Anaerobic osteomyelitis

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التهاب العظم والنقي اللاهوائي

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خلاصة : أجريت هذه الدراسة المستقبلية على 134 حالة من حالات التهاب العظم والنقي المقيَّح (الصديدي) لاستقصاء مدى انتشار الجراثيم اللاهوائية ودورها في عدوى العظام . فأخذت عينات من هذه الحالات وتم تلقيحها على الفور في غرفة العمليات أو قاعة المرضى ، ووضعت في حاضنة في ظروف هوائية أو لا هوائية من دون استعمال أوساط النقل . وتم استفراد الجراثيم اللاهوائية من 39 حالة من هذه الحالات (29%) التي تتضمن كل أنواع التهابات العظم والنقي المقيح . وكان مجموع عدد المستفردات الهوائية واللاهوائية 224 ، من بينها 50 مستفردة لا هوائية (22%) . وتبين أن العينات المرشوفة بالمحاقن كانت أفضل من تلك المأخوذة بالمسحات في عزل اللاهوائيات ، وأن معظم اللاهوائيات تم استفرادها من حالات التهاب العظم والنقي الطويلة الأمد .

ABSTRACT The prevalence and role of anaerobic bacteria in bone infection were investigated in this prospective study on 134 cases with pyogenic osteomyelitis. Specimens were inoculated immediately in the operating theatre or in the ward and incubated under aerobic and anaerobic conditions without using transport media. Anaerobic organisms were isolated from 39 of the 134 cases (29%) of all types of pyogenic osteomyelitis. The total number of aerobic and anaerobic isolates was 224, of which 50 were anaerobes (22%). Syringe-aspirated specimens were better than swab specimens for the isolation of anaerobes. Anaerobes were mostly isolated from osteomyelitis cases of long duration.

L'ostéomyélite anaérobie

RESUME La prévalence et le rôle des bactéries anaérobies dans les infections osseuses ont été examinés dans cette étude prospective englobant 134 cas de malades atteints d'ostéomyélite pyogène. Des prélèvements ont été ensemencés immédiatement dans la salle d'opération ou dans la salle d'hôpital et placés en incubation dans des conditions aérobie et anaérobie sans recourir à des milieux de transport. Des organismes anaérobies ont été isolés dans 39 cas sur 134 (29%) d'ostéomyélite pyogène tous genres confondus. Le nombre total isolats aérobies et anaérobies s'élevait à 224 parmi lesquels 50 concernaient des organismes anaérobies (22%). Les échantillons prélevés par aspiration à l'aide d'une seringue étaient meilleurs que ceux qui avaient été prélevés à l'aide d'un écouvillon pour isoler les organismes anaérobies, et ceux-ci ont été isolés dans la plupart des cas chez des patients atteints d'ostéomyélite de longue date.

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Introduction

Von Langenbeck (1844) reported the first case of osteomyelitis due to anaerobic bacterial infection. The patient died of a widely disseminated infection involving the vertebrae. The cause was unknown until the infection was recognized as being characteristic of actinomycosis [*1*]. Taylor and Davies [2] confirmed the presence of anaerobic organisms within sequestra. They showed, using a crude anaerobic technique, that anaerobes were present in up to 55% of 48 sequestra obtained from patients with chronic osteomyelitis. Those anaerobes were found more frequently inside dead bones than on the outside surfaces. Therefore, the assumption that anaerobic bacteria are a rare cause of osteomyelitis may be incorrect [*1,3–5*].

Anaerobic osteomyelitis of the feet is usually associated with diabetes mellitus, severe trauma or other underlying diseases that can cause vascular insufficiency. Some investigators have noticed that, as the duration of chronic osteomyelitis lengthens, the number of isolated species of anaerobes increases [*2,3*]. Osteomyelitis involving anaerobes is often mixed (i.e. also involves aerobes) and may typically yield between two and four anaerobic species and several aerobes on culture [*4–6*].

Materials and methods

One hundred and thirty-four (134) cases of pyogenic osteomyelitis were studied for anaerobic infection. The study was conducted in Basra University Teaching Hospital. The investigation period was from November 1992 to August 1993.

The specimens submitted for bacteriological diagnosis included: pus, live bone piece, sequestrum, infected tissue and venous blood. The specimens were either taken at the time of surgery in the operating theatre or taken in the ward. The aspiration of pus was carried out using a sterile disposable syringe. The aspirate was freed from visible air bubbles. In other cases, when there was a small amount of pus, the specimen was taken by a cotton swab. The solid specimens were transferred aseptically and immediately into a suitable liquid media (thioglycollate broth and brain–heart infusion broth). Blood cultures under aerobic and anaerobic conditions were done for eight cases with acute osteomyelitis. The important point in this study was that the inoculation of the specimens was done immediately in the operating theatre or in the ward (bedside inoculation) and incubated under aerobic and anaerobic conditions without using transport media. The proper and rapid cultivation method led to good results in the isolation of strict anaerobic bacteria.

The following media were employed for the isolation of anaerobic bacteria: blood agar, phenyl ethanol agar, bile eculin agar, egg yolk agar, Baird–Parker agar and Omata and Disraely's agar, while Shaedler broth was used for anaerobic blood culture.

Results

The cases were divided clinically into four main groups of osteomyelitis: haematogenous (44 cases), exogenous (62 cases), postoperative (21 cases) and mastoiditis (7 cases). The bacterial isolates were analysed (Table 1).

One case out of 25 cases (4%) of acute haematogenous osteomyelitis was infected with two anaerobic organisms (*Peptostreptococcus anaerobius* and *Fusobacterium nucleatum*), and that constituted 8% of the total number of aerobic and anaerobic iso-

Table 1 **Bacterial isolates from 134 patients with pyogenic osteomyelitis according to type of infection**

Bacterial isolate	No. (%) of isolates and number of patients (*n*) by type of infection											
	Haematogenous				Exogenous				Post-operative		Mastoiditis	
	Acute (*n* = 25)		Chronic (*n* = 19)		Acute (*n* = 26)		Chronic (*n* = 36)		(*n* = 21)		(*n* = 7)	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Aerobes (total)	23	(92)	14	(82)	41	(77)	57	(72)	28	(82)	11	(69)
Anaerobes (total)	2	(8)	3	(18)	12	(23)	22	(28)	6	(18)	5	(31)
B. fragilis					2	(3.8)	5	(6.3)	2	(5.9)	1	(6.3)
B. melaninogenicus					2	(3.8)	4	(5.1)	2	(5.9)	2	(12.5)
P. micros					1	(1.9)	3	(3.8)				
P. magnus			1	(5.9)							1	(6.3)
P. anaerobius	1	(4)	1	(5.9)	3	(5.7)	1	(1.3)	1	(2.9)		
F. nucleatum	1	(4)			1	(1.9)	2	(2.5)				
F. mortiferum			1	(5.9)								
Fusobacterium species							2	(2.5)			1	(6.3)
Prop. acnes					2	(3.8)			1	(2.9)		
A. israelii							3	(3.8)				
C. perfringens							1	(1.3)				
Eubacterium species					1	(1.9)						
Veillonella species							1	(1.3)				

lates (25) from the same group. Three cases out of 19 cases (16%) with chronic haematogenous osteomyelitis were infected with anaerobic organisms: one case yielded pure anaerobic growth, while the other two cases yielded mixed organisms (aerobic and anaerobic). There was one isolate (5.9%) for each of *P. magnus*, *P. anaerobius* and *F. mortiferum*, out of a total of 17 isolates.

Anaerobic microorganisms were isolated from 9 patients out of 26 cases (35%) with acute exogenous osteomyelitis; 8 cases yielded mixed growth of aerobic and anaerobic organisms, while one case yielded pure anaerobic bacteria. The anaerobic isolates constituted 23% of the total of 53 isolated organisms. There were four isolates (7.5%) for each of the *Peptostreptococcus* species, and *Bacteroides* species, two isolates (3.8%) of *Propionibacterium acnes*, and one isolate (1.9%) for each of *F. nucleatum* and *Eubacterium* species. Among 36 cases of chronic exogenous osteomyelitis, anaerobic organisms were isolated from 16 patients (44%). The anaerobic isolates constituted 28% of the total number of isolated microorganisms. All cases with anaerobic infection yielded mixed growth with aerobic bacteria. *Bacteroides* species were the most common anaerobic organisms encountered in chronic exogenous osteomyelitis (11.4%), followed by *Peptostreptococcus* species

(5.1%), *Fusobacterium* species (5.1%), *Actinomyces israelii* (3.8%), *Clostridium perfringens* (1.3%), and *Veillonella* species (1.3%).

Anaerobic bacteria were isolated from 6 patients out of 21 cases (29%) with postoperative osteomyelitis. The anaerobic isolates constituted 18% of the total number of isolates. Five cases yielded anaerobes mixed with aerobic organisms, while one case yielded pure anaerobic isolate. *Bacteroides* species were the most frequent anaerobic isolates (11.8%), followed by *P. anaerobius* (2.9%), and *Prop. acnes* (2.9%).

Seven cases presented with chronic mastoiditis of which four (57%) were infected with anaerobic organisms. The anaerobic isolates constituted 31% of the total number of isolates. *Bacteroides* species were the most commonly isolated anaerobic bacteria (18.8%), followed by *P. magnus* (6.3%), and *Fusobacterium* species (6.3%).

Therefore, from the data outlined above, anaerobic organisms were isolated from 39 cases out of 134 cases (29%) of all types of pyogenic osteomyelitis. The anaerobic organisms were mixed with aerobic organisms in 31 cases (79%), while the other 8 cases (21%) yielded anaerobic isolates only. Moreover, the total number of isolated aerobic and anaerobic bacteria, from all cases of pyogenic osteomyelitis, was 224 isolates; the anaerobes comprised 50 isolates (22%).

Sixty-five (65) specimens for bacteriological analysis were taken by syringe, of which 26 specimens yielded anaerobic organisms (40%) and 56 specimens were taken by swab, of which 13 yielded anaerobic organisms (23%). As shown in Table 2, the difference was statistically significant (SND = 2, $P < 0.05$), i.e. syringe-aspirated specimens seemed to be better than swab specimens for the isolation of anaerobic bacteria.

Table 2 **Difference between syringe and swab specimens regarding isolation of anaerobic bacteria**

Isolate	Specimen[a] obtained by				
	Syringe		Swab		Total
	No.	(%)	No.	(%)	
Aerobic	39	(60)	43	(77)	82
Anaerobic	26	(40)	13	(23)	39
Total	65	(100)	56	(100)	121

$\chi^2 = 3.88$, df = 1, $P < 0.05$

[a] Excluding nine cases which were culture-negative and four cases where the method of taking specimen was not given

Out of the total cases, 125 specimens yielded positive growth of microorganisms, of which 86 specimens yielded aerobic isolates only (with mean of duration of illness = 254 days), while 39 specimens yielded anaerobic isolates (with mean of duration of illness = 1548 days). As shown in Table 3, the difference between both duration means was statistically significant (SND = 3.9, $P < 0.01$), i.e. anaerobic organisms were more common in cases with long duration of illness than in cases with short duration of illness. Nine cases were culture-negative

Table 3 **Correlation of aerobic and anaerobic isolates with duration of illness**

Isolate	Mean duration (days)	Geometric mean ± *s*
Aerobic	254	39 ± 0.8
Anaerobic	1548	210 ± 0.99

SND = 3.9; P < 0.01

Discussion

In the present work, anaerobic organisms were isolated from 39 patients (29%) out of 134 cases with pyogenic osteomyelitis. Those 39 cases comprised all types of pyogenic osteomyelitis; 4 cases (10.3%) were among haematogenous osteomyelitis, 25 cases (64.1%) were among exogenous osteomyelitis, 6 cases (15.4%) were among postoperative osteomyelitis and 4 cases (10.3%) were among chronic mastoiditis. These results approximately coincide with those found by other investigators [*4,7*] but are not in agreement with those found by Lewis et al. [*1*]. In the latter study of 58 cases with osteomyelitis, they found that all patients affected by anaerobic organisms (23 cases) were from the exogenous group of osteomyelitis, and all cases of haematogenous osteomyelitis included in their study were infected with aerobic bacteria only. In addition, our results are not in agreement with those of Templeton et al. [*8*] who studied 61 cases of anaerobic osteomyelitis, and found that 29.5% of the cases were among the haematogenous group, 32.8% were among exogenous osteomyelitis and 37.7% were among postoperative osteomyelitis. These differences in the percentages of isolates by type of case of osteomyelites could be related to the differences in the related composition of the original cases. In our study we had a high proportion of exogenous osteomyelitis as a result of mine and shell injuries during the time of the study.

In the present study, the anaerobic microorganisms were involved in 29% of cases of osteomyelitis. Raff and Melo [*3*] reviewed a large series of cases published between 1936 and 1976; they found that only 12 cases (0.7%) out of 1603 cases were reported as anaerobic osteomyelitis. Waldvogel et al. [*9*] reported 247 cases of osteomyelitis seen at the Massachusetts General Hospital; only 2% of the cases were due to anaerobic bacteria. In a series of 257 cases studied by Kelly et al. [*10*], only 7 cases (2.7%) were due to anaerobes. In several large series reported by many investigators [*11–18*], no cases were attributed to anaerobic organisms. However, there were large numbers of negative culture cases among these studies but there was no mention of a possibility of anaerobic osteomyelitis among these negative culture cases. The failure to isolate anaerobes in these studies of bone infection could be related to the disuse of optimal transport and culture techniques for anaerobes. In addition, anaerobic cultivation was not done routinely for every case with osteomyelitis.

Lewis et al. [*1*] reported a somewhat higher incidence of anaerobic osteomyelitis than that obtained in the present work. They reported 58 patients with osteomyelitis from Wadsworth VA Hospital, of which 23 patients (39.6%) had anaerobic bone infections. They attributed this higher incidence of anaerobic osteomyelitis to the selection of cases referred to that hospital.

A lower incidence of anaerobic osteomyelitis than in this study was reported by Hall et al. [*4*]. Their study of 182 cases with osteomyelitis yielded 40 patients (22%) with anaerobic bone infections. They mentioned that those 182 cases were referred to the Mayo Clinic after failed treatment elsewhere, and they added that this was one of the reasons for the high incidence of anaerobes among their cases. Therefore, the results of the present study may indicate the actual prevalence of anaerobic osteomyelitis since all cases were admitted to one centre of orthopaedics only in Basra city and there were

no referred cases from other orthopaedic centres.

In the present work, the anaerobic isolates were 50 out of 224 isolates (22%) among cases of pyogenic osteomyelitis. These 50 isolates included 20 isolates (40%) of *Bacteroides* species, 13 isolates (26%) of *Peptostreptococcus* species, 8 isolates (16%) of *Fusobacterium* species. 3 isolates (6%) of *A. israelii*, 3 isolates (6%) of *Prop. acnes*, and one isolate (2%) each of *C. perfringens*. *Eubacterium* species and *Veillonella* species. This order of frequency was in agreement with that of Raff and Melo [3], who reported 149 patients with anaerobic osteomyelitis; the *Bacteroides* species were the most common anaerobic isolates (45%), then *Peptostreptococcus* species (21%), *Fusobacterium* species (9%), *Prop. acnes* (0.5%), and *Eubacterium* (0.5%). Ziment et al. [7] found that *Bacteroides* species were the most common anaerobic isolates (48%) among 17 cases of anaerobic osteomyelitis, followed by *Peptostreptococcus* species (30%) and *Fusobacterium* (9%). Nakata and Lewis [5] also found that *Bacteroides* species were the most common anaerobic isolates (31%) among 461 cases with anaerobic osteomyelitis, followed by *Peptostreptococcus* species (30%) and *Fusobacterium* species (15%). This order of frequency in both studies was in agreement with our results in the present study.

This study was in agreement with that of Lewis et al. [1] regarding the incidence of the following anaerobes in anaerobic osteomyelitis: *Actinomyces* species (4.7%), *Prop. acnes* (3.5%), *Clostridium* species (2.3%), and *Eubacterium* species (4.7%). However, they found in their study that the *Peptostreptococcus* species were the most frequent anaerobic isolates followed by *Bacteroides* species, an order of frequency different from our findings. The order of frequency of anaerobes in the present work also differs from that of Templeton et al. [8] and Hall et al. [4], who found that *Peptostreptococcus* species were the most common isolates in cases of anaerobic osteomyelitis, followed by *Bacteroides* species.

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