Original Article

Morbidity Pattern and Interferon Gamma Level in Sickle Cell Anemia Patients with Autosplenectomy

CI Okongwu, FA Fasola[1], AJ Adekanmi[2], AA Onifade[3]

Faculty of Clinical Sciences, [1]Department of Haematology, Faculty of Basic Medical Science, [2]Department of Radiology, Faculty of Clinical Sciences, [3]Department of Immunology, Faculty of Basic Medical Science, College of Medicine, University of Ibadan, Ibadan, Nigeria

ABSTRACT

Introduction: In sickle cell anemia patients (SCA), the spleen suffers multiple occlusion of its microvasculature causing ischemia and subsequently autosplenectomy. Among the functions of the spleen is the production of gamma interferon (IFN-γ) which has several immunological roles. This function could be impaired in these patients. Therefore, this study aimed at determining the extent to which autosplenectomy affects the immunity of SCA patients as reflected by the level of IFN-γ, frequency of infections, and crises (vaso-occlusive, hemolytic, aplastic) in these patients. **Materials and Methods:** Forty SCA patients in steady state condition were recruited into the study. A self-administered questionnaire was completed by all patients, and venous blood sample was analyzed for IFN-γ levels. All patients underwent abdominal ultrasound scan. **Results:** Autosplenectomy was observed in 20% of the sickle cell patients. Bone pain was the most frequent type of crisis in the study population; this occurred in 100% and 85% of patients with autosplenectomy and those without autosplenectomy, respectively. Infection rates of more than once a year was reported in 87.5% of SCA patients with autosplenectomy compared with 50% of SCA patients without autosplenectomy. There was no significant difference in IFN-γ plasma levels between SCA patients with autosplenectomy and those without autosplenectomy. **Conclusion:** This study showed a higher prevalence of infection and bone pain crisis among SCA patients with autosplenectomy than in SCA patients without autosplenectomy. It also showed comparable level of IFN-γ in the 2 groups of patients. Patients with autosplenectomy may benefit from early institution of drugs such as hydroxyurea to improve quality of life.

KEYWORDS: *Autosplenectomy, gamma interferon, infection, sickle cell anemia*

Date of Acceptance:
27-Jun-2018

INTRODUCTION

Sickle cell disease (SCD) is a group of hemoglobin (Hb) disorders resulting from the inheritance of the sickle globin allele (HbS).[1] The HbS allele has been sustained over time due to the protection of individuals with heterozygote inheritance afforded by the carrier state against malaria morbidity and mortality which is endemic in the sub-Saharan Africa, Mediterranean, India, and Middle East.[2] Sickle cell anemia (HbSS, SCA) in which the HbS allele is inherited in the homozygous form is the most common, with a prevalence of 3.1% in Nigeria.[3] The clinical expression of SCA is variable. Although some patients may have an almost normal life, others may experience crises which may be debilitating. The clinical manifestations result from two key pathological processes: vaso-occlusion and hemolysis.[4] The HbS forms crystals when exposed to low oxygen tension. Deoxygenated sickle Hb polymerizes into long fibers, which causes an alteration in the normal shape of red blood cell (RBC).[1] The red cells assume a sickle shape. The sickle cells, along with non-sickled RBCs, leukocytes, and platelets, form heterocellular aggregates, which adhere to the vascular endothelium, causing obstruction of the lumen of small

Access this article online

Quick Response Code:

Website: www.njcponline.com

DOI: 10.4103/njcp.njcp_217_18

Address for correspondence: Dr. FA Fasola, Department of Haematology, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan, Nigeria.
E-mail: folukefasola@yahoo.com

How to cite this article: Okongwu CI, Fasola FA, Adekanmi AJ, Onifade AA. Morbidity pattern and interferon gamma level in sickle cell anemia patients with autosplenectomy. Niger J Clin Pract 2018;21:1615-21.

blood vessels. In SCD, the spleen is prone to early injury by the sickling process. Repeated splenic microvascular obstruction occurs which results in loss of splenic tissue and progressive fibrosis.[5] The progressive splenic fibrosis causes a reduction of the spleen from its normal size of 101.6 mm (±13.7) × 46.8 mm (±8.2) × 86.8 mm (±12.7) and weight of 150–200 g in a normal adult person, to a state of being regarded as absent.[6] This disease process in the spleen culminates in autosplenectomy, which develops from the age of 6 months to 3 years.[7] By ages 6–8, anatomic asplenia secondary to autosplenectomy develops. Autosplenectomy affects the spleen both structurally and functionally.[8,9]

Quantifiable reduction in splenic function has been reported in SCA patients as early as in the first year of life.[10] The spleen and its functions in an adult sickle cell patient are greatly impaired or absent. Autosplenectomy in SCD results in impaired immunity and propensity for infection.[11] Infections are important contributors to the very high mortality in SCA, which may be as high as 25%–35%.[12] Many factors contribute to this susceptibility; splenic dysfunction is doubtless a crucial factor. The normal spleen plays an important role in defense against infection.[13] It combines the innate and adaptive immune system in a unique way. It releases an immediate innate reaction to microbial penetration and also an adaptive immune response that involves the interaction of cells that recognize a particular antigen, implicating major histocompatibility complex (MHC) molecules presented by antigen-presenting cells.[14] The spleen is a rich store of immune cells, storing about a quarter of total body lymphocytes. The immune cells are known to secrete many cytokines that help fight infections; gamma interferon (IFN)-γ is one of these many cytokines. Natural killer (NK) cells produce higher amounts of IFN-γ after stimulation with interleukin (IL)-12 and IL-18. IFN-γ is a type II INF primarily produced by T-cells and NK cells.[15,16] IFN-γ produced by innate and adaptive immune cells had been documented to be one of the key players in inflammation.[15,16] The cellular effects of IFN-γ include up-regulation of pathogen recognition, antigen processing and presentation, inhibition of cellular proliferation and effects on apoptosis, activation of microbicidal effector functions, immunomodulation, and leukocyte trafficking. It is an important activator of T-cells and macrophages and an inducer of class I MHC molecule expression,[15,16] making the study of the role of IFN-γ and SCA spleen in infectious disease pathogenesis imperative to the improvement in patient health. Ultrasonography is usually the first-line imaging modality in the evaluation of the splenic size.[17]

Ultrasonography is a cheap, non-invasive, and a non-ionizing radiological investigation. A significant percentage of patients with SCA have impaired IFN-γ production in both the steady state and infectious state.[18] Despite the high prevalence of SCD in Nigeria, studies done on this topic have evaluated autosplenectomy and immune function in isolation; hence, there is a dearth of information on the contribution of autosplenectomy to IFN-γ levels. This study is aimed at understanding the effect of autosplenectomy on immunity, as reflected by the plasma levels of IFN-γ and frequency of infections in patients with SCA.

Materials and Methods

Study design

A cross-sectional study was carried out on 40 SCA patients aged 16–50 years in steady state attending the hematology day care clinic of the University College Hospital (UCH), Ibadan.

Study site

The study was carried out at the hematology day care clinic and Department of Radiology, UCH, Ibadan. UCH, Ibadan, is a tertiary hospital of about 850 bed spaces that receives referral from the south west region of Nigeria.

Sampling strategy

Purposive sampling technique was used to select all eligible participants for this study.

Inclusion criteria

Consecutive patients with SCA patients in steady state in attendance at the clinic for routine medical checkup were recruited.

Exclusion criteria

- SCA patients in crises.
- SCA patients who had not received blood transfusion in the previous 3 months.

Statement on invasive sampling and frequency of sampling

Ten milliliters of venous blood was collected once from the anterior cubital fossa of the sickle cell patients. The samples were analyzed for hematological parameters and IFN-γ levels.

Data collection procedure

Data were collected using a questionnaire which was administered to the participants. Questionnaire had three sections: demographic and clinical data, results of ultrasonographic evaluation, and blood samples collected from patients were also recorded. Data were collected over a period of 6 months from April to September 2016.

Laboratory and imaging procedure

Five milliliters of the collected blood sample was put into Ethylenediamine tetraacetic acid (EDTA) bottles for full blood count and the other 5 ml was put into plain bottles

for IFN-γ assay. The sample for full blood count was analyzed immediately using Mindray BC-3000Plus Auto Hematology analyzer for hematocrit, red cell indices, white cell count and differentials, and platelet count. The percentage of sickled red cells (% sickled RBCs) was determined on the peripheral blood film under 100× microscopic fields. Percentage of sickled red cells was the number of sickled RBCs per 100 RBCs counted per slide. Sickled RBCs were defined by elongated RBCs with tapering of opposite ends that culminated in a point.

The blood samples in the plain bottle were allowed to clot for 1 hour after which it was spun at 3000 revolutions/min. This was done at the hematology department laboratory. The serum was decanted and stored at −20°C until analysis. The IFN-γ was analyzed at the Chemical Pathology Department, UCH, Ibadan. Enzyme-linked immunosorbent assay (ELISA) was employed for the measurement of IFN-γ concentration. The brand of human IFN-γ ELISA kit used was AssayPro LLC, USA with Catalogue No. EI1023-1. To determine IFN-γ concentration, 50 µl of IFN-γ standards and samples were added to appropriate wells and incubated for 2 hours at room temperature. Wells were washed five times with wash buffer and hit on absorbent paper to completely remove the liquid. Fifty microliters of biotinylated human IFN-γ antibody was added to each well and the mixture was incubated for 2 hours. Wells were washed five times with wash buffer and hit on absorbent paper to completely remove the liquid. Fifty microliters of streptavidin–peroxidase conjugate was added to each well and the mixture was incubated for 30 minutes. Wells were washed five times and hit on absorbent paper to completely remove the liquid. Fifty microliters of chromogen substrate was added to the wells and incubated for 15 minutes. Reaction was stopped with 50 µl of stop solution and absorbance read at 450 nm. Standard curve of log10 of absorbance against log10 of concentration was plotted using curve-fitting software. Standard curve was fit to a four-parameter logistic regression (4PL) equation and concentration of the samples extrapolated from the curve.

Ultrasonographic evaluation

Ultrasound scan was carried out at the ultrasound suite of the Radiology Department, UCH, Ibadan, to determine the splenic size of the participants.

All participants were scanned by an Ultrasonix SP ultrasound machine with a 3–5 MHz curvilinear transducer. Consenting participants were placed in the supine position. The posterior axillary line in the area of the 10th rib was located and scanned through the intercostal space to have the entire longitudinal view of the spleen with the hilum in view. In this position, maximum length was measured between the most superomedial and the most inferolateral points of the spleen.

We considered a spleen was shrunken if it was less than 5 cm long[19] and autosplenectomy, as non-visualization of the spleen or less than 1 cm long on ultrasonography.[20]

Data analysis method

Data obtained from questionnaire and laboratory results were entered and analyzed using SPSS version 21 (IBM, United States). Descriptive statistics such as frequency counts, percentages, mean, and standard deviation were used to summarize the results. Chi-square and Student's *t*-test were used to test for association between qualitative and quantitative data, respectively. Level of significance was set at *P* value of 0.05. Correlation between variables was determined using Pearson's correlation tests. Significance was assigned to *P* values less than 0.05.

Ethical consideration

Ethical approval was obtained from UI/UCH ethical approval committee; all subjects gave written informed consent before participating in the study and were duly informed of their right to withdraw from the study at any time without any consequence to their treatment.

All data collected from the participants were kept confidential. Names and other easily identifying details were kept confidential as only codes were written on forms. All data were kept in a secure place.

Results

There were 40 participants in this study (17 males and 23 females). The ages of the participants ranged from 17 to 51 years, with a mean age of 29.3 ± 8 years.

Table 1: Socio-demographic characteristics and spleen size of sickle cell anemia patients

Patients characteristics	Frequency (%)
Age (years)	
16-25	14 (35)
26-35	17 (42.5)
36-45	7 (17.5)
>45	2 (5)
Sex	
Male	17 (42.5)
Female	23 (57.5)
Level of education completed	
Secondary	14 (35)
Tertiary	26 (65)
Splenic size (cm)	
Autosplenectomy (<1)	8 (20)
Shrunken (1-4.9)	6 (15)
Reduced (5-8.4)	16 (40)
Normal (8.5-11)	5 (12.5)
Increased (>11)	5 (12.5)

Table 2: Clinical parameters in sickle cell anemia patients with and without autosplenectomy

Clinical indicators	Autosplenectomy, *n* (%)	No autosplenectomy, *n* (%)	*P*
Type of crises			
Vaso-occlusive (painful)	8 (100)	28 (87.5)	0.57
Hemolytic	0	3 (9.4)	
Aplastic	0	1 (3.1)	
Number of admission			
0	0	5 (15.5)	
1	1 (12.5)	10 (31.3)	
2	2 (25)	6 (18.8)	
≥3	5 (62.5)	11 (34.4)	0.63
Frequency of infection			
0	0	2 (6.3)	0.073
1	0	12 (37.5)	
2	5 (62.5)	6 (18.75)	
≥3	3 (37.5)	12 (37.5)	
Frequency of bone pain			
1	3 (37.5)	11 (34.4)	0.87
2	2 (25.0)	9 (28.1)	
≥3	3 (37.5)	12 (37.5)	
History of blood transfusion			
0-3	1 (12.5)	13 (40.8)	0.019
4-7	4 (50.0)	8 (25.0)	
8-10	3 (37.5)	11 (34.4)	

Table 3: Gamma interferon concentration in sickle cell anemia patients with and without autosplenectomy

Autosplenectomy	Geometric, mean±SD (ng/ml)	Interquartile range
Yes	7.28±0.54	0.08
No	7.25±0.62	4.5

SD=Standard deviation

Among the cases, 40% and 92% of participants had been diagnosed as HbSS by the 1st and 12th year of life, respectively. Autosplenectomy was observed in 20% of the patients [Table 1]. Mean age of patients with and without autosplenectomy was 30.8 ± 5.9 and 28.9 ± 8.1 years, respectively. Mean splenic length in HbSS patients without autosplenectomy was 7.9 ± 3.2 cm long.

Vaso-occlusive crises represented by painful crises were the most frequent type of crises occurring in the previous 1 year in the study population; this occurred in all (100%) and 87.5% of patients with autosplenectomy and without autosplenectomy, respectively. Fifty percent and 43.7% of participants with and without autosplenectomy, respectively, had three or more crises in the previous year [Table 2]. Treatment for fever or infection occurred more than once in the last 1 year in 87.5% of HbSS with autosplenectomy compared with only occurring more than once a year in 63% of HbSS patients without autosplenectomy; this difference, however, was not statistically significant. The mean transfusion rate among the SCA patients was 1.6 ± 0.8 units of blood. Twelve (30%) patients had never been transfused. There was a higher frequency of blood transfusion in patients with autosplenectomy [Table 2], a difference that was statistically significant. The most common chronic complication in both groups of patients was avascular necrosis of the femoral head, with a prevalence of 37.5% and 28.1% among the patients with autosplenectomy and those without autosplenectomy, respectively. The prevalence of priapism and chronic osteomyelitis was 12.5% and 12.5% in HbSS with autosplenectomy and 9.4% and 21.9% in HbSS without autosplenectomy, respectively. Other complications reported among the patients without autosplenectomy included chronic leg ulcer in 25% and retinopathy in 12.5% of the patients. Twenty-five percent of the patients without autosplenectomy had more than one complication.

IFN-γ concentration was similar in both groups of HbSS patients. The interquartile range, however, is narrower in those with autosplenectomy when compared with those without autosplenectomy [Table 3]. There was no correlation among IFN-γ levels and splenic size, number of hospital admissions, and frequency of fever/infection or blood transfusion. There was a positive correlation between the frequency of admissions and blood transfusions (r = 0.470, P = 0.012).

Discussion

The result of this study showed that 20% of adult SCA patients had autosplenectomy. This is lower than the reported incidence of 41% in Northern Nigeria, 47.8% among Sudanese patients, and 33.3% among patients in Turkey.[19,21,22] In a study of patients aged 0–21 years, 35.7% of the patients had autosplenectomy.[23] The finding of autosplenectomy in 20% of our patients with mean age of 29.3 years is contrary to our expectation of a higher incidence of autosplenectomy in adult age. An earlier study conducted in the same hospital also reported an incidence of 20%, although the ultrasonography definition of autosplenectomy for that study was splenic size of less than 6 cm.[24] However, a lower incidence of autosplenectomy, between 6.6% and 15.5%, has been reported in Eastern Saudi Arabia.[25] The less frequent incidence of autosplenectomy among the Saudi Arabian patients was attributed to the relatively higher Hb F-level

in their patients with Arab-Indian haplotype compared to the African haplotype. The intermediate prevalence in our study may suggest an admixture of the haplotypes among our patients. Better and improved supportive care may also contribute to the intermediate incidence of autosplenectomy,[23] and the use of acute and chronic transfusion therapy.

Consequent to autosplenectomy, features of hyposplenism, which increases the susceptibility of patients with SCA to infections, may indirectly impact hospital admission rates, frequency of vaso-occlusion, and blood transfusion. Patients with autosplenectomy lack the immune cells naturally resident in the spleen. This study did not show any significant difference in frequency of hospital admission and fever/infections among the patients, although a higher percentage of patients with autosplenectomy was treated for infections (P = 0.073). This observation is in support of a study which reported that mortality and bacteremic episodes did not differ between SCA patients who had splenectomy and control group.[26] Frequency of blood transfusion was significantly higher in patients with autosplenectomy than those without autosplenectomy (2.5 vs. 1.3 units of blood). This is in agreement with the finding of Helvaci *et al*.[27] who reported that less frequent transfusion was associated with reduced incidence of autosplenectomy. The significant correlation between hospital admission and blood transfusion may suggest a history of severe acute morbidity in the patients. The frequency of infection obtained for the last year may not capture those infections that were not serious or those that did not result in presentation at the clinics/hospital, or due to poor recall. The more frequent transfusion in the patients may not necessarily be a reflection of severity of disease but rather severity of acute illnesses adjudged by more long-term complications in the group of patients without autosplenectomy.

Whether hyposplenism indirectly increases the risk of vaso-occlusion or other circulatory complications remains to be determined.[7] Infection has long been recognized as one of the most common precipitants of crisis in SCD. During infection with any pathogen, changes occur at cellular level, which predispose to crises. Levels of circulating leukocytes and inflammatory cytokines increase, with elevated expression of adhesion molecules on both the vascular endothelium and leukocytes themselves.[4] Painful crises are the pathognomonic symptoms of the SCDs and therefore painful crisis was used to assess the frequency of vaso-occlusive crisis in this group of patients. In contrast to the report by Helvaci *et al*.[27] who observed a higher frequency of painful crises in a group of SCD patients with low incidence of autosplenectomy, the frequency of painful crises was similar in both groups of patients. Wright *et al*.[26] reported that painful crises were more common in the autosplenectomy group than in the control group. The study by Helvaci *et al*. suggested an inverse relationship between prevalence of autosplenectomy and severity of SCD. Although that conclusion could not be made from this study, we have observed that other types of crises and non–skeletal-related complications occurred more often in patients without autosplenectomy than with autosplenectomy.[27] Patients treated with transfusions had echogenic spleens and had a higher frequency of splenic regeneration nodules.[23]

The spleen is a rich source of monocytes, macrophages, B and T-lymphocytes, and NK cells.[28] The complex interaction of these cells with NK cell stimulatory factor or IL-12 produced by monocytes/macrophages, B cells, and possibly other accessory cell types primarily in response to bacteria or bacterial products and IL-2 are powerful stimulators of IFN-γ production.[28] In the patient with autosplenectomy, the cells and cytokines are not available to be recruited for the production of IFN-γ. The concentration of IFN-γ is similar in patients with autosplenectomy and without autosplenectomy, which suggests that autosplenectomy may not significantly contribute to the diminished concentration of IFN-γ in patients with SCA. Various explanations may be given for the similarities of IFN-γ values. The patients without autosplenectomy are not as homogenous as those with autosplenectomy. The patients without autosplenectomy had variable splenic sizes, ranging from splenomegaly to normal size spleen, reduced spleen size, and shrunken spleen. The narrower interquartile range in those with autosplenectomy compared with those without autosplenectomy suggests a role for the spleen in the generation of IFN-γ, which might have been blunted by variability in the level of production with varying splenic sizes. The similar concentration of IFN-γ in both groups of patients in steady state may further support a global splenic hypofunction irrespective of splenic size. Hypofunction has been consistently demonstrated by the absence of splenic uptake of 99mTc-sulphur colloid, the enumeration of pitted red cells, sickled erythrocytes, and Howell–Jolly bodies in SCA, even in the presence of an enlarged spleen (functional asplenia).[6] The functional impairment precedes the anatomical regression.[29] This suggests that innate (NK) and adaptive (CD4 and CD8 T) cells producing IFN-γ are reduced or not activated to generate IFN-γ. This further reiterates the finding by Rogers *et al*.[8] that size by physical examination or ultrasound does not predict spleen function. IFN-γ has been shown

to exert beneficial effects in patients with sepsis, and the incorporation of targeted immune enhancement with IFN-γ in the therapy of sepsis has been suggested.[30] Therefore, the effort to preserve INF production by the spleen is worthwhile. A study conducted in the United States by Hankins and colleagues reported that the use of hydroxyurea at the maximum tolerated dose may preserve spleen and brain function in children with SCA.[31]

Studies have also shown that blood transfusion,[7] bone marrow transplant,[32] and hydroxyurea can cause regeneration of splenic tissue and correct hyposplenia (restores its size and its reticuloendothelial function) in patients with SCD.[5] Implementation of preventive care such as hydroxyurea may reduce disease burden and morbidity due to infection from hyposplenism with better disease management. In view of the similar IFN-γ concentration in both groups of patients, preservation of splenic function rather than splenic size should be of utmost care in the defense against infection.

Conclusion

Autosplenectomy is a known complication of SCA and causes a disruption in the physiological function of the spleen, which is a major organ in the immunologic defense of the body. Our study showed no relationship between the splenic size and serum level of IFN-γ, which is an important immune marker. Research into other immune markers and methods of preserving splenic function is recommended to help reduce morbidity and improve the quality of life of SCA.

Financial support and sponsorship

The project described was supported by the Medical Education Partnership Initiative in Nigeria (MEPIN) funded by the Fogarty International Center, the Office of AIDS Research, and the National Human Genome Research Institute of the National Institute of Health, the Health Resources and Services Administration (HRSA), and the Office of the U. S. Global AIDS Coordinator under Award Number R24TW008878. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding organizations.

Conflicts of interest

There are no conflicts of interest.

References

1. Darghouth D, Koehl B, Madalinski G, Heilier JF, Bovee P, Xu Y, *et al.* Pathophysiology of sickle cell disease is mirrored by the red blood cell metabolome. Blood 2011;117:e57-66.
2. Kwiatkowski DP. How malaria has affected the human genome and what human genetics can teach us about malaria. Am J Hum Genet 2005;77:171-92.
3. Omotade OO, Kayode CM, Falade SL, Ikpeme S, Adeyemo AA, Akinkugbe FM, *et al.* Routine screening for sickle cell haemoglobinopathy by electrophoresis in an infant welfare clinic. West Afr J Med 1998;17:91-4.
4. Booth C, Inusa B, Obaro SK. Infection in sickle cell disease: A review. Int J Infect Dis 2010;14:e2-12.
5. Serjeant GR. The spleen in sickle cell disease. In: Bowdler AJ, editor. The Complete Spleen: A Handbook of Structure, Function, and Clinical Disorders. Ch. 15. Totowa, NJ: Humana Press; 2002. p. 251-7.
6. Yahuza MA, Tabari AM, Isyaku K, Suwaid MA, Umar MU, Kabo NA, *et al*. Sonographic measurement of spleen dimensions in healthy adults in North-Western Nigeria. Niger J Basic Clin Sci 2016;13:30-5.
7. William BM, Corazza GR. Hyposplenism: A comprehensive review. Part I: Basic concepts and causes. Hematology 2007;12:1-3.
8. Pearson HA, McIntosh S, Ritchey AK, Lobel JS, Rooks Y, Johnston D, *et al.* Developmental aspects of splenic function in sickle cell diseases. Blood 1979;53:358-65.
9. Brousse V, Buffet P, Rees D. The spleen and sickle cell disease: The sick(led) spleen. Br J Haematol 2014;166:165-76.
10. Rogers ZR, Wang WC, Luo Z, Iyer RV, Shalaby-Rana E, Dertinger SD, *et al.* Biomarkers of splenic function in infants with sickle cell anemia: Baseline data from the BABY HUG trial. Blood 2011;117:2614-7.
11. Claster S, Vichinsky EP. Managing sickle cell disease. BMJ 2003;327:1151-5.
12. Diggs LW. Anatomic lesions in sickle cell anemia. In: Abramson HF, Bertles JF, Withers DL, editors. Sickle Cell Disease: Diagnosis, Management, Education and Research. St. Louis: C.V. Mosby; 1973. p. 189-229.
13. United Nations General Assembly. Recognition of Sickle-Cell Anaemia as a Public Health Problem: Resolution/adopted by the General Assembly; 2009. Available from: http://www.dag.un.org/handle/11176/172628. [Last accessed on 2009 Mar 17].
14. Tiron A, Vasilescu C. Role of the spleen in immunity. Immunologic consequences of splenectomy. Chirurgia (Bucur) 2008;103:255-63.
15. Sojka DK, Plougastel-Douglas B, Yang L, Pak-Wittel MA, Artyomov MN, Ivanova Y, *et al.* Tissue-resident natural killer (NK) cells are cell lineages distinct from thymic and conventional splenic NK cells. Elife 2014;3:e01659.
16. Wang R, Jaw JJ, Stutzman NC, Zou Z, Sun PD. Natural killer cell-produced IFN-γ and TNF-α induce target cell cytolysis through up-regulation of ICAM-1. J Leukoc Biol 2012;91:299-309.
17. Vancauwenberghe T, Snoeckx A, Vanbeckevoort D, Dymarkowski S, Vanhoenacker FM. Imaging of the spleen: What the clinician needs to know. Singapore Med J 2015;56:133-44.
18. Schoenborn JR, Wilson CB. Regulation of interferon-gamma during innate and adaptive immune responses. Adv Immunol 2007;96:41-101.
19. Babadoko AA, Ibinaye PO, Hassan A, Yusuf R, Ijei IP, Aiyekomogbon J, *et al.* Autosplenectomy of sickle cell disease in Zaria, Nigeria: An ultrasonographic assessment. Oman Med J 2012;27:121-3.
20. Walker TM, Serjeant GR. Focal echogenic lesions in the spleen in sickle cell disease. Clin Radiol 1993;47:114-6.
21. Attalla BI. Sonographic fi ndings in Sudanese children with sickle cell anemia. J Diagn Med Sonogr 2010;26:276-80.

22. Balci A, Karazincir S, Sangün O, Gali E, Daplan T, Cingiz C, *et al.* Prevalence of abdominal ultrasonographic abnormalities in patients with sickle cell disease. Diagn Interv Radiol 2008;14:133-7.
23. Gale HI, Bobbitt CA, Setty BN, Sprinz PG, Doros G, Williams DD, *et al.* Expected sonographic appearance of the spleen in children and young adults with sickle cell disease: An update. J Ultrasound Med 2016;35:1735-45.
24. Ojo OT, Shokunbi WA, Agunloye AM. Splenic size in sickle cell anaemia patients in a tertiary hospital. Niger Hos Pract 2014;13:5-6.
25. Gupta P, Acharya S, Shukla S. Comparison of adverse prognostic haematological parameters in sickle cell anaemia patients with splenomegaly and without splenomegaly. IOSR J Dent Med Sci 2016;15:48-52.
26. Wright JG, Hambleton IR, Thomas PW, Duncan ND, Venugopal S, Serjeant GR, *et al.* Postsplenectomy course in homozygous sickle cell disease. J Pediatr 1999;134:304-9.
27. Helvaci MR, Acipayam C, Davran R. Autosplenectomy in severity of sickle cell diseases. Int J Clin Exp Med 2014;7:1404-9.
28. D'Andrea A, Aste-Amezaga M, Valiante NM, Ma X, Kubin M, Trinchieri G, *et al.* Interleukin 10 (IL-10) inhibits human lymphocyte interferon gamma-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. J Exp Med 1993;178:1041-8.
29. Zago MA, Bottura C. Splenic function in sickle-cell diseases. Clin Sci (Lond) 1983;65:297-302.
30. Wang Y, Kong BB, Yang WP, Zhao X, Zhang R. Immunomodulatory intervention with gamma interferon in mice with sepsis. Life Sci 2017;185:85-94.
31. Mulumba LL, Wilson L. Sickle cell disease among children in Africa: An integrative literature review and global recommendations. Int J Afr Nurs Sci 2015;3:56-64.
32. Ferster A, Bujan W, Corazza F, Devalck C, Fondu P, Toppet M, *et al.* Bone marrow transplantation corrects the splenic reticuloendothelial dysfunction in sickle cell anemia. Blood 1993;81:1102-5.