

Cytokine concentration and profile of lipid peroxidation in synovial fluids of patients with osteoarthritis and concomitant defects of articular surfaces

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The objective of the study was to evaluate cytokine concentration and profile of lipid peroxidation in synovial fluid of patients with osteoarthritis and concomitant defects of articular surfaces. **Material and methods** Synovial fluid samples were taken from 102 patients with osteoarthritis of the knee joint. Patients with rheumatoid arthritis, osteoarthritis of a post-traumatic etiology, and somatic diseases that could affect results of the study were excluded from the study. Thirty control samples originated from deceased donors of both genders. Synovial fluid was extracted in compliance with Ministry of Health Order No. 694 dtd July 21, 1978, p. 2.24 "Guidelines of forensic medical examination in the USSR". **Results** Findings of laboratory studies showed statistically significant differences in synovial fluid cytokine levels depending on absence or presence of defects on the tibial condyles. Biochemical tests revealed greater changes in lipid peroxidation in patients with articular defects. Total level of lipid peroxidation products resulting in the formation of conjugated dienes (CD), malondialdehyde (MDA) was shown to increase in both groups of patients being significantly higher in patients with defects on articular surfaces. Primary (conjugated dienes) and secondary (malondialdehyde) lipid peroxidation products accumulated in the synovial fluid of the patients with the levels being significantly increased in both groups with no changes in the CD/MDA ratio. Patients with defects on articular surfaces demonstrated increased formation of primary products, and non-defect group showed greater formation of secondary products. Antioxidant enzyme, catalase, appeared to be more active in patients of Group I. **Conclusion** The findings can be used to evaluate defects on articular surface and identify strategies of medication therapy.

Keywords: synovial fluid, osteoarthritis, cytokine, lipid peroxidation

Osteoarthritis (OA) is a multifactorial disease with complex etiologies involving mechanical, metabolic and other factors [1, 2]. Inflammation plays a leading role in pathogenesis of OA [3]. Some authors suggest that OA is a systemic disorder [4, 5, 6] and recent researches focus on lipid metabolism. Peroxide modification of low density lipoproteins accompanied by increased immunogenicity can add autoimmune

factor to pathogenesis [7]. There is a correlation between several markers of lipid metabolism and cytokine status to be suggested in OA patients.

The purpose of the study was to evaluate cytokine concentration and profile of lipid peroxidation in synovial fluid (SF) of patients with osteoarthritis (OA) and concomitant defects of articular surfaces.

MATERIAL AND METHODS

SF samples of 102 patients with OA of the knee joint were used in the study. There were 78 patients without tibial condyle defect including 13 males and 65 females aged 64.4 ± 2.8 years in Group 1 and 24 patients with tibial condyle defects including 5 males and 19 females aged 67.4 ± 3.1 years in Group 2. SF samples were collected into a sterile test glass during primary total knee replacement. Patients with rheumatoid arthritis, osteoarthritis of traumatic etiology and somatic conditions (autoimmune disease, acute exacerbation of chronic condition, allergy, HIV, HCV, HbsAg positive) that could affect results of testing were excluded from the study. All individuals

involved in the study provided written informed consent for medical intervention and publication of the findings in accordance with ethical principles of the World Medical Association Declaration of Helsinki with recent amendments (Seoul, 2008).

Control peroxidation products included 30 SF samples originated from deceased donors of both genders (22 males and 8 females) with the average age of 68.4 ± 1.92 years. No joint pathology was recorded in the groups by an expert as evidenced in outpatient/inpatient medical records, supplementary sheet and medico-legal report. Synovial fluid was extracted in compliance with Ministry of Health

Order No. 694 dtd July 21, 1978, p. 2.24 "Guidelines of forensic medical examination in the USSR".

SF concentration of IL-1 β , IL-6, IL-10, IL-8, TNF α , IFN γ was measured with enzyme immunoassay using the ELx808 enzyme immunoassay analyzer (BIOTEK Instruments Inc., USA) and chemical kit (Zao "Vector-Best", Novosibirsk).

Primary (conjugated dienes, CD) and secondary (malondialdehyde, MDA) peroxidation product content was evaluated to assess lipid peroxidation (LP). Conjugated dienes were detected with spectrophotometry by a difference in optical density between test and control samples with wavelength of 232 nm [8]. Malondialdehyde was identified with thiobarbituric acid reaction [8]. Concentration of lipid peroxidation products was calculated per

mg of SF total lipids that were evaluated with Lachema kits (Czech Republic). Cholesterol (Ch) and triglyceride (TG) concentration was measured with Vital Diagnostic kit. Anti-oxidant protection was evaluated by activity of SF catalase enzyme identified with spectrophotometry with wavelength of 410 nm using the method described with hydrogen peroxide and molybdenum forming stable coloured complexes [9].

Statistical analysis. Clinical part of the work was performed on representative samples with outliers excluded and inspected for normality of distribution. AtteStat software was used for statistical data analysis [10]. Non-parametric statistical tests were employed to analyze data assessing significant differences between the groups with Wilcoxon sign test.

RESULTS AND DISCUSSION

OA grade would be considered by most researchers measuring cytokine concentration in SF with an extent of cartilage and osseous destruction being neglected. Analysis of literature showed no physiological role of cytokines in SF [11]. According to literature sources [12] cytokines can induce LP processes with LP products having impact on several interleukins. Incidentally IL1 intensifies production of free oxygen, IL8 primes human neutrophils for enhanced production of superoxide anion generated as a by-product of oxygen utilization of aerobic organisms. Human superoxide anion is involved in stimulation of immune response phagocytes steering leukocytes to the infection site. The excessive compound and the derivatives can result in injury to the cells.

Laboratory tests showed statistically significant differences in SF cytokine concentrations depending on presence/absence of tibial condyle defect (Table 1).

IL-1 β is one of the most important osteotropic mediators of inflammation involved in destruction of

cartilage and osseous tissues. The cytokine stimulates production of reactive oxygen species causing articular cartilage degradation [12]. IL-1 β concentration in SF was significantly greater by 330 % in the group of patients with knee OA having tibial condyle defects.

Our findings showed that IL-6 content in SF was significantly greater by 122 % in Group II of patients with tibial condyle defect as compared to group I. It was likely due to the fact that IL-6 supports osteoclast differentiation and induces bone resorption. The level of IL-8 that is known to contribute to inflammation and cartilage degradation was significantly greater by 26 % in Group II.

TNF α is an osteotropic cytokine that can cause catabolism of bone tissue and inhibit restoration. TNF α and IL-1 β play a critical role in subchondral destruction of the femur. Концентрация TNF α concentration was significantly greater by 235 % in the group of patients with defects of articular surfaces.

Results of biochemical tests of the SF in both groups are presented in Table 2.

Table 1

Cytokine concentrations in SF of OA patients with/without tibial condyle defect

Item	Group I (n = 78)	Group II (n = 24)
IL-1 β	2.0 \pm 1.01	8.6 \pm 2.310.08
IL-6	125.0 \pm 42.4	278.7 \pm 49.50.01
IL-8	62.1 \pm 33.6	78.7 \pm 49.50.26
IL-10	7.32 \pm 1.94	9.41 \pm 2.560.178
TNF α	2.18 \pm 1.3	7.4 \pm 2.330.025
IFN γ	5.5 \pm 2.46	16.4 \pm 10.30.23

Note: superscript is the significance level (p) of comparison of the groups.

Table 2

Biochemical values of study groups as measured in the system LP-AOS

Parameter, unit of measure	Normal (n = 30)	Group I (n = 78)	Group II (n = 24)
Total lipids, g/L	0.71 ± 0.07	1.41 ± 0.12*	1.95 ± 0.55 ^{0.01*}
Cholesterol, mmol/L	0.46 ± 0.12	1.12 ± 0.07*	1.10 ± 0.15
Triglycerides, mmol/L	0.86 ± 0.20	0.27 ± 0.03*	0.18 ± 0.07 ^{0.05*}
Conjugated dienes, nmol/g OL	8.70 ± 1.97	13.13 ± 1.67*	18.13 ± 4.24 ^{0.01*}
Malondialdehyde), nmol/g OL	2.44 ± 0.70	5.07 ± 0.68*	4.23 ± 1.18 ^{0.05*}
Catalase, mcatal/g OB	8.72 ± 2.71	8.70 ± 1.90	6.49 ± 2.05 ^{0.05*}
CD+MDA	10.33 ± 2.20	66.97 ± 10.61*	99.40 ± 44.75 ^{0.05*}
CD/MDA	5.76 ± 2.76	3.94 ± 0.91	5.17 ± 0.97

Note: superscript is the significance level (p) of comparison between groups I and II; * – the values that are different from normal group.

Table 2 shows significant (by 100 % and 150 % in Groups I and II, respectively) increase of total lipid level in patients with the knee OA as compared to normal values. Lipid concentration was statistically higher in patients with defects of articular surface as compared to no-defect group. Cholesterol level was noted to increase in both groups and was higher in patients with defects of articular surface. Triglycerides in SF were observed to decrease by 218 % in group I and 380 % in Group II as compared to normal values. Therefore, changes in the parameters of lipid spectrum were more evident in patients with defects of articular surface.

Overall concentration of LP [CD+MDA] was shown to increase in both groups (by 548 % in Group I and by 862 % in Group II) as compared to normal values and was significantly higher in group of patients with defects of articular surfaces. Primary (CD) and secondary (MDA) LP products accumulated in SF with the statistically significant increase in both groups and absence of changes in CD/MDA ratio. Patients with defects of articular surfaces primarily showed increase in primary (CD) LP products whereas no-defect group demonstrated enhanced secondary (MDA) LP products. Antioxidant enzyme, catalase, appeared to me more active in patients of Group I.

CONCLUSION

Cytokines are one of biologically important factors regulating magnitude and duration of inflammatory and immune responses. Medications developed and introduced to treat OA in more recent years have impact on cytokine expression. A correlation between several markers of lipid metabolism and cytokine status can be suggested for patients with

knee OA. Our series showed significant increase in concentration of proinflammatory cytokines in SF of patients with defects of articular surfaces, total concentration of LP products and decrease in activity of major antioxidant enzyme, catalase. The findings can be used to evaluate defects on articular surface and identify strategies of medication therapy.

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REFERENCES

- Simakova E.S., Zavodovskii B.V., Sivordova L.E., Poliakova Iu.V., Kravtsov V.I., Zborovskii A.B. Prognosticheskoe znachenie narushenii lipidnogo obmena v patogeneze osteoartrioza [Prognostic value of lipid metabolism disorders in osteoarthritis pathogenesis]. *Vestnik Rossiiskoi Voenno-meditsinskoi Akademii*, 2013, no. 2 (42), pp. 29-32. (in Russian)
- Ali Aus Tariq, Crowther Nigel John. Health risks associated with obesity. *Journal of Endocrinology. Metabolism and Diabetes of South Africa*, 2005, vol. 10, no. 2, pp. 56-61. Available at: <http://dx.doi.org/10.1080/22201009.2005.10872117>.
- Grekhov R.A., Aleksandrov A.V., Alekhina I.Iu., Zborovskii A.B. Ispolzovanie pokazatelei kachestva zhizni pri vosstanovitelnoi terapii vospalitelnykh i degenerativnykh revmaticheskikh zabolevani [Using the indicators of quality of life in restorative therapy of inflammatory and degenerative rheumatic diseases]. *Terapevticheskii Arkhiv*, 2009, vol. 81, no. 12, pp. 51-54. (in Russian)
- Gubin A.V., Borzunov D.Y., Marchenkova L.O., Malkova T.A., Smirnova I.L. Contribution of G.A. Ilizarov to bone reconstruction: historical achievements and state of the art. *Strategies Trauma Limb Reconstr.*, 2016, vol. 11, no. 3, pp. 145-152. DOI: 10.1007/s11751-016-0261-7.

5. Branca F., Nikogosian H., Lobstein T., eds. *Problema ozhireniia v evropeiskom regione voz i strategii ee resheniia* [Obesity problem in WHO European region and strategies of its solution]. Daniia, 2009. 408 p. (in Russian). Available at: http://www.euro.who.int/_data/assets/pdf_file/0011/74747/E90711R.pdf (accessed 12.05.18)
6. Aspden R.M., Scheven B.A., Hutchison J.D. Osteoarthritis as a systemic disorder including stromal cell differentiation and lipid metabolism. *Lancet*, 2001, vol. 357, no. 9262, pp. 1118-1120. DOI: 10.1016/S0140-6736(00)04264-1.
7. Chepeleva M.V., Volokitina E.A., Karmatskikh O.L. Osobennosti immunnogo statusa patsientov s distroficheskimi zabolevaniiami tazobedrennogo sustava [Specific characteristics of the immune status of patients with the hip dystrophic diseases]. *Materialy VIII Vserossiiskogo foruma s mezhdunarodnym uchastiem im. Akademika V.I. Ioffe «Dni Immunologii v Sankt-Peterburge 2004»* [Materials of VIII All-Russian Forum with international participation "Days of Immunology in St. Petersburg 2004"]. *Meditinskaiia Immunologiya*, 2004, vol. 6, no. 3-5, pp. 407. (in Russian)
8. Orekhovich V.N., ed. *Sovremennye Metody v Biokhymii* [Modern Methods in Biochemistry]. M., Meditsina, 1977, 392 p. (in Russian)
9. Koroliuk M.A., Ivanova L.I., Tokarev V.E. Metod opredeleniia aktivnosti katalazy [The technique of catalase activity determination]. *Laboratornoe Delo*, 1988, no. 1, pp. 16-19. (in Russian)
10. Gaidyshev I.P. *Reshenie nauchnykh i inzhenernykh zadach sredstvami Excel, VBA i C/C++* [Solution of scientific and engineering problems by means of Excel, VBA and C/C++]. SPb., BKhV-Peterburg, 2004, 512 p. (in Russian)
11. Chepeleva M.V., Sazonova N.V., Kuznetsova E.I. Kontsentratsii immunoglobulinov i tsitokinov v sinovialnoi zhidkosti patsientov s osteoartrozom pri nalichii i otsutstvii klinicheskikh priznakov sinovita [Concentrations of immunoglobulins and cytokines in synovial fluid of patients with osteoarthritis in case of the presence and absence of synovitis clinical signs]. *Sibirskii Nauchnyi Meditsinskii Zhurnal*, 2015, vol. 35, no. 2, pp. 69-73. (in Russian)
12. Askarov S.E., Volokitina E.A., Sazonova N.V., Chepeleva M.V., Shved N.S. Vliianie kompleksnogo konservativnogo lecheniia na uroven syvorotochnykh tsitokinov u bolnykh s I-II stadiiami osteoartroza krupnykh sustavov [The effect of complex conservative treatment on the level of serum cytokines in patients with large joint osteoarthritis of I-II Stages]. *Genij Ortopedii*, 2009, no. 1, pp. 58-61. (in Russian)

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