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Crescent Journal of Medical and Biological Sciences
Vol. 3, No. 4, October 2016, 128–131
eISSN 2148-9696

Identification of *Staphylococcus aureus* in Synovial Fluid of Patients Suspected to Arthritis Through PCR in Urmia City

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Abstract

Objective: Synovial fluid is composed of plasma ultrafiltration and hyaluronic acid secretion by synovial cells. Synovial fluid plays a role as softener and feeding cartilages without vessels. Infectious arthritis is one of the commonest arthritis and if the disease did not cure in the first days it would injure cartilages irreversibly. The goal of this study was identification of *Staphylococcus aureus* in synovial fluid of patients suspected to arthritis through PCR in Urmia city.

Materials and Methods: In this research synovial fluid contamination with *Staphylococcus aureus* and biochemical parameters such as the amount of glucose, protein and the number of white blood cells were studied. Amplification of *nuc* gene with the length of 279 bp using PCR method was applied to confirm *Staphylococcus aureus* isolation.

Results: For this, 400 cerebrospinal fluid samples were tested from hospitalized patients with arthritis in two hospitals in Urmia city during 3 months, which out of them 109 of samples were contaminated with bacteria including: 78 of isolates were *Staphylococcus aureus*, 12 of them were coagulase negative Staphylococci, 4 of them were *Streptococcus* and 15 of them were gram negative *bacilli*. Also, results showed that the amounts of glucose in positive samples in comparison to the amount of glucose in synovial fluid were significantly decreased. The amount of protein and the number of white blood cells in synovial fluid of positive samples were significantly higher in comparison to normal synovial fluid.

Conclusion: Results showed that *Staphylococcus aureus* is the most common agent at infections arthritis, therefore it is recommended to use an experimental treatment for *Staphylococcus aureus* prior to final results.

Keywords: Synovial fluid, Arthritis, *Staphylococcus aureus*, PCR.

Introduction

Synovial membrane, made up of three layers of cells, lines the cavities of joints, tendon sheaths, and bursae except the cartilage surface. Synovial fluid is produced from ultra-filtration of plasma and secretion of hyaluronic acid by synovial cells. It has a low volume so that even large joints such as knee have not more than 4 mL. In addition to the smoothing role, synovial fluid is also responsible for nutrition of avascular cartilage. Synovial effusion refers to accumulation of synovial fluid and if not treated in the first few days in septic arthritis, it can irreversibly damage cartilage (1).

There are three ways for organisms to reach a joint: (*a*) Blood, (*b*) Wound, (*c*) Spread of infection from an adjacent focus of osteomyelitis.

Infection can cause acute or subacute inflammatory reactions in articular tissue. Exudate accumulates in the joint and it may be turbid or clearly purulent depending on the severity of septic arthritis. The result of infection varies from full recovery with normal function to complete destruction of joints associated with fibrosis and bone disorders (2).

Routine tests of synovial fluid include evaluating the color, turbidity, cell count and differentiation, gram stain and culture, and examination with polarized microscope for crystals (3).

Since bacterial infections can destroy articular cartilage in less than 48 hours, inflammatory joints should be examined for the presence of infection. Secretion of cytokines and proteases and increased intra-articular pressure are major causes of cartilage damage, therefore, rapid culture and diagnosis of disease play an important role in the treatment protocol (4).

Materials and Methods

In this study, a total of 400 samples of synovial fluid were collected from hospitalized patients with arthritis from the knee joint in two hospitals in Urmia city during 3 months. Samples were sent to the laboratory. The samples were tested microbiologically and biochemically. After puncture, synovial fluid was collected in 3 sterile tubes which labeled as 1, 2, and 3 by collection order, and were examined in terms of chemical and serological tests, microbiological test, and counting, respectively.

1- Heparin-containing tubes were used for chemical tests. They were immediately centrifuged to separate liquid

Received 14 December 2015, **Accepted** 30 August 2016, **Available online** 16 September 2016

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from cells and to prevent intracellular components to enter the fluid.
2- The tubes used for microbiology tests were simple and sterile.
3- The tube containing ethylenediaminetetraacetic acid (EDTA) was used for cell count and differential.
Glucose and protein of sample in tubes labeled 1 were measured by biochemical automatic analyzer (Autolab®, AMS®; Rome, Italy) using commercial kits (Pars Azmoon, Iran).
Samples in tubes labeled 2 were cultured in blood agar medium and incubated at 37°C for 24 hours. Then round, white, or yellow colonies were transferred and cultured in mannitol salt agar and their purity was examined. The purified colonies were subsequently gram stained and colonies with gram-positive cocci were studied by biochemical-microbial tests.
The suspected colonies were undergone catalase test, and the catalase-positive colonies were then examined by coagulase test to isolate *Staphylococcus aureus* from other types of staphylococci (5). Colonies identified as *S. aureus* were transferred to the nutrient broth and stored in refrigerator. PCR was performed on DNA extracted from colonies identified as *S. aureus.* DNA was extracted from bacteria cultured in nutrient broth medium for 24 hours using the DNA extraction kit (Fermantas, Germany). The isolates of *S. aureus* were identified through amplification of *nuc* gene (279 bp) by PCR. Sequence of used primers is shown in Table 1 (6).
For negative control, distilled water was used instead of DNA in the reaction mixture. PCR was performed in a thermocycler (Eppendorf, Germany) with the following conditions:
- Initial denaturation at 95°C for 5 minutes
- Followed by 35 cycles, each consisting of denaturation at 94°C for 60 seconds, annealing at 59°C for 30 seconds, and extension at 72°C for 90 seconds
- Final extension at 72°C for 5 minutes.
PCR products were electrophoresed on 1.3% agarose gel containing 10 mg/mL ethidium bromide at 80 V for 1 hour. The gels were observed in a transilluminator (Uvitec, Europe) and the images were recorded.
Cell count of sample in tubes labeled 3 were measured by glass hemocytometer.
All data are expressed as mean ± standard error (SE) of mean. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan test; a value of $P < 0.05$ was considered statistically significant.

Results

The following results were obtained in this study conducted during 3 months on 400 samples of synovial fluid.

The Results of Culture

The results showed that of 400 samples of synovial fluid, 78 isolates were *S. aureus*, of which 35 were isolated from patients less than 20 years and 33 from 20-50 years patients. In addition, of 78 isolates, 40 were obtained from male patients and 38 from female patients.

The Results of Biochemical Tests

Two biochemistry tests of glucose and protein measurement were performed on 78 positive samples. As can be seen in Table 1, blood glucose levels of positive cases were decreased and its difference with glucose levels of normal synovial fluid was significant ($P<0.05$). The total protein of synovial fluid of positive cases was also increased and this increment was significant compared to the levels of protein in normal synovial fluid ($P<0.05$).

The Results of Cell Counting

Cells were counted in 78 positive samples. As can be seen in Table 2, white blood cells were increased and this increment was significant compared with the number of white blood cells in normal synovial fluid ($P<0.05$). According to white blood cell (WBC) differential count, more than 90% of cells were polymorphonuclear.

The Results of PCR

Seventy-eight samples, suspected to *S. aureus* according to chemical tests and microbial culture, were molecularly diagnosed through PCR and all of them produced band (Figure 1).

Discussion

Rheumatoid arthritis refers to be articular inflammation. Infectious arthritis develops when a bacterium spread through the bloodstream to a joint (7). Acute infectious

Table 1. Descriptive Statistics of Biochemical Parameters Measured in Positive Patients and Normal People

Minimum and Maximum Amount	Mean ± Standard Deviation	Group	Unit of Measure	Parameter
1-3	1.38 ± 0.54	Normal	g/dL	Protein
6-10	4.6 ± 1.2[a]	Patient		
60-100	75 ± 4.35	Normal	mg/dL	Glucose
32-51	42.4 ± 2.56[a]	Patient		

[a] $P<0.05$

Table 2. Descriptive Statistics of Haematological Parameters Measured in Positive Patients and Normal People

Minimum and Maximum Amount	Mean ± Standard Deviation	Group	Unit of Measure	Parameter
0-150	73.6 ± 7.42	Normal	/mm^3	WBC
4750-14100	8320.5 ± 20.31[a]	Patient		

[a] $P<0.05$

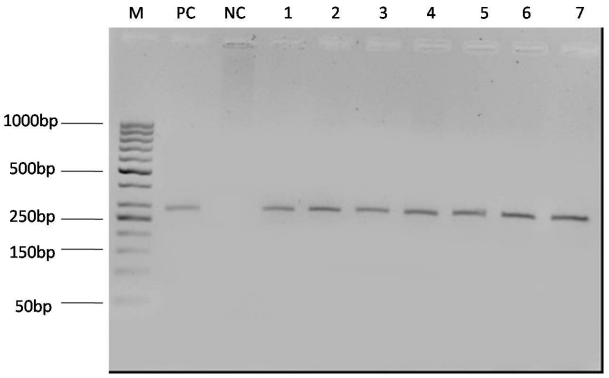

Figure 1. PCR Results. Well M: marker ; Well PC (*S. aureus ATCC29213*); well NC: negative control (distilled water); wells 1 to 7: positive samples of *S. aureus*.

arthritis is more common and is produced by *Staphylococcus* or *Streptococcus*. Chronic infectious arthritis is less common and is developed by *Mycobacterium tuberculosis* and *Candida albicans*. Knee is more likely to develop infectious arthritis (8). The present study examined the contamination of synovial fluid in people suspected to septic arthritis, and a total of 400 synovial fluid samples were tested which resulted in isolation of 78 cases of *S. aureus*. Jordan et al (9) studied synovial fluid of patients with articular infections in 2014, and of 219 samples tested, 15 were coagulase-negative staphylococci, 11 *S. aureus*, 2 *Pseudomonas*, and 3 *Enterobacter*. After studying 720 samples of synovial fluid (9), Yagupsky et al (10) isolated 15 cases of *Brucella melitensis*. Hughes et al investigated 805 samples of synovial fluid and found 45 cases of bacterial infection, with 20 cases of *S. aureus* and 11 cases of coagulase-negative staphylococci (7). In 2004, Trampuz et al examined synovial fluid in terms of leukocyte count and out of 99 samples of synovial fluid, 3 were suspected to septic arthritis in which the number of white blood cells was significantly increased (11). Jain et al collected synovial fluid of a 37-year-old man with arthritis and isolated *S. aureus* which was treated with metronidazole (12). In a study by Shirani and Zarei in 2001 in Rasoul Akram hospital in Tehran, among 140 samples of synovial fluid examined, 75, 5, 19, and 5 cases were *S. aureus*, beta-hemolytic *streptococcus*, gram-negative, and *S. epidermidis*, respectively (13). In this study, the number of synovial fluid leukocytes was 4700-137000 per mm^3 with 72% to 98% polymorphonuclear cells.

For final approval of *S. aureus* in this study, after isolation through culture and microbial tests, we amplified the *nuc* gene by PCR to produce a 279 bp fragment, and as mentioned in the results, 78 samples of synovial fluid were infected with *S. aureus* and these isolates were confirmed using molecular methods (14).

Synovial fluid was also examined biochemically in this study through measuring protein and glucose, and the biochemical results were consistent with the microbial results.

Since synovial fluid is chemically an ultra-filtrate of plasma, the values obtained from chemical tests were almost the same as serum levels. Therefore, a few numbers of synovial fluid chemical tests are clinically important. Measurement of glucose is the most common requested test. Synovial fluid glucose should be normally lower than blood glucose by a maximum of 10 mg/dL, because its significant decrease can be a marker of inflammatory and infectious diseases. In this study, synovial fluid glucose was significantly decreased and this reduction could be due to increased glucose consumption. For an accurate evaluation of synovial fluid glucose, it is necessary to measure also blood glucose in order to obtain a criterion for comparison, as a result, interpretation of synovial fluid glucose without knowing a patient's blood sugar levels has no diagnostic value; that is why the levels of blood sugar were also measured in this study (15).

The second biochemical test was the measurement of protein in the synovial fluid. Since large protein molecules could not pass across the synovial membrane, synovial fluid has a protein level of less than 3 g/dL which is equivalent to one third of its serum levels. The amount of protein increases in inflammatory and hemorrhagic diseases. In this study, protein levels were significantly increased in synovial fluid samples (16).

In this study, synovial fluid was also examined in terms of blood cells. A healthy adult has about 0-150 white blood cells per liter of synovial fluid. These cells include neutrophils, lymphocytes, monocytes, macrophages, and articular tissue cells. In differential count, neutrophils are less than 25% of the cells. Increased neutrophils indicate infection, while increased number of lymphocytes suggests non-infectious inflammation. In this study, the number of white blood cells was significantly increased compared to normal conditions, and their differential count showed that polymorphonuclear cells were predominant which reflect infectious arthritis more than other conditions.

It can be concluded from the mentioned results that biochemical tests and cell counting showed a bacterial arthritis in samples isolated from cultures.

In the end, it can be noted that no study has been performed so far regarding bacterial contamination of synovial fluid in West Azerbaijan province and the results of this study and familiarity of physicians with infectious arthritis-inducing bacterial pathogens can be very helpful for the treatment process.

Ethical Issues

We have no ethical issues to declare.

Conflict of Interests

Authors declare that there is no conflict of interest.

Financial Support

None.

Acknowledgments

None.

References

1. Lemmey AB, Patel W, Rottiers S. Efficacy of

progtressive resistance training for patients with rheumatoid arthritis and recommendation regarding its prescription. Int J Ckin Rheumatol. 2011;6(2):189-205. doi: 10.2217/ijr.11.10.
2. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-Joint Infections. N Engl J Med. 2004;351(16):1645-54. doi: 10.1056/NEJMra040181.
3. Font Vizcarra L, Garcia S, Martínez Pastor JC, Sierra JM, Soriano A. Blood culture flasks for culturing synovial fluid in prosthetic joint infections. Clin Orthop Relat Res. 2010;468(8):2238-43. doi: 10.1007/s11999-010-1254-3.
4. van der Heijden IM, Wilbrink B, Vije AE, Schouls LM, Breedveld FC, Tak PP. Detection of bacterial DNA in serial synovial samples obtained during antibiotic treatment from patients with septic arthritis. Arthritis Rheum. 1999;42(10):2198-203.
5. Carroll KC, Butel JS, Morse SA, Mietzner TA. Jawetz, Melnick, & Adelberg's Medical Microbiology. McGraw-Hill Education; 2015:985-1012.
6. Mehrotra M, Wang G, Johnson WM. Multiplex PCR for detection of genes for Staphylococcus aureus enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. J Clin Microbiol. 2000;38(3):1032-5.
7. Moussa I, Kabli SA, Hemeg HA, Al-Garni SM, Shibl AM. A novel multiplex PCR for molecular characterization of methicillin resistant Staphylococcus aureus recovered from Jeddah, Kingdom of Saudi Arabia. Indian J Med Microbiol. 2012;30(3):296-301. doi: 10.4103/0255-0857.99490.
8. Trampuz A, Piper KE, Jacobson MJ, et al. Sonication of removed hip and knee prostheses for diagnosis of infection. N Engl J Med. 2007;357(7):654-63.
9. Jordan RW, Smith NA, Saithna A, Sprowson AP, Foguet P. Sensitivities, Specificities and predictive values of microbiological culture techniques. for the diagnosis of prosthetic joint infection. Biomed Res Int. 2014;2014:180416. doi: 10.1155/2014/180416.
10. Yagupsky P, Peled N, Press J. Use of bactec 9240 blood culture system for detection of brucella melitensis in synovial fluid. J Clin Microbiol. 2001;39(2):738-9.
11. Trampuz A, Hanssen AD, Osmon DR, Mandrekar J, Steckelberg JM, Patel R. Synovial fluid leukocyte count and differential for the diagnosis of prosthetic knee infection. Am J Med. 2004;117(8):556-62. doi: 10.1016/j.amjmed.2004.06.022.
12. Jain S, Bui V, Spencer C, Yee L. Septic arthritic in a native joint due to Anaerococcus prevotii. J Clin Pathol. 2008;61(10):775-6. doi: 10.1136/jcp.2007.053421.
13. Shirani F, Zarei A. Evaluating clinical and laboratory signs of infectious arthritis in hospitalization patients. J Iran Med Sci. 2001;23:50-56.
14. Wang M, Li YX, Li JH. Epidemiology of Meningococcal meningitis in China 2008-2010. Disease Surveilliance. 2012;27(6):435-8.
15. Hughes JG, Vetter EA, Patel R, et al. Culture with bactec peds plus/F bottle compared with conventional methods for detection of bacteria in synovial fluid. J Clin Microbiol. 2001;39(12):4468-71. doi: 10.1128/JCM.39.12.4468-4471.2001.
16. Murray PR, Rosenthal KS, Pfaller MA. Medical Microbiology. Elsevier; 2015:802-25.