Study of the Bacterial Etiology of Cryptic Arthritis

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Abstract

Thirty patients showing clinical symptoms of arthritis with negative culture results were negative as done by the clinical laboratories. With cooperation of senior rheumatologist synovial fluid samples were collected .

The routine culture were repeated, serial, dilution, leucocyte lysate and biphasic cultures were done for them , in addition to direct exam .Cell-Wall Defective (CWD) colonies were found as tiny, pouched egg appearance in 13 instances: *Staphylococcus aureus* CWD, *Escherichia coli* CWD, *Streptococcus pyogenes* CWD and *Klebsiella pneumoniae* CWD. In 13 serial , dilution , lysate and biphasic culture and 2 routine culture as well as the remaining were culture-negative. Thus CWD *S. aureus* and CWD *E . coli* were the dominant arthropathic organisms.

الخلاصة :

شخص ثلاثون مريضاً يظهرون اعراض التهاب المفاصل مع حصول نتائج زرع سلبي في العمل المختبري الروتيني في المختبرات السريرية . ,وبمعونة اخصائي المفاصل جرى جمع سائل المفصل لهؤلاء المرضى .جلبت العينات الى المختبر واعيد الزرع الاولي لسائل المفصل بالطرق الاعتيادية وطرق الكائنات فاقدة الجدار والمتمثلة بالمزرعة المتسلسلة ،التخفيف وخلاصة الخلايا البيض والمزرعة ثنائية الطور بالاضافة الى الفحص المباشر . وظهر بان بكتريا فاقدة الجدار ذات مستعمرات دقيقة او شبيهه بمظهر البيض المقلي في 13 حالة . وعزلت منها *S.aureus* و *E.coli* و *S.pyogenes* و *K.pneumoniae* فاقد الجدار لمختلف تقنياتها . وكانت بكتريا *S.aureus* و *E.coli* هي السائدة في جميع العينات المدروسة .

Introduction

During health state no microbe shows any tropism for synovial membrane and / or joints. While in case of septicemia caused by an infection outside of the joints , organisms are deposited in or on the synovial membrane and only rarely proliferate to cause septic arthritis [1].

When bacteremia initiate joint infection, the initial growth of the bacteria is either in the syhovial membrane or in the adjacent bone. In another case an inflammation of the synovial membrane is quickly established and resulted in marked increase in leucocytes in the synovial fluid even though the fluid is sterile. When the bacteria have spread into joint fluid culture of the fluid, reveals the etiology of infection [1,2].

Cryptic bacterial arthritis (CBA) is the invasion of synovial membrane or the inflammatory cells by cell wall defective bacteria usually with extension into the joint space which is generally secondary to an infectious focus elsewhere in the body. The primary infection can be genital (*N . gonorrhae*), while, the cutaneous (*S. aureus)*, several other agents may be involved but in low frequency [1-3]. The present work is undertaken to

match the bacterial profile of cryptic bacterial arthritis.

Materials and Methods

The collection of synovial fluid was done by rheumatologist from 30 patients with pyogenic arthritis using standardized technique [3] . Processing of the joint fluid samples was in accordance with direct examination , routine, serial, dilution cultures, as well as biphasic culture as in [4].

Results

Monocytic (29.95%) lymphocytic (3.35%), neutrophilic (6.65%)as well as mixed infiltrates (13.35%) on the examination of synovial fluid samples were noticed, among which monocytic were the predominant. Although these infiltrates swollen spheres, swollen cylinders, swollen aggregates were matched in half of samples, routine, sequentional, leucocyte lysate as well as biphasic cultures have shown culture positive on routine media 2:30 (6.65%) with ordinary colony morphotype alone. Tiny or pouched egg appearance colony morphotype alone 7:30 (23.30%), tiny and ordinary colony morphotype 6:30 (20.05%) and 5:30 (50%) were negative by all culture methods (Tables 1,2,3,4,5,6). These were noted in six profiles. Z test was used to evaluate the different culture techniques (Table 7) .

Biochemical identification of the CWDB and ordinary bacterial isolates were showing *S aureus ,K . pneumonia, St . pyogenes* and *C . diphtheriae* .

Table 1 The culture positive in all culture methods

A- Serial culture method

Sample	B.A		M.A		V.A	
	+	-	+	-	+	-
Whole synovial fluid	2	0	2	0	2	0
Centrifuged supernate	2	0	2	0	2	0
Centrifuged sediment	2	0	2	0	2	0
Washed sediment	2	0	2	0	2	0
Lysed sediment	2	0	2	0	2	0

B-Dilution method

Dilution		B.A		M.A		V.A	
		+	-	+	-	+	-
lysing Cells supernate	1:10	2	0	2	0	2	0
	1:100	2	0	2	0	2	0
	1:500	2	0	2	0	2	0

C. Biphasic media technique

Sample	B.A		M.A		V.A	
lysing Cells sediment	+	-	+	-	+	-
	2	0	2	0	2	0

B.A :Blood agar, MA: Macconkey agar , V.A: Varient agar

Tabel 2 Culture is negative in the four first steps of serial culture method & positive for other techniques. (4 samples)

A-serial culture technique .

Sample	B.A		M.A		V.A	
	+	-	+	-	+	-
Whole synovial fluid	0	4	0	4	0	4
Centrifuged supernate	0	4	0	4	0	4
Centrifuged sediment	0	4	0	4	0	4
Washed sediment	0	4	0	4	0	4
Lysed sediment	4	0	1	3	4	0

B-dilution method

Dilution		B.A		M.A		V.A	
		+	-	+	-	+	-
Lysing Cells supernate	1:10	3	1	1	3	4	0
	1:100	4	0	1	3	4	0
	1:500	4	0	0	4	4	0

C . Biphasic media

Sample	B.A		M.A		V.A	
lysing Cells sediment	+	-	+	-	+	-
	4	0	2	2	4	0

Table 3 The culture negative for serial culture method & positive in the dilution & biphasic media methods

A- Serial technique

Sample	B.A		M.A		V.A	
	+	-	+	-	+	-
Whole synovial fluid	0	3	0	3	0	3
Centrifuged supernate	0	3	0	3	0	3
Centrifuged sediment	0	3	0	3	0	3
Washed sediment	0	3	0	3	0	3
Lysed sediment	0	3	0	3	0	3

B – Dilution technique

Dilution		B.A		M.A		V.A	
		+	-	+	-	+	-
Lysing Cells Supernate	1:10	3	0	3	0	3	0
	1:10	3	0	2	1	2	1
	1:500	3	0	1	2	3	0

C : Biphasic media technique

Sample	B.A		M.A		V.A	
lysing Cells sediment	+	-	+	-	+	-
	3	0	3	0	3	0

Table 4 The culture positive in serial culture technique except (the first step) and positive in the biphasic media technique but negative in dilution method

A . Serial culture technique

Sample	B.A		M.A		V.A	
	+	-	+	-	+	-
Whole synovial fluid	0	3	0	3	0	3
Centrifuged supernate	2	1	0	3	2	1
Centrifuged sediment	3	0	1	2	3	0
Washed sediment	3	0	1	2	3	0
Lysed sediment	3	0	3	0	3	0

B . dilution method

Dilution		B.A		M.A		V.A	
		+	-	+	-	+	-
Lysing Cells Supernate	1:10	0	3	1	2	0	3
	1:100	0	3	0	3	0	3
	1:500	0	3	0	3	0	3

C . Biphasic media technique:

Sample	B.A		M.A		V.A	
lysing Cells sediment	+	-	+	-	+	-
	3	0	3	0	3	0

Table 5 The culture negative in two first steps of serial culture method and positive for other techniques

A – Serial culture technique:

Sample	B.A		M.A		V.A	
	+	-	+	-	+	-
Whole synovial fluid	0	3	0	3	0	3
Centrifuged supernate	0	3	0	3	0	3
Centrifuged sediment	3	0	1	2	2	1
Washed sediment	3	0	0	3	3	0
Lysed sediment	3	0	1	3	3	0

B – Dilution technique

Sample		B.A		M.A		V.A	
		+	-	+	-	+	-
Lysing Cells supernate	1:10	1	2	0	3	1	2
	1:100	2	1	1	2	1	2
	1:500	1	2	1	2	1	2

C .Biphasic media method

Sample	B.A		M.A		V.A	
lysing Cells sediment	+	-	+	-	+	-
	3	0	0	3	3	0

Table 6 The culture negative for all techniques

A . Serial culture technique

Sample	B.A		M.A		V.A	
	+	-	+	-	+	-
Whole synovial fluid	0	15	0	15	0	15
Centrifuged supernate	0	15	0	15	0	15
Centrifuged sediment	0	15	0	15	0	15
Washed sediment	0	15	0	15	0	15
Lysed sediment	0	15	0	15	0	15

B- Dilution technique

Sample		B.A		M.A		V.A	
		+	-	+	-	+	-
Lysing Cells Supernate	1:10	0	15	0	15	0	15
	1:100	0	15	0	15	0	15
	1:500	0	15	0	15	0	15

C . Biphasic media method

Sample	B.A		M.A		V.A	
lysing Cells sediment	+	-	+	-	+	-
	0	15	0	15	0	15

Table 7 Comparison of routine with different culture techniques of synovial fluid using Z Test

Statistical Features	Types of culture methods					
	Comparison between centrifuged sediment with routine work	Comparison Super-nate with routine work	Washed sediment with routine work	Lysed sediment with routine work	Diluted infiltrate with routine work	Biphasic media with routine work
P1	0.16	0.23	0.26	0.40	0.23	0.50
P2	0.06	0.06	0.06	0.06	0.06	0.06
P	0.23	0.30	0.33	0.46	0.40	0.56
Zc	1.00	1.40	1.80	2.70	2.10	3.50
Zt	0.8438	0.9207	0.9649	0.9699	0.9826	0.99997
PL	0.01	0.01	0.01	0.01	0.01	0.01

P1: Possibility of any method , P2 : Possibility of routine methods (whole synovial culture) , P : Plus of P1+P2 , q : P1-P2 , Zc :Calculated Zt : Tablated Z , PL: Level of possibility.

Discussion

The culture negative in routine culture may be due to an antibiotic regmin, antibody coated bacteria, mycoplasm or CWDB or viruses [4].

Half of the studied 15:30 (50%) were possibly due to either one or more of the a formentioned causes: 4:30 samples (13.35%) were seen caused by CWDB (tables 1-5) of both gram positive and gram negative types. *S. aureus* was the dominant CWDB pathogen [5,6] followed by *E . coli* [7]. The profile of CWDB in cryptic arthritis are mostly concomitant with that on urine [8] blood [9] , chest [4] and meningitis[10], but with variable percentages. The Z test prove that leukocyte lysate and biphasic culture were the most valid among others.Thus in conclusion one may state the following:

1- Around 46 .35% of cryptic arthritis were associated with CWDB and monocytic infiltrates .
2- *S . aureus* and *E . coli* were the dominant for gram positive and gram-negative bacteria, respectively
3- Still 50% of cryptic arthritis were of negative culture and can be of virus or mycoplasma origins .

The infiltration of basophilic cells in the synovial fluid for percentage 6.65 % may be re-released to acute infection found during the chronic states [11] while the infiltration of monocytes in the synovial fluid for percentage 29.95% that of chronic state of disease. This according with many of studies revealed that the infiltration of monocytes in the synovial cavity when inter the bacterial toxins or lipopolysaccharide [12] the auto

immune disease led to chronic infection in the joint [13] .

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