients (18 males, 17 females) in the average age of 67.4 ± 3.1 years that developed knee instability within three years postoperatively. Group 2 were 35 samples of patients (16 males, 19 males) in the mean age 69.4 ± 2.8 years with stable implants. Group 3 was SF samples harvested from 30 individuals after their sudden death (22 males, 8 females) in the average age of 68.4 ± 1.92 years without any articular pathology that was recorded by an expert. Surgical interventions, SF collection and postoperative monitoring of patients were performed by surgeons and researchers of the Russian Ilizarov Scientific Center for Restorative Traumatology and Orthopaedics of the Russian Ministry of Health.

The level of oxidative modification of the protein (OMP) was expressed in units of optical density per mg of the total protein (TP) which was determined by the biuret method [15]. OMP products were recorded at 270 nm (aldehydes; OMP_{270}); 363 and 370 nm (ketones $OMP_{363+370}$).

The content of primary and secondary products of lipid peroxidation, diene conjugate (DC) and malondialdehyde (MDA), was determined, giving an overall assessment of lipid peroxidation (LPO). DC content was judged by the difference in the optical density at the wavelength of 232 nm in the experimental and control samples [16]. Malondialdehyde (MDA) was determined by reaction with thiobarbituric acid [16]. The concentration of total lipids was determined using the "Lachema" kits (Czech Republic).

Concentrations of cholesterol (CL) and triglycerides (TG) were evaluated with the help of Vital Diagnostic kits. The concentration of peroxidation products was calculated as mg per SF total lipids (TL). The antioxidant protection was assessed by the activity of catalase enzyme in SF, which was determined by spectrophotometry at a wavelength of 410 nm according to the method described [17]. The method is based on the ability of hydrogen peroxide to form a stable colored complex with molybdenum salts [17].

Statistical processing of the findings was performed by a method which is applied to small samples. The clinical part of the work was conducted on the representative samples in which the distributions of pop-up variants were excluded and tested for normal distribution. In the observation groups, the median values were calculated and the interquartile range was 0.25 and 0.75 per percentile. The reliability of differences in the comparison groups was assessed using the non-parametric Wilcoxon test according to licensed programs. Differences were considered significant at $p < 0.05$. Permission was obtained from the Ethics Committee of the RISC for RTO of the Ministry of Health of Russia for this clinical study. It was conducted in accordance with the ethical standards of the Helsinki Declaration [18].

RESULTS

Table 1 data show that the majority of SF biochemical indices of Group 1 and Group 2 patients are statistically significant and feature unidirectional difference from the control group values. In group 1 patients, the TL content and CL concentration in the synovial environment of the affected joint were triply increased. The concentration of triglycerides was decreased 9-fold. In Group 2 patients with stable implants, the TL content and the CL concentration were increased 2 times, and the concentration of triglycerides showed a 7.6-fold reduction. It was noted that the changes in the SF lipid composition were unidirectional in both groups of patients, but they were much more pronounced in Group 1.

However, the changes in the indices of LPO products were not unidirectional in the groups with stable and unstable implants. First of all, it refers to the change in the activity of catalase (AC), the main antioxidant enzyme. In Group 1 patients, its activity increased almost 2-fold, and in patients with a successful outcome, its activity was reduced

by 30 %. Also, the MDA content changed in different directions. It was four times higher in group 2 that in the controls but was decreased in Group 1.

In SF of patients with knee arthroplasty, the TP concentration showed significant changes, which consistently increased relative to the control group. The concentration of aldehydes increased also as compared with the control values. The concentration of ketones was statistically reduced in Group 1.

Table 1

LPO-AC biochemical indices in the study groups according to the results compared (medians of values and interquartile ranges)

Parameter, unit	Group 1 (n = 35)	Group 2 (n = 35)	Group 3 (n = 30)
Total lipids, g/l	$1.92^{0.05}$ (1.22; 2.76)	1.23 (0.77; 1.83)	0.69 (0.60; 0.83)
Cholesterol, mmol/l	$1.33^{0.05}$ (0.99; 1.68)	0.68 (0.42; 111)	0.42 (0.29; 0.49)
Triglycerides, mmol/l	0.11 (0.07; 0.23)	0.13 (0.02; 0.30)	0.72 (0.38; 1.18)
Diene conjugates, nmol/g TL	$7.62^{0.001}$ (5.79; 13.41)	35.29 (28.79; 49.76)	5.91 (3.94; 13.03)
Malondialdehyde, nmol/g TL	$1.48^{0.001}$ (1.31; 1.71)	8.77 (3.80; 13.24)	1.95 (1.32; 3.41)
Catalase, mcatal/gTP	$9.03^{0.05}$ (3.61; 12.97)	3.50 (2.26; 13.07)	5.00 (2.54; 12.08)
DC+MDA	$15.97^{0.001}$ (10.98; 22.15)	309.49 (109.40; 658.82)	11.52 (5.20; 44.43)
DC/MDA	$7.36^{0.05}$ (4.35; 12.04)	4.32 (3.12; 8.15)	3.02 (2.98; 3.82)

Note: superscript is the level of significance (p), comparison between the groups; underlined are the results that differ from group 3

Table 2

Biochemical indices in the study groups based according to the results of the OMB determination (median values and interquartile ranges)

Parameter, unit	Group 1 (n = 35)	Group 2 (n = 35)	Group 3 (n = 30)
Total protein, g/l	32.45 (27.33; 39.00)	22.90 (19.20; 38.80)	20.40 (12.30; 25.70)
Aldehydes , unit of optical density/g TP	0.16 (0.10; 0.19)	0.18 (0.10; 0.37)	0.05 (0.04; 0.07)
Ketones , unit of optical density/g TP	0.01 (0.01; 0.03)	0.03 (0.01; 0.06)	0.08 (0.04; 0.12)
Aldehydes +Ketones (10^{-3})	3.31 (2.17; 3.66)	5.41 (1.00; 6.01)	5.00 (1.00; 8.00)
Aldehydes /Ketones	$7.09^{0.05}$ (2.62; 18.67)	9.09 (1.33; 20.63)	0.82 (0.49; 1.58)

Note: superscript is the level of significance (p), comparison between the groups; underlined are the results that differ from group 3

DISCUSSION

Our study revealed biochemical changes in the SF of OA patients before the operation of knee arthroplasty. There were statistically significant differences in its parameters in Group I and Group 2. Having analyzed the results for their possible prognostic value, we identified those biochemical tests that changed in different directions in both groups.

First of all, we focused on the assessment of MDA in the synovial fluid. Its concentration grows if the pathological process develops. However, it was revealed only in Group 2. Its concentration was significantly lower in patients with instability than in Group 2. The same pattern was also found for the total indices of primary and secondary LPO products. Based on this, we estimate the prognostic value of these tests as high.

The increase in catalase activity also plays a significant role in determining the prognosis for knee arthroplasty. It did not show significant differences from control values in Group 2 but was significantly higher in Group 1. We also mark the indicators of primary LPO products, diene conjugates, in forecasting instability. With a unidirectional change in patients of both groups, its concentration was 4.5 times higher in Group 2 than in patients in Group 1. The concentrations of cholesterol had a 2-fold difference between the groups, which also would make a predictive contribution. It should be noted that patients with a high concentration of peroxidation products and low catalase activity would have a more favorable prognosis than the patients who show these values close to the control ones, but in who the catalase activity, on the contrary, is increased.

Thus, when the dynamic equilibrium between the accumulation of peroxidation products and the activity of catalase shifts, the risk of endoprosthe-

sis instability appears. It is known that the intensification of the oxidation process can become the starting point for many pathophysiological processes, including joint diseases [19, 13]. It was shown that osteolysis in the implantation zone is the main reason for implant adaptation failure in the body [20]. The trigger mechanism of osteolysis is its induction by attractants, which are the products (particles) of wear [21].

Bone tissue remodeling around the implant would be disturbed in the conditions of changes in the peroxidation system. In group 2 patients, we observed a typical reaction of enhanced lipid peroxidation processes in the synovial environment. They had a significantly increased amount of lipoperoxidation products (both primary and secondary) and a decreased activity of the main antioxidant enzyme, catalase.

However, a deregulation effect on the processes of free radical oxidation is exerted not only by an increase but also by a decrease in the amount of lipid peroxidation products. This mechanism of cellular functioning disorder is associated with the lack of activation of transcription factors and gene expression [13]. Proceeding from this, one can assume that patients that exhibit a decrease in the peroxidation products and an increase in the catalase activity in the synovial environment fall into a risk group what is possibly mediated genetically. In other words, the patients that have an increased activity of catalase and a decreased MDA concentration should be followed up by an attending surgeon.

CONCLUSION

1. The increase in the catalase activity and the decrease in the concentrations of secondary products of lipid peroxidation (malondialdehyde) in the synovial fluid might indicate that there is a risk of implant instability in patients with gonarthrosis accompanied by articular surface defects.

2. These biochemical parameters should be taken into account along with other laboratory tests, clinical and radiographic findings that reflect the patient's condition.

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