ORIGINAL INVESTIGATIONS

The gene expression of caspase 3, matrix metalloproteinase-9, tissue inhibitor of metalloproteinases-1, and cathepsins S and K in patients with osteoarthritis requiring large joint replacement

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Osteoarthritis (OA) is a chronic rheumatic disease that is characterized by pain and articular cartilage degradation. Pain in OA is a main clinical symptom that limits working capacity and is one of the indications for joint replacement. However, 10-40% of patients with OA also continue to experience painful sensations after surgery.

Objective: *to develop a method for searching for biomarkers to predict the dynamics of pain in the postoperative period and to determine the feasibility of arthroplasty on the basis of a retrospective analysis of relative blood gene expression prior to surgery.*

Patients and methods. *The investigators tested the blood taken from 53 OA patients (mean age, 56.5Í}8.9 years) before knee arthroplasty and from 26 healthy donors (mean age, 55Í}8.3 years). Total RNA was isolated from blood and after reverse transcription into complementary DNA was used to measure the level of relative gene expression in real-time polymerase chain reaction.*

Results and discussion. *A retrospective analysis of the expression of genes associated with central sensitization in 53 patients with OA before arthroplasty showed that the data on the expression of tumor necrosis factor-α, interleukin-1β, cyclooxygenase-2, and transforming growth factor β1 were uninformative due to their high blood expression in all the patients. The high gene expression of cathepsin S (in 17% of the patients) and cathepsin K (in 21%) and the low gene expression of tissue inhibitor of metalloproteinases 1 (TIMP-1) (in 31%) may indicate that postoperative pain can be persistent. In contrast, no post-arthroplasty pain can be expected in 43% OA patients with low caspase 3 expression and in 23% of those with low MMP-9 one.*

Conclusion. *Analysis of pre-arthroplasty blood gene expression in patients with OA seems to be a promising approach to predicting the dynamics of pain after surgical treatment.*

Keywords: *osteoarthritis; pain; gene expression; peripheral blood; prediction of arthroplasty outcome.*
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Osteoarthritis (OA) is a chronic rheumatic disease that is characterized by pain and destruction of joint tissue. Pain in OA is the main clinical symptom limiting the ability to work. Since there are currently no disease modifying drugs for the treatment of OA, nonsteroidal anti-inflammatory drugs are widely used in its treatment, and in the late stage of the disease, severe pain is one of the important indications for joint replacement surgery. Knee arthroplasty is the most common option for joint surgical treatment. The number of such operations is growing annually and may increase by 7 times by 2030 [1]. Meanwhile, in 10–40% of patients, pain is preserved after knee replacement [2]. Understanding the factors that affect the outcome of surgical treatment will contribute to a proper selection of patients for arthroplasty [3].

In OA, pain is classified as: 1) nociceptive, which occurs when damage and / or inflammation of the joint tissues is due to activation of tissue nociceptors and has a protective function, and / or 2) neuropathic, which is caused by damage or dysfunction of the nervous system and includes disturbances of peripheral and central sensitization mechanisms [4, 5]. Peripheral sensitization is accompanied by a decrease in threshold values and an increase in the sensitivity of nociceptors [6]. Central sensitization is characterized by an excessive response of central neurons to receptor signals, as well as altered signal transmission from sensory neurons, impaired function of descending anti-nociceptors, increased activity enhancing pain paths, temporary summation (wind-up) and long-term potentiation of neural synapses in the cerebral cortex [7].

Recent studies have shown a predominance of central sensitization in 30% of patients with OA, including those in the late stage of the disease [8]. This is probably the reason for the persistence of pain after arthroplasty. Due to the high cost of surgical

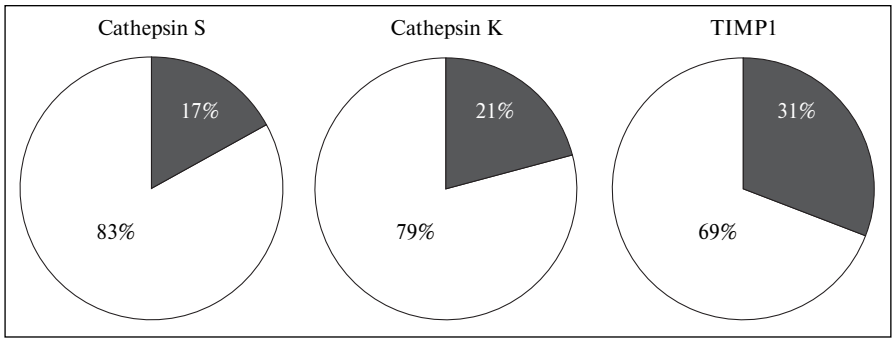

Fig. 1. *The ratio of the number of patients with high relative expression of cathepsin S and K genes (black segment) and low expression of the TIMP1 gene (black segment) in the blood of patients with OA before arthroplasty*

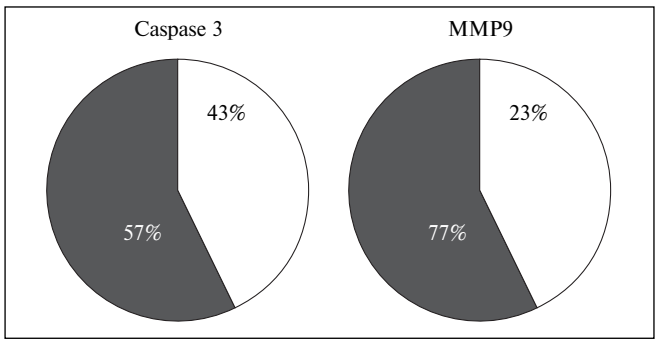

Fig. 2. *The ratio of the number of patients with low relative expression of caspase 3 genes (white segment) and MMP9 (white segment) in the blood of patients with OA before arthroplasty.*

treatment, there is a need to predict its results. The predictors of postoperative chronic pain are female gender, high body mass index, obesity, a higher level of pain before surgery, and the use of opioids or antidepressants before surgery [9–11]. In addition, it was suggested to use wind-up or conditional pain modulation methods as predictors of pain persistence [12–15].

Recent studies of central sensitization mechanisms have revealed a number of molecular markers associated with pain, including several cytokines, chemokines, calcium or glutamate transporters, caspases and proteases [16]. In particular, elevated serum CRP levels correlated with the concentration of interleukin (IL) 6 in the synovial fluid and the intensity of pain in patients with OA [17]. This suggests a promise of using some of these markers as predictors of pain persistence after surgery. Indeed, high concentrations of tumor necrosis factor (TNF) α, matrix metalloproteinase (MMP) 13 and IL6 in the synovial fluid were independent predictors of pain persistence 2 years after arthroplasty [18].

In addition, it was shown that cathepsin S contributes to the maintenance of neuropathic pain due to cleavage of the transmembrane chemokine on the surface of neurons [19, 20]. Caspase 6 is capable of regulating the neuron – microglia signaling pathway and central sensitization [21]. MMP9 is necessary for the induction of neuropathic pain, whereas MMP2 is involved in its long-term maintenance [22]. It was also shown that MMP9 can cross the blood-brain barrier. At the same time, chronic low expression of a tissue inhibitor of metalloproteinases (TIMP) in astrocytes is associated with the development of chronic neuroinflammation and hyperalgesia [23]. It is important that all these genes are expressed both in cells of the nervous tissue and in the blood, while the expression levels of cathepsins S and K correlate in the peripheral blood and articular cartilage of patients with OA [24]. This suggests a correlation of their expression in the peripheral blood and nervous cells.

The aim of this study is to develop a biomarker search method for predicting post-operative pain after arthroplasty and the feasibility of its implementation based on a retrospective analysis of the relative expression of genes in the peripheral blood before surgery.

Patients and methods

Patients

The blood of 53 patients with OA (mean age 56.5±8.9 years) was examined before knee replacement. All patients had III or IV radiological stage of OA according to Kellgren and Lawrence, experienced severe pain, and suffered from lameness. The diagnosis of OA was established according to the classification criteria ACR (American College of Rheumatology) [25]. The study protocol was approved by the local ethics committee, informed consent was obtained from all patients.

The control group consisted of 26 randomly drawn blood donors (average age 55±8.3 years) without rheumatic diseases and burdened heredity, comparable in gender and age to the group of OA patients.

Molecular biological methods

Total RNA was isolated from whole blood using the commercial RIBO-sol-A kit (InterLabService, Moscow). The reverse transcriptase reaction was carried out using the commercial Revert kit (InterLabService, Moscow). Relative gene expression was determined using a real-time polymerase chain reaction using a 7300 Applied Biosystems device and gene expression kits (Applied Biosystems, USA): caspase 3 (Hs00263337_m1), TNFα (Hs00174128_m1), MMP9 (Hs00234579_m1) (Hs00166156_m1), transforming growth factor (TGF) β1 (Hs99999918_m1), IL1β (Hs00174097_m1), cyclooxygenase (COX) 2 (Hs00153133_m1), cathepsin S (Hs00175407_m1), as described previously [26]. β-actin was used as an endogenous control.

Results

A preliminary retrospective prognostic analysis is based on our previous observations, which revealed that in 30–40% of patients with OA, pain persists after surgery. In this regard, in some patients, compared with healthy individuals, postoperative pain may be associated with increased expression of putative marker genes, which can be assessed before arthroplasty. Those genes whose expression is increased in 30–40% of patients can presumably be considered candidates for prognostic markers.

A retrospective analysis of the expression of genes associated with central sensitization in 53 patients with OA before endoprosthetics showed that data on the expression of TNFα, IL1β, COX2, TGFβ1 genes are non-informative due to their high expression in the blood of all patients.

In contrast, expression of the cathepsin S gene was high in 17% of OA patients, and cathepsin K in 21% (Fig. 1). At the same time, TIMP1 gene expression was low in 31% of OA patients (see Fig. 1), caspase 3 - in 43%, and MMP9 - in 23% (Fig. 2).

The method can be further validated using an example of gene expression analysis in individual patients with OA before arthroplasty. Analysis of the relative expression of combinations of TIMP1 and MMP9 genes showed that in patients No. 1 and No. 4, in contrast to patients No. 2 and No. 3, the expression of TIMP1 is higher than MMP9 in comparison with healthy individuals (see table). The expression of the cathepsin S and K genes in patients No. 5 and No. 8 is higher, and in patients No. 6 and No. 7 lower than in healthy individuals. In addition, the expression of the TIMP1, MMP9 and caspase 3 genes in patient No. 10 exceeded the control level, and in patients No. 9 and No. 11 it did not practically differ from the control (except for TIMP1 in patient No. 11).

Discussion

Despite technological progress in arthroplasty, the persistence of pain after surgery is a problem for both the patient and a doctor. A significant improvement in joint function and a decrease in pain that are noted in the early stages after surgery, may not be preserved in all patients in the long term. Moreover, although the goal of arthroplasty is to relieve pain and improve function, the factors determining the long-term results of arthroplasty in OA, as well as the individual perception of pain and its intensity after surgery, have not yet been sufficiently explored [27]. This may be due to the fact that pain is a subjective sensation and includes physiological, cognitive and emotional components.

Nevertheless, the reason for the search for predictors of pain persistence after surgery in the blood of OA patients prior to surgery among the genes that control metabolism is based on the knowledge of various metabolic disorders associated with pain including obesity, inflammation, comorbidity, and damage to other joints [3, 28]. In addition, the study of persistent pain in animal models showed that chemical or inflammatory stimuli can alter protein expression at the cellular level, affecting the phenotype and function of peripheral and central nociceptive neurons and glial cells [29, 30]. Therefore, the degree of violation of these metabolic processes can be estimated by the change in gene expression.

Despite the previously noted role of pro-inflammatory cytokines as prognostic markers of pain persistence after surgery [17, 18], our studies have shown that they are not enough informative, since their expression is increased in the blood of all patients with OA before surgery. Literature data indicating the prognostic role of the genetic markers studied in this work are absent.

Since increased expression of proteases and caspases is associated with central sensitization, high expression of cathepsin S genes (in 17% of patients), cathepsin K (in 21%), as well as low expression of TIMP1 gene (in 31%) may indicate a possible persistence of pain after surgery in these patients. In contrast, in 43% of OA patients with low caspase 3 gene expression and in 23% of OA patients with low MMP9 gene expression, pain can be expected after arthroplasty.

A more detailed examination of the mutual influence of genes was obtained after analysis of the expression of the MMP9 and TIMP1 genes. Moreover, since the expression of the TIMP1 gene exceeds the expression of proteinase, it can be assumed that patients No. 1 and No. 4 will not experience pain after surgery. In patient No. 2, postoperative pain is also unlikely, since his expression of these genes is lower than their expression in healthy individuals. On the contrary, in patient No. 3, pain persistence after surgery can be presumed, since the expression of the MMP9 gene exceeds the expression of the TIMP1 gene.

In addition, the expression of cathepsin S and K genes is either increased (in patients No. 5 and 8) or below the control level (patients No. 2 and 3). Therefore, pain persistence after surgery is likely to be observed only in patients No. 5 and 8. Comparison of the expression of caspase 3, MMP9 and TIMP1 genes shows that patient No. 9 might not experience pain due to the low expression of these three genes, like the patient No. 11, in which the expression of the TIMP1 gene was higher than the control while expression of the MMP9 and caspase 3 genes was low. In the patient No. 10, the expression of the examined genes was increased. He can be expected to develop pain. This might happen as although the expression of the TIMP1 gene in this patient exceeds that of the MMP9 gene, he also has high expression of the caspase 3 gene.

The proposed method is inexpensive and technically simple. However, since we conducted a retrospective study, the proposed method needs to be tested in a clinical setting not only in terms of confirming the prognostic efficacy of the proposed molecular markers, but also by expanding the set of examined genes whose expression is associated with pain.

Conclusion

Based of our studies, a biomarker search method has been proposed for predicting the persistence of pain after surgery and the relevance of arthroplasty in the individual OA patients. At the same time, identification of patients with a high risk of pain after surgery will require improving the quality of treatment for these OA patients excluding surgical intervention.

Relative gene expression in the blood of patients with OA prior to arthroplasty compared with healthy individuals (n=26)

OA patients	Relative gene expression			
	Control	NIMP1	MMP9	
№1	1	10,14		4,70
№2	1	0,78		0,93
№3	1	2,05		2,93
№4	1	3,76		1,19
		Cathepsin K	Cathepsin S	
№5	1	4,26		4,54
№6	1	0,70	0,84	
№7	1	0,28		0,63
№8	1	2,30		1,36
		TIMP1	MMP9	Caspase 3
№9	1	0,78	0,93	1,05
№10	1	36,06	24,79	38,91
№11	1	2,29	1,40	0,69

LITERATURE

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