ORIGINAL ARTICLE

Oman Medical Journal [2017], Vol. 32, No. 3: 207-213

Prevalence and Risk Factors for Sulfadoxine Antibody Among Patients Undergoing Treatment for Malaria in Benin City, Nigeria

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ARTICLE INFO

Article history:
Received: 17 December 2016
Accepted: 14 March 2017

Online:
DOI 10.5001/omj.2017.39

Keywords:
Sulfadoxine; Antibodies; Malaria; Demography; Prevalence; Benin; Nigeria.

ABSTRACT

Objectives: Because of lack of data on the prevalence of sulfadoxine antibody, this study was conducted to determine the prevalence of sulfadoxine antibodies and its possible risk factors. ***Methods***: Blood specimens were collected from 500 patients undergoing treatment for malaria at Central Hospital, Benin City, Nigeria. A structured questionnaire was used to collect information and sociodemographic data. Sulfadoxine antibodies were detected by drug absorption (DAT) and immune complex (IMC) methods. ABO, rhesus blood group, and hemoglobin (Hb) phenotype were determined by using standard technique. ***Results:*** DAT method had a significantly higher rate of detecting sulfadoxine antibodies compared to IMC method ($p = 0.019$). Age, gender, and level of education did not affect the prevalence of sulfadoxine antibodies ($p > 0.050$). Patients that were an artisan ($p < 0.001$), married ($p = 0.025$), living in a two-room apartment ($p = 0.003$), had history of drug reaction, consumed antimalarial drug (maloxine), and consumed sulfadoxine-containing drug within the last month ($p < 0.001$ each), and significantly affected the prevalence of sulfadoxine antibodies. Individuals with Hb phenotype AA had significantly higher prevalence of sulfadoxine antibodies ($p < 0.001$), and presence of rhesus D antigen was associated with sulfadoxine antibodies. ***Conclusions:*** An overall prevalence of 22.0% among the tested individuals had sulfadoxine antibodies. Prudent use of sulfadoxine containing drugs is advocated.

Malaria is an ancient disease referenced in a Chinese document from about 2700 BC, clay tablet Mesopotamia from 200 BC, Egyptian papyri from 1570 BC, and Hindu texts as far back as the sixth century BC.[1] Malaria is a major parasite disease in developing countries and particularly in sub-Saharan African.[2] Malaria is caused by five species of plasmodium parasite, the most severe being *Plasmodium falciparum,*[3] which is responsible for at least one million deaths every year.[4] Anemia is a known complication of severe malaria,[5] and over half of malaria-related deaths are attributable to severe malaria.[6] Antimalarial agents such as sulphonamide and other sulphonamide-containing drugs have been reported to cause anemia.[7] Drug–induced immune hemolysis is classified according to three mechanism of action: drug absorption (DAT) (Hapten-induced), immune complex (IMC), and autoantibody; and sulphonamide is usually reported as causing hemolytic anemia via IMC mechanism.[8] Sulphonamide is also reported to cause anemia in glucose-6-phosphate dehydrogenase (G6PD) deficient individuals.[9] There is no report on the prevalence of sulfadoxine antibodies in our environment. Hence, this study aimed to determine the prevalence of sulfadoxine (a sulphonamide) antibodies among patients undergoing treatment for malaria. The effect of demography and some possible risk factors on the prevalence were also determined.

METHODS

This study was carried out in Central Hospital, Benin City, Nigeria, among outpatients with malaria infection. Five hundred patients (223 males, 277 females) were recruited for this study. All the patients had consumed sulphonamide-containing drugs for malaria treatment at least one month prior to specimen collection. Patients who did not consume

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sulphonamide-containing drugs and confirmed not to have malaria were excluded from the study. A structured questionnaire was used to collect social demographic data. Informed consent was obtained from each patient or their parents/guardian in the case of children prior to specimen collection. This study was approved by the Ethical Committee of Central Hospital, Benin City.

Blood was collected from each patient (10 ml) and dispensed into plain and EDTA containers in 5 ml each. The samples in the plain containers were allowed to clot and the sera obtained were used to detect sulfadoxine antibodies. The samples in the EDTA container were used to determine ABO, rhesus blood group, and hemoglobin (Hb) phenotype as described by Enosolease et al.[10] To determine the ABO and rhesus blood group, a drop of each participant's blood was placed on three separate clean white tile. Each drop of blood was mixed with a drop of commercially prepared antiserum A, B, and D, and observed for agglutination.

Hb phenotype was determined by the electrophoresis method.[11] The blood samples were lysed with water and spotted on pre-labeled cellulose acetate paper. Blood specimen with known Hb phenotype (standard Hb AA, AS, SS, SC, and AC) were spotted along with the test sample. The strip was placed in the genotype tank in which one end of each compartment was filled with tris-borate-EDTA buffer (pH 8.5). The tank was covered and electrophoresis carried out for 10 minutes at a potential difference of 220 volts. After electrophoresis, the strip was removed and read macroscopically by comparing the mobility of the test with the standard Hb phenotype.

Sulfadoxine antibodies were detected using two methods DAT and IMC methods.[12] Sulfadoxine 0.5 g was dissolved into 10 ml of sterile normal saline and used for the detection of sulfadoxine antibodies using the two methods.

DAT method worked at equal volume of 10% group O washed red blood cell and sulfadoxine solutions were placed in test tube and incubated at 37 ^{0}C for 30 minutes. This was washed four times with normal saline to remove excess drug. Equal volume of patients' serum and sulfadoxine-red cell suspension was placed inside a test tube and incubated for 30 minutes at 37 ^{0}C. After incubation, the content of the test tube was centrifuged for one minute and observed for the presence of agglutination or hemolysis. When negative, the mixture was washed four times with normal saline and anti-human globulin (AHG) was added. This was centrifuged for one minute and observed for agglutination or hemolysis. The procedural control was carried out as above but consisted of patients' serum and group O red cell without sulfadoxine, and drug-red cell suspension without the patients' serum. Both were negative (no agglutination or hemolysis were observed).

IMC method worked as adding equal volume of sulfadoxine solution, patients' serum, and 5% washed group O red blood cell were placed inside a test tube and incubated at 37 ^{0}C for one hour. After the incubation period, it was centrifuged for one minute and observed for agglutination or hemolysis. When negative, the mixture was washed four times with normal saline and AHG was added. This was followed by centrifugation for one minute and observed for agglutination or hemolysis. Control was carried out as above but consisted of patients' serum, red blood cell, and normal saline without sulfadoxine solution; and red blood cell, sulfadoxine solution, and normal saline without the patients' serum. Both solutions were negative (no agglutination or hemolysis).

The data obtained were analyzed with chi-square (χ^2) test using the statistical software GraphPad InStat version 2.05 for Windows 7, GraphPad Software, La Jolla California USA, www.graphpad.com.

RESULTS

A total of 110 (22.0%) out of the 500 patients had sulfadoxine antibodies. There was a significant difference (p = 0.019) in the rate of detection of sulfadoxine antibodies by DAT and IMC methods with DAT method detecting more sulfadoxine antibodies [Table 1]. Age, gender, and level of education did not significantly affect the prevalence of sulfadoxine antibodies (p > 0.050), while

Table 1: Prevalence of sulfadoxine antibodies using different methods.

Methods	Positive (%)
Drug absorption (DAT)	44 (8.8)
Immune complex (IMC)	22 (4.4)
DAT + IMC	44 (8.8)
Total	**110 (22.0)**

p = 0.019; n = 500.

Table 2: Effect of demography on the prevalence of sulfadoxine antibodies.

Characteristics	Patients tested	Positive (%)	OR	95% CI	*p*-value
Gender			**0.786**	**0.511, 1.209**	**0.322**
Male	223	44 (19.7)			
Female	277	66 (23.8)			
Age, years					**0.141**
5–14	30	4 (13.3)			
15–21	90	16 (17.8)			
22–34	166	36 (21.7)			
35–44	172	39 (22.7)			
45–55	36	11 (30.6)			
> 55	6	4 (66.7)			
Education level					**0.519**
Illiterate	28	3 (10.7)			
Primary	82	18 (22.0)			
Secondary	230	50 (21.7)			
Tertiary	160	30 (18.8)			
Occupation					**< 0.001**
Student	126	28 (22.2)			
Artisan	23	20 (87.0)			
Trader	83	21 (25.3)			
Civil servant	93	23 (24.7)			
Driver	24	5 (20.8)			
Businessmen	40	4(10.0)			
Self-employed	70	5 (7.1)			
Applicant	41	4 (9.8)			
Marital status					**0.025**
Single	185	40 (21.6)			
Married	265	67 (25.3)			
Divorced	22	2 (9.1)			
Widow	28	1 (3.6)			
Living arrangements					**0.003**
One room	132	28 (21.2)			
One room self-contain	49	6 (12.2)			
Two rooms	115	40 (34.8)			
Two rooms self-contain	74	14 (18.9)			
Flat	130	22 (16.9)			

OR: odd ratio; CI: confidence interval.

the prevalence of sulfadoxine antibodies were significantly higher among individuals who are artisans ($p < 0.001$), married ($p = 0.025$), and living in a two-room apartment ($p = 0.003$) [Table 2].

Patients with history of drug reaction ($p < 0.001$), consumed antimalarial drug, Maloxine ($p < 0.001$) and sulfadoxine within one month prior to specimen collection ($p < 0.001$) were more likely to have sulfadoxine antibodies in their sera [Table 3].

The prevalence of sulfadoxine antibodies was not significantly affected by ABO blood group ($p = 0.499$) while rhesus D positive status was significantly associated with sulfadoxine antibodies (odds ratio (OR) = 3.738, 95% confidence interval (CI) 1.672, 6.796; $p = 0.002$). The prevalence of sulfadoxine antibodies was significantly affected ($p < 0.001$) by Hb phenotype, with Hb phenotype AA individuals having the highest prevalence of sulfadoxine antibodies [Table 4].

DISCUSSION

Sulfadoxine/pyrimethamine is the prefered first drugs of choice for the treatment of malaria in Nigeria.[13] Sulphonamides are usually reported as causing hemolytic anemia via IMC mechanism.[8]

Table 3: Features of sulfadoxine consumption associated with presence of antibodies.

Characteristics	Patients tested	Positive (%)	OR	95% CI	*p*-value
History of drug reaction			**52.757**	**28.730, 96.879**	**< 0.001**
Yes	118	89 (75.4)			
No	382	21 (5.5)			
Brand of sulphonamide					**< 0.001**
Fansidar	250	59 (23.6)			
Maloxine	106	38 (35.8)			
Metakefin	87	5 (5.7)			
Septrin	57	8 (14.0)			
Duration of last sulphonamide usage, months					**< 0.001**
1	172	49 (28.5)			
2	179	48 (26.8)			
3	103	11 (10.7)			
≥ 4	46	2 (4.3)			

OR: odd ratio; CI: confidence interval.

There is no report on the prevalence of sulfadoxine antibodies in our environment, hence, this study was conducted.

Twenty-two percent of patients in our study were at an increased risk of developing anemia, which had been reported to cause over half of malaria-related deaths.[6] The use of antimicrobial agent in Nigeria is unregulated, and over-the-counters sales of antimicrobial agents are common.[14–16] Sulphonamide-containing antimalarial drugs are mainly preferred in Benin City because it is taken as a single dose (2–3 tablets/dose) compared to other drugs that are taken more than once a day for ≥ 2–3 days. No published literature on the prevalence of sulfadoxine antibodies was found in other studies to compare the findings. This may be the first report of sulfadoxine antibodies among patients undergoing treatment for malaria with sulphonamide-containing drug in Benin City.

There was a significant difference ($p = 0.019$) in the rate of detecting sulfadoxine antibodies by DAT, IMC methods, or their combination, with DAT method having a higher rate of detection. This method does not agree with the review of Dhaliwal et al,[8] where sulphonamide was classified as causing drug-induced hemolysis by the IMC mechanism. It was observed that there were instances where DAT method detected sulfadoxine antibodies and IMC method did not, and vice versa. Therefore, both methods are recommended for the detection of sulfadoxine antibodies.

Malaria is endemic in Nigeria, and irrespective

Table 4: Effect of ABO, rhesus blood groups, and hemologin phenotypes on the prevalence of sulfadoxine antibodies.

Characteristics	Patients tested	Positive (%)	OR	95% CI	*p*-value
ABO blood group					**0.499**
A	94	19 (20.2)			
B	76	14 (18.4)			
AB	22	3 (13.6)			
O	308	74 (24.0)			
Rhesus blood group			**3.738**	**1.672, 6.796**	**0.002**
Rhesus D positive	414	103 (24.9)			
Rhesus D negative	86	7 (8.1)			
Hemoglobin phenotype					**< 0.001**
AA	339	96 (28.3)			
AS	129	11 (8.5)			
SS	32	3 (9.4)			

OR: odd ratio; CI: confidence interval.

of gender, age, and educational level, every infected patient seeks urgent relief. In Nigeria, over-the-counter drugs sales without prescription are common.[14–16] This may explain why age, gender, and level of education did not significantly affected the prevalence of anti-sulfadoxine antibodies.

The prevalence of sulfadoxine antibodies was significantly higher ($p < 0.001$) among artisan (87.0%) compared to other occupations in the study. A study on artisan and traders, knowledge, attitude, and practice of malaria in selected area of Lagos State Nigeria, revealed that 50% of the artisans, practice self-medication.[17] Since over-the-counter drugs sales are rife, artisans are more likely to engage in self-medication and sulphonamide containing drugs are possibly their first drug of choice to treat malaria.

The prevalence of sulfadoxine antibodies was significantly higher ($p = 0.025$) in married participants (25.3%) compared to the others. The provision of funds for healthcare by both parents (married) has been reported to reduce the risk of malaria.[18] It was also reported that people with higher earnings were more likely to use appropriate antimalarial drugs (sulphonamide-containing drugs), compared to those with lower earnings in Nigeria.[19] It is plausible that married couples may have higher combined earning and as such may use sulfadoxine containing drugs for the treatment of malaria because of it single dosage.

Poor housing quality, overcrowding, and household economical index have been associated with the risk of malaria.[20] People with lower economic index may result to self-medication with drugs and prefer single dose regiment, which may favor the use of antimalarial containing sulphonamide.

Participants who live in a two-room apartment (34.8%) and one-room apartment (21.2%) have significantly higher ($p > 0.003$) prevalence of sulfadoxine antibodies. People living in a house with poor wall, floor, roof, and window conditions were reported to be associated with higher prevalence of malaria.[21] People living in one- and two-room apartments may have increased cases of malaria.

Participants with a previous history of drug reaction to sulphonamide are significantly more likely to have sulfadoxine antibodies (OR = 52.757; 95% CI 28.730, 96.879; $p < 0.001$). Sulfadoxine has been reported to have side effects such as hypersensitivity and cytopenia.[22,23] Hypersensitivity reaction can be classified into four types by the Gell and Coombs classification system.[24] The immunologically medicated reaction caused by sulphonamide antibodies encompasses the entire Gell-Coombs spectrum.[25] The method used in this study detects only immunoglobulin (Ig) G and IgM antibodies.[8]

Fansidar, maloxine, and metakefin contain sulfadoxine/pyrimethamine, while septrin contain sulfamethoxazole and trimetoprim. In this study, people who consumed fansidar, maloxine, metakafin, and septrin all produced sulfadoxine antibodies, although at various prevalence. This cross-reactivity is possible among sulphonamide antibiotics, because they share similar functional group. No cross reactivity were found between sulphonamide antibiotics and non-antibiotic sulphonamides.[26,27] In this study, the prevalence of sulfadoxine antibodies was significantly higher ($p < 0.001$) in patients that consumed maloxine, and was least prevalent in patients that consumed metakefin. The reason for this finding was unclear. Manufacturing processes may increase the sulphonamide functional groups more in maloxine than other sulphonamide used in this study. This will require further investigation to verify.

There was a significant ($p < 0.001$) inverse relationship between prevalence of sulfadoxine antibodies and duration of sulphonamide use. The prevalence of sulfadoxine antibodies decreases with increasing the duration of the last sulfadoxine usage with those who used the sulphonamide-containing drugs within one month of specimen collection having the highest prevalence (28.5%). The method used in this study detected IgG and IgM antibodies that take part in type II hypersensitivity reaction.[24] This ultimately resulted in cytopenia that are dose–dependent.[28] It therefore follows that those who took sulphonamide-containing drugs within one month prior to specimen collection will have a higher dose of the drug in their system, consequently, higher titres of sulfadoxine antibodies in their system. As the time between drug consumption and specimen collection increases, the titres of the sulfadoxine antibodies will reduce.

The prevalence of sulfadoxine antibodies did not differ significantly ($p = 0.487$) among the various ABO blood group. The drug metabolite binds to circulating red blood cell irrespective of their ABO blood group to activate antibody production (DAT mechanism). Also, the drug-antibody complex

attached to normal bystander red blood cell and activate complement, which ultimately lead to destruction of red blood cell (IMC mechanism). It may appear from this study that ABO blood group antigen may not be required for drug-antibodies complex to attach to red blood cell. The presence of rhesus D antigen was significantly associated with sulfadoxine antibodies production (OR = 3.738; 95% CI 1.672, 6.796; $p = 0.002$) and may indicate that rhesus D antigen may be required for drug attachment to red cell or for drug–antibody complex to red blood cell.

Patients with Hb phenotype AA have significantly ($p < 0.001$) higher prevalence of sulfadoxine antibodies compared to Hb phenotype AS and SS. Antibody production is dependent on the immune status of an individual. Patients with SS anemia have been reported to have impaired cell-mediated immunity.[29] This may indicate that patients with Hb S genotype may not be able to respond immunological by producing antibody against sulfadoxine or its metabolite. In sickle cell anemia patients, there are abnormalities in leucocytes function, complement, Ig, and cell mediated immunity.[30]

CONCLUSION

An overall prevalence of 22.0% sulfadoxine antibodies among patients with malaria was observed in this study. Identified risk factors for presence of sulfadoxine antibodies included being an artisans, married, living in an apartment, having a history of drug reaction, consuming sulphonamide-containing drugs within one month, being rhesus D positive, and having Hb phenotype AA. Prudent use of sulphonamide-containing antimalarial is advocated.

Disclosure

The authors declared no conflicts of interest. No funding was received for this study.

Acknowledgements

The authors acknowledge with thanks the Management of Central Hospital, Benin City, Nigeria, for permission to carry out this study.

REFERENCES

1. Cox FE. History of the discovery of the malaria parasite and their vector. Parasites Vectors 2010 Feb; 3(1):5.
2. Doua JY, Matangilla J, Lutumba P, Van Geestroyden JP. Intermittent preventive treatment: efficacy and safety of sulfadoxine-pyrimethamine and sulfadoxine-pyrimethamine plus piperaquine regimens in schoolchildren of the Democratic Republic of Congo: a study protocol for a randomized controlled trial. Trails 2013 Sep; 14:311.
3. Kantele A, Jokiranta S. Plasmodium knowlesi–the fifth species causing human malaria. Duodecim 2010 Jan;126(4):427-434.
4. Prugnolle F, Durand P, Ollomo B, Duval L, Ariey F, Arnathau C, et al. A fresh look at the origin of Plasmodium falciparum, the most malignant malaria agent. PLoS Pathog 2011 Feb;7(2):e1001283.
5. Oladeinde BH, Omoregie R, Osakue EO, Onaiwu TO. Asymptomatic malaria among blood donor in Benin City, Nigeria. Iran J Parasitol 2014 Sep;9(3):415-422.
6. Murphy SC, Breman JG. Gaps in the childhood malaria burden in Africa: cerebral malaria, neurological sequelae, anemia, respiratory distress, hypoglycemia, and complications of pregnancy. Am J Trop Med Hyg 2001 Jan-Feb;64(1-2)(Suppl):57-67.
7. Salama A, Göttsche B, Mueller-Eckhardt C. Autoantibodies and drug- or metabolite-dependent antibodies in patients with diclofenac-induced immune haemolysis. Br J Haematol 1991 Apr;77(4):546-549.
8. Dhaliwal G, Cornett PA, Tierney LM Jr. Hemolytic anemia. Am Fam Physician 2004 Jun;69(11):2599-2606.
9. Fanello CI, Karema C, Ngamije D, Uwimana A, Ndahindwa V, Van Overmeir C, et al. A randomised trial to assess the efficacy and safety of chlorproguanil/dapsone + artesunate for the treatment of uncomplicated Plasmodium falciparum malaria. Trans R Soc Trop Med Hyg 2008 May;102(5):412-420.
10. Enosolease ME, Bazuaye GN. Distribution of ABO and Rh-D blood groups in the Benin area of Niger-Delta: Implication for regional blood transfusion. Asian J Transfus Sci 2008 Jan;2(1):3-5.
11. Omoregie R, Ogefere HO, Omokaro EU, Omorogbe E. Distribution of ABO and rhesus blood group and haemoglobin phenotypes among tuberculosis patients in Benin City, Nigeria. J Med Lab Sci 2002;11(1):68-70.
12. Petz LD. Auto immune and drug induced immune hemolytic anemia. In: Rose NR, Friedman H, Fahey JL, editors. Manual of Clinical Laboratory Immunology. 3rd ed. American Society for Microbiology: Washington D.C; 1986.
13. Adeneye AK, Jegede AS, Mafe MA, Nwokwhan EE. Community Perception and home Management of Malaria in selected rural communities of Ogun State, Nigeria. Int J Malaria Res Rev 2013 Aug;1(3):22-34.
14. Okeke IN, Lamikanra A, Edelman R. Socioeconomic and behavioral factors leading to acquired bacterial resistance to antibiotics in developing countries. Emerg Infect Dis 1999 Jan-Feb;5(1):18-27.
15. Omoregie R, Eghafona NO. Urinary tract infection among asymptomatic HIV patients in Benin City, Nigeria. Br J Biomed Sci 2009 Jan;66(4):190-193.
16. Ogbolu DO. Impact of ESBLs and CREs the Nigeria experience. APUA New Letter 2013 Sep;31(2):15-16.
17. Okwa OO, Soremekun BM, Adeseko O, Raheem AM. Artisans and traders knowledge, attitude and practices of malaria in selected areas of Lagos, Nigeria. Global Adv Res J Med Med Sci 2012 April;1(3):68-74.
18. Okebe J, Mwesigwa J, Kama EI, Ceesay SJ, Njie F, Correa S, et al. A comparative case control study of the determinants of clinical malaria in the Gambia. Malaria J 2014 Aug;13 (1):306.
19. CHESTRAD. Malaria, Poverty and Health. Centre for Health Sciences Training Research and Development International. 2000.
20. Koram KA, Bennett S, Adiamah JH, Greenwood BM. Socio-economic risk factors for malaria in a peri-urban area of The Gambia. Trans R Soc Trop Med Hyg 1995 Mar-Apr;89(2):146-150.
21. Sonko ST, Jaiteh M, Jafali J, Jarju LB, D'Alessandro U, Camara A, et al. Does socio-economic status explain the

differentials in malaria parasite prevalence? Evidence from the Gambia. Malaria J 2014 Nov;13:449.

22. Leddy JP, Wilkinson SL, Kissel GE, Passador ST, Falany JL, Rosenfeld SI. Erythrocyte membrane proteins reactive with IgG (warm-reacting) anti-red blood cell autoantibodies: II. Antibodies coprecipitating band 3 and glycophorin A. Blood 1994 Jul;84(2):650-656.
23. Cribb AE, Pohl LR, Spielberg SP, Leeder JS. Patients with delayed-onset sulfonamide hypersensitivity reactions have antibodies recognizing endoplasmic reticulum luminal proteins. J Pharmacol Exp Ther 1997 Aug;282(2):1064-1071.
24. Riedl MA, Casillas AM, Adrian M, Castilas MD. Adverse drug reactions: types and treatment options. Am Fam Physician 2003 Nov;68(9):1781-1790.
25. Cribb AE, Lee BL, Trepanier LA, Spielberg SP. Adverse reactions to sulphonamide and sulphonamide-trimethoprim antimicrobials: clinical syndromes and pathogenesis. Adverse Drug React Toxicol Rev 1996 Mar;15(1):9-50.
26. Brackett CC, Singh H, Block JH. Likelihood and mechanisms of cross-allergenicity between sulfonamide antibiotics and other drugs containing a sulfonamide functional group. Pharmacotherapy 2004 Jul;24(7):856-870.
27. Shakoor MT, Ayub S, Ayub Z. Sulfa allergy, cross-reactivity versus multiple concurrent allergies. Am J Infect Dis 2013 Dec;9(4):148-154.
28. Klinker KP, Harbilas JW, Johns TE. Drug-Induced hematologic disorders. Pharmacotherapy. A pathophysiologic approach. 5th ed. McGraw-Hill: New York; 2002.
29. Sanhadji K, Chout R, Gessain A, Sasco AJ, Yoyo M, Mezard F, et al. Cell-mediated immunity in patients with sickle cell anaemia. Thymus 1988-1989;12(4):203-213.
30. Serjeant GR, Serjeant BE. Sickle cell disease. Oxford. Oxford University Press. 3rd ed. 2001.