

Bacteriological and serological study on synovial Fluid in septic arthritis patients

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ABCTRAT

Background

Septic arthritis is the microbial infection of the joint constituents. Various microorganisms can be involved in arthritis like bacteria, viruses, fungi and other microorganisms like Mycoplasma. bacterial pathogens are the most significant.

Aims of the study:

- 1- To study the most important macroscopic and microscopic characters of synovial fluid samples in patients with septic arthritis.
- 2- Isolation and identification of bacteria involved in septic arthritis.
- 3- To perform some serological tests for patients with septic arthritis, like (CRP, ASOT, RF).
- 4- To study of white blood cell count, sugar and protein in synovial fluid of patients with septic arthritis.

Materials & Methods:

One hundred patients from both sexes and different age groups (1-80) years were included in this study. They were attending Department of Orthopedics and Joints Diseases in Ramadi General Hospital and Private Clinics of Orthopedics and Joints diseases during the period from May 2008 to January 2009. synovial fluid aspirates and blood specimens were taken from each one , these specimens were manipulated soon following bacteriological ,serological and biochemical required tests.

Results:

Twenty five (25) patients were suffering from septic arthritis and seventy five (75) of them were affected with aseptic type, males represented 67% of total patients.

The highest rate of arthritis was found within age group (19-49) years old while septic arthritis was higher within Age group (50-80) years old patients. Synovial fluid from 22 patients showed poor mucin while that from 23 patients had low viscosity and 10 patients showed purulent synovial fluid. Gram positive bacteria were most common pathogens, regarding single infections *Staphylococcus aureus* took the first rank of isolation (11 isolates) with (55%) followed by *Staphylococcus epidermidis* and *Escherichia coli*. Other bacteria were showing lower rate of isolation. patients with septic arthritis were showing more WBCs in their synovial fluid. The mean values of C- reactive protein within age groups (19-49) and (50-80) years of septic arthritis were 26 mg/L and 27 mg/L respectively. (17) patients with aseptic arthritis and one patient of septic arthritis showed positive rheumatoid factor test.

One child and another one patient within the same age group (19-49) years old were showed positive titer of ASOT test.

Mean sugar values in synovial fluid of septic type were lower than that of a septic type while all patients were showing higher protein values in synovial fluid.

Keywords: Septic arthritis, Bacterial arthritis, Arthritis

INTRODUCTION

The joint cavity is usually sterile space with synovial fluid and cellular matter including few White blood cells. Bruschi, L. J., (2005).

Septic arthritis is the inflammation of joint constituents. It means the infection of the lining membrane of the joint called the synovium. The knee is the most commonly involved joint accounting for about 50% of the cases. Livolsi, V. A., *et al.* (1993).

Various microorganisms may be involved in arthritis like bacteria, viruses, fungi and other microorganisms like Mycoplasma. Although any infectious agent may cause arthritis, bacterial pathogens are the most significant. Dale, C. D., and Federman, D. D. (2003).

The most common mode of spread of septic arthritis is haematogenous with the predisposing factors like intravenous drug use, presence of indwelling catheters and immunocompromised status. The other predisposing conditions are rheumatoid arthritis, gout or osteoarthritis. Ryan, M. J. *et al.* (1997).

Gram positive and gram negative bacteria are imposed in septic arthritis like *Staphylococcus aureus*, *Staphylococcus epidermidis*, *pyogenes*, *Neisseria gonorrhoeae*, *gram negative bacilli*, and *Myco tuberculosis*. Ryan, M. J. *et al.* (1997) and Dickie, A. S. (1986).

Septic arthritis must be diagnosed quickly and treated with antibiotics, surgery is more effective especially if pus is thick then a repeat aspiration may be required depending on the clinical signs and symptoms. Laurent, R. and Gleeson, R. (2000). In our community this conflict still needs more investigation so this study is devoted.

MATERIALS AND METHODS

Synovial fluid aspirate was taken from (100) patients attending the Department of Orthopedics in Ramadi General Hospital and Private Orthopedic Clinics during the period extended from May 2008 to January 2009. Synovial specimens were taken by senior Orthopedist from patients of both sexes and age groups (1- 80) years. Synovial fluid was aseptically transported to the lab in a cool box as soon as possible for processing physical characters (color, viscosity) were reported soon after aspiration.

Three ml of venous blood were taken aseptically from each patient and transferred to clean and dry plane tube (without anticoagulant) for serum collection after clotting and centrifuged the blood at 2500 rpm for 5 minutes pooled serum was kept in Epindrof tubes at -20°C. to be used.

Synovial fluid investigation:

Physical characters of Synovial fluid (color, viscosity), Biochemical tests on synovial fluid include (Mucin clot test, Sugar and protein estimation and PH) were performed following Davidson, I. and Henry, B. J. (1974).

Bacteriological study was done following Washington *et al.* 2006, by direct smears examination stained with Gram stain and Ziehl-Neelsen stain and inoculation aerobically on Blood agar and MacConkey agar plates and incubated at 37°C for 24 hours. Inoculated chocolate agar plates were incubated under 5-10% of CO₂. blood agar plates were incubated anaerobically using anaerobic Gaspack system Oxoid (France) for 48hr at 37°C. Bacterial identification by using different biochemical tests as (Oxidase test, Catalase test, Coagulase test, Api system (Api 20 E Aerobic, and Api system (Api 20 Anaerobic). Serological tests in this

work included C- Reactive protein test (CRP), Antistreptolysine O titer test (ASOT) and Rheumatoid factor (RF) test, white blood cell count was done for Synovial fluid samples following Davidson, I. and Henry, B. J. (1974).

Statistical analysis

Data qualitative of this study had been analyzed statistically using Chi (X²) square test to find the significance of probability level (P) value of the increase or decrease for all studied

factors. Data had been analyzed statistically using SPSS program.

RESULTS

One hundred patients from both sexes and different age groups were included in this study. Twenty five (25) of them were suffering from septic arthritis and (75) were suffering from other types of arthritis. Regarding sex distribution, males were showing higher rate of arthritis than females (table 1) (table 2).

(Table -1) Distribution of age and sex among patients with arthritis.

Age groups (Years)	Males		Females		Total	
	No	%	No	%	No	%
≤18	6	85.7	1	14.3	7	7%
19-49	38	60.3	25	39.7	63	63%
50-80	23	76.6	7	23.4	30	30%
Total	67	67%	33	33%	100	100%

(Table -2) Distribution of patients according to the type of arthritis.

Age Groups (Years)	Septic arthritis		Aseptic arthritis		Total	
	No	%	No	%	No	%
≤18	0	0.0	7	9.3	7	7
19-49	11	44	52	69.3	63	63
50-80	14	56	16	21.4	30	30
Total	25	100%	75	100%	100	100%

Majority of specimens (23) out of (25) were showing low viscosity with low mucinase activity and all of them were turbid, pH of samples range was between (6-7) (table 3). Out of 25 gram

stained smears 16 smears were showing gram positive organisms, while 9 of them were gram negative organisms, all specimens showed negative AFB test.

(Table -3) Physical characters and pH of synovial fluid in septic arthritis.

Physical characters of synovial Fluid		No. of samples
1- Mucin clot	poor	22
	Moderate-poor	2
	Moderate	1
2- Viscosity	High	2
	Low	23
3- PH	6	13
	7	12
4- Colour	Turbid yellow	4
	Purulent	10
	Turbid	6
	Gray	5

Regarding single infections *Staphylococcus aureus* which took the first rank of isolation (11 isolate) with (55%) followed by *Staphylococcus epidermidis* and *Escherichia coli* (10%) for each one, other species, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*,

Peptostreptococcus sp., *Klebsiella sp.*, and *Neisseria gonorrhoeae*, were showing (5%) equally in (table 5). *Staphylococcus aureus* showed the highest rate of mixed isolation with other bacterial types like with *E coli* (40%), with *S. epidermidis*, and *Proteus sp.* (20%) for each (in table 4).

(Table -4) Types and numbers of mixed bacterial isolates in patients with septic arthritis.

Types of M.O	No of isolates	%
Staphylococcus aureus and Staphylococcus epidermidis	1	20
Staphylococcus aureus and Escherichia coli	2	40
Klebsiella and Pseudomonas aeruginosa	1	20
Staphylococcus aureus and proteus species	1	20
Total	5	100%

White blood cells mean values in synovial fluid specimens from patients with septic arthritis showed higher values than aseptic type (table 5). The mean values of C- reactive protein within age groups (19-49)

and (50-80) years of septic arthritis were 26 mg/L and 27 mg/L respectively, while the mean values for individuals from the same age groups suffering from aseptic arthritis were 21 and 16 mg/L respectively.

(Table -5) WBCs values in synovial fluid of patients with septic and aseptic arthritis.

Age groups (Years)	WBCs cell/cmm3 in Septic arthritis	WBCs cell/cmm3 in Aseptic arthritis
≤18	Rang 0 Mean 0	Rang (8000-45000) Mean (15714)
(19-49)	Rang (45000-90000) Mean (58636.36)	Rang (1100-50000) Mean (8382.692)
(50-80)	Rang (60000-90000) Mean (73214.29)	Rang (1000-20000) Mean (5631.25)

* Number of patients with septic arthritis =0.

Patients within aseptic arthritis groups were showing more positive results of Rheumatoid factor than those suffering from septic type. While one patient from each septic and aseptic type of arthritis were showing high ASOT result (table 6).

Biochemical investigations of synovial fluid in patients with septic arthritis showed lower mean sugar value in there synovial fluid while all patients were showing higher protein values in Synovial fluid (table-7).

(Table -6) CRP, RF, ASOT values in patients with septic and aseptic arthritis.

Age group (years)	Septic arthritis			Aseptic arthritis		
	CRP Mg/L	RF IU/ml	ASOT IU/ml	CRP Mg/L	RF IU/ml	ASOT IU/ml
≤18	0	0	0	R(12-36) M(17) (7 cases)	(2 case) *	(onecase) *
19-49	R(18-36) M(26) (11 case)	(onecase) *	(onecase) *	R(12-42) M(21) (52 case)	R(16-128) M(38) (15 case)	0
50-80	R(18-36) M(27) (14 case)	0	0	R(12-18) M(16) (16 case)	0	0

*RF value in septic arthritis (64 IU/ml) , ASOT value in septic arthritis (800 IU/ml)

* RF values in aseptic arthritis (32 IU/ml) , ASOT value in aseptic arthritis (400 IU/ml).

(Table- 7) Sugar and protein results from patients with septic and aseptic arthritis.

Age groups (years)	Septic arthritis		Aseptic arthritis	
	Sugar(mg/dl)	Protein(g/L)	Sugar(mg/dl)	Protein (g/L)
≤18	No any case	No any case	R(36-90) M(68) ±SD (20.16)	R(19-45) M(28) ±SD (9.47)
19-49	R(20-32) M(26) ±SD (3.12)	R(40-56) M(42) ±SD (5.01)	R(36-95) M(63) ±SD (13.83)	R(13-59) M(28) ±SD (9.00)
50-80	R(20-30) M(27) ±SD (3.86)	R(31-62) M(48) ±SD (8.76)	R(46-95) M(70) ±SD (10.38)	R(20-48) M(26) ±SD (4.97)

DISCUSSION

Arthritis is multifactorial disorder and one of these factors is infection so we found 25% of cases were septic type. Livolsi, V. A. (1993). Males showed the highest rate (67%) of arthritis among all studied groups. This was due to the physiological status of males and their exposure to the Occupational risk. This result was in agreement with (Geirsson, *et al.*, 2008), who found that 62% of males were affected with arthritis. But this result was disagree with 10 (Caksen and his Colleagues, 2000), who found that 22 (55%) were males. Wars in Iraq increased the risk of trauma and injuries for males more than females in this category. Although arthritis was more frequent among aged individuals due to the high risk factors of arthritis among them but we found the highest rate of arthritis within age group (19-49) years.

This might be due to many factors like sampling method which was unable as to get samples from aged individuals because of restrictions and difficulty of mobility of such group to reach clinics due to bad traffic and security in this city because of risky war or other risk factors like habitual, familial, nutritional, occupation of individuals that predispose arthritis among them early. This was in agreement with (Al-Baiatti, 2004), who found that the highest rate of arthritis was in (35-40) age group. Septic arthritis was not recorded in children in this study; this was absolutely due to sampling method. So we can not match this observation with other references who recorded different rates of septic arthritis among children.

In this study the highest rate of septic arthritis (56%) was found within age group (50-80) years as this age group often has Preexisting Medical Conditions (e.g., Diabetes mellitus) and other age related joint disorders. Septic arthritis almost occurs in the extremities of age, despite in depth research into the pathophysiology and treatment of septic arthritis, the morbidity and mortality are still significant. Vincent, G.M. and Amirault, J.D. (1990). Gavet, F. *et al.* (2005).

High rate of septic arthritis among patients over 50 years old was in agreement with (Brusch 2005), who found the highest

percentage of cases among over 50 years patients.

Intra articular infections lead to many pathological and physiological changes which cause shift of synovial fluid physical and chemical characters such as decreased viscosity, decreased mucinase activity, abnormal color, pus tinged, and that was observed in this study, Davidson, I. and Henry, B. J. (1974). Mucin clot test in most of the samples was poor degree due to the enzymes released from inflamed cells, Gaffney, K. *et al.* (1995). Viscosity test used as an indicator of disease activity, low degree of viscosity character due to inflammatory fluid the clump of hyaluronate-protein complex (mucin) will fragment easily reflecting the loss of integrity of hyaluronan, Sweiss, N. *et al.* (2009). Turbid color of synovial fluid was due to the number of WBCs and pus cells which determines the turbidity of inflammatory SF, also lipids, crystals and debris that accumulates in destructive forms of arthritis. Gatter, R. A. (1984). Low PH of studied Synovial fluid specimens was due to inflammatory processes and sepsis, which leads to increase hydrogen ions (H⁺) via lactate enzyme release in Synovial fluid, Dorwart, B. B. and Schumacher, H. R. (1975).

Microscopical examination of Synovial fluid specimens showed negative acid fast bacilli results during direct smear examination with Ziehl-Neelsen stain. This might be due to sampling from non tuberculosis patients. *Staphylococcus aureus* took the first rank of isolation (55%), 11 out of 20 cases bacterial isolates, this was in agreement with the ratio observed by many references like. Geirsson, J. A. *et al.* (2008). & Le Dantec, L. F. *et al.* (1996). and Al-Mashhadani S. S. (2003).

Higher rate of *S. aureus* isolation was observed by Munoz, G. and Raycraft, W. E. (2006), they found that *S. aureus* isolation rates from septic arthritis were (60% & 70%) respectively. This high ratio of *Staphylococcus aureus* isolation from the specimens of this study may be due to many factors related to this organism like virulence factors of this bacteria, as well as pathogenicity factors such as protein A, coagulase and biofilm formation capacity Brooks, G, F. (2004). *Staphylococcus*

epidermidis is opportunistic pathogen causing infection accidentally as in case of septic arthritis in studied patients Mahmoud, D. A. R., (2004). Unfortunately *S. epidermidis* may reach joints due to bad skin disinfection and low precautions during joint centesis and other joint manipulations Sweiss, N. *et al.*(2009). Infection with gram negative bacteria may be due to long hospitalization, migration from microorganisms of the neighboring infected foci or from bones, haematogenous route, postoperative complications, arthroscopies and therapeutic joint injections Canal, S. T. *et al.* (1998). Our results were similar to, Le Dantec, L. F. *et al.* (1996) and Mahmoud, D. A. R. (2004), who found that 35% cultivation results were gram negative organisms and 65% of them were gram positive organisms. In spite of the similarity in genera of gram negative bacteria isolated from patients in this study with results of other references like, Caksen, H. *et al.* (2000).; Al-Mashhadani S.S. (2003), and Mahmoud, D. A. R. (2004), but different rates of gram negative isolation were found between this study and them. Regarding mixed infection, isolation of *E coli* and *S. aureus* from the same specimen was logical because they are compatible in their growth and resist the antagonizing effect of each. Shirtliff, E. M. and Mader, J. T. (2002). This observation was also mentioned by (Brusch, 2005), who found 5% of cases anaerobic due to trauma or abdominal infection. In this study 75 of specimens showed negative cultivation results .This agreed with (Lyon & Evanich 1994), who found that 70% of the cases were culture negative . This high rate of negative cultivation results was attributed to either actual absence of infectious agent in synovial fluid, or to the fact that there is true pathogen requiring more microbiological investigation to detect them like *Brucella species*, *Chlamydia*, *Mycoplasma*, *Spirochetes* and viral infection, Washington *et al.* (2006). and Brooks, G. F. (2004).

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Bacterial infection induces an influx of WBCs at the site of joint infection, Roitt, I. *et al.* (2001)., this leads to an increase of WBCs counts in synovial fluid of patients with septic arthritis in this study. This was in accordance with (Brusch, 2005). Regarding CRP values in sera of our patients with septic arthritis, they were similar to that of (McCune & Golbus., 2001).

Increased acute phase proteins like CRP was ought to the effect of IL-6 released from liver, Roitt, I. *et al.* (2001).; Scott, L. D. and Kingsley, H. G. (2008).

We did not find significant differences among patients groups of this study for different types of arthritis, this confirms CRP as supportive non specific test in this category. Geirsson , J. A. *et al.* (2008).; Kherani, B. R. and Shojania, K. (2007).

Decreased glucose values in synovial fluid of patients with septic arthritis was due to the glycolytic activities of the white blood cells and utilization of sugar by invading bacteria, Brooks, G. F. *et al.*(2004). Our findings were in accordance with the findings of (6) who found that glucose levels will be lower in Synovial fluid in comparison to serum sugar values in patients with septic arthritis.

At the same time increased values of protein in Synovial fluid of patients with both types of arthritis was due to leakage of protein through the blood vessels and attracted microbial cells, Cohen, A. S. *et al.* (1975).

We can conclude from this study that physical and chemical characters are helpful in Synovial fluid manipulation. *Staphylococcus aureus* and *S. epidermedis* as well as *E coli* are considered as important pathogens in arthritis *S. epidermedis* could be considered in joint infections in patients with joint implants. Sugar values are useful for the differentiation of septic arthritis form other types of joint affections.

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ARABIC SUMMARY

دراسة بكتيرية ومصلية على السائل المفصلي لمرضى التهاب المفصل الخمجي

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خلفية الدراسة:

التهاب المفصل الخمجي هو خمج جرثومي لمكونات المفصل. هناك جراثيم مختلفة تسبب التهاب المفصل مثل البكتريات , الفيروسات والفطريات وجراثيم أخرى مثل المايكوبلازما. البكتريات هي الأكثر تأثيراً .
أهداف الدراسة:

- 1 - دراسة الخصائص العينية والمجهرية لسائل المفصل لمرضى التهاب المفصل الخمجي
- 2 - عزل وتوصيف الجراثيم المسببة لخمج المفصل
- 3 - إجراء اختبارات مصلية لمرضى التهاب المفصل الخمجي
- 4 - دراسة تعداد كريات الدم البيضاء و مستوى السكر والبروتين للسائل المفصلي لنفس المرضى .

المواد وطرائق العمل :

أجريت هذه الدراسة على مائة مريض من كلا الجنسين ولأعمار مختلفة تراوحت بين أقل من 18 عاماً إلى 80 عاماً . هؤلاء المرضى يعانون من التهاب المفاصل المختلف السبب راجعوا قسم المفاصل والكسور في مستشفى الرمادي العام والعيادات الخاصة لأطباء المفاصل والكسور في مدينة الرمادي غرب العراق للفترة من الأول من مايس 2008 إلى كانون الثاني 2009 .

سحبت عينات من السائل المفصلي من المرضى من قبل الأطباء الاختصاص وكذلك عينات دم من كل مريض وفحصت هذه العينات حالاً بإتباع الطرائق البكتريولوجية والسيرولوجية والكيمائية اللازمة لكل اختبار.
النتائج:

تبين أن خمسة وعشرين مريضاً من مجموع مائة مريض كانوا مصابين بالتهاب المفصل الخمجي بينما كان خمسة وسبعون مريضاً منهم يعانون من التهاب المفصل غير الخمجي وكانت نسبة الذكور 67% من مجموع المرضى . كانت أعلى نسبة إصابة بالتهاب المفصل الخمجي ضمن الفئة العمرية 50-80 عاماً وكانت أعلى نسبة من التهاب المفصل غير الخمجي ضمن الفئة العمرية 19-49 عاماً . كان السائل المفصلي من 23 مريضاً قليل اللزوجة وكان سائلاً متقيحاً لعشرة مرضى .

كانت الجراثيم الموجبة لصبغة كرام هي السائدة وجاءت المكورات العنقودية الذهبية بالمرتبة الأولى (55%) من العزل جاءت بعدها الملتويات العنقودية البيضاء ومن ثم الإشريكية القولونية وجاءت الجراثيم الأخرى بنسبة عزل أقل.

كان تعداد كريات الدم البيض في سائل المفصل لمرضى التهاب المفصل الخمجي أعلى من ذلك لمرضى التهاب المفصل غير الخمجي وكانت نسبة البروتين النشط نوع سي (CRP) أكثر في مرضى التهاب المفصل الخمجي (26 ملغ / لتر و 27 ملغ / لتر) للفئات العمرية (19-49) و (50-80) عاماً على التوالي .

كانت نتائج العامل الرثوي موجبة لسبعة عشر مريضاً كان احدهم يعاني من التهاب المفصل الخمجي بينما كانت نتائج اختبار الضد للستربتولايسن نوع سي (ASOT) موجبة لمريضين فقط احدهما كان طفلاً والأخر ضمن الفئة العمرية 19(49-) عاماً . كانت قيم السكر الكلوكوز في سائل المفصل من مرضى خمج المفصل أكثر من مرضى التهاب المفصل غير الخمجي وكانت نسبة البروتين عالية لجميع عينات السائل المفصلي من المرضى .