Immunohematological characteristics of Nigerian sickle cell disease patients with osteomyelitis

Osteomiyeliti olan Nijeryalı orak hücre hastalığına sahip kişilerin immünohematolojik özellikleri

Elkanah Ayodele Orimolade[1], Lateef Salawu[2]

[1]Department of Orthopaedics & Traumatology, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria
[2]Department of Haematology & Blood Transfusion, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria

Abstract

Objective: We aimed in this paper to investigate some immunohematological characteristics of Nigerian sickle cell anemia (SCA) patients with osteomyelitis.

Material and Methods: Thirty SCA patients with osteomyelitis (SO) and 30 SCA patients without osteomyelitis (S) were investigated. The PCV, WBC and platelet count were done on automated counter, while the erytorocyte sedimentation rate (ESR) was determined by Westergren's technique. C3 activator, C1-INH, IgA, IgG and IgM were estimated by the single radial immunodiffusion method.

Results: The SO patients weighed less (z =1.943, p<0.055) and were shorter (z = -2.064, p<0.039). High serum levels of IgG, IgM, C1-INH and C3 activator were also found in the SO group. ESR correlated positively with hematocrit (r=0.371, p<0.04) and C3 activator (r=0.468, p<0.03) in SO. Similarly, WBC correlated positively with C1-INH (r=0.806, p<0.01), while we also noted positive correlation between C1-INH and C3 activator (r=0.525, p<0.02). In SO, ESR correlated positively with both IgM (r=0.531, p<0.02) and C3 activator (r=0.449, p<0.05).

Conclusion: This study suggests some derangement in immune status in Nigerian SCA patients with osteomyelitis and that C1-INH and C3 activator may be useful markers of immune status in SCA patients. *(Turk J Hematol 2008; 25: 145-8)*

Key words: Bone, bacterial infection, hemoglobinopathy, osteomyelitis, sicle cell anemia.

Received: November 19 , 2007   Accepted: January 9, 2008

Özet

Amaç: Osteomiyeliti olan Nijeryalı orak hücre anemisine sahip kişilerin immünohematolojik özelliklerinin araştırılması.

Gereç ve Yöntemler: Osteomiyeliti olan 30 Sickle Cell Anemi (SCA) hastası (SO) ve osteomiyeliti olmayan 30 SCA hastası (S) araştırılmıştır. Eritrosit sedimentasyon hızı (ESR), Westergren tekniğiyle belirlenirken PCV, WBC ve trombosit sayımı otomatik bir sayaçla yapılmıştır. C3 aktivatör, C1-INH, IgA, IgG ve IgM tek radial immünodiffüzyon yöntemi ile hesaplanmıştır.

Bulgular: SO daha hafif (z = 1,943, p<0.055) ve daha kısadır (z = -2,064, p<0.039). SO'da IgG, IgM, C1-INH ve C3 aktivatör serum seviyeleri yüksektir. SO'da ESR, hematokrit (r=0,371, p<0,04) ve C3 aktivatör (r=0,468, p<0,03) ile pozitif olarak koreledir. Benzer şekilde WBC, C1-INH (r=0,806, p<0,01) ile pozitif olarak koreledir. Ayrıca C1-INH ve C3 aktivatör (r=0,525, p<0,02) arasında da pozitif bir korelasyon belirledik. SO'da ESR, IgM (r=0,531, p<0,02) ve C3 aktivatör (r=0,449, p<0,05) ile pozitif olarak koreledir.

Sonuç: Bu çalışmaya göre, osteomiyeliti olan Nijeryalı SCA hastalarının immün durumu biraz karışıktır ve C1 INH ile C3 aktivatör, SCA hastalarındaki bağışıklık durumunun faydalı belirteçleri olabilir. *(Turk J Hematol 2008; 25: 145-8)*

Anahtar kelimeler: Kemik, bakteriyel enfeksiyon, hemoglobinopati, osteomiyelit, orak hücreli anemi.

Geliş tarihi: 19 Kasım 2007   Kabul tarihi: 9 Ocak 2008

Address for Correspondence: Dr. Lateef Salawu, Obafemi Awolowo University Teaching Hospitals Complex, Ile-ife, Nigeria
Phone: +234 8033884177 E-mail: lsalawu2002@yahoo.co.uk

Introduction

Bone involvement in the form of painful vaso-occlusive crisis, osteomyelitis, bone necrosis, chronic arthritis, and impaired growth are common manifestations of sickle cell disease (SCD) [1]. The microvascular circulation of the bones is a common site for sickled red cells to lodge, leading to thrombosis, infarction, and necrosis of the bone [2,3]. Infarcted bones are easily colonized by bacteria following an episode of bacteremia [4], which could result from intravascular sickling of the bowel vessels as found in other tissues of patients with SCD. This may lead to ischemic infarction and devitalization of the bowel, thereby permitting loss of mucosal barrier [5] with consequent bacteremia. Other factors predisposing the SCD patients to infections, especially from encapsulated organisms, include abnormal antibody production, defective splenic function, and defects in the alternate complement pathway, leukocyte function, and cell-mediated immunity [6,7].

Clinical signs and symptoms include pain, swelling and tenderness over the affected bone area and fever [8]. These same clinical signs and symptoms also feature prominently in vaso-occlusive crisis and as such make the diagnosis of osteomyelitis in SCD subjects sometimes difficult. The objective of this study was to investigate some laboratory parameters that may aid in the diagnosis of osteomyelitis in sickle cell anemia (SCA) patients.

Materials and Methods

SCA patients with osteomyelitis who presented to the Orthopedic and Hematology Clinics of the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, between November 2003 and March 2005 were recruited into the study. Age- and sex-matched SCA patients in steady state without osteomyelitis were enrolled as controls.

Each patient was assessed clinically for features of osteomyelitis. These include the presence of painful skeletal swelling, warmth, tenderness and decreased motion of the affected part of the limb. Some demographic 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. Wound swabs and tissue biopsies were taken under sterile theater condition to avoid culturing contaminants. Aseptic techniques were also observed in blood sample collection to avoid culturing skin commensals. The characteristic radiological features were also noted. Blood samples were also taken from both the subjects and controls, in appropriate bottles, for blood counts packed cell volume [PCV], white blood cells [WBCs] 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). Hematological parameters were estimated within six hours of sample collection, while serum for Igs and complement protein samples were stored at -20ºC and estimated in batches.

The PCV, WBCs and differentials, and platelet counts were done on automated counter (ADVIA-60 Bayer Corporation, New York), while ESR was determined by Westergren's technique [9]. C3 activator, C1-INH, IgA, IgG and IgM were estimated by the single radial immunodiffusion method of Salimonu et al. [10], using monospecific antisera (Dade Behring, Germany).

Data are presented as means and standard deviation (means ± SD). Mann-Whitney U test was used to test the significance of differences between mean values. Spearman's correlation coefficient were computed where necessary. Probability (p) value greater than 0.05 was considered insignificant.

Results

Demographic Variables

Sixty patients with SCA were investigated. Their clinical and immunohematological characteristics are as documented in Table 1. They included 30 SCA subjects with osteomyelitis (SO) and 30 age- and sex-matched SCA subjects in steady state without osteomyelitis (S). Of the 30 SCA subjects with osteomyelitis, male to female ratio was 1:1. The 30 age- and sex-matched SCA patients without osteomyelitis included 12 males and 18 females, with a male to female ratio of approximately 1:1.5. The SCA patients without osteomyelitis were significantly heavier (30.7 ± 15.4kg and 38.8 ± 16.1kg, respectively; $z = 1.943$, $p < 0.055$) and taller (126.9 ± 35.9cm and 144.6 ± 25.1cm, respectively; $z = -2.064$, $p < 0.039$) than those with osteomyelitis.

Immunohematological Characteristics

The hematocrit was not significantly different between SCA subjects with ($22.7 \pm 3.8\%$) and without ($22.2 \pm 4.7\%$) osteomyelitis. The total white cells count was higher in the SCA subjects with osteomyelitis (13235 ± 6296 per cubic millimeter [/cmm] and 11716 ± 4594/cmm, respectively), but the difference was not significant ($z=0.745$, $p<0.451$). The platelet counts (265000 ± 72246 and 197517 ± 82706/cmm, respectively) and the ESR (70 ± 44.8 mm/hr and 36.9 ± 34.7 mm/hr, respectively) were significantly higher in SCA subjects with

Table 1. Demographic and laboratory parameters of SCD patients with (SO) and without (S) osteomyelitis

Parameters	SO (n=30)	S (n=30)	z-value	p-value
Weight (kg)	30.7 ± 15.4	38.8 ± 16.2	1.943	0.055
Height (cm)	126.9 ± 35.9	144.6 ± 25.1	-2.064	0.039
PCV (%)	22.7 ± 3.8	22.2 ± 4.7	0.416	0.678
WBC (/cmm)	13235 ± 6296	11716 ± 4594.3	0.754	0.451
Platelets (10e9/L)	265 ± 72	197 ± 827	3.688	0.0002
ESR (mm/hr)	70 ± 44.8	36.9 ± 34.7	3.196	0.0014
IgG (mg/dl)	700.6± 179.4	644.5 ± 171.2	1.042	0.297
IgA (mg/dl)	217.3 ± 78.3	249 ± 94.9	-1.036	0.300
IgM (mg/dl)	123.1 ± 130.6	93.4 ± 100.7	1.008	0.314
C1-INH (mg/dl)	32.2 ± 23.8	27 ± 20.2	0.894	0.371
C3 act (mg/dl)	25.7 ± 13.8	17.1 ± 7.4	1.984	0.0147

SO: Sickle cell with osteomyelitis, **S:** Sickle cell without osteomyelitis, **PCV:** Packed cell volume, **WBC:** White blood cell, **ESR:** Erythrocyte sedimentation rate, **Ig:** Immunoglobulin, **C1-INH:** C1 esterase inhibitor, **C3 act:** C3 activator, **cmm:** /cmm = per cubic milliliters, mm/hr = milliliters in the first one hour, mg/dl = milligrams per deciliter.

osteomyelitis (z = 3.688, p<0.0002 z = 3.196, p<0.0014, respectively). The ESR and PCV showed negative correlation in both groups (SO: r = -0.371, p<0.04; S: r = -0.364, p<0.05). In the SCA patients with osteomyelitis, a strong positive correlation was found between white cells count and C1-INH (r=0.806, p<0.01), and between ESR and C3 activator (r=0.468, p<0.03), while in the SCA subjects without osteomyelitis, the ESR showed significant positive correlation with IgM (r=0.531, p<0.02) and C3 activator (r=0.449, p<0.05). It is also interesting to note the positive correlation between C3 activator and C1-INH (r=0.525, p<0.02) in SCA subjects with osteomyelitis. The mean serum levels of IgG (SO = 700.6 ± 179.4 mg/dl, S = 644.5 ± 171.16 mg/dl), IgM (SO = 123.1± 130.6 mg/dl, S = 93.4 ± 100.7 mg/dl), and C1-INH (SO = 32.2 ± 23.8 mg/dl, S = 17.1 ± 7.35 mg/dl) were found to be higher in SCA subjects with osteomyelitis but differences were not significant. Conversely, serum IgA (SO = 217.3 ± 78.3 mg/dl, S = 249 ± 94.9 mg/dl) was found to be higher in SCA subjects without osteomyelitis, but the difference was also not significant. The mean serum level of C3 activator (SO = 25.7 ± 13.8 mg/dl, S = 17.1 ± 7.35 mg/dl) was significantly higher in SCA subjects with osteomyelitis (z = 1.984, p<0.047).

Wound Cultures

The majority of cultures grew Staphylococcus aureus (57.7% or 15/26). Pseudomonas species were isolated in 26.9% (7/26), while the remaining 4 (15.4%) isolates were identified as *Escherichia coli*. Interestingly, none of the cultures yielded Salmonella species.

Discussion

Osteomyelitis is regarded as the commonest orthopedic disease in developing countries, including Nigeria [11]. The chronicity of the illness in most cases decreases the quality of life, especially when it afflicts SCD patients who already have to cope with other forms of medical problems peculiar to the disorder, most especially bone pain. This combination has both psychosocial and financial implications on the patients and/or their relations.

The SCA subjects with osteomyelitis were significantly shorter and weighed less than those without osteomyelitis (z = -2.064, p<0.039; z = 1.943, p<0.055, respectively). The SCA patients with osteomyelitis also had slightly higher total leukocytes and neutrophil differentials. The increased leukocytes could be a result of increased mature neutrophils, which is usual in SCD [12] and which could be further enhanced by infection (osteomyelitis). The thrombocytosis could result from autosplenectomy, as the spleen could pool between 20-40% of the total body platelets [12] that become available in the circulation when the spleen is nonfunctional. Leukocytosis and thrombocytosis are part of the acute phase changes [13] that may accompany infection, trauma, surgery, cancer, burns, tissue infarction, and various immunologically mediated and crystal-induced inflammatory conditions.

Erythrocyte sedimentation is slow even with marked anemia in SCD subjects due to the abnormal shape of the sickled cells, which prevent rouleaux formation [14], a process that enhances red cell sedimentation. The significantly high ESR recorded in SCA patients with osteomyelitis could therefore only be explained on the basis of infection, which is also a recognized cause of elevated ESR [15]. However, the study confirms the negative relationship between ESR and hematocrit in both subjects and controls.

The complement protein C1 esterase inhibitor (C1-INH) is an acute phase protein [13], the plasma level of which can possibly increase in acute phase responses. Although the higher values recorded in SCA patients with osteomyelitis were not statistically significant when compared to those without osteomyelitis, there was a strong positive correlation between C1-INH and white cell count in patients with osteomyelitis (r=0.806, p<0.01), another marker of infection [13], thereby suggesting its possible usefulness as a marker of infection. The mean serum level of C3 activator was found to be significantly high in SCA patients with osteomyelitis compared to those without osteomyelitis (z = 1.984, p < 0.047). C3 activator, also known as factor B, is a positive acute phase protein [13], the serum level of which is expected to rise with inflammatory conditions. Interestingly, a positive correlation was also found between ESR, an acute phase response marker, and C3 activator in SCA subjects with osteomyelitis (r=0.468, p<0.03), suggesting that this complement protein could also be a marker of infection. The possible usefulness of these two complement proteins (C1-INH and C3 activator) as independent markers of infection is further supported by the positive correlation that was found between the two proteins (r=0.525, p<0.02) in SCA patients with osteomyelitis.

The majority of our isolates were identified as *Staphylococcus aureus.* This is in line with some recent reports from Nigeria [16-18]. Similar to other reports from Nigeria [16, 17], no Salmonella organisms were isolated. The decreasing incidence of Salmonella species isolation may be due to the over-diagnosis of typhoid fever based on Widal test and treatment for the same by many private medical practitioners.

It could be concluded that the higher serum levels of IgG, IgM, C1-INH and C3 activator in SCA patients with osteomyelitis coupled with the strong positive correlation found between the traditional markers of infection (elevated ESR and leukocytosis) and IgM, C1-INH and C3 activator reinforces their being classed as acute phase response markers. These findings also suggest that they may be useful markers of infection/osteomyelitis in a clinical setting suggestive of bone infection, thereby suggesting early aggressive management in such patients to prevent chronic osteomyelitis, which is difficult to treat. The type of isolates from most of our patients, as observed recently, is also informative as to the choice of antibiotic before culture reports are available.

References

1. Almeida A, Roberts I. Bone involvement in sickle cell disease. Br J Haematol 2005;129:482-90.
2. Bennett OM, Namnyak SS. Bone and joint manifestation of sickle cell anaemia. J Bone Joint Surg (Br) 1990;72B:494-9.
3. Sherman M. Pathogenesis of disintegration of the hip in sickle cell anaemia. South Med J 1959;52:632-7.
4. Adeyokunnu AA, Hendrickse RG. Salmonella osteomyelitis in childhood. Arch Dis Child1980;55:175-84.
5. Burnett MW, Bass JW, Cook BA. Etiology of osteomyelitis complicating sickle cell disease. Paediatrics 1998;101:296-7.
6. Falcao RP, Donadi EA. Infection and immunity in sickle cell disease. AMB Rev Assoc Med Bras 1998;35:70-4.
7. Overturf GD. Infection and immunizations of children with sickle cell disease. Adv Pediatr Infect Dis 1999;14:191-218.
8. Chambers JB, Forsythe DA, Bertrand SL, Iwinski HJ, Steflik DE. Retrospective review of osteoarticular infections in a pediatric sickle cell age group. J Pediatr Orthop 2000;20:682-5.
9. Dacie JV, Lewis SM, editors. Practical Haematology. 7th ed. Edinburgh: Churchill Livingstone, 2005.
10. Salimonu LS, Ladipo OA, Adeniran SO, Osunkoya BO. Serum immunoglobulin levels in normal, premature and postmature babies and their mothers. Intl J Gynaecol Obstet 1978;16:119-23.
11. Ofiaeli RO. Radiological features of chronic osteomyelitis in long bones and the effects of diaphyseal sequestration on linear growth. Orient J Med 1991;3:20-2.
12. Ashutosh L, Vichinsky EP. Sickle cell disease. In: Hoffbrand AV, Catovsky D, Tuddenham EGD, editors. Postgraduate Haematology. 5th ed. London: Blackwell Publishing, 2005. pp. 104-18.
13. Gabay C, Kushner I. Acute phase proteins and other systemic responses to inflammation. N Engl J Med 1999;340:448-54.
14. Salawu L, Durosinmi MA. Erythrocyte sedimentation rate and plasma viscosity in health and disease. Niger J Med 2001;10:11-3.
15. Austin MS, Ghanem E, Joshi A, Lindsay A, Parvizi J. A simple, cost-effective screening protocol to rule out peri-prosthetic infection. J Arthroplasty 2008;28:65-8.
16. Nwadiaro HC, Ugwu BT, Legbo JN. Chronic osteomyelitis in patients with sickle cell disease. East Afr Med J 2000;77:23-6.
17. Gbadegesin SAA, Laiyemo EA, Nnodu. Osteomyelitis in sickle cell disease, NOHI experience. Nigerian J Orthop Trauma 2004;3:77.
18. Thanni LOA, Ogunfowora OB, Olanrewaju DM. Pattern of musculoskeletal infection and haemoglobinopathy in children. Nigerian J Orthop Trauma 2004;3:75.