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Original Research Article

"OUTCOME OF ANAEROBIC CULTURE IN POSTOPERATIVE OSTEOMYELITIS CASES USING ROBERTSON'S COOKED MEAT BROTH-A PROSPECTIVE OBSERVATIONAL STUDY"

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ABSTRACT

INTRODUCTION: Osteomyelitis remains as a disastrous outcome postoperatively which is associated with increased morbidity and high economic burden. Although bacterial oraganisms have been identified as the major causative agents, role of anaerobic bacteria is discussed infrequently. Understanding the occurrence, type of osteomyelitis and its causative agents helps in treating osteomyelitis effectively.

OBJECTIVES: to analyze the occurrence of different types of osteomyelitis and the contributing factors to the condition. It mainly emphasis on screening the postoperative osteomyelitis specimen for anaerobic bacteria by culture using Robertson's Cooked Meat Broth (RCMB) medium and interpreting its outcome to aid in treatment.

METHODS: This prospective observational study was conducted for the period of 2 years from august 2018 to September 2020 in department of orthopaedics at AJ shetty institute of medical sciences, mangalore. 50 Patients with osteomyelitis occurring post fracture fixation or wound debridement were included in this study after obtaining informed consent. patients were evaluated clinically and radiologically. The infected specimens were processed for gram staining and anaerobic culture using robertson's cooked meat broth. Association between Variables was analyzed by using Chi-Square test for categorical Variables. Level of significance was set at 0.05.

RESULTS:

This hospital-based observational study included 50 patients with postoperative osteomyelitis. Majority (53.3%) presented with chronic osteomyelitis, with tibia being the most common bone involved(74%).Organisms were isolated via culture where

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aerobic bacteria were majority constituting 75.6% followed by 17.8% of facultative anaerobes. Only a single patient was found to have anaerobic infection in RCMB medium which was of bacteroides species. The Chi square value for type of osteomyelitis and type of infection was 31.177, p = 0.000, showing that there is statistically significant association between the two and depicting the occurrence of anaerobic infection in chronic osteomyelitis most commonly.

CONCLUSION:

Incidence of anaerobic infection in postoperative osteomyelitis remains less compared to aerobic organisms and Robertson's cooked meat broth medium remains an vital culture medium in isolating anaerobic organisms.

KEYWORDS: Anaerobic Culture, Osteomyelitis, Robertson's.

INTRODUCTION

Osteomyelitis infections are relatively common occurrences despite advancements in medical science and technology. Osteomyelitis refers to the infection of bone involving inflammatory process eventually causing bone death. Acute osteomyelitis evolves over days to weeks and characterized by inflammatory bone changes. Chronic osteomyelitis persists over months to years and usually associated with dead bone and reactive changes.

Since the coining of term osteomyelitis by nelaton in 1840, a variety of pathogens have been identified as causative agents, which includes bacteria, fungi and mycobacteria[1]. Among them, bacterial pathogens are known to form a large chunk which includes both aerobes and anerobes[2].since the reporting of first case of anaerobic osteomyelitis by von lagenbeck in 1844, various literatures have been followed with anaerobes being the causative agent[3].

The spread of pathogens may occur via open fractures, haematogenous spread in perioperative period or poorly managed surgical and post-surgical procedures[1,2].It usually results in chronic bone infection with organism surviving in necrotic bone tissues with increased survival. Aerobic and anaerobic bacteria presents clinically similar, which make prescribing specific antibiotics difficult, where culturing the specimen and its antibiotic specificity yields greater advantage.

Postoperative Osteomyelitis related morbidity is observed in developing countries due to improper or a lack of proper antimicrobial treatment of surgical wounds and limitations in the diagnosis procedures. Osteomyelitis is treatable with the timely and correct diagnosis of the condition and proper intervention through effective medical treatment. Thus, the present study aims to analyze the occurrence of different types of osteomyelitis and the contributing factors to the condition. It mainly emphasis on screening the postoperative osteomyelitis specimen for anaerobic bacteria by culture using Robertson's Cooked Meat Broth (RCMB) medium and interpreting its outcome to aid in treatment.

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MATERIAL AND METHODS:

This prospective observational study was conducted for the period of 2 years from august 2018 to September 2020 after obtaining institutional research board clearance and approval from the human ethics committee. Patients of all age with osteomyelitis occurring post fracture fixation or wound debridement were included in this study after obtaining informed consent. Patients previously on broad spectrum antibiotics, patients with co morbidity and immunocompromised patients were excluded.

Patients with post operative osteomyelitis were grouped as acute and chronic based on duration[2]. A pre-tested semi-structured questionnaire was used for the collection of data. A complete history, clinical examination, hematological and radiological investigations were made in all patients. Patient was clinically examined for the wound characteristics, type of discharge (serous, erythematous, purulent and separation of deep tissues), presence of sinuses, bone thickening and other signs of osteomyelitis.

Following the suspicion of osteomyelitis, patients underwent laboratory investigation for CBC(complete blood count),ESR(erythrocyte sedimentation rate) ,CRP(c-reactive protein) and radiological assessment using xrays. xrays were screened for signs of acute and chronic osteomylitis and were grouped as per cierny mader classification for chronic osteomyelitis[4].

Prior to obtaining specimens for culture, patients were confirmed not be on any antibiotics. Specimen which includes pus aspirate, drain fluid, bone tissues and biofilms were obtained either in operation theatre or in the wards as per the discharge (fig-1). Care was taken to avoid misdiagnosis by obtaining multiple deep samples in aseptic manner and preventing surface contamination while obtaining specimens.

RCMB medium was prepared in microbiology laboratory as required using fresh bullock heart, vitamin k, yeast extract and other nutritional suppliments with a Ph of 7.2 to 7.5,having a shelf life of 3 months(fig-2). The specimens were inoculated immediately into RCBM medium intraoperatively or bedside (fig-3). The inoclulated RCMB medium along with the specimen was transported to laboratory in less than 30 minutes and specimen was processed for gram staining and additionally for aerobic culture using mcconkey agar and 5% sheep blood agar. gram staining was used to identifying and confirming the organism along with culture. The inoculated RCM broth were incubated in an anaerobic workstation using automated gas flushing system anoxomat for seven days and inspected daily for anaerobic growth.

Results

50 patients were included in this hospital-based observational study as per inclusion and exclusion criteria and were followed up for 2 years. In our study, highest proportion of patients included were middle aged between 31-40 years (28%) followed by those belonging to 21-30 years of age (24%). The average age was 37±12 years. we had a male predominance who constituted 66% of our study group.

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As per figure 4,the distribution of participant in accordance to the bone involved in infection can be seen. Tibia was encountered as most infected in 74% of the participants. Femur was found infected in 12% of the participants. Other bones included fifth metatarsal, radius, humerus, and fibula.

As shown in fig 5, chronic osteomyelitis was reported in majority of the participants (62%) while 38% presented with acute osteomyelitis. Majority of the study participants exhibited increased ESR(86%), while all of the study participants reported elevated C-reactive protein (CRP) levels. Radiologically 82 % of study subjects showed osteomylitis changes which had medullary involvement in 22%, superficial in 8%, localized in 32% and diffuse in 30%(table -6).

Organisms were isolated via culture where aerobic bacteria were majority constituting 75.6% followed by 17.8% of facultative anaerobes. Only a single patient was found to have anaerobic infection in RCMB medium which was of bacteroides species(table-7).

Besides the frequency descriptive, the statistical measures of Chi-square and Phi and Cramer's were also calculated for the crosstabulation, as shown in Table 4.9. The Chi-square value for age and type of osteomyelitis was 7.277 with p=0.122, thereby showing they do not share a statistically significant relationship. Moreover, Chi-square value of 2.441 with p=0.118, for gender and osteomyelitis also showed no statistically significant association between the two. Further the Chi square value for type of osteomyelitis and type of infection was 31.177, p = 0.000, showing that there is statistically significant association between the two. Also, the Phi = 0.790, p = 0.000 showed strong strength of association between the two variables. Table 8 shows that aerobic infections were found in acute osteomyelitis cases, while facultative anaerobes and anaerobes were found only in chronic osteomyelitis cases.

DISCUSSION

Osteomyelitis has remained as a disastrous outcome postsurgery, inspite of advances in the medical field and has been associated with long term morbidity and high economical burden[5,6]. Even though newer antibiotics have been effective in treating postoperative osteomyelitis, emergence of antibiotic resistant strains has been a strenuous factor which is making treatment more difficult[7]. Understanding the associated factors and Isolating the organism and administering the specific sensitive antibiotic continues to be the best solution for the above problem[7].

The study results showed the highest proportion of cases from the age group of 32-40 years of age, followed by equal distribution among young and older adults. Kaur et al. (2008) encountered the highest incidence of osteomyelitis in the age group 16-30 years[8]. The involvement of the comparatively young age groups can be attributed to the greater likelihood of compound and trauma fractures occurring in this group which was the

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common predisposing factor[8].Concerning the type of bone involved, Tibia was affected in most of the patients. Researcher have reported similar findings where hematogenous osteomyelitis commonly affects the metaphysis of long bones such as tibia[9,10].Tibia also remains the most common site of open fracture[11,12]. The tibial shaft also presents an additional challenge of relatively low blood supply, which lowers the healing from infections, thereby making it a common site of osteomyelitis[13.]Khonglah et al. encountered femur as the common site of hematogenous chronic osteomyelitis,while tibia remained the common site for post-traumatic osteomyelitis[10.]

For the type of osteomyelitis, a higher proportion of patients presented with chronic osteomyelitis (53.3%), while the remaining were cases of acute osteomyelitis. Acute hematogenous osteomyelitis is most commonly seen in children, while chronic osteomyelitis predominates in adult age group[9.]The relatively low occurrence of chronic osteomyelitis in children could be attributed to improved care, timely administration of antibiotics and less incidence of open fractures[9]. As the upper end of tibia and lower end of femur present the regions of greater growth, thus these remain more prone to infections in children [8.]However in adults, chronic osteomyelitis is more common, especially in postoperative cases[15]. Hematogenous seeding of bacteria often remain the common source of contamination during surgeries, in addition to percutaneous sutures, suction drains and indwelling urinary catheters, which can serve as metastatic source of infection[13.]Open fractures and implant in situ are the main risk factors in case of postoperative osteomylitis[16,17].

All the patients in our study had higher than normal CRP levels, while only 14% of the patients had normal ESR levels(pre-operative).Michail et al. also recorded high serum inflammatory markers CRP and ESR, indicating its usage in diagnosis and follow-up[18.]

As per the study's primary aim to survey the incidence of anaerobic growth in post-operative infective patients of acute and chronic osteomyelitis, the results showed anaerobic bacteria's incidence only in 1 of the total 50 cases. However, other studies show comparatively greater incidence of anaerobic bacteria in chronic osteomyelitis. Shenoy et al. (2020) reported anaerobic growth in 39.2% of total cases,while 61% of the cases showed aerobic growth, amongst the cases of chronic osteomyelitis in coastal Karnataka[19]. Another study by mousa et al reported anaerobes in 22% of the cases, while aerobes constituted majority of the cases[20].Consequently,the overall incidence of anaerobes remains low as compared to aerobes. Statistically significant association was found between incidence of anaerobic infection and chronic osteomyelitis, denoting the most common occurrence of anaerobic infection in chronic osteomyelitis.

Number of factors may affect the growth of anaerobes in the RCMB medium like maintenance of pH, constituents of the broth, maintenance of anaerobic condition. Maintenance of inoculated medium in oxygen free environment becomes vital for which

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the automated gas flushing instrument, anoxomat was used in our study[21]. The automated gas flushing system helps achieve anaerobic atmosphere within minutes thereby helping shorten the exposure of inoculate plates to air[22].

The patient with anaerobic growth presented with chronic osteomyelitis and the association between type of osteomyelitis and infection causing agent was significant with $p < 0.05$.Al-habib et al in their study involving 184 patients, showed a prevalence of 8% for pure anaerobic infection which was found to be associated with chronic osteomyelitis as in our study.They even encountered facultative anerobes of 18 % as against 48 % of our study. *Bacteroides* and Peptostreptococcus were the more common organisms involved in their study while we encountered bacteroides in our only anerobic growth.

Limited anerobic growth in our study may be attributed to small sample size.The study also remains limited in terms of patient demographics and geographic area.Involving larger sample sizes with a diverse sample population may help in generalization of the results. The different culture media could also be tested for growing anaerobic bacteria which may yield additional growth that remains restricted with RCMB medium.

CONCLUSION

Statistically significant association between incidence of anaerobic infection and chronic osteomyelitis, denotes the occurrence of anaerobic infection most commonly in chronic osteomyelitis. Although incidence of anaerobic infection in postoperative osteomyelitis remains less compared to aerobic organisms, screening for anaerobic osteomyelitis via culture remains vital. RCMB medium remains an vital culture medium in isolating anaerobic organisms.

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FIG/TABLE-1 RCMB medium prepared in laboratory

FIG/TABLE-2 ASPIRATION OF PUS UNDER ASPECTIC PRECAUTION

FIG/TABLE-3 TRANSFER OF ASPIRATED MATERIAL INTO RCMB MEDIUM

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FIG/TABLE 4 - Distribution of participants based on bone infected

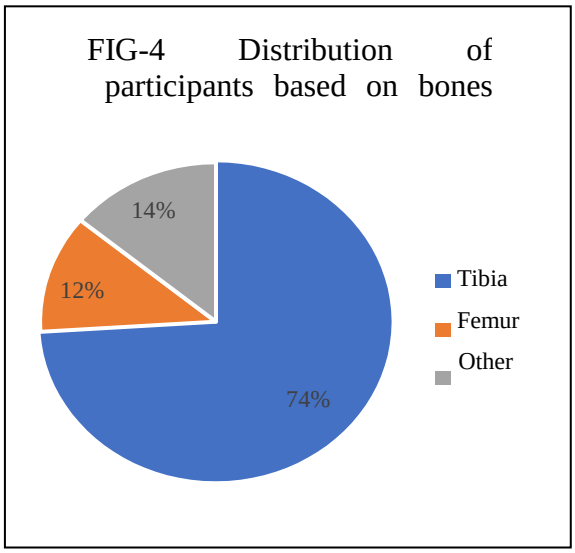

Figure 5- Distribution of participants based on osteomyelitis duration

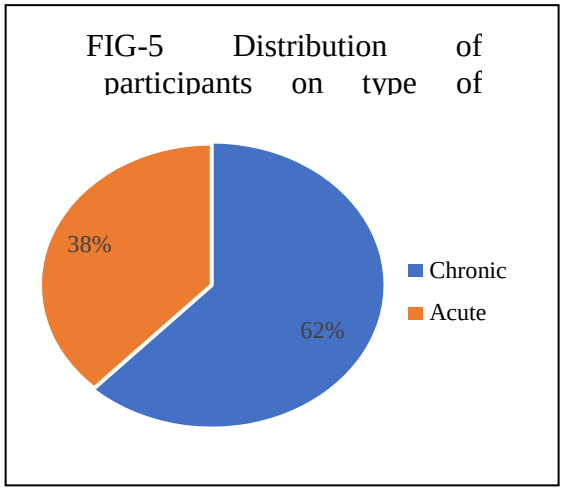

Table 6 Distribution of participants based on radiological characteristics

	Absent		Present	
	Frequency	Percentage	Frequency	Percentage

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Medullary	38	76.0	11	22.0
Superficial	46	92.0	4	8.0
Localized	34	68.0	16	32.0
Diffuse	35	70.0	15	30.0

Table 7 Distribution of participants based on the type of causative bacteria

	Frequency	**Percent**
Aerobic	23	46
Anaerobic	1	2
Facultative Anaerobe	24	48
No growth	2	4

Table 8 Crosstabulation between type of osteomyelitis and type of infection

		Culture Report					
		Aerobic	Facultative Anaerobe	Anaerobic	No growth	Chi square value	P-value
Type of Osteomyelitis	Acute	89%	0	0	11%	31.177	0
	Chronic	19%	77%	3%	0		