DOI 10.18019/1028-4427-2019-25-2-188-192

Comparative biochemical analysis of synovial fluid constituents in infected cases following total knee replacement

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Introduction Biochemical parameters of synovial fluid constituents were reviewed in patients prior to total knee replacement (TKR). The diagnostic value of certain biochemical tests was identified as prognostic factors in the development of infection. **Purpose** Comparative biochemical analysis was performed for pathogenic microflora free synovial fluid of patients grouped according to characteristics of infectious complications prior to TKR. **Material and methods** Synovial fluid samples with isolated strains of *Staphylococcus aureus*, *Klebsiella sp*. and aerobic gram-positive bacilli were excluded from the study. Total protein and its fractions, electrolytes, lipid peroxidation and catalase levels were measured in the synovial fluid. Electrolytes, lipid peroxidation and catalase were determined in the synovial fluid with exclusion of samples with identified aerobic gram-positive bacilli in bacterial culture. **Results** Preoperative assessment of synovial fluid showed changes in cholesterol concentration and systemic electrolyte index in TKR patients later grouped into periprosthetic infection and non-infection cases. No changes were seen in protein panel. Levels of total protein and albumin were elevated in both groups. **Conclusion** Our findings suggest that biochemical parameters of synovial fluid can serve as a prognostic tool for infection following TKR.

Keywords: joint replacement, synovial fluid, purulent complications

INTRODUCTION

One of the most concerning complications associated with a total knee replacement (TKR) surgery is an infection that is threatening, difficult to treat, leading to higher medical costs. A positive outcome with maintained limb function is a dubious question. The overall rate of infection following primary TKR is 1.6–2.5 % [1, 2]. Russian authors report infection rate of 5–6 % [3]. Difficulties in diagnosis are ascribed to non-specificity of diagnostic tests and polymorphic clinical picture [3, 4]. Early infectious complications are often caused by Staphyloccocus aureus and Staphyloccocus epidermidis due to postoperative contamination or direct spread from postoperative hematoma or infected skin. Delayed infection can result from hematogenous dissemination of other microbes [5]. Synovial fluid (SF) microbiological test is important for diagnosis of infectious complications. Detection of bacterial pathogens in synovial fluid is a contraindication to elective surgery of TKR. Synovial cytogram count is a valuable diagnostic tool in addition to microbiological examination. Degenerative disorders of joints are often characterized by cytosis in synovial fluid with prevailing lymphocytes count and increase in covering cells. However, diagnosis based on SF cytology has a merely descriptive nature. C-reactive protein (CRP) concentration is a biological marker used in clinical practice to identify inflammation or infection in the joint.

The purpose of the study was to make comparative biochemical analysis of pathogenic microflora free synovial fluid prior to TKR in patients who developed infection and who did not.

MATERIAL AND METHODS

SF of 467 patients with degenerative joint disorders of the knee was examined prior to TKR. There were 101 males and 366 females. Periprosthetic joint infection (PJI) was observed in 6.6 % (n = 31). PJI group (Group I) consisted of 22 female and 9 male patients with the mean age of 62.7 ± 1.61 years. Non-PJI group (Group II) included 344 female and 92 male patients with the mean age of 60.4 ± 2.09 years. The patients were followed up to 10 years. Positive synovial fluid cultures were excluded and no surgery was offered for the patients. All patients provided a written informed consent on medical intervention and publication of the findings. Control samples originated from 65 deceased donors (34 males and 31 females) with the mean age of 54.3 ± 0.9 years and no articular pathology identified. SF was extracted within a short period of

Matveeva E.L., Naumenko Z.S., Spirkina E.S., Gasanova A.G., Talashova I.A., Rakhmatulina A.A. Comparative biochemical analysis of synovial fluid constituents in infected cases following total knee replacement. *Genij Ortopedii*, 2019, T. 25, No 2, pp. 188-192. DOI 10.18019/1028-4427-2019-25-2-188-192. (In Russian)

time in compliance with RF Ministry of Health Order No. 161 dtd April 24, 2003 "On Guidelines of forensic medical examination". Aragose gel electrophoresis was used to measure SF proteins with PARAGON kit and APPRAISE multiprocessor densitometer. Total protein (TP) was measured with colorimetric method.

Oxidative protein modifications (OPM) (primary aldehydes, secondary ketones) of articular fluid were determined as 2.4-dinitrophenylhydrazine protein-bound reaction measured in optical density unit per one milligram of TP [6]. Conjugated dienes (CD), primary lipid peroxidation products, were detected with spectrophotometry by a difference in optical density between test and control samples. Secondary peroxidation product (malondialdehyde, MDA) was identified with thiobarbituric acid reaction and incubation for greater sensitivity and accuracy of assessment of MDA in human biological fluid [7]. Concentration of lipid peroxidation primary and secondary products was calculated per mg of SF total lipids (TL). Anti-oxidant protection was evaluated by activity of SF catalase enzyme [8]. Electrolyte imbalance was evaluated with spectrophotometry measuring inorganic phosphate, total calcium (Ca), magnesium (Mg) and chloride ions levels. SF acid phosphatase (AcP) and alkaline phosphatase (AlP) activity was measured with kinetic method.

The formula was used to calculate systemic electrolyte index (SEI):

$$SEI = (Ca \times Mg \times Cl) / PO_4,$$

where *Ca*, calcium concentration (mmol/L); *Mg*, magnesium concentration (mmol/L); *Cl*, chloride concentration (mmol/L); PO_4, phosphate concentration (mmol/L).

Phosphatase index (PI) was calculated with the formula:

PI = Acid phosphatase / Alkaline phosphatase.

SF and wound discharge were microbiologically tested. Aerobes and facultative anaerobes were cultured and isolated using Levin medium, blood agar with 5 % of bovine blood, egg yolk salt agar and incubated at 37o C during 24–48 hours. Bacteria were identified with routine methods and ATB Expression microbiological culture analyzer ("BioMerieux", France).

The median and the interquartile range were calculated for each group. The Wilcoxon rank-sum test was used to evaluate statistically significant differences in the findings comparing two related samples. Non-parametric statistics and normality test were conducted with Microsoft EXCEL-2010 and eStat 1.0 was employed for sample distribution. The study was approved by Ethics Committee of the Russian Ilizarov Scientific Centre "Restorative Traumatology and Orthopaedics", Kurgan, Russia and conducted in compliance with informed consent of the patients.

RESULTS AND DISCUSSION

The review of major components of mineral metabolism and enzyme activity in bone remodeling revealed a number of changes presented in Table 1.

Increase in bone resorption (collagenase and alkaline phosphatase) was noted PJI group. Statistically significant differences in SF SEI were observed preoperatively in the groups of comparison. Mineral metabolism characteristics and enzyme activity in bone remodeling were studied by researchers for identification of metabolic disorders in patients with infectious complications following arthroplasty of major joints [9]. However, statistically significant differences were noted in integral parameter only, namely, SEI. SF lipid spectrum demonstrated several deviations in lipid peroxidation measurements as compared to controls (Table 2).

Statistically significant differences in cholesterol level were observed in PJI and non-PJI groups. Infection rate is statistically higher in patients with lipid metabolism disorders [10, 11] which is likely due to a higher risk of hardware instability with greater loading on the prosthetic joint. The risk of infectious complications in patients with lipid metabolism disorders can be largely associated with the lipid spectrum status, SF cholesterol, in particular, rather than the patient's excessive weight. Compensatory reaction can be suggested in successful TKR cases with increased cholesterol level compared to that in controls, and when this compensatory reaction is inadequate patients are at greater risk of infection.

SF protein measurements in the knee joint of PJI and non-PJI patients are presented in Table 3. Acute phase proteins, immunoglobulin and interleukins are conventional inflammation markers. Serum IL-6 has been shown to have high diagnostic value for recurrence in patients with periprosthetic infection of the hip joint [12]. High levels of SF total protein and albumin being associated with degenerative changes in the SF were recorded in both groups prior to TKR [13]. Various changes in protein spectrum were observed in groups of comparison in absence of statistically significant differences between the groups.

Table 1

Measurements of SF minerals and phosphatases in the knee joint of PJI and non-PJI patients

Parameter	Control group (n = 65)	PJI group I (n = 31)	Non-PJI group II (n = 436)
Ca, mmol/L	1.89 (1.64;2.31)	1.60 (1.22;1.82)*	1.50 (1.05;1.90)*
PO_4, mmol/L	2.01 (1.59;2.43)	1.28 (1.09;1.74)*	1.24 (0.86;1.62)*
Ca/P	0.99 (0.74;1.20)	0.95 (0.46;1.42)	1.28 (0.77;1.75)*
Mg, mmol/L	0.79 (0.74;0.88)	0.77 (0.72;0.80)	0.77 (0.70;0.84)
Cl, mmol/L	65.7 (53.9;77.85)	89.20 (86.98;94.80)*	88.50 (83.05;94.90)*
AcP, U/L	1.85 (1.35;2.93)	2.00 (1.70;3.35)	3.60 (2.00;5.98)
AlP, U/L	20.30(14.20;25.20)	46.45 (39.13;55.20)*	44.60 (31.10;62.50)*
PI	8.26 (5.70;13.58)	21.45 (9.78;31.74)	13.86 (6.58;28.13)
SEI	50.99(34.63;72.77)	60.27 (33.41;88.13)*#	94.12 (70.89;136.05)*

Note: * – significant differences in the variables in comparison with controls at $p \leqslant 0.05$; # – significant differences in the variables in comparison with PJI and non-PJI groups at $p \leqslant 0.05$.

Table 2

Measurements of SF lipid peroxidation in the knee joint of PJI and non-PJI patients

Parameter	Control group (n = 65)	PJI group I (n = 31)	Non-PJI group II (n = 436)
TL, g/L	0.69 (0.60;0.82)	1.52 (0.96;2.48)*	1.53 (0.93;2.67)*
MDA, nmol/L	1.95 (1.32;3.41)	3.45 (2.05;4.93)	2.87 (1.59;5.16)
CD, nmol/L	5.91 (3.94;13.03)	8.07 (5.90;13.63)	8.28 (3.85;17.90)
Catalase, mkatal	5.00 (2.54;12.08)	6.45 (3.23;12.49)	6.48 (3.26;11.35)
OPM: aldehydes	0.05 (0.04;0.07)	0.16 (0.07;0.20)*	0.10 (0.05;0.18)*
OPM: ketones	0.08 (0.04;0.12)	0.02 (0.01;0.03)*	0.02 (0.01;0.04)*
Cl, mmol/L	0.42 (0.29;0.049)	0.73 (0.52;0.92)#	1.11 (0.70;1.54)*
Tg, mmol/L	0.72 (0.38;1.18)	0.06 (0.03;0.26)*	0.19 (0.09;0.33)*

Note: * – significant differences in the variables in comparison with controls at $p \leqslant 0.05$; # – significant differences in the variables in comparison with PJI and non-PJI groups at $p \leqslant 0.05$.

Table 3

SF protein fractions in the knee joint of PJI and non-PJI patients

Protein spectrum		Control group (n = 65)	PJI group I (n = 31)	Non-PJI group II (n = 436)
TP	L	18.95 (8.38;27.73)	25.30 (22.10;29.40)*	25.50 (20.75;31.80)*
Albumin	%	64.20 (61.80;69.85)	68.90 (66.70;71.9)*	67.60 (62.85;70.95)
	L	11.71 (5.07;18.74)	15.94 (14.84;20.26)*	16.59 (13.48;21.70)*
Globulin	%	35.80 (30.15;38.20)	31.10 (28.10;33.30)*	32.40 (29.05;37.15)*
	L	6.55 (3.14;8.81)	8.42 (7.36;9.26)	8.25 (6.51;10.86)*
α1-globulin	%	3.90 (2.98;6.18)	2.70 (2.30;3.00)*	2.90 (2.50;3.50)*
	L	0.58 (0.41;0.98)	0.68 (0.57;0.71)	0.77 (0.56;1.04)
α2- globulin	%	5.05 (4.03;6.00)	4.40 (4.10;5.30)	4.80 (3.90;6.35)
	L	0.92 (0.42;1.73)	1.09 (0.85;1.52)	1.25 (0.92;1.74)*
β- globulin	%	9.75 (8.65;12.80)	11.10 (10.50;11.60)	11.00 (9.60;12.90)
	L	1.76 (1.00;2.52)	2.93 (2.40;3.32)*	2.79 (2.09;3.78)*
γ - globulin	%	13.75 (10.93;17.18)	12.00 (10.60;14.90)	12.80 (10.60;15.85)
	L	2.61 (1.32;3.41)	3.49 (2.92;3.67)*	3.19 (2.41;4.56)*
Albumin / Globulin		1.78 (1.55;2.31)	2.22 (1.99;2.55)*	2.08 (1.68;2.44)

Note* – significant differences in the variables compared with controls at $p \leqslant 0.05$.

Preoperative prophylaxis of PJI in TKR patients includes nasal, oropharyngeal and inguinal decolonization protocol [14]. Patients with isolated SF microbial pathogen are refused an elective surgical intervention. Rheumatoid arthritis, excessive weight, diabetes mellitus are risk factors for infectious complications [15]. Patients with metabolic disorders revealed through homeostatis parameters and SF measurements are at greater risk of hematogenous spread or reactivation of latent infection. Statistically significant differences in SF SEI and cholesterol parameters were seen in PJI cases.

CONCLUSION

SF metabolic changes are a risk factor for infectious complications. Clinical, radiological and laboratory tests are integrated in the algorithms of preoperative prophylaxis. SF microbial and biochemical examination can contribute to infection reducing surgical practice. Our findings suggest that SF biochemical parameters can serve as a prognostic tool for infection following TKR.

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Received: 27.08.2018

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