Clinical and laboratory features of Nigerian patients with osteomyelitis

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ABSTRACT

Introduction: The aim of this study was to investigate the clinicopathological characteristics of Nigerian patients with osteomyelitis.

Methods: 30 patients with osteomyelitis and 30 apparently-healthy age- and sex-matched controls were investigated. The packed cell volume (PCV), white blood cells (WBC) and differentials, and platelet counts were measured using an automated counter, while the erythrocyte sedimentation rate (ESR) was determined by Westergren's technique. C3 activator, C1 esterase inhibitor (C1-INH), IgA, IgG and IgM were estimated by the single radial immuno-diffusion method. Wound swabs, blood cultures and biopsies were taken and sent for microscopic, culture and sensitivity analysis.

Results: Patients with osteomyelitis had elevated total leucocytes, neutrophils, and platelet counts compared to the controls. There was also significant anaemia (t equals 3.17, p-value equals 0.002) and a significantly elevated ESR (t equals 3.75, p-value equals 0.000). Serum levels of C3 activator were significantly higher in patients with osteomyelitis (t equals 6.29, p-value equals 0.000). Although serum levels of C1-INH, IgG and IgM were higher in osteomyelitis, they were not significantly so. Serum levels of IgA were reduced in patients with osteomyelitis. Significant correlations between PCV and ESR (r equals -0.486, p-value equals 0.006), ESR and total WBC count (r equals +0.542, p-value equals 0.002), ESR and platelet count (r equals 0.445, p-value equals 0.013) and total WBC count and IgG (r equals 0.507, p-value equals 0.019) were noted .

Conclusion: Nigerian patients with osteomyelitis have similar clinical and laboratory features already described in literature, with some noted immune dysfunctions.

Keywords: bone infection, immune dysfunction, osteomyelitis

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INTRODUCTION

Osteomyelitis is the infection of the bone, which could result from haematogeneous spread, direct inoculation of pathogenic organisms following trauma, or complications arising from septic arthritis. The most common organism implicated in acute haematogeneous osteomyelitis in children is *Staphylococcus aureus*, while *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Escherichia coli* are commonly isolated in cases of chronic osteomyelitis.[1] Acute osteomyelitis is associated with discharge of pus, positive bacterial culture from bone or blood, and the presence of classic signs and symptoms of inflammation. In chronic osteomyelitis, there are also draining sinus tracts, deformity, impaired range of motion and neurological deficiency. In addition to the presence of the causative microbial agents, the risk of osteomyelitis is also increased by the presence of host factors such as neutrophil dysfunction, abnormal humoral and cell-mediated immunity.[1] Studies have also shown that *Staphylococcus aureus*, the major causative agent of osteomyelitis, produces human-specific inhibitory proteins which prevent opsonisation, phagocytosis and killing of the pathogen.[2] The aim of this study was to investigate some immunohaematological characteristics of Nigerians with osteomyelitis.

METHODS

Subjects with osteomyelitis that presented at the orthopaedic clinic at the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, and Ladoke Akintola University of Technology Teaching Hospital, Osogbo, between November 2003 and March 2005 were enrolled into the study. Age- and sex-matched apparently-healthy controls were also recruited. Each patient was assessed clinically for features of osteomyelitis. These include painful skeletal swelling, warmth, tenderness, and decreased motion of the affected part of the limb. Some

demographical data (age, gender, weight and height) of both the subjects and controls were also documented. Diagnosis of osteomyelitis was confirmed by isolation of the infecting organism(s) from the wound and/or tissue biopsy culture, blood culture and characteristic radiological features.

Blood samples were taken from both the subjects and controls, in appropriate bottles, for blood counts (packed cell volume [PCV], white blood cells [WBC] and differentials, platelets, erythrocyte sedimentation rate [ESR]), serum immunoglobulins (IgG, IgM, and IgA) and complement regulator proteins (C1 esterase inhibitor [C1-INH] and C3 activator). Haematological parameters were estimated within six hours of sample collection, while serum for immunoglobulins and complement protein samples were stored at a temperature of -20°C and estimated in batches. The PCV, WBC and differentials, and platelet counts were measured using an automated counter (ADVIA-60 Bayer Corporation, New York, USA), while the ESR was determined by Westergren's technique.[3] C3 activator, C1-INH, IgA, IgG and IgM were estimated by the single radial immunodiffusion method of Salimonu et al,[4] using monospecific antisera (Dade Behring Marburg GmbH, Marburg, Germany). Data is presented as mean and standard deviation (means ± SD). Student's *t*-test was used to test the significance of differences between mean values. Spearman's correlation coefficient and multiple regression analysis were computed where necessary. A probability (p) value of greater than 0.05 was considered insignificant. The Statistical Package for Social Sciences version 11.0.1 (SPSS Inc, Chicago, IL, USA) software was used for all statistical analyses.

RESULTS

A total of 60 subjects were investigated, comprising 30 subjects with osteomyelitis and 30 apparently-healthy age- and sex-matched controls. The subjects with osteomyelitis are made up of 20 male and ten female patients (i.e. male-to-female ratio 2:1), while the controls were made up of 14 male and 16 female subjects (i.e. male-to-female ratio 1:1). There was no significant difference in their mean ages. The clinical features at presentation are documented in Table I. All patients aged 15 years and above (23/30; 76.7%) presented with chronic osteomyelitis, while acute osteomyelitis (7/30; 23.3%) was found mainly in those younger than 15 years of age. Unlike the patients with chronic osteomyelitis, subjects with acute osteomyelitis did not present with multiple bone, involvement, bone deformity or had sinus formation.

The laboratory characteristics of patients with osteomyelitis at presentation are shown in Table IIa. The haematocrit was significantly reduced, while the ESR was significantly elevated in subjects with osteomyelitis, compared to the controls (t = 3.17, p = 0.002; t = 3.75, p = 0.000, respectively). Although the total WBC and neutrophils differential and the platelet counts were higher in the subjects with osteomyelitis, they were not significantly different from that of the controls. The C3 activator was also found to be significantly higher in the osteomyelitis group (t = 2.46, p = 0.019). Serum IgG, IgM and C1-INH, though higher in the osteomyelitis group, were not significantly different. On the other hand, serum IgA level was reduced in the osteomyelitis group. A comparison of acute with chronic cases showed a significantly elevated ESR, WBC, and platelet count (t = 2.42, p = 0.02; t = 2.63, p = 0.01; t = 2.17, p = 0.039, respectively), and a significantly low PCV (t = 2.43, p = 0.02), in those with acute osteomyelitis. IgG, IgM, C3 activator, and C1-INH, though higher in acute cases, were however not significantly different in the two groups (Table IIb).

Table I. Clinical features at presentation of patients with osteomyelitis.

	No.	%
Fever (≥ 38°C)	10	30.0
Bone pain	14	46.7
Swelling	21	70.0
Discharging sinus	16	53.3
Bone deformity	6	20.0
Joint arthritis	6	20.0
Fracture	6	20.0
Multiple site involvement	5	16.7
Chronic osteomyelitis	23	76.7
Acute osteomyelitis	7	23.3

The different organisms isolated are shown in Table III. Of the 26 wound-swab cultures carried out, 80.8% (21/26) grew *Staphylococcus aureus*, while the rest grew *Klebsiella* spp., *Pseudomonas* spp., *Escherichia coli* and gram-negative rods. The four tissue biopsies grew *Staphylococcus aureus*, while no organism was isolated from their blood. *Staphylococcus aureus* and *Escherichia coli* were the main organisms isolated from acute osteomyelitis. Radiographs showed various pathological changes, including sclerotic changes, involucra, sequestra, and pathological fractures. In some cases, no bone lesion was observable radiologically. The degree of involvement includes whole-bone involvement, metaphyseal, diaphyseal, or a combination of metaphyseal and diaphyseal, involvement.

DISCUSSION

Osteomyelitis is a serious infection of the bone that is usually caused by pyogenic organisms, and which results in inflammatory bone destruction, bone necrosis

Table IIa. Laboratory parameters of patients with osteomyelitis and the controls.

Parameters	Patients with osteomyelitis	Controls	t-value	Significance (p-value)
PCV (%)	34.0 ± 5.5	38.5 ± 5.5	3.17	0.002
WBC	7,590 ± 2,897.4	6,543 ± 2,886	1.40	0.166
Neutrophils (%)	48.7± 16.9	43.0 ± 11.4	1.53	0.131
Platelets	215,172 ± 39,255	205,500 ± 38,107	0.97	0.337
ESR (mm/hr)	56.8 ± 35.59	27.9 ± 22.8	3.75	0.000
IgG (mg/dL)	689.5± 169.7	660.6 ± 169.7	0.54	0.591
IgA (mg/dL)	170.9 ± 86.7	179.9 ± 51.9	0.35	0.730
IgM (mg/dL)	79 ± 40.87	71.8 ± 69.4	0.39	0.701
C1-INH (mg/dL)	29.7 ± 10.47	25.8 ± 2.66	1.31	0.200
C3 activator (mg/dL)	32.0 ± 11.59	10.6 ± 4.9	2.46	0.019

Table IIb. Laboratory parameters of patients with acute osteomyelitis and chronic osteomyelitis.

Parameters	Acute osteomyelitis	Chronic osteomyelitis	t-value	Significance (p-value)
PCV (%)	30.3 ± 6.0	35.7 ± 4.9	2.43	0.02
WBC	9,885.7± 3,707.8	6,891 ± 2,264	2.63	0.01
Neutrophils (%)	44± 20.0	50 ± 16.1	0.82	0.42
Platelets	243,333 ± 55,406	207,826 ± 31,478	2.17	0.04
ESR (mm/hr)	83.1 ± 47.8	48.7 ± 27.5	2.42	0.02
IgG (mg/dL)	840.7± 161.7	664.3 ± 169.2	1.68	0.11
IgA (mg/dL)	94.0 ± 46.7	183.8 ± 87.3	1.74	0.97
IgM (mg/dL)	92.0 ± 39.8	76.8 ± 41.	0.59	0.56
C1-INH (mg/dL)	32.0 ± 13.0	29.3 ± 10.3	0.41	0.69
C3 activator (mg/dL)	32.0 ± 13.8	10.6 ± 4.9	0.01	1.00

and new bone formation. The main objective of this study was to investigate the clinical and laboratory features at presentation in a cohort of Nigerian patients. In this study, the majority of our patients (76.7%) presented with chronic osteomyelitis, associated with bone deformity and sinus formation, which are signs of chronicity.[5] Misdiagnosis, ignorance, and poverty are some of the risk factors that have been identified as being responsible for the late presentation to a healthcare facility, and the progression to a chronic stage.[6] In addition, the belief in traditional healers[7-9] and their patronage before seeking orthodox medical care are contributory. Acute osteomyelitis is more common in the paediatric age group,[1] and this is consistent with our findings.

Table III. Isolated organisms from patients with osteomyelitis.

	Wound swab no. (%)	Tissue biopsy no. (%)	Blood
S. aureus	21 (80.8)	4 (100)	–
Klebsiella spp.	1 (3.8)	–	–
Pseudomonas spp.	1 (3.8)	–	–
Gram-negative rods	1 (3.8)	–	–
E. coli	2 (7.7)	–	–

Significant anaemia was recorded in our patients, compared to the controls. Chronic infections are known to cause several changes in the haematopoietic system, including shortened red blood cell survival, decrease in the amount of iron that is available for erythropoiesis, and a decrease in bone marrow activity, all predisposing to anaemia. However, easy access to antibiotics without prescription is prevalent in our community, and its prolonged usage could have also contributed to the anaemia recorded in this study. Antibiotics as a cause of anaemia has been documented by Senneville et al[10] in the case of Linezolid. ESR is useful in predicting the presence[11] and effectiveness of therapy in osteomyelitis.[12,13] In this study, the mean ESR was significantly higher in the patients, confirming its diagnostic usefulness in osteomyelitis. The significant positive correlations between ESR and WBC count ($r = +0.542$, $p = 0.002$) and between ESR and platelets ($r = +0.455$, $p = 0.013$) reinforce the usefulness of these parameters as surrogate markers of disease activity.[14,15] ESR was also found to be greater than 50 mm in the first hour in 50% of the patients, compared to 20% among the controls. Although the higher values in the subjects are significant ($z = 2.05$, $p = 0.04$) compared to the controls, the high ESR values documented in some of the apparently-normal controls could be a result of some endemic bacterial and parasitic infections in the tropics, especially malaria and intestinal worm infestation, which could cause some occult inflammatory response.

This study also revealed elevated WBC and differential counts in the subjects, though the difference is not significant. It is however an important finding as neutrophilia has been found to be associated with an increased risk of development of chronic osteomyelitis, ambulatory disability, limb-length discrepancy, and abnormalities of bone growth in patients with pyogenic arthritis.[16] Microbial infections could result in haemolysis; and in haemolytic anaemias, the levels of erythropoietin (which also has thrombopoietic activity[17,18]) are generally raised, and could account for the higher platelet count found in the significantly anaemic patients with osteomyelitis. Further subdivision of the subjects into acute and chronic osteomyelitis revealed significant elevation of the ESR, leucocytes, and platelet counts in acute cases, confirming their usefulness in the diagnosis and monitoring of acute infections,[19-21] while the significant reduction in PCV is not unexpected as bacterial infections are known causes of (haemolytic) anaemia.[22]

The fundamental function of the immune system is the protection it provides against infectious microorganisms. In individuals with serious infection, such as osteomyelitis, management of the infection should not only involve antibiotic therapy and care of the wound, but attention should also be directed at defining immunological abnormalities that may predispose such individuals to microbial infections or factors that may prevent early resolution of the infection. Investigating such abnormalities may suggest alternative therapeutic modalities which could decrease the incidence of microbial infections and improve survival in this category of patients. Previous studies have reported the weakening of protective immunity in subjects with osteomyelitis involving abnormalities, such as reduction in the serum levels of the three major immunoglobulins (IgG, IgA, and IgM), reduction in IgG subclasses, decrease in B lymphocytes and T suppressor cells counts, and hypoproteinaemia.[23-28]

Our present study showed a non-significant increase in the serum levels of IgG and IgM. This increase might suggest an indication of differential response of patients with osteomyelitis to infection, as IgG acts as an opsonin to aid phagocytosis of microbial pathogens, and also, both IgG and IgM are important activators of the classical complement pathway which also lead to the generation of another form of opsonin (C3b) or membrane attack complex, which is important in complement-mediated cytolysis of the pathogens.[29] On the other hand, a non-significant reduction in serum IgA level was detected in osteomyelitis. Since IgA provides mucosal immunity, the sub-normal level recorded could be due to the body's diversion of immunoglobulin production to more of serum immunoglobulins to combat the haematogeneous organisms responsible for the bone infection. Sub-division of the osteomyelitis group into the acute and chronic showed that patients with acute osteomyelitis had non-significant higher serum levels of IgG and IgM, but low levels of IgA, suggesting that these parameters are useful acute phase reactants in monitoring the effectiveness of therapy in this group of patients, similar to findings from studies of other disease conditions.[14,30] The possibility is further suggested by the positive correlation found between IgG and WBC ($r = +0.507$, $p = 0.019$).

The alternative pathway C3 activator, also known as factor B, is a regulatory protein which combines with unstable C3b to form the more stable C3bBb that is capable of activating more C3 in the alternative pathway. Activation of the alternative pathway of the complement system is associated with a decrease in the serum level of the C3 activator. In this study, serum levels of the C3 activator were significantly higher in subjects with osteomyelitis compared to the controls, suggesting a defective alternative complement pathway. Some pathogens are known to produce specific inhibitor proteins, which inhibit complement-mediated phagocytosis from killing and opsonising the organism.[31] The staphylococcus specific inhibitor protein from *Staphylococcus aureus* prevents efficient cleavage of C2 and factor B, and thus affects the formation of C4bC2a and C3bBb, which in turn may be responsible for

the high value recorded and probably explain the likely defect in subjects with osteomyelitis.

In this study, more than 80% of the cultures grew *Staphylococcus aureus*. Complement component, C1-INH, is an important regulator of many plasma mediator pathways, including the complement system. Jiang et al showed that C1-INH inhibits alternative pathway activation by inhibiting the activities of factor B and the cleavage of C3 indirectly through C3b, and that the removal of C1-INH remarkably restored the activities of the pathway.[32] Our study showed a non-significant higher serum C1-INH in patients with osteomyelitis compared to the controls, also suggesting a defective alternative complement pathway in Nigerian subjects with osteomyelitis. It is also noteworthy that both C3 activator and C1-INH are slightly higher in acute osteomyelitis, which is also in agreement with their possible classification as an acute-phase protein.[33]

From our findings, it may be concluded that the clinicopathological features of Nigerians with osteomyelitis is not different from that which has been reported elsewhere. We, however, noted the usefulness of some laboratory parameters, including WBC, platelet counts, and ESR, as surrogate markers of inflammation, which may be used in monitoring the disease. Nigerians with osteomyelitis were also found to have some immune dysfunction, which may necessitate the use of immunotherapeutic agents, as has been done elsewhere.[23]

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