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THE CHANGE IN THE LEVEL OF CYTOKINES IN BLOOD AND SYNOVIAL FLUID IN THE POSTTRAUMATIC PERIOD OF PATIENTS WITH DEFORMING OSTEOARTHROSIS AS CONTINUOUS CHRONIC DISEASE

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Pain in the different departments of the bottom extremities of such damage is the most frequent reason for seeking medical attention [1]. Acute pain syndrome causes not only a sharp deterioration in the quality of life of patients due to movement restrictions, but the reaction of all physiological systems. Expressed activation of sympathoadrenal system in acute pain leads to impaired function of the respiratory, cardiovascular, gastrointestinal, urinary systems [2-4].Constant sharp pain leads to increased levels of adrenocorticotropic hormone (ACTH)and cortisol, catecholamines, interleukin-1, low levels of insulin, water-electrolyte disorders (delay Na+, liquid) [3]. Moreover, a sharp pain leads to violation of regeneration processes, changes in the activity of the immune system and hypercoagulable, raising the risk of thrombus formation. [3]. Patients may develop psychoemotional disorders - anxiety and/or depression. And these disorder, in turn, can aggravate the pain syndrome.

It is well known, that anxiety-depression conditions lead to lower pain thresholds (the change in the perception of pain)that is associated with overexpression of substance P to change the currency of serotonin, etc. to increase muscle tension, which also increases the severity of pain. Domestic and foreign researchers proved that at the different stages of traumatic disease, as in deforming arthrosis, expressed by painful reaction forces to suffer all the organs and systems, the most significant changes relate to the immune system is affected all links from stem cell to cell-effectors, and the fact of development of immunodeficiency is of no doubt. [3]

The imbalance of cytokine regulation launches a consecutive chain of reactions leading to the violation of circulation, the occurrence of hypoxia, alveolar and interstitial edema of the lungs, damage to the metabolic functions of the lungs. [2,12]

The actual problem of the modern approach to the management of patients with traumatic injuries with the purpose of forecasting, prevention and treatment of post-traumatic conditions, is to evaluate in this period, the dynamics of cytokine profile circulating in synovial fluid, and the patterns of their production by mononuclear cells of the victims of this profile.

Research objective

To determine the effectiveness of diagnosis and treatment of post-traumatic arthrosis of the knee-joints with the definition of proinflammatory cytokines in deforming osteoarthrosis in blood serum, and also directly in synovial fluid, assess their importance to the objective of making the diagnosis, stages of deforming osteoarthrosis and to assessing the quality of treatment.

Material and methods

In a clinical study involved patients, with their informed consent, patients treated in the Department of General surgical intensive care and trauma Department of the Moscow urban clinical hospital № 14 named after V.G. Korolenko, and № 54 in the period from 2010 to 2013 (table 1).

In accordance with the purpose and objectives of this study were examined 144 patients with post-traumatic arthrosis (with a history of traumas of a various nature) in age from 23 to 64 years (average age 43,2±5,7). Out of all examined patients men was 63 (43.7 per cent), women - 81 (56,3%).

As the control group, 18 relatively healthy people aged from 30 to 57 years (average age 45,5±4,3) were examined. Table №1.

All of the examined person, depending on the stage of post-traumatic arthrosis, (acute and chronic classification) were divided into 4 groups.

The 1st group consisted of 48 patients with 1st stage of deforming osteoarthrosis, 2nd - 38 patients with stage 2nd stage of deforming osteoarthrosis, 3-th - 35 patients with 3rd stage of deforming osteoarthrosis, 4th - 23 patients with 4th stage of deforming osteoarthrosis.

Table № 2. Stage of deforming arthrosis was determined in accordance with classification [7].

Table 1. **Distribution of patients according to sex and age.**

	Age	Average age	%
Men 63 people	23-64	43.2±5.7	43.70%
Women 81 people	23-64	43.2±5.7	56.30%
Test group 18 people	30-57	45.5±4.3	100%

Table 2. The distribution of patients depending on the stage of post-traumatic arthrosis

The stages of post-traumatic arthrosis	Number of people
Patients with DOA I-st	48
Patients with DOA II-st	38
Patients with DOA III-st	35
Patients with DOA IV-st	23
Test group	18

Investigations were carried out at the time of hospitalization prior medical interventions. During the research the following methods were used: clinical, physical, instrumental, follow-up, radiation (MRI, CT, R-graphy), endoscopic, laboratory.

The synovial fluid from the affected knee was received during medical diagnostic puncture. The definition of Pro-inflammatory cytokines: IL-1, IL-6 and TNF-a in the blood serum and synovial fluid of the knee joint performed by ELISA on the immunofermental analyzer Abbott AXSYM" using standard kits ProCon ("Protein contour", St. Petersburg, Russia). Data were processed statistically using Student's t-test.

Results

The tables present the results of the IL-1, TNF-a (table 3) and IL-6 (table 4) in the serum of patients with deforming osteoarthrosis at different stages of the pathological process. As can be seen from table 1, the content of IL-1 and TNF-a in the blood serum of the control group averaged 58,4±9,41 PCG/ml and 46.5±11,4 PCG/ml, respectively. Analysis of the results of determination of IL-1R in the serum of patients DK showed that in all stages of the disease, in addition to IV, the contents of this cytokine exceeded the control values in varying degrees of severity.

The highest levels of IL-1R in blood serum was revealed in patients with 1st stage of deforming osteoarthrosis. Then downwards in the following order : in patients with 2nd , 3rd and finally, 4th stage of disease on average, respectively, he was 234,4±12,4; 198,7±16,6; 88,2±6,15 (in all cases $p<0.05$) and 47.4±4,45 PCG/ml.

While the levels of IL-1R in the serum of patients with the 1st stage of the disease was 4.0 times; stage 2 - 3.4 times; the third stage is 1.5 times higher than in control. And only in patients with stage 4 of deforming osteoarthrosis contents IL-1R did not differ from the control. A similar pattern was observed when determining the contents of TNF-a in the serum of patients with deforming osteoarthrosis, and only in contrast to the level of IL-1R in patients with 4th stage of deforming osteoarthrosis contents of TNF-and remained significantly higher than in control. So, the content of TNF-a in patients with of deforming osteoarthrosis of the I, II, III and IV stage of disease on average, respectively, amounted to 330,7±24,5; 210,5±17,3; 123,4±15,3 and 98,5±12,7 PCG/ml (in all cases $p<0.05$) (table no. 3).

Table 3. **The results of determination of IL-1, TNF-a in the serum of patients with deforming osteoarthrosis at different stages of the pathological process**

Subgroup(n=144)	IL-1 (pcg/ml)	TNF(pcg/ml)	Veracity
Test group(n=21)	58,4±9,41	46,5±11,4	p<0,05
Patients with DOA I-st (n=48)	234,4±12,4	330,7±24,5	p<0,05
Patients with DOA II-st (n=32)	198,7±16,6	210,5±17,3	p<0,05
Patients with DOA III-st (n=27)	88,2±6,15	123,4±15,3	p<0,05
Patients with DOA IV-st (n=16)	47,4±4,45	98,5±12,7	p<0,05

The table № 4 presents the results of determination of IL-6 in blood serum of patients with deforming osteoarthrosis at different stages of the pathological process. As can be seen from pic., the

content of IL-6 in blood serum in the control group averaged 4,2±0,77 PCG/ml the Results of determination of IL-6 in blood serum in patients with deforming osteoarthrosis have shown that at the early stages of the disease (stage I and II), this figure was significantly exceeded the control values, while in the later stages of deforming osteoarthrosis excess of contents IL-6 revealed no significant differences. The highest levels of IL-6 in blood serum was revealed in patients with 2nd stage of deforming osteoarthrosis (12,4 within (2.5 PCG/ml; p<0.05). In patients with 1st , 3rd and 4th stage of disease it on average, respectively, amounted to 9.2±1,74 (p<0.05); 5,7±1,12 and 5.1±0,63 PCG/ml (table 2).

Table 4. **The results of determination of IL-6 in blood serum of patients with deforming osteoarthrosis at different stages of the pathological process**

Subgroup (n=144)	IL-6(pcg/ml)	Veracity
Test group(n=21)	4,2±0,77	p<0,05
Patients with DOA 1st stage (n=48)	9,2±1,74	p<0,05
Patients with DOA 2nd stage (n=32)	12,4±2,5	p<0,05
Patients with DOA 3rd stage (n=27)	5,7±1,12	p>0,05
Patients with DOA 4th stage (n=16)	5,1±0,63	p>0,05

Table 5. The content of proinflammatory cytokines in the synovial fluid of the patients with different stages of deforming osteoarthrosis (M±m)

Groups	n	IL-1, pcg/ml	IL-6, pcg/ml	TNF-a, pcg/ml
1st	2	634,3±34,7	7,7±1,76	998,9±41,1
2nd	5	722,1±123,4	9,3±1,54	546,3±21,5
3rd	10	117,8±39,2	4,04±1,21	168,2±9,72
4th	17	91,2±7,4	4,47±0,97	136,9±7,36
Test group	18	16,1±6,3	6,2±0,9	35,2±4,6

Note. n - is the number of the examined persons

The table. № 5 shows the results of determination of IL-1, IL-6 and TNF-a in the synovial fluid of patients with different stages of deforming osteoarthrosis. As seen from table, the highest values of cytokines in synovial fluid was noted in patients with 1st and 2nd stages of deforming osteoarthrosis, and in advanced cases, this figure is decreasing. Comparative analysis of results of definition of cytokines in synovial fluid and serum showed that in the knee joint of patients with deforming osteoarthrosis levels of IL-1 and TNF- were higher and IL-6 levels were even lower than in serum. So, in patients with 1st stage of deforming osteoarthrosis the content of IL-1 in synovial fluid was 2.7 times higher, and in patients with 2nd , 3rd and 4th stage DKK 3.6; 1,3 and of 1.92 times respectively higher than in the serum (in all cases p<0.05). The content of IL-6 in synovial fluid, unlike IL-1, was below the corresponding values in the serum. So, in patients with 1st stage of deforming osteoarthrosis 1.2 times (no clear results), and in patients with 2nd , 3rd and 4th stage of deforming osteoarthrosis 2.7 times; 2,98 and 5.2 times respectively (in all cases p<0.05). Similar results were observed when determining the contents of TNF-a in the synovial fluid and serum.

Analysis of the obtained results showed that the content of Pro-inflammatory cytokines in blood serum varies depending on the stage of deforming osteoarthrosis. So, the content of IL-1R in the serum of patients with deforming osteoarthrosis, except persons with 4th stage of disease was significantly higher than the corresponding control. The level of IL-6 in blood serum of patients with deforming osteoarthrosis significantly exceeded the targets only in the early stages of the pathological process (I and II stage), while the contents of TNF-a in all stages of the disease was significantly exceeded the control value. Comparative analysis of the contents of proinflammatory cytokines in blood serum and synovial fluid showed that in the hearth of the pathological process (in the knee-joints in patients with deforming osteoarthrosis) levels of IL-1 and TNF-a were much higher than the corresponding values in circulating blood, but the number of IL-6 on the contrary was lower.

Based on the data, it is clear that in the initial stages of development of deforming osteoarthrosis proinflammatory cytokines play a key role in determination of the destructive potential of effector cells of the inflammatory process of the affected joint, probably through their autosimulator, and first of all of neutrophilic granulocytes [2] and may possibly be a key marker for early detection of damage. By reducing inflammatory potential in the later stages of the pathological process, in the lesion of the knee joint, in fact, the processes of fibrogenesis become stronger. A very important point, that supports this thesis is that there is a reduction of the

contents of the system of Pro-inflammatory cytokine IL-6 *in situ*, that is, in the synovial fluid of the affected joint in the later stages of deforming osteoarthrosis.

Thus, on the basis of the results of our research and literary data, we can say that the presence of Pro-inflammatory cytokines in the affected joint indicates pathological restructuring of the extracellular matrix and its loss [13].

Conclusion

The results obtained in a comparative study of contents of proinflammatory cytokines (IL-1, IL-6 and TNF-a) in the blood serum and synovial fluid at various stages of deforming osteoarthrosis showed that the determination of the spectrum of cytokines in the hearth of the pathological process, in our opinion, is the most perspective and can be a key marker for early detection of damage and organizations of the active preventive neasures of deformation processes in the joints in the early post traumatic period of the accident victims, mainly affecting the lower limbs.

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Material and Methods. In accordance with the purpose and objectives of this study 144 patients with post-traumatic arthrosis (with a history of traumas of a various nature) in age from 23 to 64 years (average age 43,2±5,7) were examined. Out of all examined patients men was 63 (43.7 per cent), women - 81 (56,3%). As the control group, 18 relatively healthy people aged from 30 to 57 years (average age 45,5±4,3) were examined. All of the examined person, depending on the stage of post-traumatic arthrosis, (acute and chronic classification) were divided into 4 groups. The 1st group consisted of 48 patients with 1st stage of deforming osteoarthrosis, 2nd - 38 patients with stage 2nd stage of deforming osteoarthrosis, 3-th - 35 patients with 3rd stage of deforming osteoarthrosis, 4th - 23 patients with 4th stage of deforming osteoarthrosis. Investigations were carried out at the time of hospitalization prior medical interventions. During the research the following methods were used: clinical, physical, instrumental, follow-up, radiation (MRI, CT, R-graphy), endoscopic, laboratory. The synovial fluid from the affected knee was received during medical diagnostic puncture. The definition of Pro-inflammatory cytokines: IL-1, IL-6 and TNF-a in the blood serum and synovial fluid of the knee joint performed by ELISA on the immunofermental analyzer Abbott AXSYM" using standard kits ProCon ("Protein contour", St. Petersburg, Russia). Data were processed statistically using Student's t-test.

Results and Discussion. Analysis of the results of determination of IL-1R in the serum of patients DK showed that in all stages of the disease, in addition to IV, the contents of this cytokine exceeded the control values in varying degrees of severity. The highest levels of IL-1R in blood serum was revealed in patients with 1st stage of deforming osteoarthrosis. Then downwards in the following order : in patients with 2nd , 3rd and finally, 4th stage of disease on average, respectively, he was 234,4±12,4; 198,7±16,6; 88,2±6,15 (in all cases $p<0.05$) and 47.4±4,45 PCG/ml. While the levels of IL-1R in the serum of patients with the 1st stage of the disease was 4.0 times; stage 2 - 3.4 times; the third stage is 1.5 times

higher than in control. And only in patients with stage 4 of deforming osteoarthrosis contents IL-1R did not differ from the control. A similar pattern was observed when determining the contents of TNF-a in the serum of patients with deforming osteoarthrosis, and only in contrast to the level of IL-1R in patients with 4th stage of deforming osteoarthrosis contents of TNF-and remained significantly higher than in control. So, the content of TNF-a in patients with of deforming osteoarthrosis of the I, II, III and IV stage of disease on average, respectively, amounted to 330,7±24,5; 210,5±17,3; 123,4±15,3 and 98,5±12,7 PCG/ml (in all cases $p<0.05$)

Conclusion. The results obtained in a comparative study of contents of proinflammatory cytokines (IL-1, IL-6 and TNF-a) in the blood serum and synovial fluid at various stages of deforming osteoarthrosis showed that the determination of the spectrum of cytokines in the hearth of the pathological process, in our opinion, is the most perspective and can be a key marker for early detection of damage and organizations of the active preventive measures of deformation processes in the joints in the early post traumatic period of the accident victims, mainly affecting the lower limbs.

Key words: cytokine interleukin (s), tumor necrosis factor, synovial fluid, deforming osteoarthrosis