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Review Article

The Role of Histopathology in the Diagnosis of Chronic Osteomyelitis: A Retrospective Histopathological Review from a Tertiary Hospital in Northern Ghana

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Abstract

Background: The role of histopathology as a tool in diagnosing chronic osteomyelitis (COM) in Ghana has not been evaluated. The aim of this study was to evaluate the importance of histopathology as a tool in diagnosing and describing the clinicopathological features of COM in sequestrectomy and other small biopsy specimens. ***Material and methods:*** The following data on 75 patients with COM were collected: age, gender, primary and associated symptoms, history of prior trauma and the use of herbal treatment. The data was analysed using SPSS software version 26.0 (Chicago). Descriptive statistics were used to describe the data. Fisher's exact test was used to compare clinical variables of significance. Results: The median age of the patients was 12.0 years, with a male: female ratio of 2:1. The commonest primary presentation was a painful swelling 55 (73.3%, p<0.001). The sites commonly involved were: tibia (34.7%), femur (22.7%) and hip joint (10.7%). Approximately, 84.8% (p<0.001) of those with additional complains had a discharging sinus. A total of 27 (36.0%) patients had history of prior trauma, while, 41 (54.6%) had history of prior use of herbal treatment. There was a significant association between discharging sinus tract and the use of herbal medicine (P= 0.009). There was also an association between trauma and herbal medicine usage (p = 0.011). Conclusion: Chronic osteomyelitis commonly affect young males, with the long bones of the axial skeleton being the common site of infection. Prior trauma and the use of herbal medicine for fracture managements were the common risk factors.

Keywords: Chronic osteomyelitis, discharging sinus, trauma, herbal medicine, Northern Ghana, Tamale.

INTRODUCTION

Chronic osteomyelitis (COM) is a progressive inflammatory process caused mostly by pathogens, resulting in bone destruction and sequestrum formation.[1, 2] The infection may be limited to the cortex of bone, and/or extends into the bone marrow, the periosteum and the surrounding soft tissues.[1,3] COM is normally characterised by the lack of tell-tale clinical signs and symptoms.[4] It is a component of a spectrum of bone infection called osteomyelitis, the other components being acute, subacute, and acute chronic osteomyelitis.[4,5]

Decades ago, osteomyelitis was considered to be a lethal condition and the patients who survived had severe comorbidities.[6] The subsequent significant decline in osteomyelitis mortality and morbidity was attributed to optimal use of antibiotics that causative organisms were sensitive to, with or without surgery management.[6] In recent years, there is a substantial rise in osteomyelitis rate despite advanced diagnostic and treatment modalities. This rise is associated with major shifting in causes and risk factors of osteomyelitis where posttraumatic osteomyelitis was found to be the commonest variant.[4,7,8] Other researchers have documented sickle cell disease, orthopedic implants and diabetes as important risk factors for this disease.[7,8] For instance, Bahebeck *et al.,* [7] in their study with a relatively higher level of evidence, documented sickle cell disease (SCD) (Hemoglobin SS or SC) as a risk factor for chronic osteomyelitis.

Chronic osteomyelitis is normally characterised by the lack of tell-tale clinical signs.[4] In a given proportion of cases, painful bone swelling, chronic bone pain, erythema around the affected bone, swelling and bone tenderness, chills, low grade fever and general malaise are some of the commonly reported clinical symptoms. [1-4,7,8]

The diagnosis of osteomyelitis is based primarily on the clinical findings, with data from the initial history and physical examination, supported by laboratory investigations using full blood count with differentials, sedimentation rate and C-reactive protein level as well as blood and wound swab for culture and sensitivity testing. [4,9,10] However, not only are these investigative procedures, skill and time dependent, particularly for many developing countries, but it has also been reported that leukocytosis and elevations in the erythrocyte sedimentation rate and C-reactive protein level may not be informative. [10,11] Again, blood cultures are positive in up to one half of children even with acute osteomyelitis. [10,11] The situation is even worse in patients with COM which may present with periods of quiescence of variable duration and in which case blood and wound cultures may be negative. [10-12] Therefore, the role of culture and sensitivity methods as gold standards in the diagnosis of chronic bone infection are questionable and hence the need for an alternative method.[12]

Among all diagnostic modalities, histopathology plays the most crucial role in the management of many conditions like bone tumours and metastatic lesions.[13] Histopathology as an investigative method involves the macroscopic (quantitative) and microscopic (qualitative) examination of tissues from the human body for the purpose of arriving at a diagnosis, and or confirming a clinical diagnosis. It is quite easy to set it up at the level of a teaching hospital or even at the regional hospitals with just a pathologist and few biomedical staff. It does not rely on the isolation of organisms, but the presence of tissue reactions to the pathogenic agent, even antibiotic therapy has been started, or in cases of contaminations by non-pathogenic organisms. Results in many instances are available within 4 to 7 days. In very few instances, special stains may be required for the final diagnosis. Again, the cost may be a factor, but with a functioning health insurance card, patients can assess this service in many parts of Ghana.

The role of histopathology in the diagnosis and management of chronic osteomyelitis in Ghana has not been evaluated. The aim of this retrospective study was to evaluate the importance of histopathology examination as a tool in diagnosing and describing the clinicopathological features of chronic osteomyelitis in sequestrectomy and other small biopsy specimens.

METHODOLOGY

Research design: This was a retrospective histopathological review, using material and data from 1st January 2017 to 31st December, 2021.

Study Site: The Department of Pathology in the Tamale Teaching Hospital (TTH) was the study site. TTH is the only tertiary referral hospital serving the five regions in northern Ghana and beyond, particularly, neighboring Burkina Faso, as reported in previous studies.

Case selection: This was based on the following:

1. Clinical history and diagnosis of osteomyelitis by the attending clinician.
2. Sequestrectomy and small bone biopsies.
3. Histopathology examination of the tissue with confirmed diagnosis of osteomyelitis: bone changes, soft tissue changes and inflammatory infiltrates.

Data collection, entry and Analysis: We retrieved all histopathology request forms and the completed histopathology reports of all bone samples submitted to the department during the study period. All cases of bone infections were selected (n= 98). Cases of chronic osteomyelitis (n = 75) were extracted. Data were collected on the age at presentation, relevant clinical history (primary symptoms, duration, anatomic site and type of operation) and type of surgical specimens. We also collected data on associated symptoms, history of trauma and herbal medicine usage and other risk factors. The data was entered into and analysed using SPSS software version 26.0 (Chicago). Descriptive statistics of frequencies were used to describe the data.

Fisher's exact test was used to test for association. The results were presented in bar charts and frequency tables.

An algorithmic histopathologic tool designed based on the progression of osteomyelitis from acute to chronic with subcategories was used in this study. Thus, the histopathology features for making the diagnosis of chronic osteomyelitis were descriptive.

Inclusion Criteria [10]

1. Tissue section with osteonecrosis approximately constituting 2/3 of the filed at low power.
2. Soft tissue changes: Granulation tissue in the inter-trabecular spaces, mature granulation tissue and fibrous tissue seen in soft tissues adjacent to bone fragments.
3. Diffuse infiltration by mixed inflammatory cells consisting predominantly of lymphocytes, macrophages and plasma cells. (Figure 1).

Exclusion:

1. Tissue sections with focal necrosis.
2. Tissue sections with acute suppuration or increased neutrophil infiltrates.

RESULTS

Age and gender characteristics of patients with chronic osteomyelitis (COM)

A total of 75 histologically confirmed chronic osteomyelitis cases constituted the sample size for this study. Their ages ranged 6 – 85 years with a mean of

32.86±22.10 years, and median of 12.0 years. Many were within the age group of 10 - 19 (26.7%) years, followed by 20 -29 years (22.7%) (Figure 2). There were 50 (66.7%) males and 25 (33.3%) females (P<001), with a male: female ratio of 2:1.

Primary symptom and duration of COM at presentation

The commonest primary presentation of COM was a painful swelling (n = 55, 73.3%, P<0.001) (Table 1). A total of 74 (98.7%) patients had available data on the duration of symptoms at presentation and these ranged from 1 – 120 months with a mean of 17.35± 24.74 and a median of 12.0 months respectively. Approximately, 29.8% presented to a health facility after 12 months of onset of the disease (Table 1). COM was commonly diagnosed in sequestrectomy samples (Table 1).

Anatomical sites of COM among the study population

The sites commonly involved by chronic osteomyelitis were: tibia (34.7%), femur (22.7%), hip joint (10.7%), humerus (8.0%), Knee joint (8.0%) and the lumbar spine (5.3%) (Table 2).

Patients with associated complains of COM (n=46)

A total of 46 (61.3%) patients had additional complains at presentation. These were discharging sinus tracts 39 (84.8%) and inability to walk 7 (15.2%), (P<0.0001), (Table 1; Figure 3).

The ages of patients (n=39) with discharging sinus ranged from 6 – 75 years, with a mean of 30.32±19.6 and a modal age group of 10 – 19 (18.2%) years (Table 3). Approximately, 59.0% were younger than 30 years of age. Many (n = 23, 59.0%) were males (p=0.1739). A total of 38 (97.4%) patients had stated duration at presentation, of which 17 (44.7%) patients presented after 12 months of noticing the disease (Table 3). Approximately, 43.6% had previous history of trauma, while 56.4% had no prior trauma. Many (66.7%) had stated history of herbal medicine usage, compared with those (33.3%) without this history. The common bone sites of discharging sinus tracts were: tibia (46.1%), femur (20.5%) and humerus (10.2%) (Table 3).

Other relevant clinical history of chronic osteomyelitis

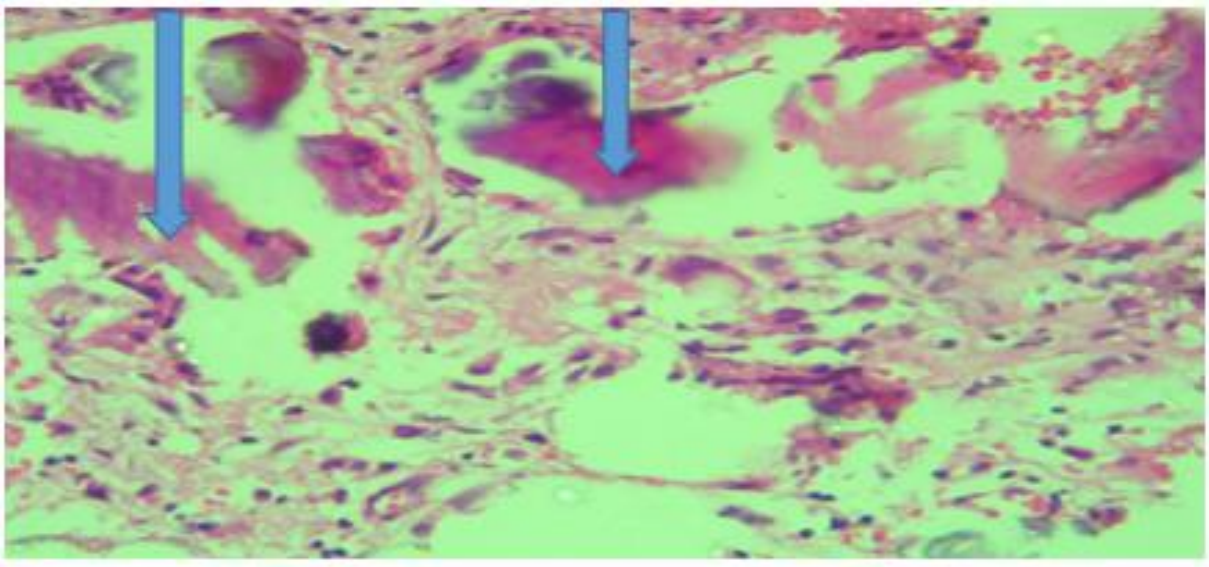
The history of previous trauma was recorded in 27 (36.0%) cases (Table 1). The mean age was 31.9±25.7 years, many (n = 10, 37.0%) were aged 10 – 19 years (Table 3). Great majority (n = 25, 88.9%) were males (p<0.001) (Table 3). The bone commonly involved was the tibia 11 (40.7%) (Table 3).

Again, 41 (54.6%) cases had a form of herbal treatment for their conditions before presenting to a health facility (Table 1). The mean age of this group of patients was 34.0±23.6 years with a modal age group of 10 – 19 (24.4%) years. Approximately, 61.0% were males (P= 0.077) (Table 3). The type of bone site of osteomyelitis for which herbal medicine was commonly used was the tibia (n = 15, 36.6%) (Table 3).

There were 6 (8.0%) cases of sickle cell, 5 (6.7%) with diabetes mellitus, 4 (5.3%) tuberculosis and 1 (1.3%) of retroviral infections.

Associations between Discharging Sinus Tract, Trauma and Herbal Medicine Usage

There was a significant association between discharging sinus tract and herbal medicine usage (p= 0.009), however, there was no significant association between discharging sinus and trauma (p=0.105). Similarly, there was a significant association between prior trauma and herbal medicine usage (P=0.011)



X40

Figure 1a: Osteonecrosis in a 19-year-old male with chronic osteomyelitis (*blue arrows*)

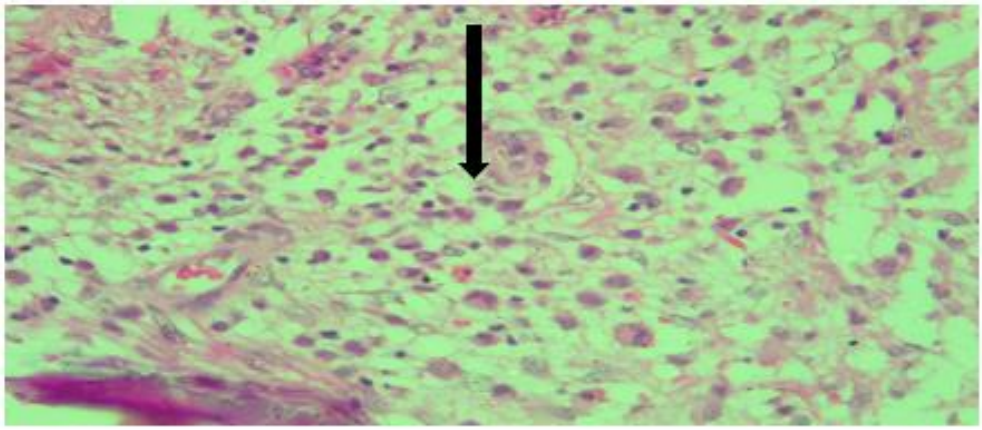


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Figure 1b: Diffuse mixed inflammatory cells; plasma cells and lymphocytes (*black arrow)* in a 19-year-old with chronic osteomyelitis

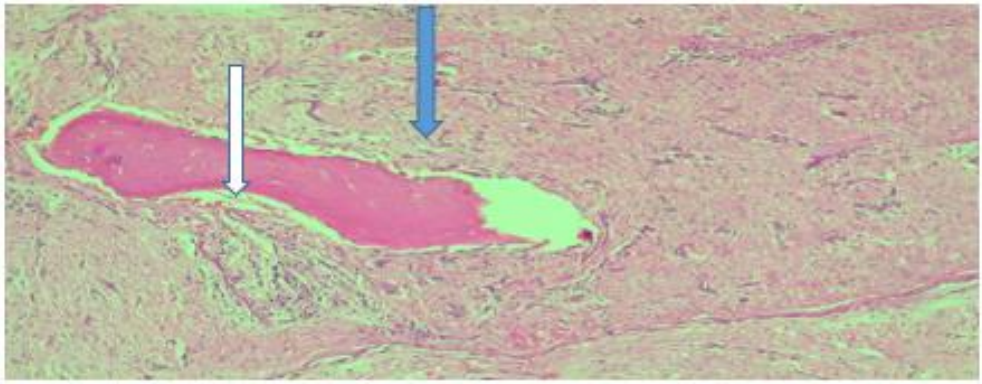

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Figure 1c: Fibrous tissue (Blue arrow) seen in soft tissues adjacent to a viable bone fragment (white arrow) from a 19-year-old male with chronic osteomyelitis

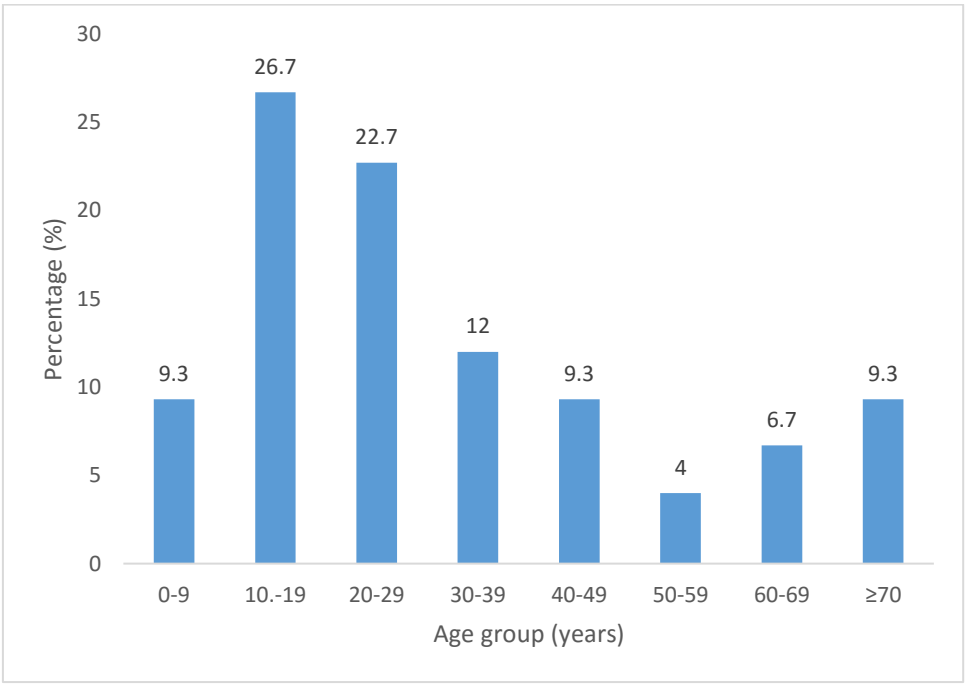

Figure 2: Age distribution of patient histologically confirmed chronic osteomyelitis

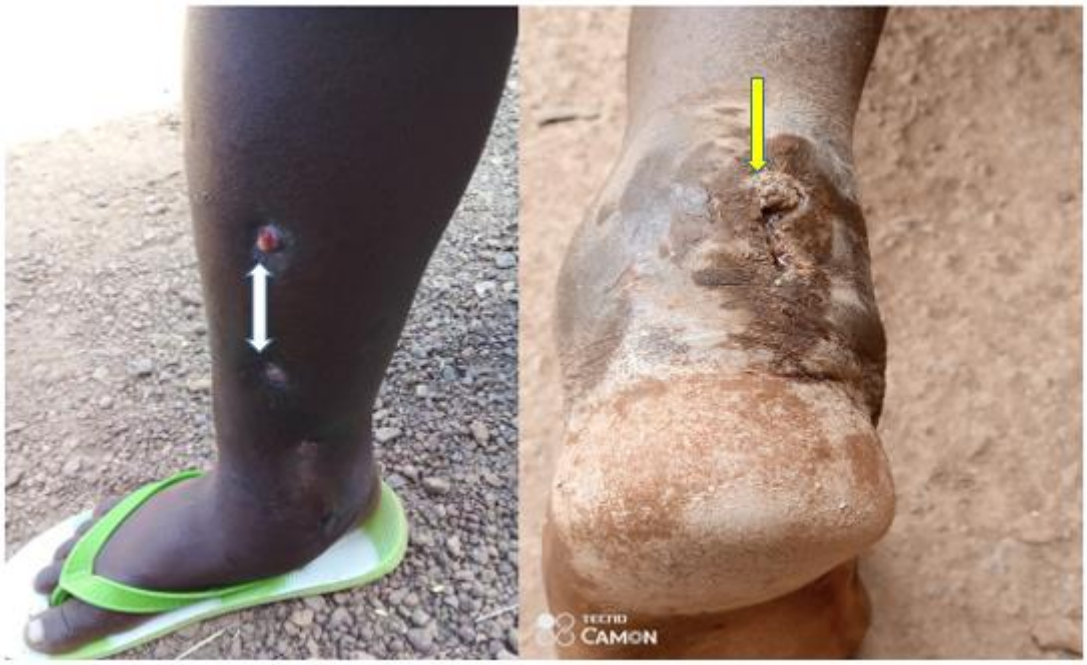

Figure 3a: Discharging multiple sinus tracts in a 12-year-old adolescent male without prior trauma (*double headed white arrow*)

Figure 3b: Discharging multiples sinus tract in a 22-year-old male following road traffic accident *(yellow arrow)*

Table 1: The clinical features of patients diagnosed with chronic osteomyelitis in a tertiary referral hospital in northern Ghana

Clinical parameter		Frequency (n)	Percentage (%)	p-value
Primary symptom (n = 75)	Painful swelling	55	73.3	0.0001
	Bone pain	15	20	
	Fracture	1	1.3	
	Swelling	4	5.3	
Duration at presentation (n = 74)	0 - 4	18	24.2	0.0001
	5 - 9	19	25.7	
	10 - 12	15	20.3	
	>12<24	15	20.3	
	>24	7	9.5	
Additional complains (n = 46)	Discharging sinus tract	39	84.8	0.0001
	Inability to walk	7	15.2	
History of previous trauma(n=75)	Yes	27	36.0	0.0010
	No	48	64.0	
History of herbal medicine usage (n=75)	Yes	41	54.7	0.3272
	No	34	45.3	
Type of surgical sample (n= 75)	Sequestrectomy	34	45.3	
	Excision biopsy	17	22.7	
	Core needle biopsy	16	21.3	
	incision biopsy	8	10.7	

KEY: p-value <0.05 as statistically significant

Table 2: Types of bones and frequency of involvement by chronic osteomyelitis

Location	Frequency (n)	Percentage (%)
Tibia	26	34.7
Femur	17	22.7
Hip joint	8	10.7
Humerus	6	8.0
Knee joint	6	8.0
Lumbar spine	4	5.3
Elbow joint	3	4.0

Location	Frequency (n)	Percentage (%)
Shoulder joint	1	1.3
Sternum	1	1.3
Radius	1	1.3
Middle finger	1	1.3
Foot bones	1	1.3
Total	**75**	**100.0**

Table 3: Age, gender, site of involvement and duration of patients with discharging sinus, trauma and history of herbal treatment

Character	Discharging sinus (n/%)	Prior trauma (n/%)	Herbal treatment (n/%)
Age group (years)			
1 - 9	3(7.7)	4(14.8)	6(14.6)
10 - 19	11(28.2)	10(37.0)	10(24.4)
20 - 29	9(23.1)	3(11.1)	7(17.1)
30 - 39	5(12.8)	3(11.1)	6(14.6)
40 - 49	5(12.8)	0(0.0)	3(7.3)
50 - 59	1(2.6)	2(7.4)	1(2.4)
60 - 69	2(5.1)	1(3.7)	3(7.3)
≥70	3(7.7)	4(14.0)	5(12.2)
Total	39(100.0)	27(100.0)	41(100.0)
Mean age	30.3±19.6	31.9±25.7	34.0±23.6
Gender			
Male	23(59.0)	24(88.9)	25(61.0)
Female	16(41.0)	3(11.1)	16(39.0)
p-value	0.1739	<0.0001	0.0766
Anatomic site			
Tibia	18(46.2)	11(40.7)	15(36.6)
Femur	8(20.5)	6(22.2)	9(22.0)
Humerus	4(10.3)	3(11.1)	5(12.2)
Knee	3(7.7)	2(7.4)	3(7.3)
Lumbar spine	3(7.7)	0(0.0)	2(4.9)
Hip	1(2.6)	3(11.1)	4(9.8)
Sternum	1(2.6)	0(0.0)	0(0.0)
Shoulder	1(2.6)	0(0.0)	1(2.4)
Radius	0(0.0)	1(3.7)	1(2.4)
Foot	0(0.0)	1(3.7)	0(0.0)
Elbow joint	0(0.0)	0(0.0)	1(2.4)
Total	39(100.0)	27(100.0)	41(100.0)
Duration (months)			
0 – 12	22(56.4)	20(74.1)	25(61.0)
>12	17(43.6)	7 (25.9)	16(39.0)
p-value	0.3652	0.0009	0.0766

KEY: p-value <0.05 as statistically significant

DISCUSSION

Histopathology examination of bone specimens removed from humans plays a crucial role in the diagnosis of neoplastic and non-neoplastic conditions of bones as indicated in the literature.[13] It is also a more objective and reliable method in bacterial infections, for it does not depend on the isolation of the infectious agent, but description of tissue reactions to the agent. However, the existing literature is directed more at microbiological method as the gold standard for the diagnosis of bone infections, which may be negative particularly in cases where an antibiotic treatment has already been started or in those involving low virulent bacteria, or due to growth of contaminants.[3,4,9-14] The usefulness of histopathology as a diagnostic tool in bone infection particularly the chronic forms, must be looked at in developing countries. This is so because, in most developing countries more often than not, most patients usually come to the hospital after they have tried treating with some antibiotics which may affect the microbiological test outcome, or encourage the growth secondary bacteria. Currently, data and relevant clinico-pathological features on chronic osteomyelitis (COM) from northern Ghana is scanty. To address this, we conducted a retrospective histopathology review of 75 cases of patients with COM in the largest referral

hospital in northern Ghana and explored the clinical characteristics of COM in this catchment area and beyond.

In this current study, COM was found to be common in young patients, particular those within the 10 – 19-year age group. This differs greatly from reports of previous studies that found the disease to be common among adults. [15,16] For instance, Xianzhi *et al.,* [16] in their study found COM to commonly affect people within the age group of 41–50 years. Osteomyelitis also was reported in this study to be significantly common among male patients (P<0.0001). This is in accordance with studies that reported the condition to be male predominant.[17,18] For instance, Kremers *et al.,* [15] reported a male to female ratio of 4:1, while the current study in northern Ghana found the ratio to be 2:1. The age and gender characteristics found in this study conducted in northern Ghana, can be attributed to the increasing incidence of road traffic accidents in Ghana, [17] and more so in the study areas. The major contributory factor in Ghana, particularly within the northern sector is the increasing commercialization of tricycles, locally called "yellow - yellow" in the hands of inexperienced young adolescents who do not have driving license and mostly of low level of education. Victims of road traffic accidents commonly sustain multiple bony fractures with massive soft tissue destructions, which are more difficult to treat and hence frequently lead to infection. Also, victims with minor soft tissue or bony injuries seek care from traditional bonesetters and eventually get infections of the bone leading to COM. For instance, Kremer et al. [15] in their study argued that increasing road and industrial accidents contributed to the greater number of male patients with COM, since this group of individuals are more likely to engage in riding of motor cycles, driving and or heavy physical labour. This is equally true in this current study, where approximately, 36.0% of the study population had history of prior trauma, although this may be an under estimation. Furthermore, our finding supports previous studies that reported trauma as the commonly identified risk factor for chronic osteomyelitis in many countries. [18,19]

Chronic osteomyelitis is normally characterised by the lack of tell-tale clinical signs.[4] In a given proportion of cases, painful bone swelling, chronic bone pain, erythema around the affected bone, swelling and bone tenderness, chills, low grade fever and general malaise are some of the commonly reported clinical symptoms.[1-4,18-21] The clinical presentation of COM in this current study in descending order were painful bone swelling, bone pain, bone fractures and painless swelling. The clinical features reported in this current study are thus in line with those reported in previous studies across the globe.

Tibia (34.7%), femur (22.7%), hip joint (10.7%), humerus(8.0%), knee joint (8.0%) and the lumbar spine (5.3%) were the common anatomical sites involved by COM in this study. This pattern of bone involvement differs from what was reported in some previous studies. [16,22,23] For instance, Xianzhi *et al.,* [12] in their study reported the common sites as: femur (30.2%), tibiofibular (25.9%) and hip joint (15.3%). Our findings are however consistent with reports of other studies across the globe. [24,25] For instance, Wang *et al.,* [24] in southwest China, reported the top two infection sites as tibia (57.5%) and femur (26.8%) in their study. Similarly, Jiang *et al.,* [25] in southern China, found the common sites to be tibia (39.00%), followed by the femur (24.46%). The argument is that, anatomically, these bones are vulnerable to trauma with multiple fractures, open and contaminated wounds. This is more so with the tibia bone which is very superficial, surrounded by a thin soft tissue and lack of adequate blood supply and this increases the likelihood of open fractures, open wounds complicated by acute infections followed by chronic osteomyelitis. Thus, tibia osteomyelitis has the highest incidence in many countries.

Chronic osteomyelitis as an inflammatory disorder must be promptly diagnosed and the appropriate treatment started. Delay in initiating the correct treatment regimens may result in a variety of complications and long term disability.[26,27] In cases with apparently 'successful' treatment rates, the incidence of relapse remains high, making it not only a management challenge for the clinician, but equally affects the quality of life of the affected patients and their families as started in the literature.[28] In this current study, the great majority (84.8%) of patients who had additional complains at presentation were those with discharging sinus tracts (P<0.0001), many (44.7%) presented late (after 12 months of illness) to a health facility for treatment. Again, 43.6% had history of previous trauma. Furthermore, 66.7% of the patients had stated history of herbal medicine usage. There was a strong positive statistical association between discharging sinus tract and the history of herbal medicine usage (P=0.009). The history of trauma preceding COM, complicating as a discharging sinus tract in our study is in line with studies that found trauma as a major risk factor for COM.[22,,23, 29,30,31] It has been reported in previous studies that, patients with long-standing sinus drainage are not only at risk of pathological fractures, but may progress to metaplasia, dysplasia, and neoplasia, notably squamous cell carcinoma (Marjolin's ulcer), over time.[22,23,26,27,31] In fact, patients presenting with long standing discharging sinus tract are described in the literature as having a neglected disease.[20,31,32] At this stage, not only does the disease pose a major financial burden to the patient and health facilities, but it substantially affects the quality of life of the affected patients and their families.

The use of herbal medicines by traditional bonesetters in the management of traumatic

musculoskeletal injuries is an age-old practice in most developing countries.[33,34] In Ghana, the practice of TBS is very common in the Northern part, due to some cultural beliefs and practices, in addition to the generally high poverty level of the people.[34,35] Easy accessibility, familiarity, affordability and the fear of amputation have been reported in the literature as other reasons for the high patronage.[36,37] for TBS services in Africa. Again, payment by instalments or in kind offered by the TBS is also an attractive package to most low-income earners in developing countries. [36,37] This is in contrast to the bulk payments usually required for treatment in the hospitals, especially in the absence of health insurance. However, most of these patients, later return to orthopaedic hospitals with several complications mostly COM, limb gangrene, non-union, malunion, and joint stiffness. [34,35,36,37,38] This normally leads to increase load on the orthopaedic surgeons in developing countries, for some preventable amputation must be carried out. The complications also pose a management challenge to orthopaedic surgeons resulting in poor outcomes of fracture treatment. [38,39,40] In the current study conducted in northern Ghana, majority (54.6%) of the cases had a form of herbal treatment for their conditions before presenting to a health facility. Secondarily, 66.7% with discharging sinus had stated history of herbal medicine usage. Furthermore, there was a strong significant positive association between discharging sinus tract and the herbal usage (P= 0.009). Similarly, there was a significant association between trauma and the likelihood of herbal medicine usage (P=0.011). These findings are in keeping with previous studies that identified prior trauma and the use of herbal medicine following musculoskeletal injuries as the major determinants of bone infection and the development of discharging sinus tract as a long-term complication in many developing countries. [34,35, 38, 39, 40].

The current study has heighted the importance of histopathology as a diagnostic tool or aid in the initial work-up and management of patients with classical and atypical symptoms of chronic osteomyelitis as follows;

1. Histopathological examination can be used as an added investigatory tool in the diagnosis of osteomyelitis with atypical clinical symptoms: For instance, blood cultures are positive in less than 50.0% of children even with acute osteomyelitis, in which case a biopsy or sequestrectomy sample for histopathological diagnosis will be very necessary.
2. Histopathological examination may be the only available method for biopsy or sequestrectomy samples from patients with long duration of discharging sinuses, but with negative blood and wound cultures.
3. Again, that histopathology is useful not only in the diagnosis of chronic osteomyelitis, but could also be used to differential neoplastic lesions from their inflammatory counterparts.
4. Furthermore, that histopathology does not rely on the isolation of organisms, but the presence of tissue reactions to the pathogenic agent, even if antibiotic therapy has been started, or in cases of contaminations by non-pathogenic organisms.
5. That results of histopathological examination of bony samples in many instances are available within 4 to 7 days. In very few instances, special stains may be required for the final diagnosis.

CONCLUSION

Chronic osteomyelitis was found to be common among young male patients, with painful bone swelling as the commonest mode of presentation. Long skeletal bones were the common sites. Discharging sinus, previous trauma and herbal medicine usage were the other significant clinical features of chronic osteomyelitis.

RECOMMENDATIONS

1. It is recommended that, histopathology examination be conducted on all sequestrectomy and biopsies from infected bone as part of the initial diagnostic work-up for chronic osteomyelitis by clinicians, particularly patients with atypical clinical presentations.
2. Referrals of open fractures should be given serious attention and prophylactic antibiotics should be given if at all such patients are referred.
3. Training bone setters and increasing public awareness on bone infections may prevent chronic osteomyelitis.

LIMITATIONS

1. Inadequate clinical information, particularly those of axillary studies.
2. This was a single institution study and thus the data may be influenced by the availability of orthopedic surgeons.
3. The duration of the study period was relatively short.
4. The sample size was low and may be a subject of bias, regarding the interpretation of the statistical results.

Conflict of Interest: There is no conflict of interest.

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Availability of Data: The data used to prepare this manuscript shall be made available upon request by the editor-in-chief.

Author's Contributions: Der EM conceptualized the manuscript, collected and analysed data, and wrote the manuscript. Buunaim ADB and Bukari MIS, both orthopedic surgeons, cross-checked the clinical data,

read through and made academic inputs in the final manuscript and approved for publication.

Ethical Consideration/Informed Consent: Permission to write and publish the data in this manuscript was granted by the Head of Department of Pathology.

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