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Cytokine concentration in peripheral blood and synovium in patients with deforming arthritis of the knee with regard to defect size of the medial tibial condyle

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Cytokine imbalance is important for pathogenesis of osteoarthritis. **Objective** To assess serum and synovium cytokine level in patients with deforming osteoarthritis of the knee joint depending on the size of the medial tibial condyle defect. **Material and methods** Immunoenzyme technique was used to measure cytokine concentration in peripheral blood and synovium of 80 patients with grade III gonarthritis. **Results** Serum concentration of IL–1β and IL–4 were significantly higher in presence of small, moderate and extensive condylar defects than that in the control group. Concentration of IL–8, IL–10, TNFα was higher in absence of the defect than that in the controls and there was no statistically significant differences in the groups with small and moderate defects. The highest concentration of the cytokines was observed in the group with defects sized more than 10 mm. Synovium concentration of IL–1β, IL–10 was minimum in absence of condylar defect and there was a tendency of increased concentration with a defect measuring less than 5 mm and it substantially increased with defects measuring more than 6 mm. Synovium concentration of IL–8 was higher than that of the serum but there was no statistically significant differences between IL-6 and synovium TNFα concentration. **Conclusions** The magnitude of the condylar defect was shown to influence over synovium and serum cytokine concentration. Concentration of both cytokines was the highest with condylar defect sized more than 50 % of the condylar area and 6 mm deep.

Keywords: cytokine, knee joint, deforming osteoarthritis, medial condyle defect

INTRODUCTION

Deforming osteoarthritis (OA) of the knee is a highly prevalent joint disorder among degenerative abnormalities accounting for 54.7 to 69.7 %. [1, 2]. Osteoarthritis of the knee can result from dysplasia, trauma, ligament and capsule instability, meniscal injury. Defects of articular surfaces of the knee joint are associated with condylar bone loss of different extent.

Immune deficiency and cytokine imbalance, in particular are important for pathogenesis of osteoarthritis [3, 4, 5, 6, 7]. Biologically active cytokines are involved in the regulation of hematopoiesis, inflammation, support the differentiation and proliferation of immune competent cells. Cytokines have a specific effect on the interactions and communications between cells and systems. With regard to the effect cytokines have on articular and juxta-articular tissues they are subdivided into three groups, destructive (proinflammatory), regulatory (including anti-inflammatory) and anabolic (also termed growth factors) [8]. As antigen-specific factors cytokines do not allow for specific diagnosis of infection, autoimmune and allergy diseases. Cytokine can be used as diagnostic and prognostic markers at different stages of osteoarthritis. Cytokine concentration levels can be one of the most prominent biomarkers in prescribing individual biologic therapy that is successfully applied in current medical practice [9, 10, 11, 12].

Despite the great interest to the problem and substantial number of publications in the field of osteoimmunology there is no evidence of a relationship between serum and synovium cytokine level and the size of the medial tibial condyle defect in patients with gonarthritis in the current literature.

Objective The purpose of the study is to assess serum and synovium cytokine level in patients with deforming osteoarthritis of the knee joint depending on the size of the medial tibial condyle defect.

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MATERIAL AND METHODS

We evaluated peripheral blood and synovium of 80 patients with grade III deforming arthritis of the knee. The mean age of the patients was 53.9 (range, 49 to 58) years. Anteroposterior and lateral radiographs of the knee joint were used to measure tibial condyle defect. Classification of N.N.Kornilov et al. (2009) was employed to group 4 types of defects. Group I consisted of 28 patients with no medial tibial condyle defect, group II included 20 patients with minimal defect (less than 50 % of a condylar area and 5 mm deep), group III had 20 patients with moderate defect (50 to 70 % of a condylar area and 6 to 10 mm deep), group IV included 6 patients with extensive defect (more than 70 % of a condylar area and 10 mm deep).

Enzyme Immunoassay Analyzer (*BioTek Instruments Inc.*, ELx808, U.S.A., Reg. FS № 2006/2919 dtd 26.12.2006) was used to measure IL–1β, IL–4, IL–6, IL–10, IL–8, TNFα concentrations in peripheral blood and synovium with reagent kit (ZAO *Vector-Best*, Novosibirsk). Peripheral blood sample was collected from median cubital vein under fasting condition in vacutainer. Synovium was collected into sterile tube during primary total knee replacement. The study excluded patients with accompanying somatic pathology (autoimmune disease, acute exacerbation of chronic condition, allergy, HIV, HCV, HbsAg positive) that can affect immune testing. Twenty eight normal healthy volunteers of the matching age were used as controls to compare measurements of peripheral blood cytokine level. All individuals involved in the study provided written informed consent for medical intervention and publication of the findings ensuring confidentiality of personal identification.

Statistical data analysis was performed using *AtteStat* computer program integrated into Microsoft Excel and Microsoft Office. The Student's t-test was used to confirm differences in cases with normal distribution. Non-parametric statistical tests (Wilcoxon-Mann-Whitney test), was used when distribution was different from the norm. Variability was summarised by the median and interquartile range (25^{th}–75^{th} percentile).

RESULTS AND DISCUSSION

IL–1β may be an important osteotropic mediator in disturbances of bone cell function due to the expressed biological activity. It is produced by macrophages, synoviocytes, chondrocytes and osteoclasts. The cytokine is involved in cartilage damage and synovium inflammation by inducing synthesis and secretion of several cytokines, matrix metalloproteinases and enzymes. IL–1β is known to stimulate active oxygen contributing to articular cartilage degradation. In addition to that, IL–1β negatively affects gene expression regulating the circadian clock mechanism in the cartilage via an NFκB-dependent pathway [13]. IL–1β levels in the peripheral blood of patients with deforming knee OA was significantly greater that those in the control group in presence of minimal, moderate and extensive condylar defects. Group I showed no statistically significant differences with controls (**Table 1**).

Similar changes were observed with interleukin-4 (IL–4). IL–4 has many biological roles including inhibition of the production of proinflammatory cytokines, the stimulation of activated B-cells and T-cell proliferation, induction of B-cell class-switching to IgE and up-regulation of MHC class II production. IL–4 decreases the production of macrophages and dendritic cells and suppresses proliferation of synoviocytes [14, 15].

Table 1

Cytokine levels in the peripheral blood of patients with OA with regard to the size of the tibial condyle defect

Cytokine	Control group	I	II	III	IV
IL–1β	0 (0–0.5)	0 (0–1.18)	**0.42* (0.03–2.76)**	**0.9* (0.05–2.64)**	**0.1* (0.03–1.23)**
IL–4	0 (0–0.5)	0.11 (0–1.28)	**0.37* (0.18–1.12)**	**0.64* (0.17–1.11)**	**0.44* (0.1–1.0)**
IL–6	0.2 (0–0.77)	**1.58* (0.35–3.25)**	**1.81* (1.14–2.88)**	**2.73**$^{+}$ (1.4–3.79)**	**3.61**$^{++}$ (3.1–7.2)**
IL–8	1.81 (0,59–5.97)	**9.03* (3.7–15.98)**	**10.75* (7.5–15.83)**	**10.0* (4.31–19.03)**	**12.2**$^{+}$ (10.0–30.47)**
IL–10	0.21 (0–0.48)	**1.75* (0.1–3.29)**	**1.54* (0.1–3.28)**	**1.1* (0.1–3.48)**	**2.85**$^{+}$ (0.9–6.03)**
TNFα	0 (0–0.69)	**0.87* (0.38–2.46)**	**1.23* (0.1–2.46)**	**1.2* (0.4–2.93)**	**2.34**$^{+}$ (1.0–3.51)**

Note: * – $p \le 0.05$, ** – $p \le 0.01$ compared to controls; $^{+}$ – $p \le 0.05$, $^{++}$ – $p \le 0.01$ compared to group I.

There was a greater correlation detected between the bone defect size and interleukin-6 (IL–6), a key cytokine that exhibits both pro-inflammatory and anti-inflammatory activities. IL–6 is believed to play a regulatory role in osteoclast differentiation that enhances bone resorption [16]. Our findings showed a greater concentration of IL–6 in peripheral blood of OA patients as compared to controls. The serum interleukin-6 level was significantly less in patients with absent condylar defect than in those with condylar defect with the highest concentration measured in group IV.

Interleukin-8 (IL–8) is believed to be associated with inflammation and cartilage degradation [17]. The serum IL-8 level was significantly greater in patients with no condylar defect than that in controls but there was no statistically significant difference between groups II and III. The highest concentration of neutrophil-activating factor was observed in patients with extensive defect (group IV).

Interleukin 10 (IL–10) is a cytokine with potent anti-inflammatory properties that inhibits the activity of Th1-cells and blocks antigen-specific T-cell response. Osteotropic effect of IL–10 includes decrease in synthesis of proinflammatory cytokines and other mediators of active inflammation (PGE2, etc.) [18]. Our findings showed the serum IL–10 level being almost similar in patients with condylar defects measuring 10 mm and higher than that in the control group. Patients with extensive defects (group IV) showed higher serum IL-10 concentration.

Tumour necrosis factor-alpha (TNFα) is recognised as an important mediator in many cytokine-dependent inflammatory events and known to cause catabolism of bone tissue and prevent the recovery. Together with IL–1β, TNFα can cause changes in the subchondral bone playing a role in osteoclast formation and bone resorption [19]. As with IL–8 and IL–10 measurements the serum TNFα level was higher in patients with condylar defects measuring less than 10 mm as compared to controls and less than that in the group of patients who had defects of more than 10 mm.

Synovial fluid as a boundary lubrication layer between synovial membrane, cartilage and subchondral bone is sensitive to structural changes that occur in articular and juxta-articular tissues [20, 21]. The series reporting synovium cytokine levels mostly focus on osteoarthritis grading without considering ab extent of bone destruction. No evidence of physiological parameters of synovium cytokines is available in the current literature. This can be explained by ethical considerations with aspiration procedure being traumatic and painful. Only one publication reported findings on synovium cytokine levels of individuals who died because of traumatic brain injury and who showed no postmortem morphological damage to the knee joint. According to the data, physiological parameters of synovium IL–6 level measured 0.12 ± 0.01 pg/ml [22].

Our findings showed significant differences in synovium cytokine levels depending on the size of the tibial condyle defect. Synovium IL-1β and IL–10 levels were minimal in absence of condylar defect and had tendency to considerably increase in case of defects measuring 50 to 70 % of a condylar area and being 6 mm deep. Synovium IL–8 level was higher than the serum IL–8 concentration and there were no statistically significant differences depending on the extent of bone destruction. A more distinct correlation between the size of condylar defect and cytokine level was detected with IL–6. Even group I showed considerably higher measurements than normal physiological values. As with IL–6, synovium TNFα level increased with regard to the condylar defect size (**Table 2**).

Overall distinct correlation between the size of condylar defect and cytokine level was observed with serum and synovium IL–6 and synovium TNFα.

Table 2

Cytokine synovium levels in patients with OA with regard to the size of the tibial condyle defect

Cytokine	I	II	III
IL–1β	0.8 (0–5.5)	2.27 (0.1–6.5)	**10.5*$^{+}$ (1.0–12.26)**
IL–6	77.29 (28.03–170.7)	**250.4* (83.7–367.0)**	**358.0**$^{+}$ (252.8–466.6)**
IL–8	30.0 (10.9–46.1)	20.4 (10.7–46.53)	35.0 (13.1–116.4)
IL–10	5.3 (3.3–11.8)	5.1 (3.1–11.9)	**13.6*$^{+}$ (7.39–21.75)**
TNFα	1.13 (0–2.87)	**3.1* (0.9–10.1)**	**8.69**$^{+}$ (5.38–20.46)**

Notes: * – $p \le 0.05$, ** – $p \le 0.01$ compared to group I; $^{+}$ – $p \le 0.05$ compared to group II.

CONCLUSION

Therefore, greater destructive changes in osteoarthritic patients were shown to result in up-regulation of cytokines and immune mechanisms intensifying pathological changes in the articular tissues. The vicious loop made the process chronic considerably aggravating late stages of osteoarthritis. The magnitude of the condylar defect had an impact over synovium and serum cytokine concentration that showed high values with the defect measuring more than 50 % of the condylar area and being 6 mm deep. The most distinct correlation between the size of condylar defect and cytokine level was observed with serum and synovium IL-6 and synovium TNFα that could be used as an additional criterion to evaluate the extent of bone destruction in patients with deforming OA of the knee joint.

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